वार्षिक प्रतिवेदन

Annual Report

2014 - 15

राष्ट्रीय पोषण संस्थान
National Institute of Nutrition
(भारतीय आयुर्विज्ञान अनुसंधान परिषद)
(Indian Council of Medical Research)

Annual Report

2014-15

CONTENTS

SCIENTIFIC STAFF

KALPAGAM POLASA, MSc, PhD, MBA
(Scientist 'G' & Director-Incharge)

CLINICAL

R. Hemalatha, MD
(Scientist 'F')
Bharati Kulkarni MBBS, DCH, MPH
(Scientist 'E')
G. Jagjeevan Babu, MBBS, MPH
(Scientist 'E')
K. V. Radhakrishna, MBBS, DCH
(Scientist 'E')
Sadhana Gupta, MSc, PhD
Raja Sriswan Mamidi, MBBS

PATHOLOGY

P. Uday Kumar, MD
(Scientist 'F')
SSYH. Qadri, MVSc
(Scientist 'E')
M.V.Surekha, MD

MICROBIOLOGY & IMMUNOLOGY

R. Hemalatha, MD
(Scientist 'F')
M. Shiva Prakash, MSc, PhD
(Scientist 'E')
Devraj J. Parasannanavar, MSc, PhD
A.Vijayendrachary, MSc (RF)
N.Himaja, MSc (RF)
D.Vasundhara, MSc (RF)

LIPID CHEMISTRY

A.. Vajreswari, MSc, PhD
(Scientist 'F')
S.Ahmed Ibrahim, MSc, PhD
(Scientist 'E')
S. M.Jeyakumar, MSc, MPhil, PhD
P. Sujatha, MSc, PhD
Anupama Tyagi, MSc (RF)
S.Anil, MSc (RF)
Ch. Anuradha, MSc (RF)
V. Anantha Krishna, MSc (RF)
J.Sugeetha, MSc (RF)
G.Rajgopala Chary, MSc (RF)

STEM CELL BIOLOGY

V.Vijayalakshmi, MSc, PhD
(Scientist 'F')
C. Suresh, MSc, PhD
(Scientist 'E')
A. Rajanna, MSc
M.Soundarya, MSc (RF)
Himadri Singh, MSc (RF)
K Sowmya, MSc (RF)

MOLECULAR BIOLOGY

Sudip Ghosh, MSc, PhD
(Scientist 'E')
Sanjay Basak, MSc, PhD
M.Srivani, MSc (RF)
B. Shankar Anand, MSc (RF)
G. Venkateswarulu, MSc (RF)
K. Sandeep Kumar, MSc (RF)

MICRONUTRIENT RESEARCH

K. Madhavan Nair, MSc, PhD
(Scientist 'F')
P. Raghu, MSc, PhD
P. Ravinder, MSc, PhD
Swarnim Gupta, MSc (RF)
A. Kiran Kumar, MSc (RF)
N. Pallavi, MSc (RF)
Y. Sravanthy, MSc (RF)
P.Ravindranath, MSc (RF)
M. Purna Chandra, MSc (RF)
Dripta Roy Choudhary, MSc (RF)
K.Archana, MSc (RF)

WORK PHYSIOLOGY

Y. Venkataramana, MSc, PhD
(Scientist 'F')
Deethu Sara Varghese, MSc (RF)

FOOD CHEMISTRY

T. Longvah, MSc
(Scientist 'G')
K. Bhaskarachary, MSc, PhD, PGDN & DM
J.Sreenivasa Rao, MSc
S.Devendra, MSc, Mphil, PhD
R.Ananthan, MSc, PhD
J. Vahini, MSc (RF)
Naveena Natrajan, MSc (RF)
Daniella Chyne, MSc (RF)

ENDOCRINOLOGY & METABOLISM

M. Raghunath, MSc, PhD
(Scientist 'G')
Ayesha Ismail, MSc, PhD
Jitendra Kumar Sinha, MSc (RF)
Shampa Ghosh, MSc. MPhil (RF)
Mehraj-ud-din, MSc (RF)
N.Bindu, MSc (RF)
M.Kiran Kumar, MSc (RF)
K.Nagabhushan Reddy, MSc (RF)

OCULAR BIOCHEMISTRY

G. Bhanu Prakash Reddy, MSc, PhD
(Scientist 'F')
P. Suryanarayana, MSc, PhD
(Scientist 'E')
N.Saravanan, MSc, PhD
S.Sreenivasa Reddy
V.Sudhakar Reddy, MSc (RF)
Sara Sarin Jose, MSc (RF)
K.Ashok Reddy, MSc (RF)
Madhusudhan A. Patil, MSc (RF)
S.Vishwaraj, MSc (RF)
Sneha Jakhotia, MSc (RF)
K.Shruthi, MSc (RF)
T.Shalini, MSc (RF)
K.Rajesh Kumar, MSc (RF)
P.Swathi Chitra, MSc (RF)

FIELD DIVISION

A. Laxmaiah, MBBS, MPH
(Scientist 'F')
R. Harikumar, MBBS, DPH
(Scientist 'E')
N. Arlappa, MD (Comm. Med.)
(Scientist 'E')
I.I.Meshram, MD(PSM)
K. Mallikharjuna Rao, MSc, PhD
M.S. Radhika, MSc, PhD
Sylvia Fernandez Rao, MA, PhD
Ch. Gal Reddy, MSc

BIOSTATISTICS

K. Venkaiah, MSc
(Scientist 'F')
M. Vishnuvardhan Rao, MSc, PhD, MTech (IT)
(Scientist 'F')
N. Balakrishna, MSc, PhD
(Scientist 'E')

PUBLICATION, EXTENSION & TRAINING

D. Raghunatha Rao, MSc, PhD, PG Dip. FNS
(Scientist 'F')
T. Vijaya Pushpam, MA, MPhil, PhD
(Scientist 'E')
M.Maheshwar, MCOM,MA,MCJ, LLB, Mphil
G. M. Subba Rao, MA, PGDJ, PGDT, PhD
K. Damayanthi, MSc, PhD

INSTRUMENTATION

V.Vijayalaxmi, MSc, PhD
(Scientist 'F')

FOOD & DRUG TOXICOLOGY RESEARCH CENTRE (FDTRC)

Kalpagam Polasa, MSc, PhD, MBA
(Scientist 'G' & Director-Incharge)

FOOD TOXICOLOGY

Arjun L Khandare, MSc, PhD
(Scientist 'F')
S.N.Sinha, MSc, PhD
(Scientist 'E')
J. Padmaja, MSc, PhD
(Scientist 'E')
V. Sudershan Rao, MSc, PhD
(Scientist 'E')
S. Vasanthi, MSc, PhD
P. Amrutha Rao, MBBS, DPH
V.Vakdevi, MSc, PhD
Priyanka Shankar, MSc (RF)
Agatha Betsy, MSc (RF)
M.Ankulu, MSc (RF)
Alekhya, MSc (RF)
Nivedita, MSc (RF)

DRUG TOXICOLOGY

M.P. Rajendra Prasad, MBBS, MSc (AN), PhD
(Scientist 'F')
B. Dinesh Kumar, MSc (Pharma), PhD
(Scientist 'F')
K.Nirmala, MSc, PhD
Nivedita Dube, MSc (RF)
A.Kiranmayee, MPharm (RF)
V.Varsha, MSc (RF)
Anita Singh, MSc (RF)

NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

Kalpagam Polasa, MSc, PhD, MBA
(Scientist 'G' & Director-Incharge)

P. Suresh Babu, MVSc
(Scientist 'F')
N. Hari Shanker, MSc, PhD
K.Rajender Rao, MSc, PhD
N.Muralidhar, MSc (RF)

ADMINISTRATIVE STAFF
(Ministerial & Secretarial)

SENIOR ADMINISTRATIVE OFFICER
N. Sitaramanjaneyulu
Wef 6.3.2014

ADMINISTRATIVE OFFICER
A.Suryaprakash Rao
M. Ashok Raj

ACCOUNTS OFFICER
N.Murali Krishna

SECTION OFFICERS
M. Vijay Kumar
G. Ramakrishna
Abdul Rasheed Shaik
Latha Kumarsswamy
M.Siva
P.Dhanasekharan
R.C.Padmini Mohan
K.Ch.Ramayya Dora
D.Venkateswarlu (adhoc)

PRIVATE SECRETARIES
Ch. Madhulatha [A.O (NNMB)]
P.Venugopal
Sudha Srinivasan
D.V.Laxmi Rani

ASSISTANTS
V.Laxminarayana
T.Satyanarayana
K.Sivarami Reddy
M.Rajagopala Chary
M.K.Koteshwara Reddy
V.Elisha
Alice Mary
D.Sunil
A.Narayana Reddy
M.Babu
C.Kalavathi
D.Seetharamaiah
E. Syama Sundari

PERSONAL ASSISTANTS
G.Hanumantha Rao (PS adhoc)
Malini V. Rao
G.Prashanthi
V.Swayam Prabha
K.Sailaja
G.Durga Prasad

UPPER DIVISION CLERKS
Shakila Banu (Asst. NNMB)
G.R. Srinivas (Asst. Adhoc)
K.Jayamma
P. Prabhavathi
Mohd. Iliyas
Shaik Jamaluddin
C.Prabhu
Mini Pramod
T.Anuradha Jayalaxmi
A.Narsing Rao
G.S.Gautami
A.Satyanarayana Prasad
Y.Bala Narayana

STENOGRAPHER
G. Mahesh Kumar (PA Adhoc)

LOWER DIVISION CLERKS
M. Rekha
D. Ramanjaneyulu
G.Y.Anita
A. Venkataramana
U. Somayya
M. Raghuram

RECEPTIONIST-CUM-TELEPHONE OPERATOR
M. Jawahar Joshua

LIBRARY STAFF

Asst. Library Information Officer
B.Narayana
Library Information Assistant
Prakash Kulkarni
Library Clerk
Ungarala Naidu

TECHNICAL STAFF

Technical Officer "B" (Tech. staff)

1. Ramachander Chaugule
2. S. Ananda Rao
3. Ch. Gal Reddy
4. Anil kumar dube
5. Sharad kumar
6. M. Ravindranath
7. K.Yadamma (adhoc)

Technical Officer "B" (Engg. Support staff)

1. Bandam Ramulu

Technical Officer "A" (Tech. staff)

11. G. Amarendra Reddy
2. A. Kasiviswaraja Mouli
3. V. Satish Babu
4. D.Sreeramulu
5. M.Satyavani
6. Virendra Vasant Panpatil
7. M. Krupadanam
8. R. Naveen Kumar
9. B.Narahari
10. Laxmi Rajkumar
11. K.Swaroopa Rani
12. Vani Acharya
13. C. Maniprabha
14. Abhay Kumar
15. K. Vinod Reddy
16. V. Vikas Rao
17. Kasinath Lingreker
18. R. Radhakrishna Sarma
19. V.Radhakrishna Rao
20. S. Prabhakar
21. D. Pandu Ranga Vittal
22. K. Nageswara Rao
23. Ch. Nagambika Prasad
24. S. Lopamudra
25. S. Hemalatha
26. Amulya Rao
27. G. Shanker Rao
28. K. Srinivasa Rao
29. B. Pothu Raju
30. Korra Mangthya
31. R. Ravindar Naik
32. D.Vijaya Bhaskara Rao
33. P. Ajey Kumar
34. Saleem Shariff
35. G.V. Narasimha Rao
36. N. Sreenivasa Chary
37. B. Venkateswara Rao
38. P.Krishnaswamy
39. G. Chenna Krishna Reddy
40. K. Subash
41. K. Narasimha Reddy
42. T. Nagasekhara Rao
43. Ch. Ranga Rao

Technical Officer "A" (Engg. Support staff)

1. Mota Chandrasekhara Rao
2. P. Moses Ashok Kumar
3. U.D. Awasthi
4. G. Mohan Rao
5. G.B. Walter
6. L. Vijaya durga
7. B. Om Prakash
8. A. Santosh Kumar
9. N. Satyanarayana

Technical Assistant (Tech. staff)

1. K. Sharada
2. P. Sailaja
3. D. Prem Raj
4. B.R. Annapurna
5. Ch. Hanumantha Reddy
6. Ch. Krishna
7. V. Bhasker
8. P. Yadagiri Reddy
9. M. Seshacharuyulu
10. S.A. Brinda
11. K. Vasudev
12. M. Srinivas
13. K. Suryam Reddy
14. S. Laxman
15. B. Tulja
16. Narottam Pradhan
17. K. Swatantra Rao
18. Madhusudhana Chary
19. P.S.Rama Rao

Technical Assistant (Engg. Support staff)
1. G. Janardhan
2. M. Asaithurai
3. Micheal Fernandez
4. A. Anjaiah
5. T. Shyam Sunder
6. Joseph Vijaykumar
7. YVL Narasimha Rao
8. Mohd. Younus
9. G.P. Narender

Driver (Special Grade)
1. P. Mahender

Technician "C" (Tech. staff)
1. R. Raghunath Babu
2. K. Usha Rani
3. C. Sai Babu
4. P. Satish Babu
5. C. Janardhan
6. G. Govindarajulu
7. P.S. Prashanthi
8. S.P.V. Prasad
9. K. Sree Ramakrishna (on deputation NNMB)
10. P. Anitha Chauhan
11. G. Madhavi
12. B. Giri Babu
13. G. Venkataraji Reddy
14. S. Ashok
15. G.l. Stephen
16. G.A. Rabbani
17. E. Sammi Reddy
18. K. Balaji
19. M. Sripal Reddy
20. Y. Salaiah

Technician "C" (Engg. Support staff)
1. K. Pavan Kumar
2. Syed Jalaluddin Hussaini
3. R. Sahadeva
4. J. Kumaraswamy
5. A.l. Goverdhan
6. K. Srenivasa Raju
7. N. Narasimha
8. Purnachandra Beshra
9. P. Dasarath
10. S. Devendran
11. Ramavath Ramsingh
12. Sriramulu Naidu
13. E. Srinivas
14. V. Bhuvaneswaran
15. Polishetty Naidu
16. P. Narender Kumar

Technician "B" (Tech. staff)
1. N. Peddi Reddy
2. Y. Agreepa Raju
3. P. Sashidharan Pillai
4. Gandamalla Narasimha
5. P. Bheem Shanker
6. J. Pochaiah
7. M. Balram
8. S. Chandraiah
9. B. Nagender rao
10. Prabhu Raj
11. P. Nagabhashunam
12. Nigala Yadagiri
13. E. Krishna
14. Bommaka Srinu
15. Neelakanta
16. L. Dasu
17. D. Dasaratha
18. J. Nageswara Rao
19. C. Chandramouli
20. Abdul Sattar
21. N. Rajaiah.
22. K. Rama Rao
23. V. Rajkumar

Technician "B" (Engg. Support staff)
1. B. Bal Reddy
2. J. Bhujender
3. K. Parthasarathy
4. D. Ravinder
5. N. Ramesh Kumar
6. Y. Veeraiah

Technician 'A' (Engg. support staff)
1. Mahender Singh Jadav
2. E.Ganesh
3. M.Narasimha

Driver (Grade – I)
1. Zahid Ali Khan
2. K. Krishna
3. V. Kondaiah
4. Syed Mohd. Ali

Driver (Grade – II)
1. D. Amruthanathan
2. K. Jangaiah

Junior Staff Nurse
1. G. Rajakumari
2. B.V. Nancharamma
3. D. Threessamma
4. D. Rani

5. K. Venkataramana
6. S. Rojamani

Auxiliary Nurse Midwife

1. K. Santhosham
2. Ch. Anitha
3. G. Tulasi Bai
4. V. Aruna Reddy
5. E. Sheela
6. G. Vijayalakshmi

Nursing Attendant

1. R.Rajyalakshmi
2. Govada Bhavani
3. Valentina Teriscova
4. D.Swarupa

Senior Cook (Isolated Post)

1. H.S. Ramu

Attendant (Services)

1. K.B. Raju
2. C. Shankaraiah
3. Manga Narasaiah
4. M. Eshwar
5. Abdul Bhasid
6. G. Eswaraiah
7. G. Viswanatham
8. M. Suresh
9. Mohd. H. Yousuf
10. Mohd. Abdul Khader
11. Bondi Ramulu
12. J. Yadagiri
13 Syed Mohd. Iqbal
14. C. Rajaiah
15. Mabbu Ramulu
16. V. Shanker
17. Kompally Pochaiah
18. A. Narasaiah
19. Mukkera Krishna
20. Mohd. Mehboob
21. J. Lakshmaiah
22. K. Rajaiah
23. P.V. Poulous
24. Manupathi Bikshapathi
25. Dhanavath Saida
26. V. Dasaratham
27. Manmohan Meena
28. Srihari Ram
29. Ch.Guruswamy
30. Mohd. Maqbool
31. P. Shiva Shanker
32. S. Hanumantha Rao
33. K. Chandran

Attendant (Services) Contd..

34. Mirza Ghouse Baig
35. G. Yadagiri
36. Mohd. Yaseen
37. K. Balraj
38. R. Narasimhulu
39. Mohd. Chand
40. Mohd. Maulana
41. Shaik Mukthar
42. K. Kasipathi
43. M. Leela
44. Manchikanti Krishna
45. Syed Asif Ali
46. K. Gopal
47. B. Eswaraiah
48. Mohd. Issamaiah
49. K. Harinarayana
50. E. Mallesh
51. Mohd. Sabeer
52. K. Narender
53. Y. Ramulu
54. M. Somaiah
55. G. Venkatesh
56. V. Somaiah
57. E. Marthamma
58. T. Govind
59. P. Srihari
60. Mohd. Habibuddin
61. A. Venugopal
62. M. Kisan
63. B. Nageswara Rao
64. P. Nagulu
65. M. Seenu
66. B.K.Mahadevaiah

Attendant (Services) Contd..

67. A. Chandraprakash
68. M. Jayamma
69. D. Venkaesh
70. M. Satyamma
71. C. Sivaleela
72. G. Satyapual
73. A. Narsing Rao
74. A. Lakshmi
75. Majeed Shareef
76. M. Upender
77. R. Punna Reddy
78. K. Srinu
79. M. Narsing Rao
80. A. Shanker
81. P. Ravinder
82. D. Madhava Reddy
83. B.V. Sudershan Babu
84. I. Posheety
85. G. Yadagiri
86. M. Venkataiah
87. N. Bhasker
88. A. Jangaiah
89. P. Dasarath
90. S. Narahari
91. K. Venkatesh
92. P. Narasimha
93. E. Kondal Reddy
94. K. Venkat Reddy
95. G. Upender
96. M. Koumura Reddy
97. Ch. Shanker
98. G. Saraswathi
99. P. Balrajun

RESEARCH HIGHLIGHTS

1. COMMUNITY STUDIES

1.1 Childhood obesity in India: A multi-centric study on its measurement and determinants

It is a task force study of ICMR carried out at two sites in Hyderabad and New Delhi to develop reference curves of body fat (content and distribution) among Indian children aged 6 to 18 years and to identify the best possible cut-off measures of body fat that predict unacceptable cardio-metabolic risk factors for defining body fatness among Indian children. The study also aims to identify determinants of childhood body fatness that operate at different levels across five diverse socio-cultural and geographical contexts of India.

1.2 Assessment of effect of consumption of Khesari dal on human health

This epidemiological study was carried out by NIN at the request of the planning commission, with an objective to assess the effect of consumption of Khesari dal on human health in the state of Chhattisgarh, to help the government take decision on whether to lift or not to lift the ban. The mean consumption of Khesari dal was only 3.5 g/CU/day, out of 50g of total pulses consumed daily. Only nine suspected old cases of neurolathyrism have been identified in the state.

1.3 Assessment of nutritional status of children below the age of five years and performance of ICDS functionaries in the state of Haryana

The prevalence of undernutrition especially among children under the age of five years may vary geographically. Therefore, for the development of area specific intervention strategies, divisional/district level mapping of undernutrition is very essential. The prevalence of undernutrition (<Median -2SD) among <5 year children such as underweight, stunting and wasting was 28%, 34% and 11% respectively. However, the magnitude of undernutrition was higher in the divisions of Gurgaon and Hissar compared to Ambala and Rohthak.

2. MICROBIOLOGY AND IMMUNOLOGY

2.1 Vitamin D status, vitamin D receptor expression and their link with CD23/ CD21 interaction and regulatory T cell function in children with allergy

Regulatory T cells and IgE receptors (CD23 & CD21) on B cells were assessed in vitamin D deficient pregnant women. The results are as follows:

The maternal and cord blood Treg cell population and regulatory cytokines, TGF β and IL-10 were significantly lower in pregnant women with vitamin D deficiency, while CD23 and CD21 were higher. The regulatory T cell transcription factor FOXP3, vitamin D receptor (VDR) and retinoic acid receptor (RXR) expressions were down-regulated, while CD23, CD21 and VDBP expressions were up-regulated in placenta of vitamin D deficient pregnant women. Vitamin D regulating enzymes (CYP24A1, CYP2R1 and CYP27B1) expression were also altered in placenta of women with vitamin D deficiency. The current study shows that impaired maternal vitamin D during pregnancy influences the spectrum of immune cells such as regulatory T cells and B cells with IgE receptors and this in turn may be linked to allergy and asthma in neonates.

2.2 Towards the Development of edible vaccine against Helicobacter pylori with recombinant lactobacilli expressing Heparan Sulfate binding protein

Cell surface display of antigens on L. rhamnosus GG strain was developed by using signal peptide

CspI from L. plantarum and anchor peptide PrtR from L. rhamnosus having MPQTG type anchor that is specific for L. rhamnosus strains.

The food-grade host, L. rhamnosus GG with pSIP503 expression vector was used with erythromycin genes as selection marker and nisRK promoter for cell surface expression of foreign proteins. This system developed by here may be very useful as antigen delivery vehicle in oral vaccine formulation.

2.3 Characterization of vaginal lactobacilli isolated from healthy women to formulate potential probiotic for reproductive health

L. crispatus (100%) was found to be the most predominant lactobacilli species present in pregnant women with normal flora followed by L. jensenii (72%), L. gasseri (66%), L.acidophilus (32%)

L. crispatus was found in 76% of women with abnormal flora followed by L. jensenii (61%), L. gasseri (41%) and L. acidophilus (24%). Whereas, L.reuteri was more (24%) commonly associated with abnormal flora than normal flora (7%). This preliminary data might be useful for the development of probiotic formulations for reproductive health of women in India.

3. BASIC STUDIES

3.1 Innovative strategies to promote early child development among low-income rural infants and pre-schoolers in India through multiple micronutrient fortification and early learning opportunities

Project Grow-Smart, a randomized controlled interventional trial was carried out among infants and pre-schoolers in 26 villages of four state administrative blocks (mandals) from Nalgonda District in the state of Andhra Pradesh (now in Telangana), India. The infant (6-12 mo) intervention was a home-based four-arm trial consisting of two interventions of 12 months duration i.e., multiple micronutrient powder, early learning, combination and a placebo. Pre-school (36-48mo.) phase was a point-of use cluster-randomized two arm trial (MNP and placebo) stratified across low and high quality Anganwadi centres for 8 mo. The MNP intervention consisted of iron and six haemopoietic micronutrients i.e., vitamins A, C, B2, B12, folic acid and zinc and placebo only vitamin B2 based on Indian RDA for the corresponding group.

Baseline data showed iron deficiency and maternal anaemia as the major factors associated with anaemia among young children. Additionally, inflammation was associated with anaemia among pre-schoolers.

MNP intervention resulted in significant anaemia reduction in both infants (67 to 50 % in MNP against 66 to 72% in placebo) and pre-schoolers (46 to 10 % in MNP against 48 to 35 % in placebo). There was a significant improvement in biomarkers of iron in both groups. MNP and early learning improved cognition in infants. Among pre-schoolers, MNP reduced disparities between children in low and high quality anganwadis by improving development in children in low quality preschools. Findings from this home and pre-school based integrated trial can be used to guide larger-scale policy and programs designed to promote the developmental, education and economic potential of young children in rural India.

3.2 Efficacy of polyphenol-rich dietary ingredients as proteasome inhibitors and their role as anticancer agents

Dietary ingredients are rich sources of polyphenols/ flavonoids and these plant metabolites are known to act as proteasome inhibitors. Inhibition of the proteasome leads to cancer cell death and is thought to be a promising approach for cancer therapy. Murraya koenigii leaf (curry leaf) is a rich source of polyphenols. M. koenigii leaf extract (MLE) inhibited enzyme activities of the 26S proteasome and led to cell death and growth arrest in two human breast cancer cell lines. Further, the leaf extract resulted in growth suppression of breast tumor xenografts. Reduction in tumor growth was associated with decrease in proteasomal enzyme activities in the treated groups. Increased caspase-3 activity and TUNEL-positive cells indicated enhanced apoptosis with MLE treatment. Decreased expression of

angiogenic and anti-apoptotic gene markers is indicative of inhibition of angiogenesis and promotion of apoptosis in the leaf extract treated tumors. In addition, increased phosphorylation of p38 and decrease in phosphorylation of Akt may contribute to the anti-proliferative and pro-apoptotic effect of M. koenigii leaf extract.

3.3 Reduced longevity in WNIN/Ob rats: Possible role of changes in neuronal metabolism and neurochemical profile

Volume changes in the brain determined in the WNIN/Ob obese and normal WNIN rats of comparable age using Magnetic Resonance Imaging (MRI) did not show any significant differences between the age matched obese and control rats in various brain regions analyzed. This falls in line with our previous observations of wet brain weights, which although were lower in WNIN/Ob obese rats than WNIN normal control rats, the differences were not statistically significant. Further, our findings on neurochemical profile indicate that at a young age itself the neurochemical profile of these animals is getting altered and resembles the changes seen in the control rat brains at later age (e.g. 15-18 months, further corroborating the accelerated ageing of the WNIN/Ob obese rats. Also, it was observed that neuronal glucose metabolism was somewhat lower in WNIN/Ob rats compared to controls of comparable age further corroborating accelerated ageing of the WNIN/Ob obese rats.

3.4 Possible utility of human umbilical cord blood or placenta derived mesenchymal stem cells for Toxicity studies

The in vitro micronucleus test is a well-known test for the screening of genotoxic compounds. However, until now, most studies have been performed on either human peripheral lymphocytes or established cancer cell lines. It is perhaps demonstrated for the first time that the use of a normal diploid umbilical cord-mesenchymal stem cells can be used as an alternative to the conventional micronucleus test.

Umbilical cord mesenchymal stem cells (UC-MSCs) were grown on the coverslips eliminating the cumbersome technique involving hypotonic treatment, fixation and preparing smears required for suspension culture (lymphocytes). A platform was created for simultaneous testing of cytotoxicity and genotoxicity of pharmaceuticals. The cytotoxic and genotoxic effects of two known mutagens, mitomycin-C and hydrogen peroxide (H2O2), on UC-MSCs, lymphocytes and A549 cells were inspected. Treatment with mitomycin-C and H2O2 demonstrated drastic differences in the degree of cytotoxicity and genotoxicity suggesting a constitutional difference between normal and cancer cells. In addition, two solvents, dimethyl sulfoxide (DMSO) and ethanol, and two drugs, metformin and rapamycin were tested. DMSO above 1% was found to be cytotoxic and genotoxic, whereas ethanol at same concentration was neither cytotoxic nor genotoxic, indicating the minimal non-toxic level of the solvents. This study thus offers UC-MSCs as a better substitute to peripheral lymphocytes and cancer cell lines for high throughput screening of compounds and reducing the animal studies.

3.5 Status of vitamin-D in type 2 diabetes patients with and without retinopathy

In this cross-sectional case–control study, the status of vitamin D (VD) in type 2 diabetic (T2D) patients with and without diabetic retinopathy (DR) has been reported. While mean plasma VD levels were significantly lower in T2D patients without (NDR) and with DR groups compared with the control group, there were no significant differences between the diabetes groups. The prevalence of VD deficiency (VDD) was higher in NDR and DR groups (66% and 63%) compared to age-matched controls (45%), suggesting that prevalence of VDD was higher in diabetic groups, irrespective of presence or absence of retinopathy.

3.6 Ellagic acid ameliorates diabetic retinopathy by inhibition of AGE formation in rats

Hyperglycemia in diabetes leads to accumulation of AGE which activates VEGF through the receptor for advanced glycation end products (RAGE) to trigger angiogenesis in retina. AGE also induces apoptosis of pericytes through interaction with RAGE and induction of VEGF. Using a range of in vitro protein glycation models, we reported that ellagic acid (EA), present in many dietary sources,

prevents AGE formation. In this study we demonstrated that EA inhibit AGE formation and activation of RAGE in retina under hyperglycemic conditions. Further, AGE-RAGE mediated cellular events and subsequent functional abnormalities are modulated by EA which may throw light on the potential of therapeutic avenues for treatment of diabetic retinopathy.

3.7 Response of small heat shock proteins in diabetic rat retina

Small heat shock proteins (sHsp) play a critical role under stress conditions to maintain cellular homeostasis. The hyperglycemia in diabetes may impose cellular stress on retina. In this study, we reported that increased expression of sHsp: αA-crystallin (αAC), αB-crystallin (αBC) and Hsp22 in diabetic retina; There was increased phosphorylation of αBC under diabetic conditions. Moreover, diabetes activated the p38MAPK signaling cascade by increasing the p-p38 MAPK in retina. These results suggest that specific sHsp are crucial for neuronal protection in diabetic retinopathy (DR) and may aid in developing therapeutic strategies for DR.

3.8 Altered ubiquitin-proteasome system leads to neuronal cell death in a spontaneous obese rat model

Obesity is associated with various progressive age-related diseases, including neurological disorders. However, underlying molecular basis for increased risk of neurodegeneration in obesity is unknown. Using a spontaneous obese rat (WNIN/Ob) model we have described neurodegeneration due to obesity. Altered ubiquitin-proteasome system (UPS), existence of ER stress, up-regulation of apoptotic markers and apoptosis was found in the cerebral cortex of obese rats. This study highlights the role of altered UPS in neurodegeneration due to obesity.

3.9 Role of growth hormone in podocyte injury and depletion: Implications in proteinuria

Loss of glomerular podocytes is a critical event in the pathogenesis of proteinuria during diabetic nephropathy (DN). Increased level of growth hormone (GH) is implicated as a causative factor in the development of nephropathy in type 1 diabetes. However, the molecular basis for the effects of GH on podocyte depletion is not understood. Our studies reveal that GH increases expression of transforming growth factor-beta-induced protein (TGFBIp) and also increased secretion of extracellular TGFBIp. Both GH and TGFBIp induced apoptosis and epithelial mesenchymal transition (EMT) of podocytes. Administration of GH to rats induced EMT and apoptosis in the kidney. Therefore, GH-dependent increase in TGFBIp in the podocyte could be one of the mechanisms responsible for podocyte depletion in DN.

3.10 Effect of reactive oxygen species on macrophage signalosome: Impact on antigen presentation function and T cell priming responses

Although reactive oxygen species (ROS) are important for induction of cytotoxic effects against invading pathogens, it is not very clear whether excess ROS production can actually underweigh the beneficial outcome of the innate immunity. Oxidative stress has been implicated in manifesting detrimental effects of various disorders like cancer, aging, diabetes, atherosclerosis and infection. Interestingly, most of these pathophysiological disorders are found to be associated with severe immune suppression, indicating that excessive production of ROS could be one of the factors responsible for development of immune suppression in these situations. Therefore, it was aimed to understand whether oxidative stress has a direct link with the observed immunosuppression in pathophysiologcal conditions, in other words whether it has any actual direct effect on the T cell priming responses and the macrophage signaling cascades required for development of subsequent adaptive immune response.

It was found that excess ROS can inhibit MHC Class I and Class II presentation of exogenous ovalmumin and inhibit processing of antigens by professional APCs like macrophages. Even presence of physiological concentrations of ROS could significantly inhibited MHC Class II-mediated antigen processing and presentation. ROS was found to target c-rel transcription factors in macrophages as over-expression of c-rel was found to ameliorate antigen processing and presentation

inhibited by ROS. Interestingly, excess exogenous ROS was not found to significantly affect surface expression of co-stimulatory molecules like MHC-I, MHC-II and CD80, considered to be important for antigen presentation. ROS was also found to inhibit calmodulin expression. Our results indicate that inhibition of antigen presentation by ROS might is probably due to modulation of calmodulin-c-rel signaling pathway, rather than weakened co-stimulatory signaling.

4. PUBLICATION, EXTENSION AND TRAINING DIVISION

4.1 Promoting the use of food label information among school-going adolescents

A nutrition education intervention study evaluated the impact of a label information reading kit on usage of food labels among adolescents for promoting healthy food choices. The kit titled 'Read-B4-U-Eat' was developed and theories of social-cognition and shared-learning were used to develop the module's five components – interactive sessions for guided learning; booklet for self-learning; 9 posters for shared learning; animation film (4 min.) for edutainment; and notes for teachers for reiteration. The kit was efficacious nutrition education module to inculcate label reading skills among adolescent consumers.

5. PATHOLOGY

5.1 Knowledge, Attitude and Practices [KAP] of medical practitioners in Hyderabad regarding food allergy (FA) - a pilot study.

A total of 300 medical professionals including gastroenterologists, pediatricians, dermatologists, pulmonologists, general practitioners and homeo-physicians (50 from each category) were contacted and administered a questionnaire. More than 50% knew that FA is a serious problem but did not responded correctly to questions on difference between food allergy and intolerance. About 54% had very little knowledge about prevalence and published literature on food allergy. Over a third of them (37.6%) reported that they rarely refer such patients for further evaluation. The medical practitioners were not sufficiently knowledgeable regarding the diagnosis, treatment and risk factors for food allergies and related anaphylaxis.

6. FOOD CHEMISTRY

6.1 Bioactive phytochemicals in Indian foods

There is a considerable evidence for the role of antioxidant constituents of fruits and vegetables in the maintenance of health and disease prevention. Studies also showed that phytochemicals some of them not necessarily antioxidants but are also bioactive and help in preventing many chronic degenerative diseases. This research project made us to standardize the separation of many polyphenolic constituents of foods. To appreciate and to understand important minimal constituents of plant foods to our health, the composition of bioactive phytochemicals in cereals, pulses, fruits, vegetables, and their processing effects were studied. This project generated database on polyphenolic compounds in commonly consumed Indian foods.

6.2 Studies on gastro-protective effects of 'King chilli'

Studies on gastric protection and mineral absorption using Naga King Chilli was carried out in ethanol induced ulcer model for a period of 24 days (sub-chronic) and 90 days (chronic). Experiments were conducted in male SD rats using ethanol induced ulcer model. Findings of this study demonstrated the gastroprotective effect of Naga King chili against gastric mucosal damage induced by ethanol. The observed gastroprotection is possibly mediated to a major extent by the local inflammatory mechanism followed by antioxidant mechanism. Results clearly shows that increased mucosal content and CGRP were higher in the groups treated with capsaicin or Naga king chili followed by ethanol. Elevated levels of antioxidant enzymes in the capsaicin+ethanol and Naga king chili+ethanol groups clearly demonstrates the ability of restoration of epithelial cells by capsaicin and Naga king chili. Therefore, the combined effect of antioxidant and other complimentary mechanism such as CGRP and PGE are accompanied to protect gastric ulcer induced by ethanol. The present

findings of gastro-protection by Naga king chili not only supports the traditional consumption of chili but also stress the need for the development of potential therapeutic drugs.

6.3 Prebiotic effect of legume raffinose family Oligosaccharides

The analysis of oligosaccharide content of commonly consumed legumes showed the presence of raffinose family of sugars with varied concentration. These sugars cannot be hydrolyzed and absorbed in the intestine, due to the lack of -galactosidase activity in the small intestine but undergo anaerobic fermentation by bacteria in the large intestine, which may result in the production of flatus gases (H2, CO2 and small amount of CH4). The effect of different house hold processing methods led to decrease in the levels of raffinose family sugars.

Oligosaccharide fermentation in the caeco-colon by the bacteria can give many positive health benefits as prebiotics. The prebiotic potential of legume oligosaccharides in animal model were shown decrease in blood glucose level, improved lipid profile, increased mineral absorption, improved body mass composition and inflammatory markers. The ceacum sample analysis showed that increase in the gut bacterial colonies of lactobacillus, bifidobacteria, enterobacillus and bacteroides by decrease in the pathogenic putrificative bacterial counts. There was reduction in inflammatory cytokines like IL-1ß, IL-6, TNF-α and INF-γ and CRP in experimental animals supplemented with legume prebiotics. No such changes were seen in control group. Further research is required to understand the role of legume prebiotics to prevent or control diabetes, obesity, cardiovascular diseases, irritable bowel syndrome and other health benefits.

7. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

7.1 Serotyping RNA virus to study molecular epidemiology of dengue supplementing emergency preparedness and capacity building in metro cities of Karnataka, India

Geographic data was used to correlate genetic analysis to disclose the spread of dengue virus lineages in urban and metro areas of city. It suggests that a pattern of dispersion is consistent with dispersion rate mostly human accidental transport. The collection of samples in endemic areas averaged within a diameter of 3-4 kilometers. The data stressed the importance of mosquito and human circulation; do play an important role in dispersal of viruses. As the egg of the vector mosquito Aedes aegypti are resistant to desiccation, it may have important implications for cryptic maintenance of viral strain. Therefore, viral strain not detected in any season can re-emerge in the next rainy season due to trans-ovarian transmission. So, availability of sample serotypes in any particular urban/metro area of Bengaluru and Mangalore could not be ascertained..

7.2 Empowerment of farm women in mitigating the pesticide residues at the farm and household levels

About 300 vegetables/fruits were analysed for various pesticide residues at different stages of household processing (1. raw;2. after soaking in distilled water; 3. after rubbing and again soaking in distilled water and 4. after soaking in 2% sodium chloride solution).

A significant reduction in the residue levels was observed after subjecting to the rubbing and again soaking in distilled water. No significant difference was found in the mineral contents viz., Ca, Mg, K, P, Na, Cu, Mn and Zn in the vegetable/fruit samples after subjecting to any processing stage.

Further, extensive educational activities were undertaken for both the farmers and farm women. Under the translational researchwork, novel cost effective protective devices were developed for the farmers and farm women to be used while working in the agricultural farms.

7.3 Quantitative detection of heavy metals and phthalates in toys

This study was formulated to determine the levels of heavy metals namely Lead (Pb), Cadmium (Cd), Chromium (Cr), Arsenic (As) and Mercury (Hg) in toy samples obtained from four different geographical regions (North, South, East and West) of India. The study also aimed to estimate the

phthalates Di butyl phthalate (DBP), Benzyl butyl phthalate (BBP), Di ethyl hexyl phthalate (DEHP), Di–n– octyl phthalate (DNOP), and Di-isononyl phthalate (DINP) content in the sampled toys. To compare the level of heavy metals and phthalates in toy samples collected from rural as well as urban areas and between the three different categories of toys – local, unbranded and branded. The results revealed that 95% of toy samples were found to have lead level within permissible limits (90 mg/kg); 98% of toy samples were found to have Cadmium level within the permissible limits (75 mg/kg); chromium level was detected below 60 mg/kg (permissible limit) in 96.5% of the samples. Arsenic level was within 25 mg/kg (permissible limit) in 97.8% of the samples. Most of the samples exceeding this limit were those collected from south zone. Mercury was within the permissible limit (60 mg/kg) in all the samples.

7.4 Evaluation of the impact of genetic polymorphism on pharmacodynamic activity of commonly prescribed antihypertensive drugs (thiazide diuretics, ace inhibitors, CCBS and β-blockers)

The prescription profile of anti-hypertensive drugs was assessed and it revealed that monotherapy is chosen over the combination therapy. The pattern of prescription adhers to JNC VII except for diuretics. The antihypertensive potential of drugs were reflected only in 30-40% of subjects, therefore, calling for an investigation to assess role of genetic variations along with nutrient interactions.

8. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

8.1 Effect of environment and microorganisms in the development of obesity in WNIN Obese rats

The species of Firmicutes detected by FAME (Fatty Acid Methyl Ester) analysis in obese rats (cecal samples) were Staphylococcus-cohnii and Staphylococcus epidermidis. Of these two species identified, Staphylo-coccus-cohnii was detected in more number of samples when compared to Staphylococcus-epidermidi. In Lean rats Bacillus-subtilis, Bacillus-GC group 22 and Paenibacillus-validus were identified. Of these three species, Bacillus-subtilis was found in more number of samples followed by Paenibacillus-validus and Bacillus-GC group 22.

The species of Bacteroidetes detected by FAME analysis in obese rats cecal samples were Prevotella-melaninogenica, and Eikenella-corrodens. Among these Prevotella-melaninogenica was found more in obese rats cecal samples when compared to Eikenella-corrodens. However, in Lean rats cecal samples among the two species identified Prevotella-melaninogenica and Eikenella-corrodens, Eikenella-corrodens was found more than Prevotella-melaninogenica.

The data supported the key idea that the gut microbiota can contribute to the patho-physiology of obesity. This could be considered when developing strategies to control obesity and its associated diseases by modifying the gut microbiota. This is a preliminary data of pilot experiment to get the lead for taking up a full fledged study. With additional studies this work could lead to identification of microbial markers that make up a kind of obesity or leanness profile in animals a vital-stats sheet of the gut world that would help understanding how people are likely to respond to microbes. Beyond that, the knowledge gained from this study can be applied to agriculture science and we could grow foods that are specifically designed to provide the optimal balance of nutrients and energy for various life stages.

9. PRE-CLINICAL TOXICOLOGY

9.1 Pre clinical safety evaluation of vegetables cultivated using deoiled Karanja seed cake

The study assessed the safety of vegetables (Amaranthus, Onion, Tomato) cultivated using Karanja seed cake (Expelled and deoiled cake) in rats. There was no mortality in any group of animals which received test material (Amaranthus- T1, T5 & T10 ,Onion - T1, T5 & T10 , Tomato - T1, T5 & T10) cultivated using karanja seed cake in SD Rats for 90 consecutive days it did not show any adverse effects on feed and test material intake, body weights, cage side activities, clinical chemistry and heamotology profile. Histopathological study showed organ changes in various groups but it may not be attributed to test material given at various doses and for whole study period.

9.2 Pre-clinical evaluation of iron filings in CTC-Tea and its brew

Safety profile of CTC tea was assessed in Rats. There was no mortality in groups of animals fed IF directly and CTC with Iron filling (10mints) which is 10 times more than permissible limits daily for 28 days. The early pre-terminal mortality was recorded in orthodox, CTC (5mints) and CTC+IF (5 mints.) tea brew perhaps due to technical errors in feeding process. The post exposure study results of live phase activity, clinical observations, chemistry and hematology and various histopathology observations did not appear to be due to exposure to test material, serum and liver ferritin, gross necropsy investigation of vital organs didn't suggest any abnormal finding in spite of exposure to CTC with iron filings more than 10times of permissible limit (250ppm) in experimental conditions.

I COMMUNITY STUDIES

1. CHILDHOOD OBESITY IN INDIA: A MULTI-CENTRIC STUDY ON ITS MEASUREMENT AND DETERMINANTS

According to the report of International Obesity Task Force (IOTF), in the year 2000 globally about 10% of the young people aged 5-17 years were overweight, out of which 2-3% were obese**.** In a recent review from developed countries, the prevalence of overweight youth (10-16 years) was > 15% (up to 30%) in the North American countries (Canada, USA), Great Britain and some South Western European Countries (Greece, Italy, Malta, Portugal, Spain), while in developing countries, it is limited especially for older children (> 5 years old) and adolescents. In Egypt 14% of adolescents and 25% of 6-11 year old children in Cyprus were reported to be overweight or obese. In a review of WHO global database, higher prevalence of overweight among preschool children was reported from Latin America, Caribbean, Middle East and North African countries. But, Asian countries had highest absolute number of overweight children (about 60%) hailing from the developing nations. Especially, it is ever increasing in India, since 2000. Latin American countries such as, Mexico and Brazil also reported high prevalence of overweight and obese (>25%) children. In the given context, a study is proposed to be carried out the above study in Southern India with the following objectives:

PRIMARY OBJECTIVES

It has two components; the quantitative (measurement of childhood obesity) and the qualitative (determinants of childhood obesity).

Quantitative component (PART I Study)

- To develop reference curves of body fat (content and distribution) among Indian children aged 6 to 18 years in Delhi and Hyderabad (2 regions),
- To identify the best possible cutoff measures of body fat (content & distribution) that predict unacceptable cardio-metabolic risk factors for defining body fatness among Indian children.

Qualitative component (PART II Study)

- To identify determinants of childhood body fatness that are operating at different levels across five diverse socio-cultural and geographical contexts of India (5 regions),
- To estimate the influences of these determinants at family, community, school, regional level across five geographic regions of India, and at national level(5 regions).

SECONDARY OBJECTIVES

- To build capacity of researchers in the realm of childhood obesity-related health research in India.

A detailed proposal was provided in the Annual report of 2010-11.

METHODOLOGY

This proposal is a part of 'ICMR Task Force' multi-centric study by Indian Council of Medical Research aimed for undertaking a comprehensive program on childhood obesity. The quantitative part was carried out by the Institutes i) All India Institute of Medical Sciences, New Delhi, and ii) National Institute of Nutrition, Hyderabad. The qualitative part was carried out by five Institutes; i) All India Institute of Medical Sciences, New Delhi, ii)

National Institute of Nutrition, Hyderabad, iii) Government Medical College, Srinagar, iv) MP Shah Medical College, Jamnagar, and v) North Eastern Indira Gandhi Institute of Health and Medical Sciences, Shillong.

Study design: It was a cross sectional and Institutional study. The children were recruited between 6-18 years of age from the select schools of Rangareddy district, Telangana.

Computation of Sample size

Developing reference curves of body fat for Indian children aged 6-18 years

For drawing comprehensive information on the dispersion of the body fatness and identifying the abnormal values, LMS (least mean square method) was used to construct normalized body fat standards and smoothened centile curves. About 100 boys and 100 girls in each one-year age band were recruited. The results of previous study indicate that a sample of 100 in each age and sex band shall be robust enough to give us the desired information. The age group under study is 6-18 years i.e., 13 age bands. Therefore, the total children covered were 2600 children (1300 boys and 1300 girls) from both the sites (Hyderabad and Delhi).

Correlating measures of body fat to cardio-metabolic risk markers: Literature reports correlations between body fatness measures and cardio-metabolic risk factors varying between 0.1-0.7. A minimum correlation of 0.3 between body fatness (total fat content/distribution) and health outcomes was considered to have reasonable biologic, public health and clinical significance. Multiple cardio-metabolic risk factors will be measured and correlated with body fat variables either alone or as combinations arrived at through factor analysis.

A sample size of 100 boys and 100 girls in each age band will allow us to detect a correlation of 0.3 between the cardio-metabolic risk factors and body fat measures at 5% level of significance with 80% power. Thus sampling strategy and size proposed for the study for the reference curves development will allow us to determine optimal cut offs for each age band. Since we shall be relating *combinations* of health outcomes (e.g. blood pressure, apo-lipoprotein A & B, glucose and insulin) to dependent variables (e.g. body fat), correlation is likely to be >0.3 and power $\approx$ 90% after controlling for covariates.

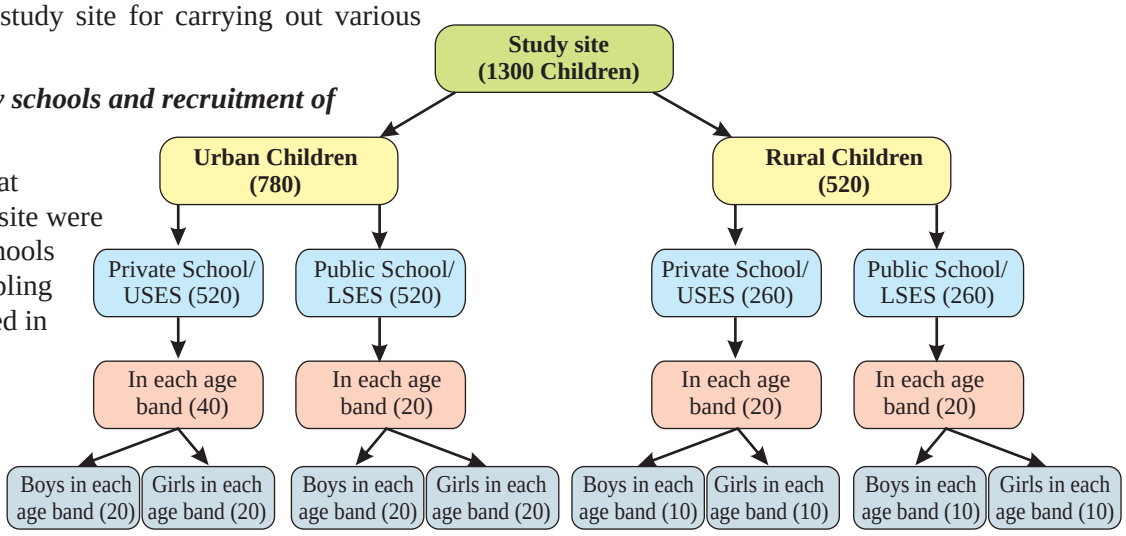
Correlation between body fat estimation using DXA and BIA:

DXA, the currently accepted standard technique for indirect estimation of body fat, is expensive, not widely available and hence unsuitable for use in large studies. On the other hand, BIA is a safe, simple, portable and highly acceptable technique which does not require highly skilled manpower. Thus, our first step will be to derive sex specific BIA equations for estimating body fat in Indian children with DXA as standard measure of body fat. In the next step, we are utilizing BIA technique for the validation of simpler anthropometric measures.

The reported correlations between DXA and BIA as techniques for body fat estimation vary between 0.6-0.9. Considering that a correlation of 0.7 is likely, detecting this at a 5% level of significance and 90% power, with adjustment for at least two covariates (age and pubertal age), requires 28 (14 boys and 14 girls) children. We intend to perform DXA scans in 32 children (16 boys and 16 girls) at each study site. However, we have covered DEXA in 80 children in each study site for carrying out various sub-sets analysis.

Selection of study schools and recruitment of children

Children at Hyderabad study site were recruited from schools based on the sampling scheme as depicted in figure-1.



Investigations

Biochemical estimations: The Cardiac Biochemistry Laboratory at AIIMS was carried out all the proposed biochemical investigations and the results were shared with INCLEN for analysis and preparation of final report.

Exclusion criteria

Children suspected/documented to having medical problems (e.g. nephrotic syndrome, cushing's syndrome, known genetic disorders associated with obesity or cachexia, thyroid disorders, malignancy, etc) which may have influence on the nutritional status and body composition were not recruited for the study.

Investigations

Baseline socio-economic and demographic parameters, significant past medical illness and current state of health was obtained through a structured, pre-tested study tool/instrument.

Anthropometric measurements including, weight, height, skin fold thickness (triceps, biceps, sub-scapular, trunk/supra-iliac), body circumferences (waist, hip and mid arm) was measured by trained research staff using standard equipment and procedures.

Resting blood pressure and heart rate was recorded in the right arm using automated BP measuring instruments.

Pubertal status, defined as pre-puberty, early puberty, late puberty and post-puberty were determined based on breast and pubic hair stages for girls and pubic hair stages for boys according to Tanner criteria for pubertal staging (Table 1). Pubertal stage assessment of the girls and of the boys was done by the respective gender investigators (Doctors/public health nurses).

Body fat measurements: Body fat content was estimated in all children recruited (1300 children) in the study site by using Bio-impedance Analysis (BIA) equipment. Apart from BIA, 32 children (16 boys and 16 girls) were measured their body fat by the Dual-energy X-ray Absorptiometry (DXA).

Blood collection and laboratory work up: For the biochemical analysis parameters, 7 ml blood in plain vacutainer and 3 ml blood in EDTA vacutainer, was collected from each child in the morning after 12 hours of last meal. After collection, samples were stored immediately in ice-filled insulated containers. The samples were centrifuged, aliquoted within 2 hours of collection and transported to the designated laboratory for the following investigations:

- Fasting Blood Sugar: Fasting blood glucose was analyzed by standard enzymatic method on Autoanalyzer.
- Fasting Insulin level: Fasting insulin was analyzed by RIA using commercially available kits.
- Apolipoproteins: Apolipoprotein A1 and B was measured by immunoturbidimetric method on autoanalyzer.
- Serum uric acid: Serum uric acid was analysed by uricase method on autoanalyzer.
- Adiponectin and Leptin levels: Adiponectin and leptin levels were also at AIIMS, New Delhi.

Ethical issues

The proposal was approved the ethical committee of NIN, Hyderabad. After obtaining approval from the ethics committee, the study will be rolled out at the study site.

Data entry and data processing

The collected data schedules were sent to the Central Coordinating Office (CCO), at INCLEN, New Delhi.

Data analysis and report writing:

Data analysis is being done by the CCO in coordination with ICMR and guidance from the ICMR Expert Group Members. Hyderabad site (NIN, Hyd) has collected the data as per the protocol including blood samples and sent to the designated centers for analysis and report writing.

2. ASSESSMENT OF NUTRITIONAL STATUS OF BELOW FIVE YEAR CHILDREN AND PERFORMANCE OF ICDS FUNCTIONARIES IN THE STATE OF HARYANA

GENERAL OBJECTIVE

To assess the current nutritional status of under <5 year children and infant & young child feeding practices of mothers of <3 year children in the rural areas of Haryana.

SPECIFIC OBJECTIVES

- To assess nutritional status of under <5 year children in terms of anthropometry and clinical examination for signs of nutritional deficiencies,
- To assess the prevalence of current morbidities among these children covered for anthropometry during preceding fortnight,
- To assess infant and young child feeding (IYCF) practices of mothers of <3 years children,
- To assess knowledge and practices of mothers with respect to health and nutrition, and
- To assess the knowledge and practices and performance of ICDS functionaries in the state.

The prevalence of undernutrition especially among under five year children may vary geographically. Therefore, for the development of area specific intervention strategies, divisional/district level mapping of undernutrition is very essential. At the request of DWCD, Government of Haryana, the National Institute of Nutrition, Hyderabad, has carried out the present study to estimate the prevalence of undernutrition among under five year children and infant and young child feeding practices. It was a community based cross-sectional study carried out using systematic random sampling procedure. A total of 4226 HHs were covered from 204 Anganwadi Centers, including 4727 children for the present study.

About 76% HHs were using sanitary latrine, the proportion of which was higher in Hissar and Rohtak divisions (80%) and lower in Ambala and Gurgaon divisions (71-72%). About two thirds (64%) of HHs were using adequately iodized salt (≥15 ppm), the proportion of which was higher in Gurgaon division (74.9%) and lower in Hissar division (42.5%).

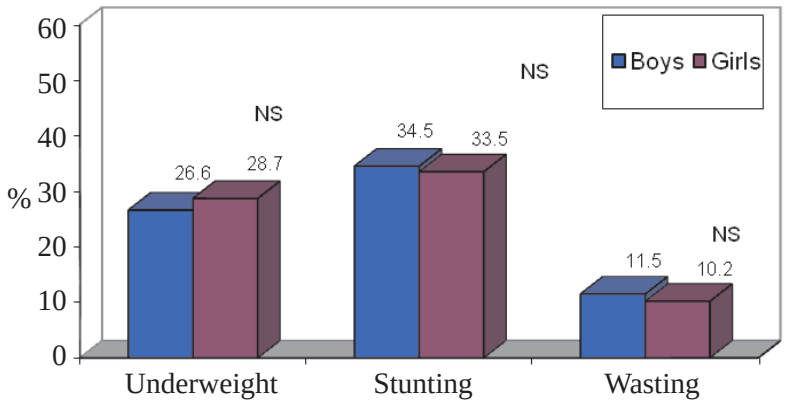
Almost all (96.4%) the pregnant women reportedly undergone Antenatal care (ANC) during last pregnancy, of which about 77% had ≥3 ANCs. Majority (92.7%) of pregnant women received IFA tablets during pregnancy, 57% received ≥90 tablets and about 44% reportedly consumed ≥90 tablets. The proportion of women consuming ≥90 IFA tablets was higher in Rohtak division (49.2%) and lower in Gurgaon division (38.2%). About 85% of deliveries were conducted in government (51.6%) or private hospitals (33.4%), ranging from 93% in Ambala division and 75% in Gurgaon division. Birth weight was recorded in 88% of infants, while records were available for 86% infants, as per the record the prevalence of low birth weight was 20%.

Majority of the mothers (93.4%) fed colostrum to their newborn. About 46% of mothers initiated breastfeeding within 1 hour, while 24% initiated 1-3 hours of delivery. The initiation of breastfeeding within 1 hour of birth was higher in Rohtak division (51.8%) and lower in Ambala division (38.2%). About 16% initiated breast feeding after 24 hours of delivery. Pre-lacteal such as glucose water, cow/buffalo milk, honey, etc. were given to 39% of the infants. The use of pre-lacteal was more in Ambala division (47.4%) and low in Gurgaon division (31.8%).

About 79% of 6-11 months children received complementary feeding in addition to breast milk, while only 14% received the same at 6 months of age, and 58% children received after 6 months of age. About 14% of children did not start complementary feeding. The proportion of children receiving complementary feeding in addition to breast milk was higher in Ambala division (85.9%) and lower in Gurgaon division (73.8%).

About 41% of children reportedly had one or more morbidities such as fever, ARI and diarrhoea during the preceding fortnight. About 54% children (6-59 months) were participating in ICDS supplementary feeding programme, while 31% were participating regularly (>20 days/month).

Majority (89%) of the children (12-24 months) were fully immunized, and ranged from a high 94% in Ambala and Hissar divisions and lower in Gurgaon division (81.5%). About 49% of 12-59 months children received at least one dose of Vitamin A during the preceding year. The prevalence of undernutrition (<Median -2SD) among <5 year children such as underweight, stunting and wasting was 28%, 34% and 11% respectively.



The study revealed a significant association between nutritional status and different socioeconomic variables. The prevalence of underweight, stunting and wasting was significantly associated with cast, type of family, size of family, literacy status and major occupation of parents, per capita income, type of house, type of cooking fuel used, presence of electricity, sanitary latrine, separate kitchen and history of morbidity proceeding 15 days.

Therefore, the study emphasises the need to impart health and nutrition education to the pregnant and lactating mothers through effective information, Education, and Communication (IEC) activities coupled with behaviour change communication (BCC) on infant and young child feeding practices, especially initiation of breast feeding within one hour of delivery and initiation of complementary feeding at 6 months. Since, the coverage under the national nutrition intervention programmes was poor, there is need to encourage the community to participate in the same. The poverty alleviation programme and other income generating activities may be strengthened to improve the household food security. The infrastructural facilities in AWCs need to be improved in terms of separate kitchen, toilet facilities and storage space etc. Community participation through mothers groups needs to be increased in terms of their knowledge on access and outreach of ICDS services and monitoring the quality of food supplementation to increase its acceptance and active participation.

3. ASSESSMENT OF EFFECT OF CONSUMPTION OF KHESARI DAL ON HUMAN HEALTH

Lathyrus sativus (Khesari dal), a legume (family *Fabaceae*) commonly grown for human consumption and livestock feed, if consumed as a large part of the diet for prolonged period is believed to cause neurolathyrim, a neurodegenerative disease that causes paralysis of the lower limbs. The neurotoxin, β-N-Oxalyl- aminoalanine (BOAA) present in the pulse is considered the cause of lathyrism.

Therefore, at the request of ICMR, the National Institute of Nutrition has taken up an epidemiological study, to assess the effect of consumption of Khesari dal on human health, being carried out in Bilaspur, Raipur and Durg districts of Chattisgarh state the districts with the highest cultivation of Khesari Dal. The objectives and methodology were presented in the 2014 Annual Report.

During the reporting year, the data collection in all the districts was completed and the data entry into computer and data analysis was carried out, and the khesari dal samples were analyzed for ODAP, and the preliminary results were presented in the expert committee meeting held in ICMR headquarters on 23rd January 2015.

RESULTS

Coverage: A total of 13,129 rural and 4,626 urban individuals were covered for anthropometry and clinical examination for signs and symptoms of nutritional deficiencies and neurolathyrism in the present study from 151

villages and 60 wards, respectively from the districts of Bilaspur, Durg and Raipur, where the Khesari dal cultivation was high. Out of the total 5769 HHs, 1602HHs were cultivating Khesari dal, 1791 HHs were non-cultivators and 2,376 HHs were agriculture labourers and other labourers. Twenty four hour recall method of diet survey was carried out in all the HHs and information on knowledge and practices of khesri dal consumption was collected from the head of the household/housewives from 4,490 rural HHs and 1,221urban HHs, olds. A total of 380 Khesari dal samples were collected from rural (213nos) and urban areas (167nos) for the estimation of β-N-Oxalyl-aminoalanine (BOAA).

Cultivation and consumption of khesari dal: About 27-29% of the study population reported cultivation of Khesari dal, with a mean duration of more than three decades. The mean consumption of Khesari dal among the districts ranged between 2-6gm/cu/day in rural and urban areas respectively. The consumption in the urban HHs is less (about 1gm) as compared to rural areas (2-7g). The mean Khesari dal consumption constituted to 8% of the total pulse consumption. Average intake of protein per day is about 54g/cu and the protein contribution from Khesari dal to the total protein intake was only 1-2%.

Pulse and legumes are mostly consumed 2-3 times a week. Some of them include these once a week.

About 9% of the rural HHs and 16% of the urban HHs were consuming Khesari dal either daily or 4-5times a week. However, a majority of the HHs were consuming either 2-3 times a week (33% Rural vs 25% Urban) or once a week (20% Rural vs, 18% Urban). Khesari dal is usually consumed along with the other pulses, mostly 2-3 times a week or once a week. It is consumed mostly as plain cooked gravy dal, gravy dal with vegetables and green vegetables, snacks, roti/chapathi and Khadi. The quartile distribution of the mean consumption of Khesari dal at HH level indicated that it ranged from 1.78g in the 1st quartile to 29.9g in the 4th quartile, which is almost half of the recommended levels by the ICMR. The proportion of HHs consuming Khesari dal varied between the cultivators, non-cultivators of Khesari dal and others (Low socio-economic groups, SC & ST). The proportion of HHs consuming Khesari dal is higher among cultivators of Khesari dal in all the three districts as compared to rest of the two categories of study population. While the mean intake levels among the consumers belonging to three categories of study population is essentially similar at 45-48g/cu/day.

Nutritional Status

The overall prevalence of underweight, stunting and wasting among 1-5 year children was 37%, 43% and 17% respectively among the study districts. This prevalence is less than that of NNMB figures reported for Madhya Pradesh, in the survey carried out in 2012. The overall prevalence of CED (BMI <18.5) was 24% among men and 26% among women, which is less than the figures reported in the NNMB survey in Madhya Pradesh carried out in 2012. Overall prevalence of obesity was 14% among men and 16% among women, which is marginally higher compared to the NNMB figures of Madhya Pradesh.

Clinical signs and lathyrism among adults

The prevalence of symptoms and signs of lathyrism are observed in few of the subjects in all the three districts. A majority of the subjects experienced agonizing pain in calf muscles especially during nights, the prevalence of which is high (0.64%) in Bilaspur district as compared to other study districts.

Knowledge and practices about Khesari dal and lathyrism

About 60% of the rural HHs and 26% of the Urban HHs surveyed were reportedly consuming Khesari dal for more than 10 years. The average quantity consumed per HH/month is ranging from 2.7kgs in Urban Bilaspur to 11.6Kgs among Urban HHs in Durg district. The commonest reason for consumption of KHESARI DAL is the low price, easy availability and long standing habituation. Only 2-6% of HHs were reportedly following the ideal method of cooking the Khesari dal, like washing, boiling, and draining.

About 15% of the HHs was aware of health effects of consumption of Khesari dal. Pain in legs was the commonest symptom reported.

Estimation of BOAA (mg/100gm) levels in the Khesari dal samples:

The mean ODAP levels (mg/100gm) of the Khesari dal samples from three districts was 0.635mg/100gms, however but It is observed that the study population in Bilaspur is consuming Khesari dal with higher mean BOAA

levels as compared to other two districts. About 10% of the rural HHs and 18% of urban HHs from Bilaspur were currently consuming Khesari dal with an average intake of 42g/day with mean BOAA of 0.635mg/100gms dal. A similar proportion of HHs from rural and urban HHs from Durg districts were consuming at a lower intake levels of 34gms of Khesari dal with mean BOAA of 0.652mg/100gms. While, in the district of Raipur,6% of the rural and 22% of the urban HHs were consuming at a higher intake of 87gms/day of Khesari dal with a mean BOAA of 0.604mg/gms dal.

Summary of case histories of nine suspected cases of Neurolathyrism

In preliminary screening 24 suspected cases were identified by project staff, with signs and symptoms of paralysis of lower limbs, of which13 cases were excluded based on history, which were due to cerebro vascular accidents, poliomyelitis and tuberculosis of spine, by the supervisory staff of NIN, Hyderabad. Finally a total of eleven of cases were confirmed by supervisory staff of NIN, physician and neurologist in Bilaspur, and Durg districts. Of the eleven cases, eight were cases were from *Masabhat* village, two cases were from Saoni village of Durg district and one case was from *Ranigaon* village of Bilaspur. The two cases from Saoni village who had history of intake of Khesri dal and were also having spastic paraplegia have not consented for investigations and consultation with the Neurologist, while, the rest of the nine cases were subjected to investigations and consulted neurologist for their opinion.

All the suspected cases had history of consumption of Khesari dal during the periods of drought. All the cases were more than 50 years of age with an average duration of history of illness of more than 35 years. All the nine of them were old cases with long history of illness and no new cases with recent history were found during the study. A majority of them have been cultivating Khesari dal in their farms and have a long history ranging from 5-35yrs and have history of Khesari dal consumption ranging from 30-250gms/day, as rotis and gravy dal. The current consumption frequency is mostly occasional. Three cases have classical scissors gait and others have a range of other clinical signs and symptoms of Neurolathyrism. Agriculture is major occupation for all nine suspected cases. The period of consumption before the symptoms appeared ranged from 1-10 years. The biochemical investigations revealed that the erythrocyte sedimentation rate is high in all the cases, one of the subject is anaemic with Haemoglobin level at 7.7g/dl, four subjects are having high eosinophil count (10-40cells), one subject is reactive for VDRL and all the suspected cases are HIV negative. The six HHs with suspected cases are still consuming Khesari dal at least once in a week, with a mean intake of Khesari dal 14/CU/day while the rest were consuming at 39gms/day.

Baisaku Ram
M-60, Masabhat, Durg

Profile:

Period of consumption before these symptoms appeared: 3 years
H/o Illness: 35 years
Past consumption of KD-50g/Day
Current frequency-once a week
Intake on day of visit- Nil

Complaints:

Difficulty in walking
Spasms and pain the legs in night
Severe back pain

Clinical findings:

Scissors gait
Increased tone in both lower limbs
Extensor plantar responses
Very brisk knee and ankle tendon reflexes

AVERAGE CONSUMPTION OF FOOD STUFFS (g/CU/day) – POOLED

Particulars		Cereals & Millets	Khesari Dal	Other Pulses & Legumes	Green Leafy Veg.	Other Veg.	Roots & Tubers	Fruits	Fish	Other Flesh Foods	Milk & Milk Prod.	Fats & Oils	Sugar & Jaggery
(g/CU/day) (n=4549)	**Mean**	**465**	**4**	**45**	**45**	**97**	**63**	**54**	**3**	**4**	**21**	**12**	**15**
	SD	**156**	**17**	**46**	**88**	**101**	**64**	**59**	**23**	**30**	**48**	**8**	**12**
% RDI	<50	4.0	-	35.6	72.1	36.4	35.8	-	-	-	90.7	48.8	63.1
	50-70	13.2	-	4.3	0.5	1.5	5.3	-	-	-	4.0	22.9	15.9
	‡70	82.8	-	60.1	27.3	62.1	58.9	-	-	-	5.3	28.3	21.0
(g/CU/day) (n=1209)	**Mean**	**397**	**0.7**	**52**	**37**	**88**	**66**	**55**	**6**	**7**	**33**	**14**	**15**
	SD	**136**	**5**	**49**	**71**	**92**	**66**	**60**	**32**	**31**	**56**	**10**	**14**
% RDI	<50	9.2	-	24.3	74.0	37.9	32.8	-	-	-	85.5	42.6	60.4
	50-70	20.0	-	5.5	0.2	2.2	6.0	-	-	-	6.9	19.4	21.0
	‡70	70.8	-	70.1	25.7	60.0	61.3	-	-	-	7.6	38.0	18.6
(g/CU/day) (n=5758)	**Mean**	**450**	**4**	**46**	**43**	**95**	**64**	**54**	**4**	**5**	**24**	**12**	**15**
	SD	**155**	**15**	**46**	**85**	**99**	**65**	**59**	**26**	**31**	**50**	**9**	**12**
% RDI	<50	5.1	-	33.2	72.5	36.7	35.2	-	-	-	89.6	47.5	62.5
	50-70	14.6	-	4.6	0.5	1.7	5.5	-	-	-	4.6	22.2	17.0
	‡70	80.3	-	62.2	27.0	61.6	59.4	-	-	-	5.8	30.3	20.5
RDI		**460**	**-**	**40**	**40**	**60**	**50**	**-**	**-**	**-**	**150**	**20**	**20**

DISTRIBUTION OF HOUSEHOLDS (%) BY FOOD FREQUENCY

Food Groups	Frequency	Bilaspur		Durg		Raipur		Pooled	
		Rural	Urban	Rural	Urban	Rural	Urban	Rural	Urban
Khesari dal	**Daily**	**5.1**	**14.5**	**7.1**	**11.0**	**2.9**	**10.0**	**4.9**	**11.8**
	4-5 times a week	**5.3**	**3.7**	**3.0**	**7.9**	**3.2**	**11.6**	**3.8**	**4.4**
	2-3 times a week	32.4	26.0	37.6	15.0	30.3	33.1	33.3	24.6
	Weekly once	16.2	13.4	18.6	17.0	23.2	23.7	19.7	18.1
	Once in fort night	8.4	6.2	6.9	13.0	13.5	4.9	9.9	8.1
	Monthly	11.1	14.8	7.4	22.1	11.3	10.7	10.0	15.9
	Quarterly	7.7	5.5	4.5	7.2	4.0	0.3	5.2	4.3
	Occasional	13.8	15.8	14.9	6.9	11.6	15.6	13.3	12.7
Other Pulses	**Daily**	**4.3**	**0.0**	**2**	**1.2**	**0.8**	**0.0**	**1.9**	**0.3**
	4-5 times a week	**10.0**	**5.1**	**3.4**	**3.5**	**8.5**	**0.9**	**7.6**	**3.1**
	2-3 times a week	52.8	35.7	47.0	18.8	45.1	32.1	48.4	29.5
	Weekly once	20.8	36.7	41.0	62.4	38.7	52.1	32.8	50.0
	Once in fort night	4.1	4.1	5.8	9.4	5.6	10.1	5.1	7.9
	Monthly	3.8	9.2	2.1	3.5	1.1	4.6	2.4	5.8
	Quarterly	0.9	2.0	0.0	0.0	0.1	0.0	0.4	0.7
	Occasional	3.4	7.1	0.5	1.2	0.2	0.0	1.5	2.7

II MICROBIOLOGY AND IMMUNOLOGY

1. VITAMIN D STATUS, VITAMIN D RECEPTOR EXPRESSION AND THEIR LINK WITH CD23/ CD21 INTERACTION AND REGULATORY T CELL FUNCTION IN CHILDREN WITH ASTHMA

Asthma is a chronic in?ammatory disorder of the airways that causes an increase in airways hyper responsiveness, leading to recurrent episodes of wheezing, breathlessness, and coughing that are associated with variable air?ow obstruction. In the developing countries like India, asthma is the most common cause of childhood emergency department visits, hospitalizations, and missed school days. Increasing prevalence of asthma involves vitamin D deficiency there are limited data on Vitamin D levels in children with asthma, as well as on what features of asthma are associated with Vitamin D levels. A recent study on children with asthma from Costa Rica showed a signi?cant inverse association between Vitamin D levels and use of anti-in?ammatory medication (either ICSs or leukotriene inhibitors) in the previous year, To our knowledge, the prevalence of Vitamin D insufficiency/de?ciency is unknown for children with asthma recently, studies have identified additional influences of vitamin D on the immune system and several lines of evidence suggest a possible influence of vitamin D on prevalence of allergic diseases even though results are still conflicting. In addition, more information is needed regarding the speci?c clinical and therapeutic variables associated with lower vitamin D levels in children with childhood asthma.

OBJECTIVES

- To determine the vitamin D levels, VDR expression and their link with CD23/CD21 expression and Treg cell function in children with allergy.
- To determine the correlation of Th1, Th2 cytokines and CD23/CD21 expression with vitamin D, VDR expression and Treg cell function in allergic asthma children.

METHODOLOGY

About 60 children, aged 2 to 5 years with symptoms of asthma were recruited. Age and sex matched 60 children without history of allergic asthma or atopic dermatitis were enrolled as controls. Blood samples were collected for Treg cell function, CD23 and CD21 expression, vitamin D concentration. In addition, cytokine were done.

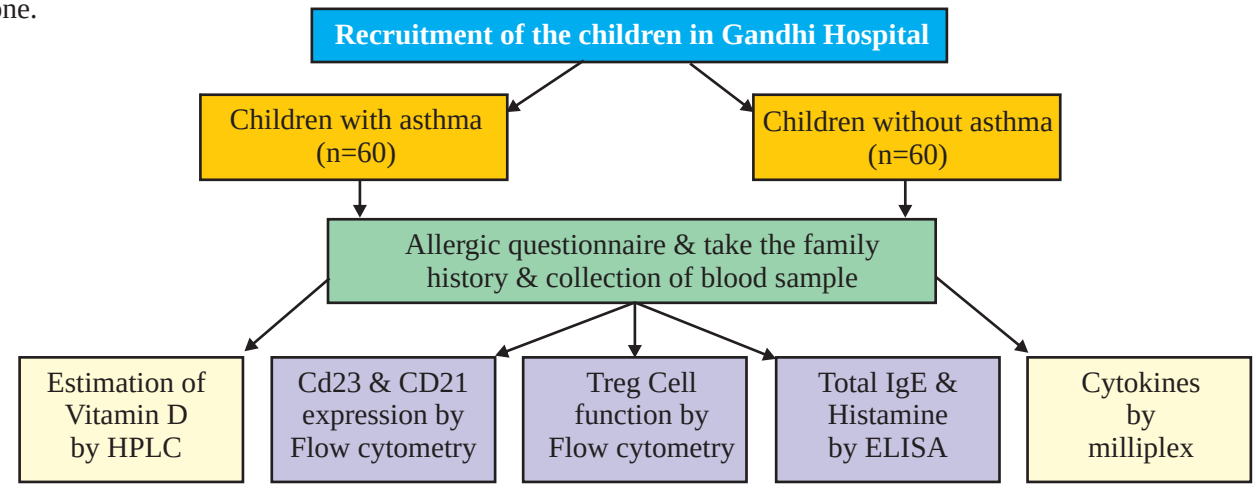


Table 1

Parameters	Controls	Asthma children
Subjects	60	60
Boys	33	39
Girls	27	21
SES SCALE:		
Upper	0	0
Upper middle	12	17
Lower middle	11	9
Upper lower	33	32
Lower	4	2
Age in months	46.95 - 14.22	48.87 - 12.96
WT (kg)	14.36 - 4.54	16.40 - 2.42
HAZ (SD)	0.18 (1.98)	-0.45 (1.48)
WAZ (SD)	-1.19 (1.85)	0.02 (1.11)
BMIZ (SD)	-1.92 (3.07)	0.43 (1.83)
WHZ (SD)	-2.16 (2.97)	0.40 (1.89)
Family history of asthma (%)	-	18.3
Asthma levels		
Severe persistent	-	4 (6.66)
Moderate persistent	-	26 (43.33)
Mild persistent	-	20 (33.33)
Mild intermittent	-	10 (16.66)

Values are expressed as Mean ± SD, Otherwise no. of subjects

RESULTS

Demographic information: Maximum number of children in the study population belonged to lower middle class as assessed by modified Kuppuswamy's socioeconomic status (SES) scale.

The mean±SD of age and weight were 4.3±1.4 years, 3.8±1.4 kg respectively. The severity of asthma in this group of patients was presented as severe persistent in 6.66%, moderate persistent in 43.33%, mild persistent in 33.33% and mild intermittent in 16.6%. The serum calcium concentration was comparable between asthma (9.11±2.1 mg/dL) and control children (9.11±2.1 mg/dL). Similarly, the haemoglobin concentrations was also not different between asthma (10.68±2.1 gm/dL) and control children (10.97 ± 1.9 gm/dL).

***25(OH)D3* levels in asthma and control children:** Based on 25(OH)D3 concentrations subjects were categorized in to two groups; 25(OH)D3 sufficient (≥15 ng/mL) and 25(OH)D3 deficient (<15ng/mL) as previously recommended [IOM 2013]. 25(OH)D3 concentration was insufficient in 19 (31.66%) and deficient in 41 (68.33%) asthma children. In control children, insufficient levels were found in 29 (48.33%) and deficient levels were found in 31 (51.66%).

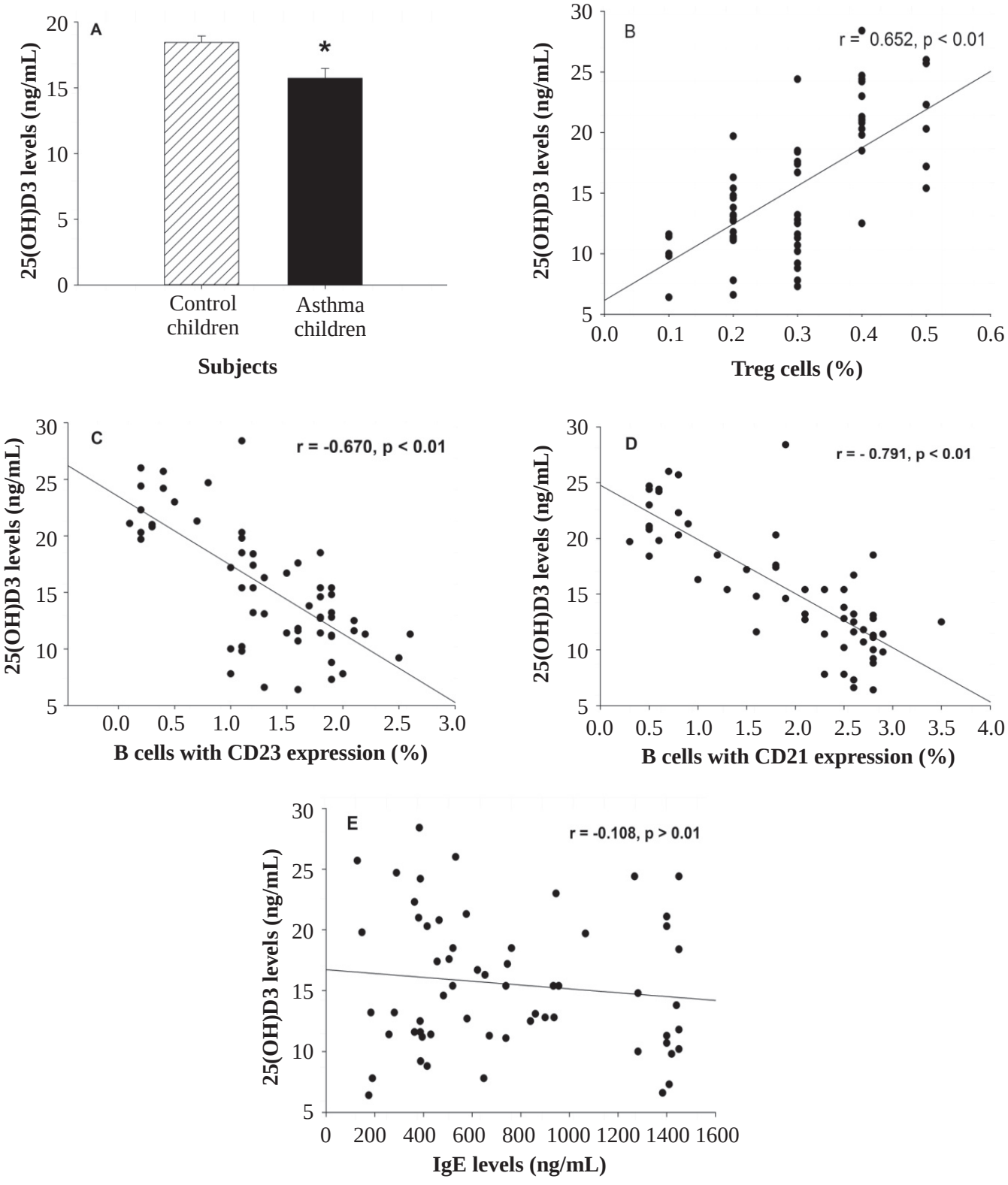
The mean 25(OH)D3 concentrations (ng/mL) were significantly ($P < 0.05$) lower in asthma children (15.54 ± 0.71) than in control (18.44 ± 0.49) children. 25 (OH) D3 was directly correlated with Treg cell population ($n = 60$, $p < 0.01$ $r = 0.652$) and inversely correlated with total IgE and B cells with CD23 ($n = 60$, $p < 0.01$ $r = -0.670$) and CD21($n = 60$, $p < 0.01$; $r = -0.791$) (Fig. 1).

Regulatory T cell (CD+/CD25+/CD127-/FOXP3) population: The proportion of Treg cell population were evaluated within the CD4+ population, Treg cell population ranged from 0.1 to 0.5% and 0.2 to 0.9% in asthma and control children respectively. The mean (%) of Treg cell population were significantly ($P < 0.05$) lower in asthma children (0.29 ± 0.01) compared to control children (0.38 ± 0.02). Treg cell population was inversely correlated with B cells with CD23 ($n = 60$, $p < 0.01$; $r = -0.456$) and CD21 ($n = 60$, $p < 0.01$; $r = -0.504$) (Fig. 2).

B cells with CD23/CD21 expression: The percentage of B cells with CD23 expression ranged from 0.2 to 2.6 and 0.1 to 0.4 in asthma and control children respectively. The mean (%) of B cells with CD23 expression was significantly ($P < 0.05$) higher in asthma children (1.30 ± 0.08) than in control (0.18 ± 0.01) children. Similarly, the percentage of B cell with CD21 expression ranged from 0.3 to 2.9 and 0.2 to 0.9 in asthma and control children respectively. The mean (%) of B cell with CD21 expression was significantly ($P < 0.05$) higher in asthma children (1.89 ± 0.11) compared to control (0.84 ± 0.03) children. B cell with CD23 population was significantly correlated with B cell with CD21 population ($n = 60$, $p < 0.01$; $r = 0.729$) (Fig. 3).

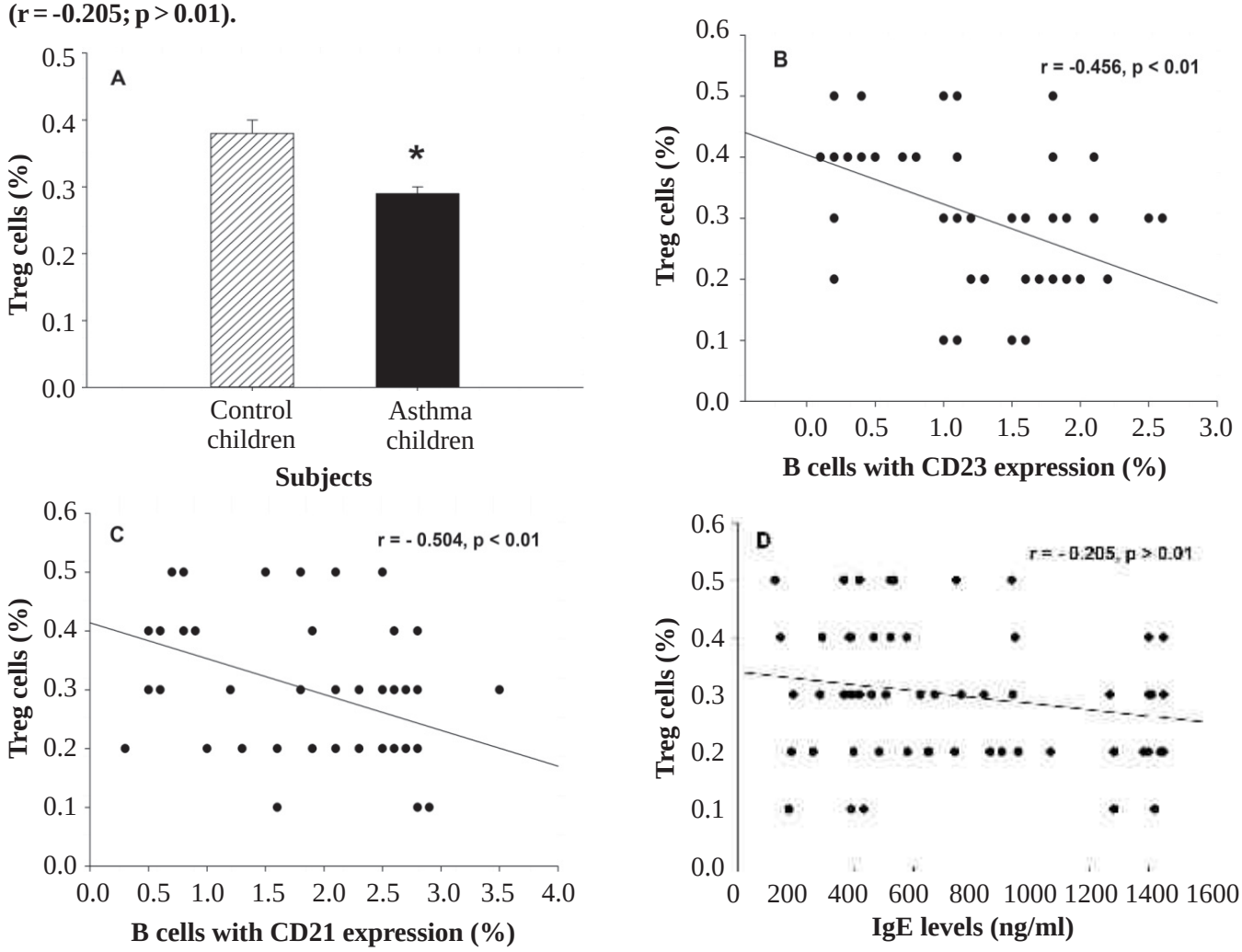
Regulatory cytokines (IL-10 & TGF β): IL-10 (pg/mL) ranged from 2 to 20.29 and 1.5 to 19, TGF β concentrations ranged from 2129 to 7180 and 1572 to 5856 in asthma and control children respectively.IL-10 was significantly ($P < 0.05$) lower in asthma children (3.4 ± 0.46) compared to control (5.06 ± 0.84) children. TGF β concentrations was significantly ($P < 0.05$) higher in asthma children (4330.46 ± 162.56) compared to control children (3338.45 ± 168.17). We did not find any correlation between IL 10 and Treg cells ($n = 60$, $p > 0.01$; $r = -0.068$), TGF β and Treg cell ($n = 60$, $p > 0.01$; $r = 0.196$) population (Fig. 4).

Fig. 1 A) 25(OH)D3 concentrations (ng/mL) in asthma and control children. B) Spearman's Rank correlation of 25(OH)D3 and Treg cells (r = 0.652; p < 0.01). C) Spearman's Rank correlation of 25(OH)D3 and B cells with CD23 expression(r=-0.67; p<0.01). D) Spearman's Rank correlation of 25(OH)D3 and B cells with CD21 expression (r = -0.791; p < 0.01) E) Spearman's Rank correlation of 25(OH)D3 and IgE levels (r = -0.108; p > 0.01).



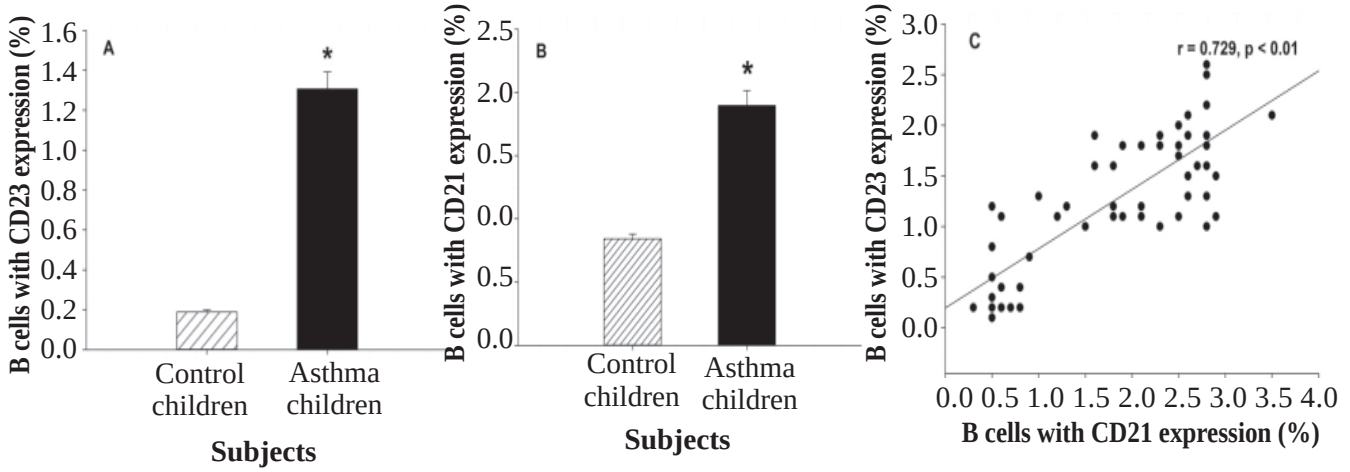
Values are expressed as mean±SE.

Fig 2. A) Treg cell concentrations (%) in asthma and control children, B) Spearman's Rank correlation of Treg cells and B cells with CD23 expression (r = -0.456; p < 0.01). C) Spearman's Rank correlation of Treg cells and B cells with CD21 expression (r = -0.504; p < 0.01). D) Spearman's Rank correlation of Treg cells and IgE levels (r = -0.205; p > 0.01).



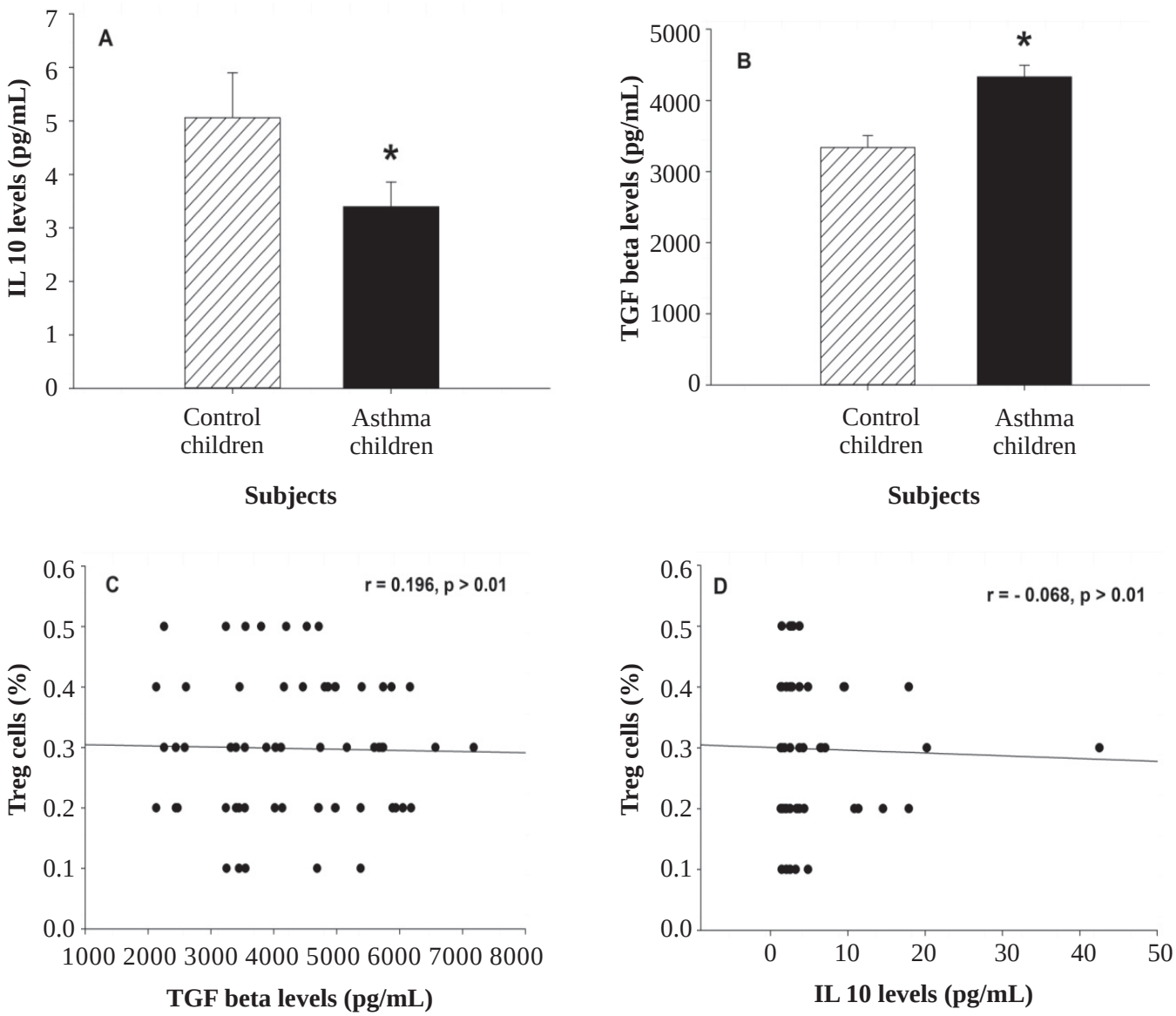
Values are expressed as mean±SE.

Fig 3. A) B cells with CD23 expression in asthma and control children B) B cells with CD23 expression in asthma and control children. C) Spearman's Rank correlation of B cells with CD23 and CD21 expression (r = 0.729; p < 0.01).



Values are expressed as mean±SE.

Fig 4. A) Regulatory cytokine IL 10 levels in asthma and control children. B) Regulatory cytokine TGF beta levels in asthma and control children. C) Spearman's Rank correlation of Treg cells and TGF beta (r = 0.196; p > 0.01). D) Spearman's Rank correlation of Treg cells and IgE levels (r = -0.068; p > 0.01).



Values are expressed as mean±SE.

Total IgE levels: The IgE concentrations ranged from 56 to 586 ng/mL and 128 to 1450 ng/mL in asthma and control children respectively. As expected, total IgE levels were significantly ($p < 0.05$) higher in asthma children (748± 55.8 ng/mL) compared to control children (362.88 ± 38.53 ng/mL). No correlation was found between the IgE levels and Treg cell population ($n = 60$, $p > 0.01$; $r = -0.205$), IgE levels and 25 (OH) D3 levels ($n = 60$, $p > 0.01$; $r = -0.108$).

Serum Calcium and hemoglobin Assay: Serum calcium levels (ng/mL) were comparable between asthma (8.3± 0.05) and control children (8.5±0.08). Similarly, hemoglobin levels (mg/dL) were comparable between asthma (10.3±1.98) and control children (11.6 ±1.87).

CONCLUSION

Children with asthma have impaired vitamin D 23(OH)D3, and impaired regulatory T-cell function and regulatory cytokines.

2. TOWARDS THE DEVELOPMENT OF EDIBLE VACCINE AGAINST HELICOBACTER PYLORI WITH RECOMBINANT LACTOBACILLI EXPRESSING HEPARAN SULFATE BINDING PROTEIN

Lactic acid bacteria (LAB) are microorganisms that are extensively used in the production of fermented foods enzymes, metabolites and nutraceutical mainly due to their GRAS (Generally Regarded As Safe) status and are known for their functional effects on the health of humans and animals. Oral administration of live recombinant lactobacilli showed mucosal as well as a systemic immune response against antigens. The display of heterologous proteins on the cell surface of lactobacilli is an exciting and emerging research area that has great promise for a variety of biotechnological applications, including the developments of live vaccine delivery system, diagnostics, peptide library screening and whole-cell biocatalysts.

Lactobacillus strains that have been developed successfully for Genetically engineered live vaccine include L. casei, L. plantarum, L. johnsonii, and L. acidophilus. However, the use of non-food-grade vectors restrict their application in humans. Therefore, developing food-grade vaccine delivery systems is important for escalating the human usefulness of Genetically engineered live vaccine . Expression of antigens on the surface of lactobacilli is attractive for vaccine design, especially because the peptidoglycan layer of some strains appears to exhibit natural immunoadjuvanticity. Most pathogens enter the body through mucosal surfaces, so the development of protective vaccines at such sites should be an effective means of preventing a wide range of infectious diseases.

The presence *of H. pylori* in human gastric mucosa is now established as the aetiological agent of chronic gastritis and most cases of peptic ulcer and gastric adenocarcinoma world wide and is considered as a class I carcinogen by WHO . Previous studies have shown that *H. pylori* expresses cell surface- associated proteins (outer-membrane proteins, OMP) and haparan sulphate binding protein HSBP) which have affinity for the glucosaminoglycan hepran sulphate. In the present study, by using the CspI signal peptide of lp_2940 from *L. plantarum* and MPQTG motif of PrtR anchor peptide from *L. rhamnosus*, we have developed a novel display system in *L. rhamnosus* and also to modify food-grade pSIP503 vector for surface display of Hsbp from *H. Pylori* on *L. rhamnosus* GG (LGG) and to bestow with an alternative antigen delivery vehicle in oral vaccine formulation.

AIMS AND OBJECTIVES

The present research work has been carried with the following objectives.

- Expression of *H. pylori* immunogenic heparan sulphate binding protein (Hsbp) on the surface of probiotic *Lactobacillus* strain.
- Exclusion of *H. pylori* from enterocytes by recombinant *Lactobacillus* strain expressing Hsbp.
- Assessment of protective immune response of Hsbp expressed by recombinant *lactobacilli* against *H. pylori* in mice.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

The bacterial strains used in this study are *L. rhamnosus* GG for surface display of Hsbp protein and *E. coli* XL-1 Blue cloning purpose. The *H. pylori* 26695 strain was provided by Dr. Niyaj Ahmed, Hyderabad Central University, India. *Lactobacillus* specific expression vector pSIP503 was generously gifted by Dr. Lars Axelsson, Nofima, Norway. *Lactobacillus* strains were cultured in Man-Rogosa-Sharpe (MRS) broth at 37°C without aeration. Erythromycin (Erth) was used at a concentration of 200 µg/ml for *E. coli* and 50 µg/ml for *lactobacilli*. *H. pylori* strains was cultured in Difco™ Columbia broth at 37°C in anaerobic condition.

Synthesis of fusion gene *for cell surface display*

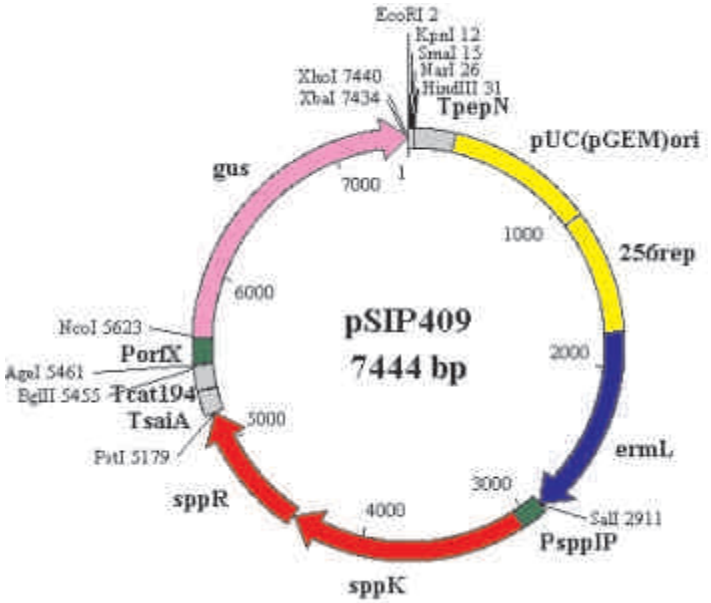
The *lactobacillus* genomic DNA was extracted as per Pospiech & Neikmann (1995) method. All PCRs were carried out using high fidelity Q5 DNA polymerase (NEB). A 66 base pair gene fragment which covered the signal peptides region of the surface protein CspI (YP004890527) between amino acids 1 and 22 was PCR amplified from

genomic DNA of *L. plantarum* 21 using primers CspS_F and Csp_R. The MPQTG motif gene fragment which covered the sequence of amino acids 1303 to 1480 of cell envelope proteinase PrtR (AJ496666.1) was PCR amplified from genomic DNA of *L. rhamnosus* GG using primer PrtR_F and PrtR_R. The gene encoding signal peptide from *L. plantarum* and anchor motif containing MPQTG from *L. rhamnosus* GG were amplified by overlap PCR to obtained fusion product SR, using primer CspS_F and PrtR_R. Fusion gene was purified from agarose gel to clone into the pJET1.2 cloning vector for nucleotide sequence analysis.

Construction of plasmid for cell surface display

The purified fusion construct was double digested with Nco1 and Hind III restriction enzymes and ligated with pre-digested pSIP503 vector with same enzymes which resulted into pSIP503CMR plasmid. Purified digested PCR products of Gus gene was inserted into pre-digested pSIP503SR with Nde1 and Xho1 restriction enzymes. Plasmid pSIP503SR (Fig. 1) contain the 'backbone' elements of the expression vectors, the replication determinants for *E. coli* (pUC(pGEM) ori), *Lactobacilli* (256rep), an erythromycin resistance marker (ermL) and the pepN terminator. It also contains the nisA promoter followed by NcoI site, CspI signal, a multicloning site (MCS), thrombin specific proteolytic cleavage site and PrtR anchor.

Fig. 1 pSIP503 CMR expression vector map



Amplification of Gus and Hsbp Gene

The heparan sulphate binding protein VacA (HsbpV) gene was PCR amplified from the genomic DNA of *H. pylori.* All PCRs were carried out using high fidelity DNA polymerase (Fermentas). The primer pairs HsbpF/HsbpR were used to amplify Hsbp gene. The gene Gus was also PCR amplified from the original plasmid pSIP503-Gus vector using primers GusF and GusR. The resulting purified PCR products were double digested with Nde1 and Xho1 restriction enzymes. Digested PCR products were purified from agarose gel and further used for cloning in pSIP503CMR expression vector for surface display on lactobacillus strains.

Electroporation of *L. rhamnosus* GG

The protocol used for electro transformation of *lactobacilli* (Heravi et al., 2012) Briefly, an overnight culture of lactobacillus was used to inoculated in 12 ml MRS broth containing 2% glycine and 0.5M sucrose and incubated at 37°C until the cells were attained in the middle of the exponential phase (OD= 0.2- 0.4). The bacterial cells were harvested by centrifugation at 6000g for 10 min at 4°C and washed twice with 10 ml of cold distilled water and followed by 5 min incubation in 50 mM EDTA. After washing with sterile distilled water and 0.3M sucrose, the cells were resuspended in 75ul of 0.3M sucrose as electroporation buffer. Electroporation was carried out by mixing of 75µl of competent cells with 0.3 -0.5 µg plasmid DNA. The suspension was transfer into a disposable cuvette with 0.1cm electrode gap and subjected to an electric pulse at 1.5 kv, 129 µF and 2.5 Ω using BTX electroporator. The elctroporated cells were immediately diluted in 0.5 ml of MRS broth and incubated at 37°C for 2 h. The transformed cells were plated on MRS agar containing erythromycin and incubated for 2 to 3 days.

Expression of GusA in *L. rhamnosus* GG

For the expression of heterologous protein in *L. rhamnosus* GG under the control of nisA promoter, the recombinant *L. rhamnosus* GG strain was grown in MRS medium at 37°C. Nisin induction of recombinant *L. rhamnosus* GG strains was first performed as described by Pavan et al., 2000 with some modification. Briefly, bacteria were grown at 37°C to an OD_{600} of 0.3 was induced with different concentrations (0, 15, 25, 50 and 100 ng/ml), and cultures were further grown for at 37°C. Optimization of the protocol for *L. rhamnosus* GG led to the following modifications. Bacteria were grown at 37°C with an overnight culture. Bacteria were again sub-culture

and incubated for 2 h of growth (beginning of the exponential growth phase), nisin was added at a concentration of 50 and 100 ng/ml and cells were propagated for 2, 4, 5, 6 and 8 h at 37°C before harvest.

β-glucuronidase assay

β-glucuronidase assay was performed using micotiter plate based protocol by the method described by Sigrid et al, 2006 with some modification. After appropriate induction, 5 ml of bacterial cells was harvested by centrifugation (10 min, 5,000g) and resuspended in 1 ml of PBS buffer. Bacterial cells (20 µl), mixed with 90 µl of GUS buffer (50 mM NaHPO4 (pH 7.0), 14.3 mM β-mercaptoethanol, 1 mM Na_2-EDTA, 0.1% Triton X-100, 0.1% sodium laurylsarcosine, 25 mM *p*-nitrophenyl-β-D-glucuronic acid (PNPG) (Sigma). The mixture was incubated at 37°C. The reaction was stopped by adding 50 µl of a 1 M Na2CO3 solution after sufficient yellow color had developed. The reaction was stopped at least three different times (replicates) to ascertain that enzyme activity was still linearly increasing with incubation time. Identical treatments were performed with Na-phosphate buffer but without cells as controls. Activity was calculated as described by Miller (1972) and expressed as Miller Unit equivalents (MU).

SDS-PAGE Analysis and Western Blot Analysis

Overnight cultures of Lactobacillus spp. harboring the recombinant pSIP503M-Hsbp expression vectors (Table 1) were diluted in MRS medium containing 200µg/mL erythromycin. The cultures were induced by adding the inducing peptide nisin at OD_{600} 0.3 as described previously. Cells were harvested 8 hours after induction by centrifugation at 6000g for 10 min at 4°C, after which the supernatants were filtered (0.22 µm) and PMSF was added to 1 mM final concentration. The supernatant samples were run on 10% SDS-PAGE.

The cells were washed with ice-cold 0.85% (w/v) NaCl. To extract surface proteins, washed cells were resuspended in 2 M Guanadine-HCl (Gu-HCl) and incubated for 2 hr at 37°C. The cells were pelleted by centrifugation and supernatant was dialyzed against water at 4°C for overnight to remove the salts. The precipitated proteins were collected by centrifugation for 20 min at 10,000×g at 4°C and lyophilized. The lyophilized proteins dissolved in PBS buffer and electrophoresis was done on 10% SDS-PAGE. Separated proteins were transferred to PVDF membrane, the appropriate proteins identified by western analysis.

RESULTS

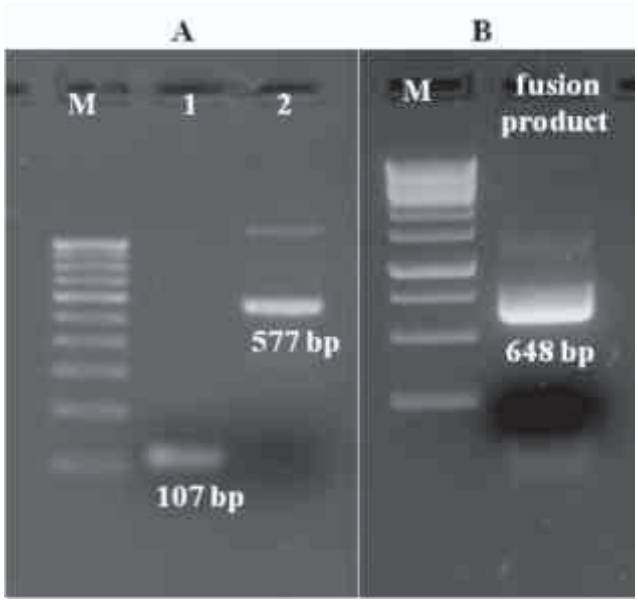
Anchoring of target protein on the surface of *lactobacillus* including a strong signal and anchoring domains are essential for development of live vaccines.

Construction of Plasmids for Cell Surface Display

The vectors constructed in this work are shown in Fig 1 The CspI signal sequence with size 107 bp (Fig. 2) amplified from genomic DNA of *L. plantarum* using primer pair CspF and CspR. PCR amplicon of anchor region of PrtR from genomic DNA of *L. rhamnosus* GG was amplified using primer pair PrtRF and PrtRR having size 577 bp (Fig. 2a). After confirming the amplification of both signal and anchor regions, the amplified products were purified from agarose gel. The purified product was used as template for overlap PCR for amplification of fusion product using primer pair CspF and PrtRR (Fig. 2b).

The double digested fusion gene with Nco1 and Hind III restriction enzyme were ligated with pre-digested pSIP503 vector with same set of enzymes. The ligation mixture containing the recombinant

Fig 2. Agarose gel showing PCR amplification of (A) Csp signal from genomic DNA of *L. plantarum* and PrtR Anchor from Genomic DNA of *L. rhamnosus* GG, Lane: 1. Csp signal, 2. PrtR anchor, M. 50 bp DNA Ladder, (B) Overlap PCR amplification fusion product using CspF /PrtRR, M. 1kb DNA Ladder.



plasmid was transformed in to competent cells of *E. coli* Dh5α. The recombinant plasmids isolated from the erythromycin positive transformants were further confirmed by restriction digestion. We had used GusA as a control to evaluate the efficiency of the constructed signal sequence CspI and cell surface anchor sequence prtR to display foreign proteins on *lactobacilli* surface.

Amplification of Gus and Hsbp Genes

Amplification of hsbp genes was carried out using primer pairs HsbpF/ HsbpR. Size of PCR product of hsbp gene was 1.5 kb as shown in Fig. 3. In the present study GusA was used as control to evaluate the efficiency of plasmid vector for surface display of proteins.

The purified recombinant plasmids pSIP503 CMR-GusA were transformed in to competent *L. rhamnosus* GG by electroporation at 15000V/cm electric field and a time constant of approx 5 msec. After transformation positive clones were selected on erythromycin-agar plates (200µg/ml) resulted recombinant *L. rhamnosus* GG transformants.

Gus activity

To determined the maximum level of expression of the pSIP503SR-GusA system was optimized by adding different concentrations of nisin (0.1 to 100ng/ml) to recombinant *L. ramnosus* GG cultures harboring pSIP503SR-GusA plasmid. Initially the strains were induced at different OD_{600} from 0.1 to 0.6 with different nisin concentration. The highest activity (791.66 MU) was observed while bacterial culture was induced at OD_{600} 0.3 with 50 ng/ml of nisin. We examined that when nisin concentrations was increases (75 ng/ml and 100ng/ml) than GusA activity was decreased (666.66 MU and 500MU respectively) (Fig. 4.).

Expression of GusA in *L. rhamnosus* GG

Induction using 50 ng/mL of nisin for maximum expression was screened by using cells in a defined growth phase for GUS activity measurements.

(B). Western blot analysis of expressed Hsbp protein. Lane: 1. Induced LGG-pSIP503-Hsbp cell lysate, 2. Induced LGG-pSIP503-Hsbp cell surface proteins

The induced bacterial culture by adding nisin at OD_{600} 0.3 and cells were harvested 8 hours after induction for surface layer protein by GuHCl method. The extracted surface protein was analyzed on 10% SDS-PAGE electrophoresis as shown in Fig. 6a. The

Fig 3. Agarose gel showing A) Amplification of fusion gene of the 648 bp product by colony PCR, B) Restriction digestion of recombinant clone pSIP-Csp-M-PrtR with Nco1 and Hind III enzymes

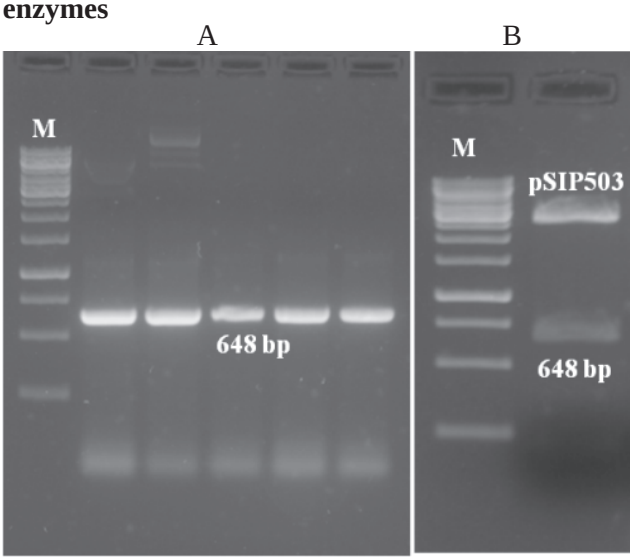


Fig 4 . Agarose gel showing amplified product of Hsbp gene from genomic DNA of *H. pylori*

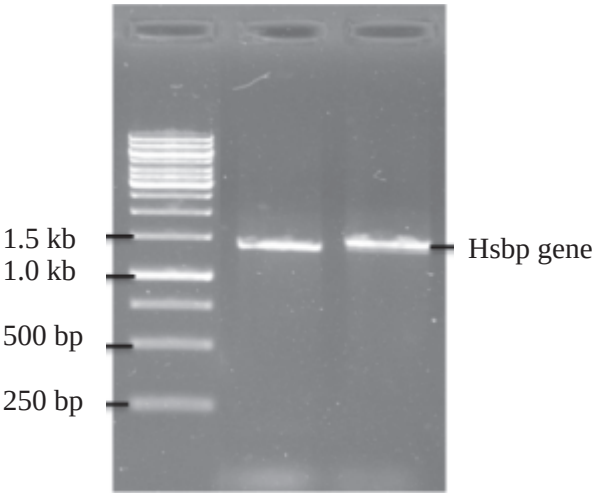
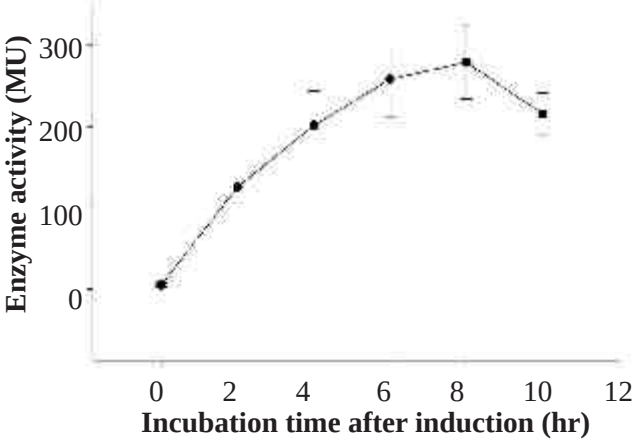


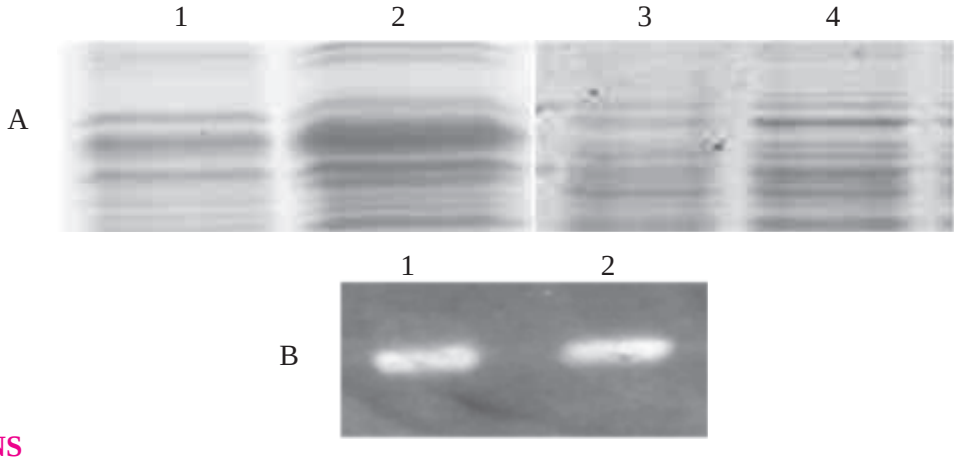
Fig 5. Beta-glucuronidase activity of recombinant LGG-pSIP503SR-Gus



western blot analysis of separated proteins anti-hsbp antibody demonstrated specific interaction with expected protein band, resulting a successful confirmation of 72 kDa Hsbp protein (Fig. 6b).

In other experiment we have examined the GusA activity at different incubation (6, 8 and 12 h) time after induction. The culture harvested at 8 h incubation showed highest GusA activity 777.78 MU.

Fig 6. A. SDS-PAGE showing the expression profile of extracted Hsbp protein induced with nisin, Lane: 1. Un-induced LGG-pSIP503 cell lysate, 2. Induced LGG-pSIP503-Hsbp cell lysate, 3. Un-induced LGG-pSIP503 cell lysate, 4. Induced LGG-pSIP503-Hsbp cell surface proteins.



CONCLUSIONS

In conclusion, a new system of cell surface display in lactic acid bacteria has been developed by using signal peptide CspI from *L. plantarum* and anchor peptide from *L. rhamnosus* PrtR having MPQTG type anchor that is specific for *L. rhamnosus* strians. The food-grade host is a plasmid-free derivative of *L. rhamnosus* GG. The food-grade vector was constructed using erythromycin genes as selection markers and the regulatory regions of nisRK regulatory genes for cell surface expression of foreign proteins. The proposed system is an alternative antigen delivery vehicle in oral vaccine formulation.

3. CHARACTERIZATION OF VAGINAL LACTOBACILLI ISOLATED FROM HEALTHY WOMEN TO FORMULATE POTENTIAL PROBIOTIC FOR REPRODUCTIVE HEALTH

Bacterial vaginosis (BV) is a common vaginal infection caused by imbalance of indigenous microbiota. When indigenous beneficial *Lactobacillus* spp are replaced by a mixture of other indigenous bacteria, these include *Gardenerella vaginalis Prevetella, Mobilincus,* and *peptostreptococcus* causes BV. Many women of child-bearing age experience bacterial vaginosis, although most of them are unaware of the condition. BV is prevelent in 20 to 40% of women in India as well as globally. BV increases predisposition to sexually transmitted diseases, including gonorrhoea, chlamydia, syphilis, trichomoniasis, human immunodeficiency virus (HIV) and human papilloma virus (HPV). In pregnancy, BV increases the risk of post-abortal sepsis, early miscarriage, recurrent abortion, late miscarriage, preterm prelabor rupture of membranes, spontaneous preterm labor, histological chorioamnionitis, and postpartum endometritis. Therapy of BV involves oral or local administration of metronidazole or intra-vaginal clindamycin, and varies in efficacy. The long-term cure rate is low, BV recurs in up to 40% of women within 3 months after initiation of antibiotic therapy and in up to 50% of women after 6 months. There are several unpleasant side-effects and disadvantages associated with these therapies, including severe infections by pathogenic microorganisms, susceptibility of lactobacilli to clindamycin in local application, and disturbance of gut flora occurs when treated by oral supplementation. Moreover, vaginal pathogens, particularly *G. vaginalis* and anaerobic bacteria, are showing increasing drug resistance in recent past. In this context, lactobacilli administered orally or locally may be an effective alternative therapy, which would re-establish the indigenous *Lactobacillus* to prevent BV

and associated complications. Hence, a better understanding of the indigenous species composition of vaginal ecology may help to develop better prophylaxis against BV. With this background the present study was conducted to undertake Lactobacillus profile in healthy pregnant women with normal and disturbed vaginal flora.

Ten different vaginal *Lactobacillus* spp profile were screened in 47 women to identify their abundance and distribution by multiplex PCR profile of 16S rDNA gene amplification. *Lactobacillus crispatus*, *Lactobacillus jensenii*, and *Lactobacillus gasseri* were found predominantly in the vagina of pregnant women, out of which *L. crispatus* was present in all the samples (27/27) of women with normal flora, whereas in women with abnormal flora it was found in 16 of 21 samples (76%). *L. jensenii* was also predominantly distributed in women with normal flora (20 of 27) when compared with abnormal flora (13 of 21). But *L.geserii* was found to be more predominant in women with abnormal flora (66.6%) when compare to normal (44%). Other *Lactobacillus* spp particularly *L.reuteri* and *L.acidophilus* were found in 20 and 32% of samples respectively in women with normal flora, while both were prevalent in 24% in women with abnormal flora. Lactobacillus profile by culture dependent method also showed *L.crispatus* and *L. jensenii* in 61 and 39% of samples respectively.

Based on our observation it may be suggested that *L.crispatus* alone or in combination with *L.jensinii* may be evaluated for probiotic properties for treating bacterial vaginosis (BV) by oral or local application.

METHODS

Sampling procedure: This study was performed with approval from the ethics committee of the National institute of nutrition, Hyderabad, India. Written informed consent was obtained from all study participants. In total, 48 vaginal samples (vaginal exudates) were collected for identification of Lactobacillus species from pregnant women. The first swab was used to prepare a smear on a glass slide for the purpose of grading as described by Nugent et al [29]. The second swab was transferred to a sterile phosphate buffer saline (PBS) tube for the purpose of DNA extraction.

Sample groups: The samples were classified into groups according to the Nugent score criteria [29] women with Nugent score (NS) 1-3 were classified as normal, and women with NS 4-6 classified as abnormal flora or intermediate flora. Vaginal smears were Gram stained and graded on a 10 point scale based on the presence of lactobacilli and other anaerobes, for normal microbiota (score 0–3), abnormal or intermediate microbiota (score 4–6) and BV (score 7–10). In brief, a score of zero to four is given individually for the presence of Lactobacilli and for Gardnerella/Bacteroides morphotypes and a score of zero to two for curved Gram variable rods such as mobilincus. The sum of all scores is a representative of the BV score. The presence of epithelial cells covered with bacteria (clue cells), a criteria for BV, was also noted. Nugent score (NS) 1-3 is represented in this manuscript as normal Nugent score or normal flora or normal microbiota and intermediate Nugent score (NS) 4-6 is written as either abnormal flora or intermediate flora or intermediate microbiota or disturbed flora.

Extraction of DNA from Lactobacillus and Polymerase chain reaction (PCR): Vaginal swab samples collected in 1ml PBS in ependoff tube and centrifuged at 6000 RPM for 15 minutes at 4^0C. 500 µl of freshly prepared lysozyme was added to the pellet and incubated at 37^0C for 1 hr (For 1 ml of SET buffer 5 mg of lysozyme). Then added 50µl of 10% SDS and inverted the sample for 5-6 times before incubated for 30 minutes, then added 200µl of 5 molar NaCl along with protein kinase and Rnase and incubated for 30 min. Subsequently added 800µl of phenol: chloroform: isoamylalcohol and incubated for 30 minutes then Inverted the sample for every 5 min in 30 min of duration. Centrifuge at 4500 rpm for 15 mins at 30^0C (two layers will be formed only transfer upper part of aqueous solution in a new 1.5 ml ependoff). Then Added ice cold isopropanal to double the volume of aqueous layer part from above part and inverted the sample for 4-5 mins then centrifuged at 12000 RPM for 15 mins at 4^0C (discard supernatant make sure the DNA pellet is not thrown away). Finally washed the pellet with 500µl of 70% ethanol and kept the sample for 2-4 mins and centrifuged at 12000 rpm for 10 mins and discarded the ethanol without pellet is discarded, and kept open in incubator for 10-15 min to make sure that no alcohol is left in the tube. To the final pellet TE buffer (this is an elution buffer) was added. DNA concentration and purity was checked by nano drop and agarose gel electrophoresis.

The polymerase chain reaction (PCR) is carried out for isolated DNA samples, Each sample was initially identified to the genus level by amplification with genus specific primers, using programme as initial denaturation at 94^0C for 5 min, followed by 30 cycles of denaturation at 94^0C for 30 sec, annealing at 55^0C and extension at 72^0C for 30 sec and 5min and genus was identified on agarose gel. Each isolate was positive for genus were then identified to the species level by amplification with specific primers, forward primer and reverse as mentioned above. Multiplex programm was used for identification of 10 lactobacillus spp, these were divided into groups a. Using programme as initialization at 94^0C for 5 min, followed by 30 cycles of denaturation at 94^0C for 30 sec, annealing at 55^0C for 30

seconds and extension at 72^{0}C for 35 sec final extension at 72^{0}C for 5min and hold temperature at 4 ^{0}C and finally species were identified on agarose gel base on product size.

Cultivation of vaginal swabs in MRS broth: The vaginal swabs were vortexed in 1ml sterile PBS (pH 7.4) to prepare bacterial suspensions. 100 µl of sample was used to inoculate in the test tube containing the 5 ml of freshly prepared sterile MRS broth medium (Himedia). After incubation for 48 hours under anaerobic condition at 37 ^{0}C, samples positive for growth were used for DNA extraction. 1ml MRS broth was taken in 2 ml ependoff tube and centrifuged at 6000 RPM for 15 minutes at 4^{0}C. Further DNA extraction and PCR was followed as described above.

RESULTS

In total, 48 women were recruited for the study. At the first study visit, weight, age, height, haemoglobin levels along with information regarding pregnancy history were collected. Mean of age, weight, height, BMI, birth weight, haemoglobin levels, and gestational age at recruitment and delivery were taken and categorised as shown in Table 1. All the measurements such as age, weight, height, BMI, haemoglobin levels were similar in normal and women with abnormal flora. But mean SD of birth weight was 2.7 kg in normal verses 2.52 kg in women with BV. Similarly, gestational age at delivery was 38.3 weeks in normal verses 37.8 weeks in women with BV. Out of 48 pregnant women, *L. crispatus* was identified in 88% of women followed by *L. jensenii* 66%, *L. gasseri* 55%, *L.acidophilus* 28%, *L.reuteri* 22%, *L salivarias* in 13%. The Lactobacillus species in pregnent women with normal flora and pregnent women with abnormal flora are shown in figure 1. *L. crispatus* (100%) was the most predominant lactobacilli species present in pregnant women. All the women with normal flora had *L.crispatus* followed by *L. jensenii* (72%), *L. gasseri* (66%), *L.acidophilus* (32%), and *L.reuteri* (20%) (Fig.1). While women with abnormal flora *L. crispatus* was found in 76%, followed by *L. jensenii* (61%), *L. gasseri* (41%) and *L.acidophilus* (24%). Whereas *L.reuteri*, which was more (24%) in abnormal flora than normal flora (7%), however all the lactobacillus spp were lower in women with abnormal flora (Fig.1)**,** *L. crispatus* (27/27) and *L. jensenii* (20/27) were more frequently detected in the normal samples than in the abnormal group.18.5% of the pregnant women with normal flora had *L.Crispatus* alone and another 18.5% had *L.Jensenii* along with *L.Crispatus*. 15% had *L.Crispatus, L.Jensenii,* and *L.gasseri* together, a similar combination was observed less frequently in women with intermediate microbiota (9%) (Table 2).

Interestingly, *L.crispatus* alone was found only 4.7% in women with intermediate flora. Though one or more *Lactobacillus* were identified by PCR only 18 of 48 (37.5%) samples had growth in MRS broth, while the rest were negative for lactobacillus growth. Lactobacilli profile was compared between culture dependent and culture independent method in all the 18 samples. In culture independent method *L. crispatus* was positive in 94% subjects, followed by *L. jensenii* (83%), *L. gasseri* (28%), *L.acidophilus* (22%), *L.reuteri* (17%) and *L salivarias* was (33%) in culture independent methods (Fig.2). Whereas in, culture dependent method L. *crispatus* was found only in 61%, followed by L. *jensenii* 39%, *L. gasseri* 50 %, *L.acidophilus* 5.5%, and *L.reuteri* was 28%. Interestingly, *L. gasseri* and *L.reuteri* were more frequent in culture dependent method, while *L salivarias* was frequent both in culture dependent and culture based methods (Fig 3).

Table 1. Demographic parameters in pregnant women with normal verses abnormal vaginal flora

Nugent Score	Normal (N) (NS 1-3)	Intermediate (I) (NS 4-6)	Total (N+I)
Number of subjects	27	21	48
Gest age at recruitment*	28.07- 2.63	27.52- 3.26	27.83 - 2.90
Age in Years	22.34- 2.74	21.88- 3.50	22.14- 3.07
Height in centimetres	151.91- 4.29	151.91- 4.2	152.39- 4.46
Weight in kilogram(kg)	50.25- 5.96	49.84- 8.51	50.10- 6.84
BMI	22.59- 2.22	21.71- 2.14	22.20- 2.21
Haemoglobin g/dl	10.35- 1.52	10.52- 1.47	10.43- 1.49
Birth weight in Kg	2.7- 0.46	2.52- 0.45	2.52- 0.46
Gest age at delivery*	38.33- 1.16	37.5- 1.63	37.8- 1.40

**Gestational age in weeks*

Table 2. Different combinations of *Lactobacillus* species distribution and percent in the pregnant women with normal and intermediate nugent score lactobacilli identified by species-specific multiplex PCR profile

S.No	Different combinations of Lactobacillus spp*	Normal nugent score (N=27)	Intermediate (N=21)
1	c, J , a, r, s	1 (3.7%)	1 (4.7%)
2	c, j , g, s	1 (3.7%)	0
3	c, J, ,a, s	0	1 (4.7%)
4	J, g, a, s	0	1 (4.7%)
5	c,g,a,s	0	1 (4.7%)
6	c, J, g,a,	3 (11%)	0
7	c, J, g, r	1 (3.7%)	2 (9.5%)
8	c,j, g	4 (15%)	2 (9.5%)
9	c,j,a	2 (7.4%)	0
10	c,j,r	3 (11%)	1 (4.7%)
11	j,g,r	0	1 (4.7%)
12	c,j	5 (18.5%)	2 (9.5%)
13	j,g	0	2 (9.5%)
14	c,g	2 (7.4%)	2 (9.5%)
15	c	5 (18.5%)	1 (4.7%)
16	J	0	1 (4.7%)

**L.Crispatus-c, L.Jensenii-j, L.gasseri-g, L.acedophilus-a, L. reuteri-r, L.Salivaris-s*

Fig 1. Percent of the most frequent vaginal *Lactobacillus* species in women with normal flora and intermediate or abnormal flora as determined by multiplex PCR profile

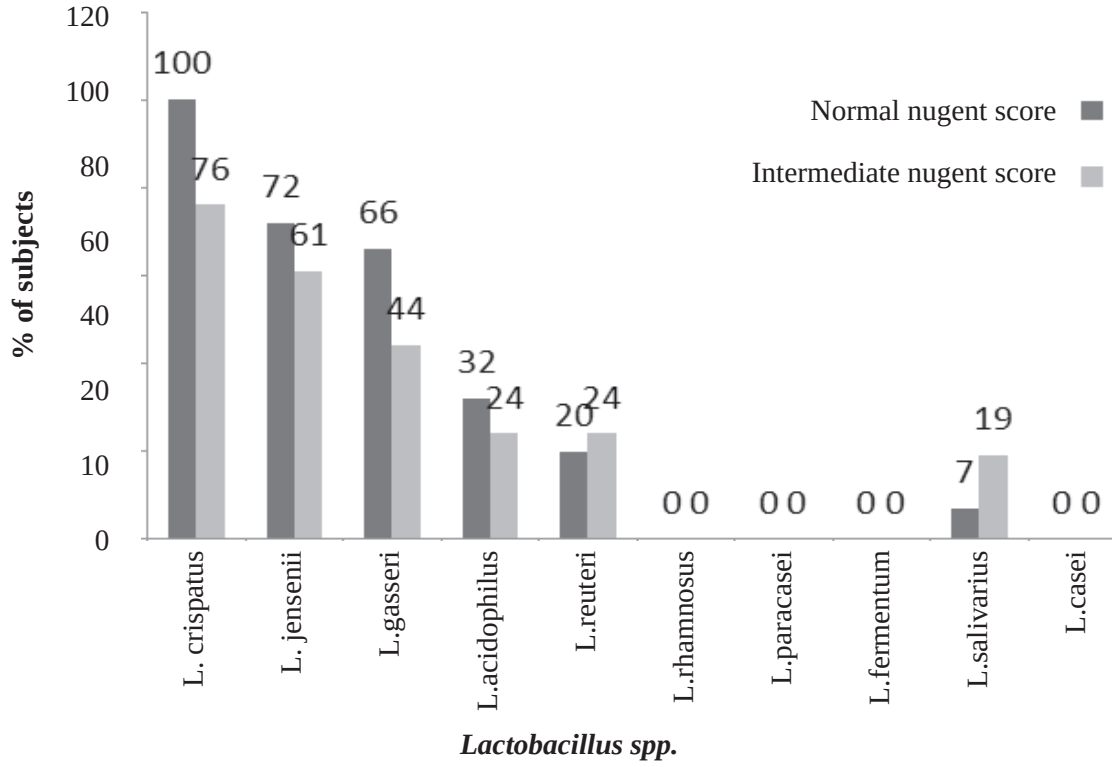


Fig 2. Gel pictures shows : *Lactobacills* genus confirmation followed by *Lactobacillus* spp. profile: Species-specific PCR products of the 10 species were identified after genus confirmation of 48 samples. The representative of this figure shown the profiles of 8 samples of the genus confirmation 250bp, followed by 3 species confirmation (*L.Crispatus, L.Jensenii, L.gasseri)* viewed on 2 percent agarose gel. *L. gasseri*, 360bp, *L. crispatus*, 522bp *L. jensenii*, 700bp.

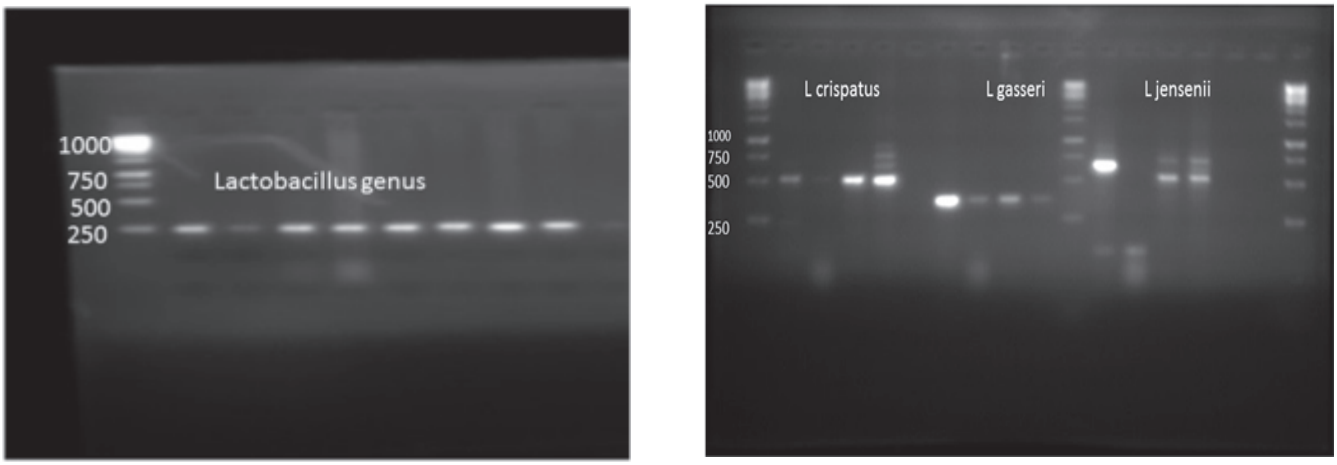
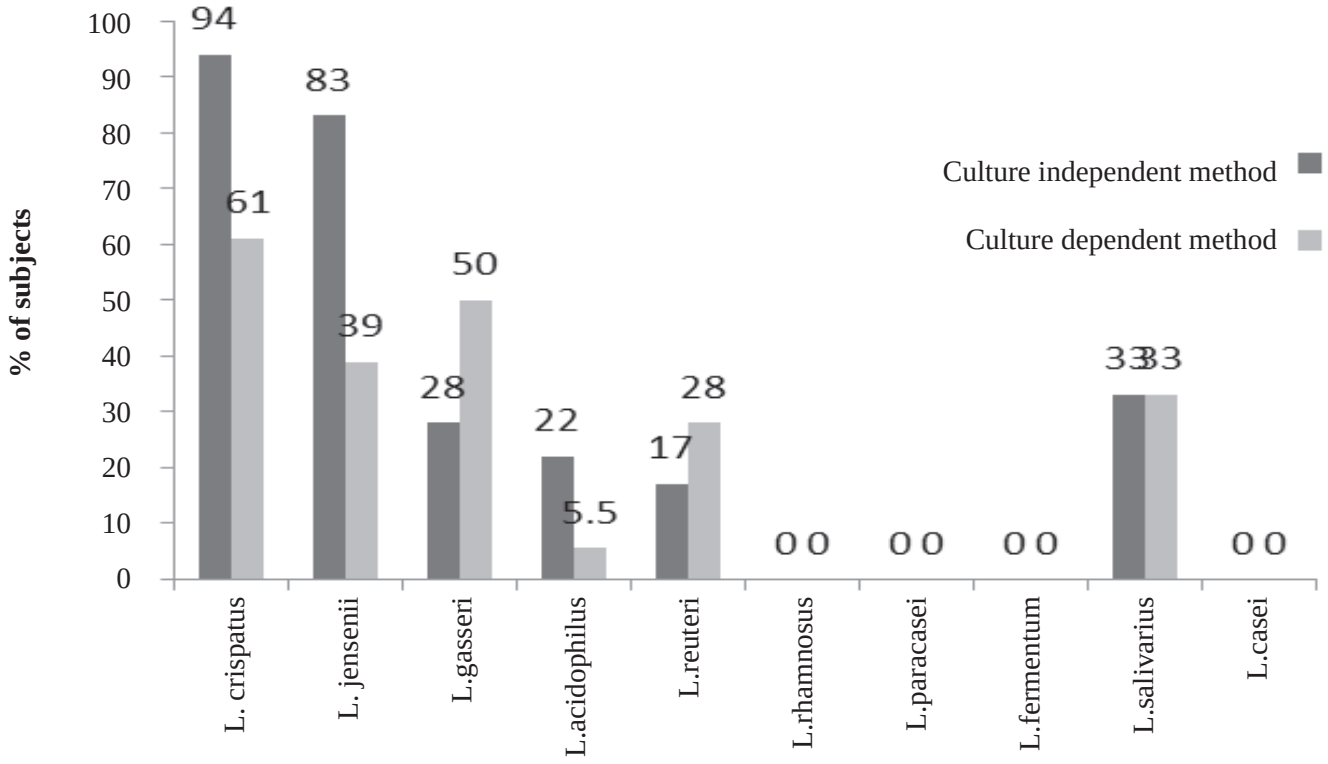


Fig 3. Vaginal Lactobacillus species obtained by culture dependent and culture-independent method



CONCLUSION

In conclusion, the study showed *L.crispatus* to be the predominant lactobacilli present in the pregnant women (with normal vaginal flora) of Indian origin. This information might be useful for the development of probiotic formulations for reproductive health of women in India or Indian origin throughout the globe. This study also made possible to understand how normal flora of vaginal lactobacillus shifted into intermediate flora before going into complete BV state.

III BASIC STUDIES

1. INNOVATIVE STRATEGIES TO PROMOTE EARLY CHILD DEVELOPMENT AMONG LOW-INCOME RURAL INFANTS AND PRESCHOOLERS IN INDIA THROUGH MULTIPLE MICRONUTRIENT FORTIFICATION AND EARLY LEARNING OPPORTUNITIES

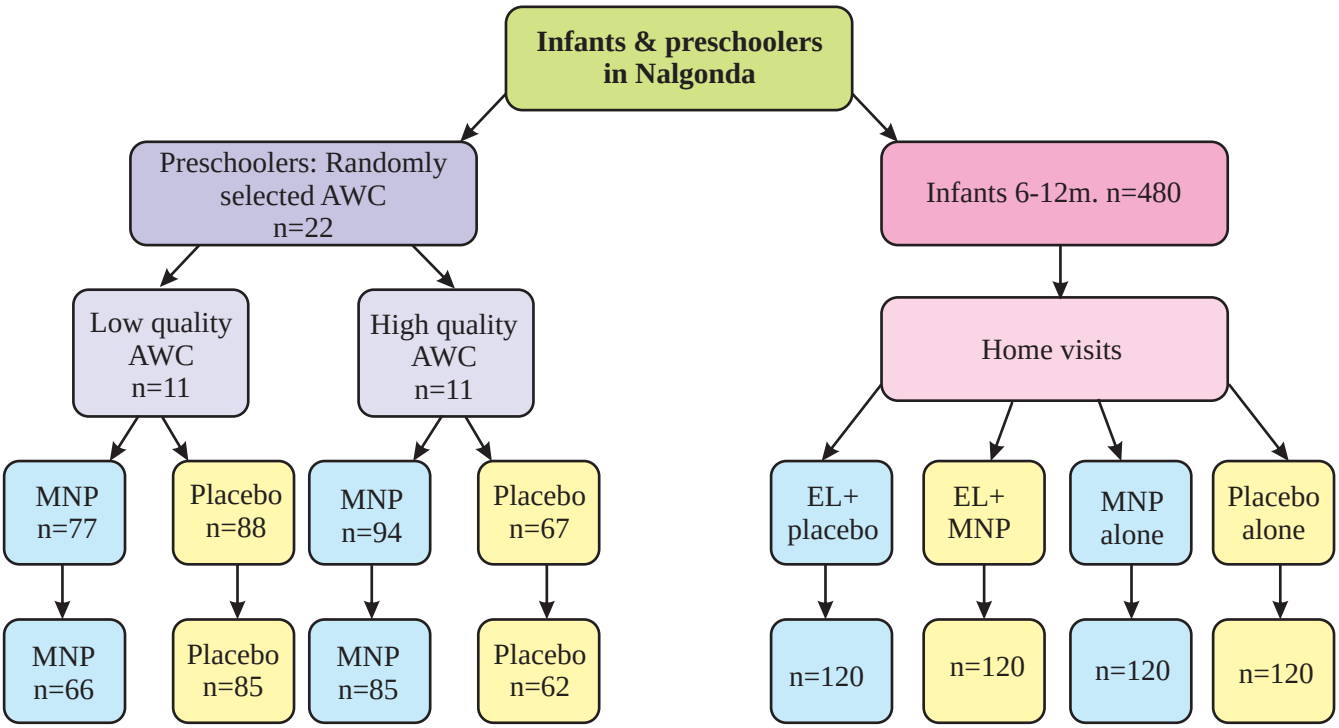
Micronutrient deficiencies and limited learning opportunities contribute to the loss of developmental potential among millions of children. Early developmental loss contributes to low academic performance, limited economic capabilities, and lifelong disparities. Integrated nutrition and early child development interventions may be efficient and optimal strategies to promote early child health and development.

AIMS AND OBJECTIVES

To evaluate the effects of an integrated micronutrient powder (MNP) and Early Learning (EL) intervention on micronutrient status and development of infants and preschoolers.

METHODS

Setting: Rural areas, Nalgonda District, Telangana (formerly Andhra Pradesh), India. The study design is given in fig.1.



Infant Phase *(age 6-12 months):* Randomized controlled trial with four arms: 1) MNP + EL, 2) MNP alone, 3) placebo + EL, 4) placebo alone. Village level workers conducted home visits biweekly for one year and delivered MNP/placebo sachets and early learning intervention. The MNP and placebos were mixed with infant's food by caregivers. Education for caregivers was based on care for child development from UNICEF/WHO.

Preschool phase *(age 36–48 months)*: Cluster-randomized trial in Anganwadi centers (AWCs), stratified by AWC quality (High/low) and randomized into 1) MNP and 2) placebo. MNP/placebo packets delivered biweekly for 8 months and mixed into the children's mid-day meal.

MNP formulations were based on Indian RDAs and prior efficacy trials, and were produced specifically for the study by M/s Primal Healthcare Mumbai, India (Table 1). Evaluations for infants were conducted at enrollment, 6-months, and 12-months; evaluations for preschoolers were conducted at enrollment and 8-months. Primary outcome measures: were 1) anemia and micronutrient status (hemoglobin, iron, zinc, folate, and vitamin B12); 2) child development (cognitive, motor, social emotional development, executive functioning, and parent-child interactions).

Table 1. Nutrient composition of MNP fortification products

	Grow smart 1 (6-12 months of age)	Grow Smart 2 (13-24 months of age)	Grow Smart 3 (36+ months of age)	Placebo (all age groups)
Iron (encapsulated ferrous fumarate), mg	8	10	13	0
Vitamin A (retinyl palmitate), µg	200	200	150	0
Vitamin C (ascorbic acid), mg	20	20	20	0
Folic acid, µg	20	20	20	0
Zinc (zinc gluconate), mg	5	5	5	0
Vitamin B_{12} (cobalamin), µg	0.5	0.5	0.5	0
Vitamin B_2 (riboflavin), mg	0.5	0.5	0.5	0.5
Maltodextrin	Filler			

RESULTS

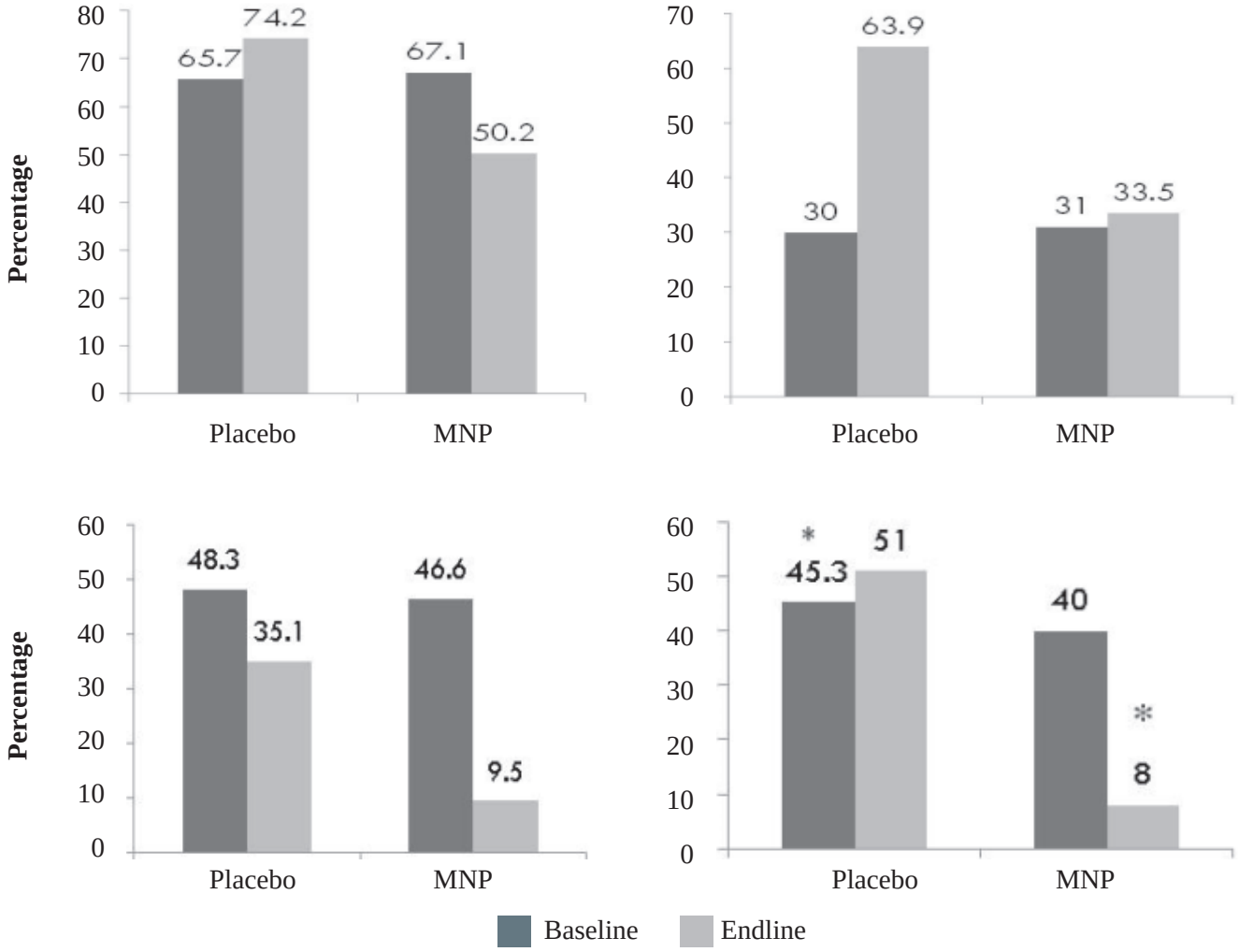
Impact on biomarkers: Anemia in infants reduced from 67% to 50% with MNP. There was an increase in anemia prevalence (66% to 72%) with placebo. Iron deficiency increased from 31% to 34% with MNP and increased from 30% to 64% with placebo.

Anemia in preschoolers reduced from 46% to 10 % with MNP. The placebo group also showed anemia reduction from 48 % to 35 %. Iron deficiency reduced from 40% to 8% with MNP and increased from 45% to 51% with placebo (Fig. 2).

Impact on development: Infants who received Early Learning intervention had better cognition at 6 m post intervention. Infants who received no intervention had the lowest scores. No additional benefit was observed from giving MNP & Early Learning together.

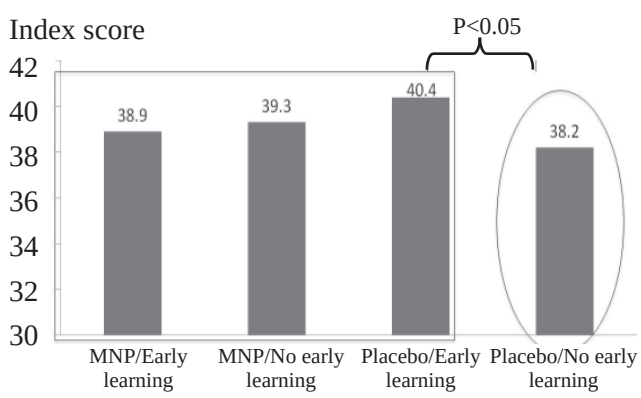
Among preschoolers, MNP significantly improved children's cognitive skills in low quality AWCs. No difference was observed in High Quality AWCs. MNP reduced disparities in development with respect to Anganwadi quality.

Fig 2. Impact of intervention on anemia and iron status among infants (A&B) and preschoolers (C&D).
Panel A. Impact on anemia among infants B. Impact on Iron deficiency among infants C. Impact on anemia among preschoolers D. Impact on iron deficiency among preschoolers

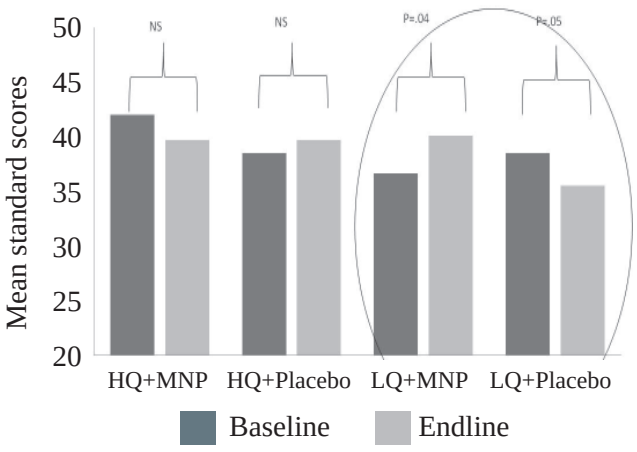


* Significantly different from baseline P<0.05

Fig 3. Impact on development (Expressive language) among infants (A) and preschoolers (B). A. Interaction for *Expressive Language (EL) among infants. B.* Interaction for *Expressive Language(EL) among preschoolers*



Adjusted for baseline



CONCLUSION

Findings from this home (infant) and preschool (Anganwadi Centre) based integrated trial can be used to guide larger-scale policy and programs designed to promote the developmental, education, and economic potential of young children in rural India.

2. EFFICACY OF POLYPHENOL-RICH DIETARY INGREDIENTS AS PROTEASOME INHIBITORS AND THEIR ROLE AS ANTI-CANCER AGENTS

Regular consumption of fruits and vegetables rich in polyphenolic content has been associated with a reduced cancer risk in humans. The mechanisms by which polyphenols/flavonoids impart their anti-cancer effects are beginning to emerge. Among these are the anti-inflammatory and free radical scavenging activities. In recent years, it has been reported that polyphenolic compounds could inhibit proteasome activity. Proteasome activity plays a critical role in the degradation of signaling molecules, tumor suppressors, cell-cycle regulators, anti-apoptotic proteins and transcription factors, thereby implying its role in a number of diseases. Upon proteasome inhibition these critical signaling cascades are dysregulated ultimately resulting in apoptosis**.** Since transformed cells have faster proliferative rates and defective cell-cycle checkpoints they are more vulnerable to pro-apoptotic stimuli compared to normal cells. Therefore, proteasome has emerged as an attractive molecular target for cancer therapy. Preliminary screening of commonly consumed fruits, vegetables and spices in our laboratory has shown that some of these dietary sources are highly rich in polyphenols and further these ingredients were found to possess proteasome-inhibitory activity. The present proposal was aimed at testing the efficacy of Murraya *koenigii* (curry leaves) leaves rich in phenolic content for proteasome inhibitory activity and further its anti- cancer property.

METHODOLOGY: This project work was divided into two phases:

Phase 1: *Cell culture studies* – using two breast carcinoma cell lines namely MDA-MB-231 and MCF-7; and WI-38 a normal lung fibroblast cell line.

Phase 2: *Animal Experimentation – In vivo* studies using nude mice implanted with human breast cancer xenografts.

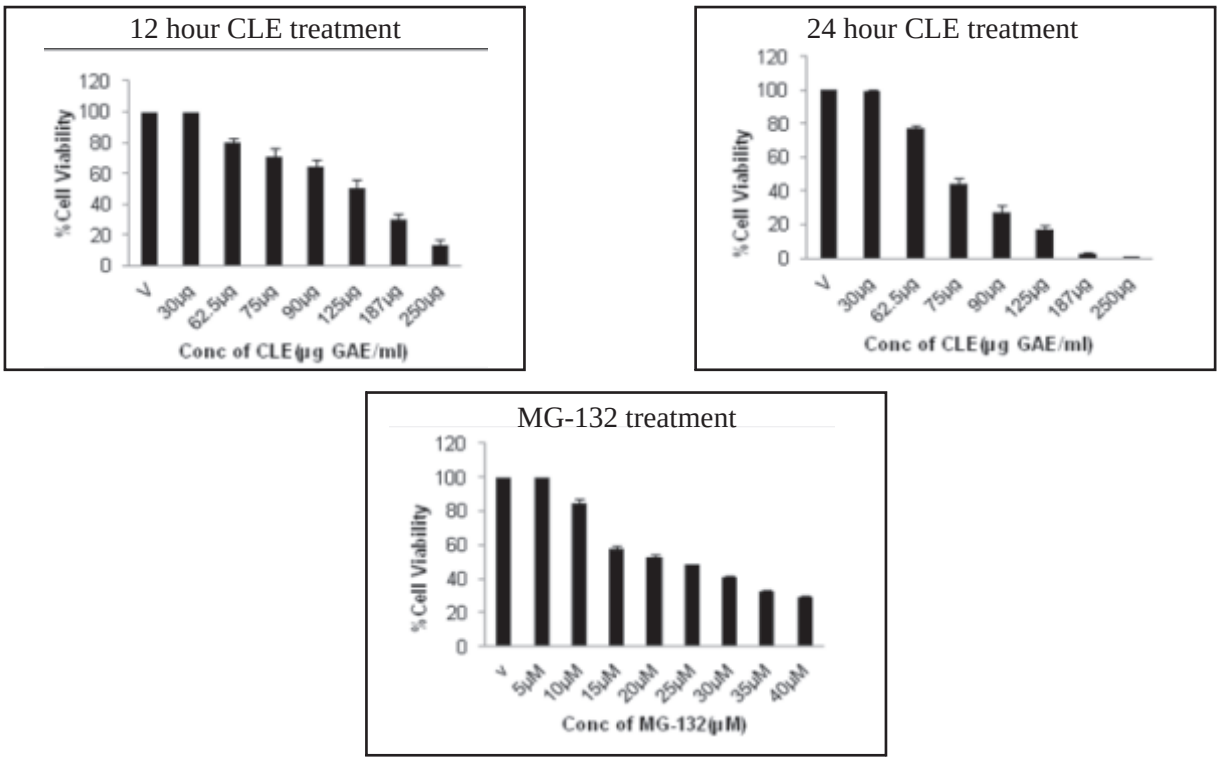
Phase 1: An 80% aqueous-methanolic extract of curry leaves (CLE) was prepared and its total phenolic content [TPC] determined by, the Folin-Ciocalteau's method. Cytotoxicity of the CLE was assessed by the MTT assay. We studied the effect of CLE on growth kinetics using colony formation assay. Growth arrest was assessed by cell cycle analysis and apoptosis by Annexin-V binding using flow cytometry. Inhibition of the endogenous 26S proteasome was studied in intact cells and cell extracts using substrates specific to 20S proteasomal enzymes.

RESULTS

- The TPC of the methanolic extract of M. *koenigii* leaves was 5µg of GAEs/µl of the CLE. MTT assays were performed with different concentrations of CLE (GAE) in both the cell lines at the 12h and 24h time points to assess the effect of the extract on cell viability. There was a significant ($p<0.05$) time and dose-dependent decrease in cell viability in both the cell lines (Fig 1). As a positive control, the effect of MG-132- a specific inhibitor of the proteolytic activity of the 26S proteasome was also assessed on cell viability in both the breast cancer cell lines. 24h post treatment with MG-132, a 50% reduction in cell viability was observed in both MDA-MB-231and MCF-7 cells (Fig 1).
- To test the effect of CLE on growth kinetics, MCF-7 and MDA-MB-231 cells were seeded at a lower density and treated with different concentrations of the CLE. After incubation for a week, it was observed that at a dose of 25µg GAE of CLE, no colonies were found in either MDA-MB-231(Fig 2) or MCF-7 cells (Fig 2). In line with our observations on cell viability (MTT assay), MDA-MB-231 cells appeared to be more sensitive than MCF-7 cells.
- Cell cycle experiments were done to determine whether CLE treatment arrested growth in MDA-MB-231 and MCF-7 cells. In both the breast carcinoma cell lines CLE treatment showed a dose-dependent arrest in the S

Fig 1. Curry leaf extract decreases cell viability of breast cancer cells: Top panel shows results of MTT assay after 12h and 24h treatment with CLE and MG-132 treatment, a specific proteasome inhibitor in MDA-MB-231 cell line. Bottom panel shows data from MCF-7 cell line. The data represents mean+/- SEM of three independent experiments.

MTT in MDA-MB-231 cells



MTT in MCF-7 cells

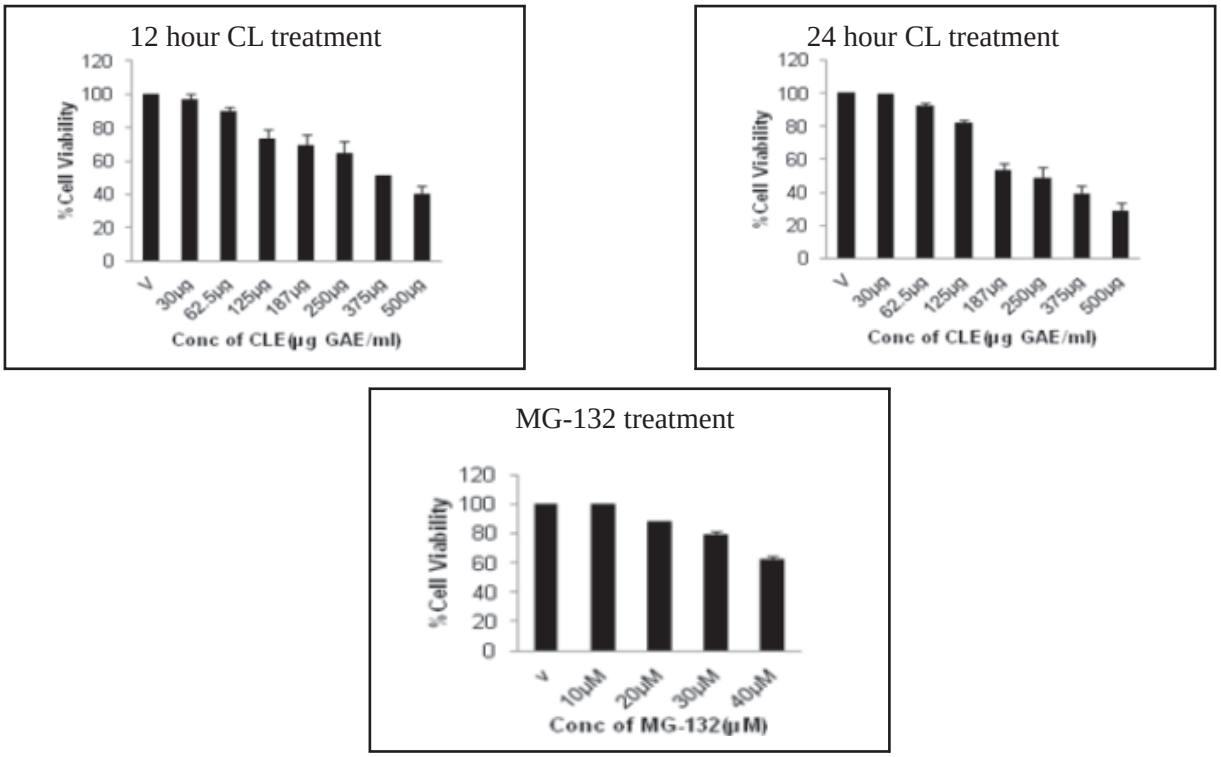
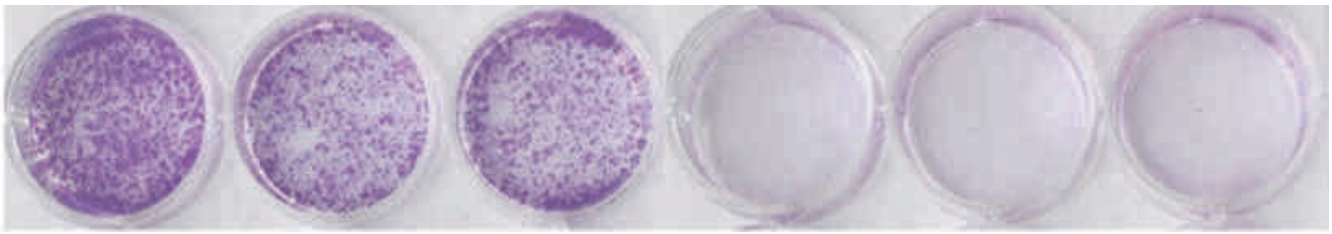


Fig 2. Curry leaf extract alters growth kinetics of breast cancer cells: Top panel depicts results of colony formation assay in MDA-MB-231 cell line, while the bottom panel depicts results of colony formation assay in MCF-7 cell line. Cells were grown in 6-well plates and treated with various concentrations of the CLE (0- 50µg GAE). After a week cells were stained with crystal violet and photographed.

MDA-MB-231 Cells

Vehicle 12.5mg 18mg 25mg 37.5mg 50mg

MCF-7 Cells



Vehicle 12.5mg 18mg 25mg 37.5mg 50mg

Table 1. Differential sensitivity to cell cycle arrest by CLE in breast cancer and normal fibroblast cells: Cell cycle analysis of cells treated with the CLE for 24h was done by flow cytometry.

Cell cycle analysis in MDA-MB-231 cells

Cell phase	Vehicle	0.6mg	1.2mg	2.5mg	5mg
G0-G1	43.98	54.15	44.53	33.9	37.3
S	52.07	37.26	53	66.10	62.6
G2-M	3.95	8.59	2.48	0.0	0.0

Cell cycle analysis in MCF-7 cells

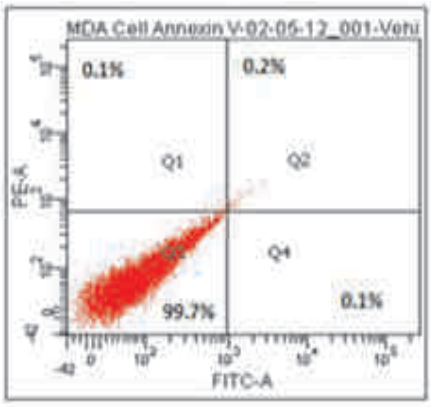
Cell phase	Vehicle	2.5mg	5mg	7.5mg	10mg
G0-G1	63.51	52.24	56.42	35.62	44.46
S	27.89	38.22	34.68	64.38	55.54
G2-M	8.60	9.59	8.89	0.0	0.0

Cell cycle analysis in WI-38 cells

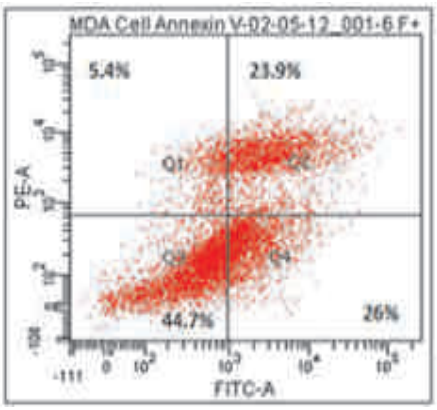
Cell phase	Vehicle	2.5mg	5mg	10mg
G0-G1	48.7	55.83	48.57	52.8
S	50.35	44.16	51.43	47.20
G2-M	0.87	0.00	0.00	0.00

Annexin V in MDA-MB-231 cells

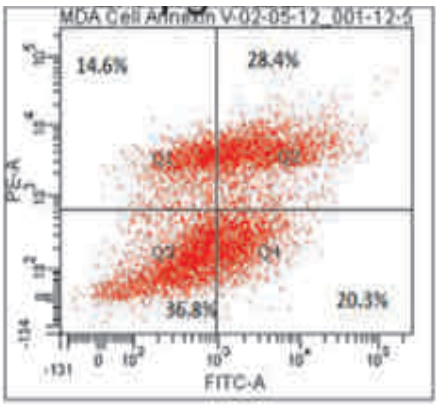
Vehicle



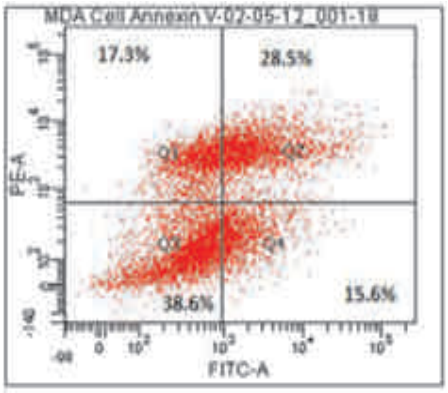
1.2mg CLE



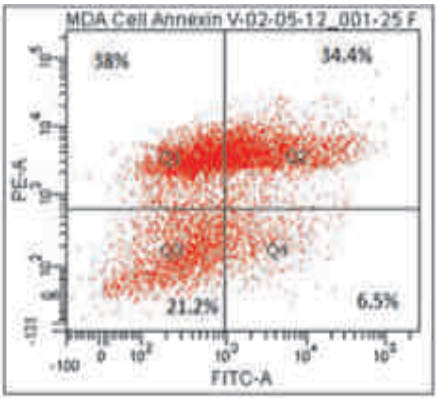
2.5mg CLE



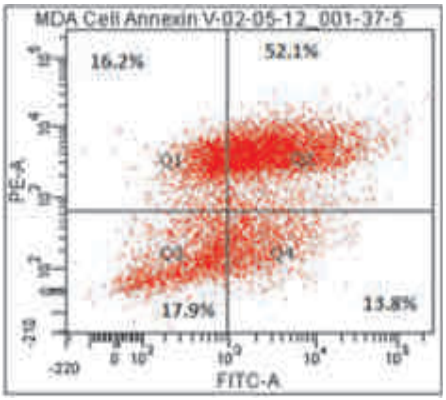
3.6mg CLE



5mg CLE

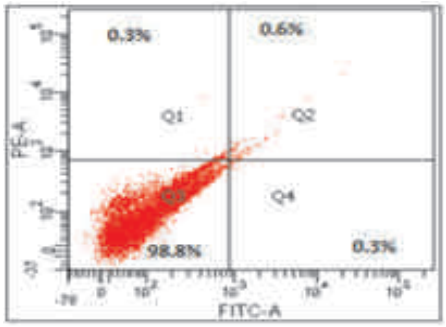


7.5mg CLE

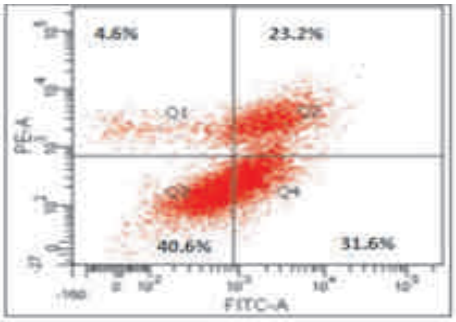


Annexin V in MCF-7 cells

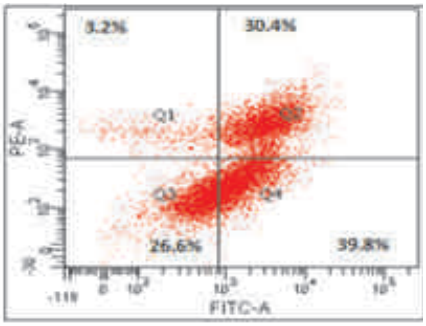
Vehicle



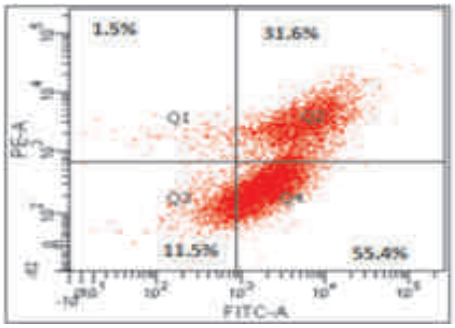
2.5mg CLE



5mg CLE



10mg CLE



phase of the cell cycle resulting in complete inhibition of cell proliferation (Table 1). Interestingly, CLE had no effect on cell cycle in the normal WI-38 cell line at any of the concentrations tested (Table 1), indicating that CLE could arrest growth only in cancer but not normal cells.

- Further, Annexin-V binding experiments were conducted in both cell lines to determine the probable mechanism of cell death. That 6µg CLE resulted in 45% of live MDA-MB-231 cells whereas, a dose of 12.5µg CLE was required for a similar effect in MCF-7 cells not only confirms the greater sensitivity of MDA-MB-231 than MCF-7 cells but also suggests apoptosis to be the probable mechanism of cell death. This is confirmed by our finding that CLE demonstrated a dose dependent increase in the % of apoptotic cells in both MDA-MB-231 and MCF-7 cells (Fig 3).
- We then tested whether or not CLE inhibited the activity of the purified 20S rabbit proteasome in a cell-free system. Indeed CLE decreased the chymotrypsin-like activity of the 20S proteasome in a dose-dependent manner (data not shown). Whether the CLE also inhibited the activity of the 26S proteasome in living cancer cells was assessed next in both MCF-7 and MDA-MB-231 cells. Similar to its effects on the purified 20S rabbit proteasome, CLE showed a significant ($p<0.05$), dose-dependent decrease in the chymotrypsin-like activity of the 26S proteasome in intact cancer cells (Fig 4). On the other hand, CLE did not inhibit the chymotrypsin-like activity of the 26S proteasome at any of the concentrations tested in the normal WI-38 cells (Fig 4) indicating the specificity of the effect to cancer cells.

Fig 3. Exposure to CLE induces apoptosis in breast cancer cells: Phosphatidylserine levels were detected by annexin V-FITC binding. a.MDA-MB-231 or b.MCF-7 cells were treated with varying concentrations of the CLE for 24h and stained with FITC-conjugated annexin V and propidium iodide (PI).

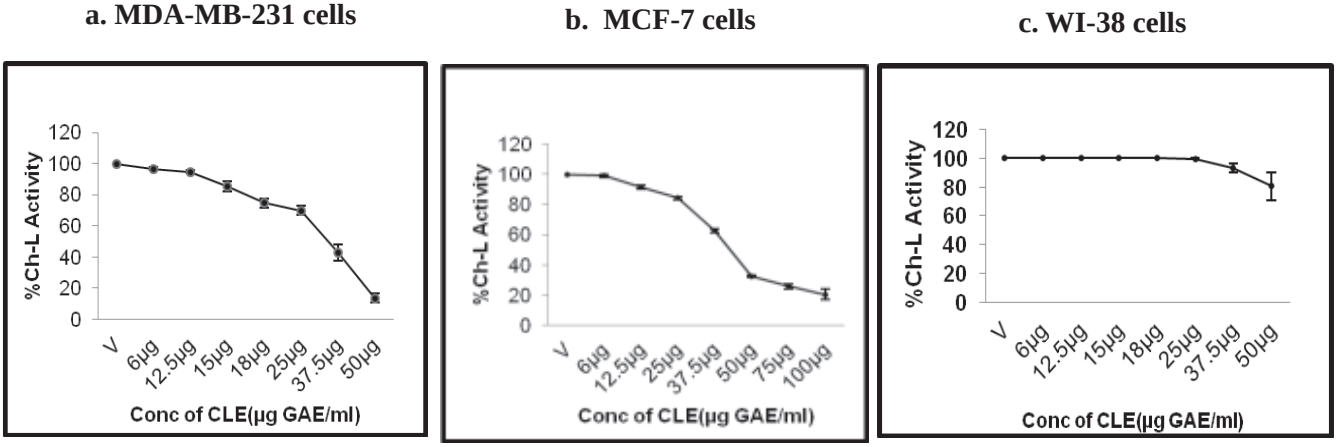
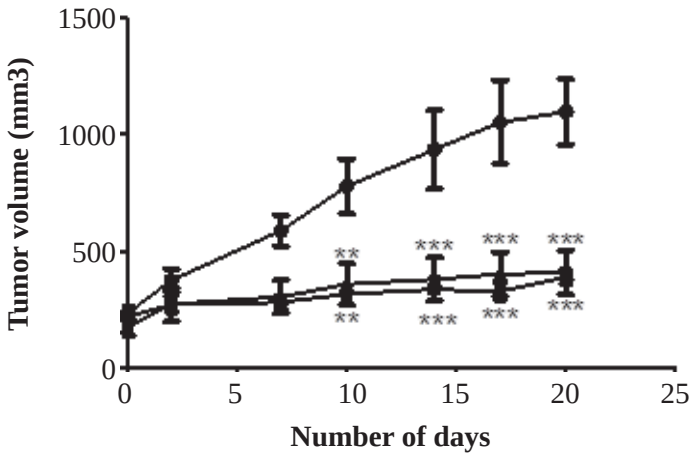


Fig 4. Effect of CLE on the Ch-L activity of the endogenous 26S proteasome in intact breast cancer and normal cells



- M. *koenigii* leaves are known to be a rich source of alkaloids and flavanoids. In order to test the cytotoxic and proteasome inhibitory potential of the alkaloid component, a total alkaloid extract (TAE) was prepared from the leaves. The TAE dose-dependently decreased the cell viability of MDA-MB-231 cells (data not shown). Further the TAE inhibited the Ch-L activity in both intact cells and cell extracts prepared from MDA-MB-231 cells (data not shown). These data indicate that M. *koenigii* leaf alkaloids possess both proteasome inhibitory and cytotoxic activity towards breast cancer cells.
- Cytotoxicity and proteasome inhibition of the CLE was also tested in a panel of solid human tumor cell lines namely Caco-2 (colon), HeLa (cervical), HepG2 (liver) and LNCaP (prostate). There was a dose-dependent decrease in cell viability and proteasome activity in all the cell lines tested (data not shown). There appeared to be a differential response to the CLE in the different cell lines with CLE treatment in the parameters tested.

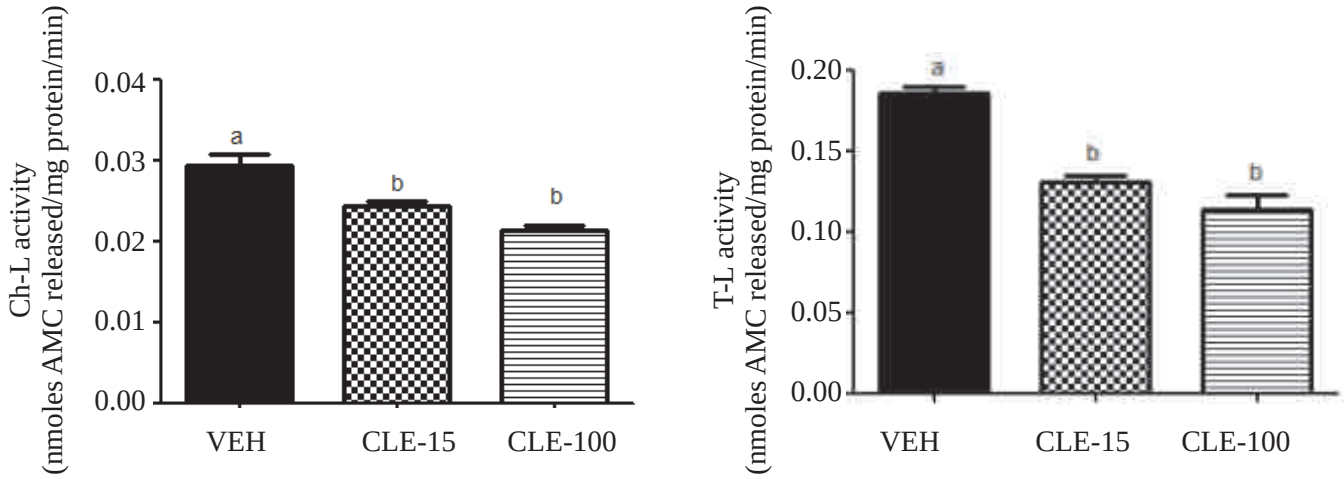
Phase 2

Female nude mice (*nu/nu*) aged 4week, were housed under pathogen-free conditions in accordance with Institutional animal care guidelines. Mice were fed on regular chow diet and had free access to de-ionized water. After one week of acclimatization, human breast cancer MDA-MB-231 cells (1 x 10^7) suspended in 0.2ml serum-free DMEM media were injected subcutaneously in the right flank of each mouse. When the xenografts reached sizes of ~200 mm^3 on average, the mice were randomly divided into three groups ($n = 6$). One group was given the control (1% CMC), while the second group received 15mg MLE/100g and the third group was administered 100mg MLE/100g body weight. Doses were administered orally through gavage on a daily basis. The tumor size was measured every alternate day using callipers, and the tumor volumes were calculated according to a standard formula: $\underline{(\text{length x width})^2 \text{x } 0.5}$. Mice were sacrificed after 21 days of treatment. The tumors were dissected out, photographed and the tumor tissues were frozen in liquid nitrogen. They were stored at -80°C for subsequent analysis. A part of tumor tissue was fixed in 10% formalin for histo-pathological analysis.

RESULTS

- To determine whether MLE could inhibit tumor growth *in vivo*,mice were treated with two different concentrations of MLE and compared with the control groupTumors from control group grew to an average of1099±127.9mm^3,while the low dose (15mg MLE/100g BW) treated tumor grew to 389.6 ±26.7 mm^3 and high dose (100mg MLE/100g BW)treated tumor grew to 409.5± 22.4 mm^3.There was 64% inhibition in tumor growth in 15mg/100g MLE treated group and 62.8% inhibition was seen in the 100mg MLE/100 g treated group which was significantly different ($P<0.001$) from the control group (Fig 5). This data suggests that MLE possesses potent antitumor effects *in vivo*.

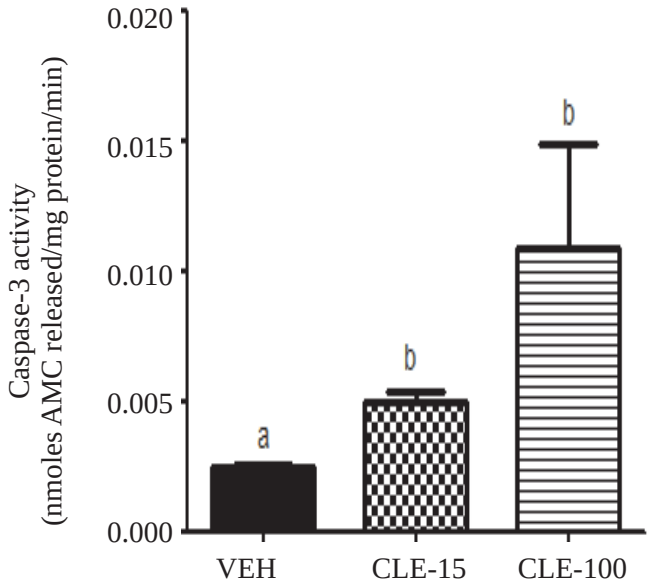
Fig 5. Effect of M. *koenigii* extract on human breast cancer cell tumor xenografts: Tumor volumes were measured twice a week for three weeks.



Data are represented as mean ± SEM of n=6

- To investigate whether the observed antitumor effects of MLE are associated with proteasome-inhibitory activities *in vivo*, tumor extracts from control or MLE treated tumors were used to estimate the proteasomal Ch-L and T-L activities. The proteasomal Ch-L activity was decreased by 17.5% in tumors that received the low dose and by 27.5% in the high dose (P<0.001)treated tumor tissues when compared to control group (Fig 6). Similarly, there was a dose dependent decrease in the T-L activity(29.4% in low dose group) and (38.6% in the high dose group) which was significantly (P<0.01) different from the control group (Fig 6).

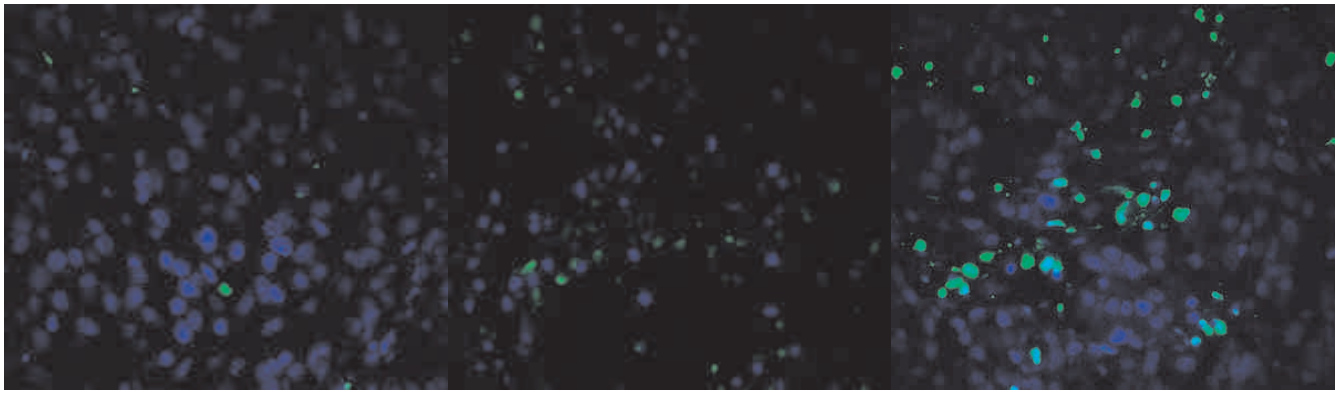
Fig 6. Effect of M. *koenigii* extract on proteasomal catalytic activity of human breast cancer cell tumor xenografts: Chymotrypsin-like and trypsin-like enzymatic activities of the 26S proteasome was measured using fluorogenic substrates in tumor extracts.



Data is represented as mean ± SEM of n=6

- To determine whether suppression of tumor growth is due to induction of apoptosis, caspase-3 activity was monitored in tumor extracts. There was a significant (P<0.01) increase in the caspase-3 activity in the MLE treated groups as compared to control group (Fig 7). To further confirm that apoptosis is responsible for the reduction in tumor size, TUNEL assay was performed. Increased TUNEL positive cells in MLE treated tumor tissues were observed when compared to the control group (Fig 7). Increased expression of p27 and p21 (cell cycle inhibitors) was observed in the MLE treated group as compared to control (Fig 8) indicating arrest in cell cycle. Decrease in VEGF protein was also seen in MLE treated groups as compared to controls (Fig 8).
- Cancer cells are known to express anti-apoptotic and angiogenesis related proteins at high level.To determine if the inhibition of tumor growth was due to down regulation of anti-apoptotic, angiogenic and FoxM1 transcription factor genes, expression of anti-apoptotic markers Survivin and XIAP and angiogenic marker genes VEGF and VEGFR were analyzed in tumor extracts. There was a significant (P<0.001) decrease in expression of angiogenic genes in both the MLE groups compared to control. Expression of survivin was also observed to be significantly lower in both the low (P<0.01) and high dose (P<0.001) MLE treatments. On the other hand, expression of XIAP gene was decreased (P<0.001) only in the group treated with high dose of MLE as compared to control, while FoxM1 expression remained unaltered between the groups (Fig 9).

Fig 7. Effect of M. *koenigii* extract on breast tumor apoptosis: Apoptosis was assessed by measuring the caspase-3 activity and TUNEL assays in tumor extracts. Caspase-3 activity was measured using a specific fluorogenic substrate while TUNEL assay was done using a kit.



- In order to assess the effect of MLE treatment on pathways involved in cell proliferation, the PI3k/Akt and p38/MAPK pathway protein levels were investigated by western blot. As shown in Fig 10 the levels of pAkt were decreased in MLE treated groups than control group. On the other hand, both total p38 and pp38 levels were increased in MLE treated groups. The expression of the pro-apoptotic protein Bax was increased in the MLE treated groups as compared to control group (Fig 10) and was in accordance with the data showing increase in caspase-3 activity and TUNEL positive cells.

Fig 8. Effect of M. *koenigii* extract on expression of p21, p27 and VEGF in breast tumor xenografts: Immuno-histochemistry of p21, p27 and VEGF in the different groups of nude mice.

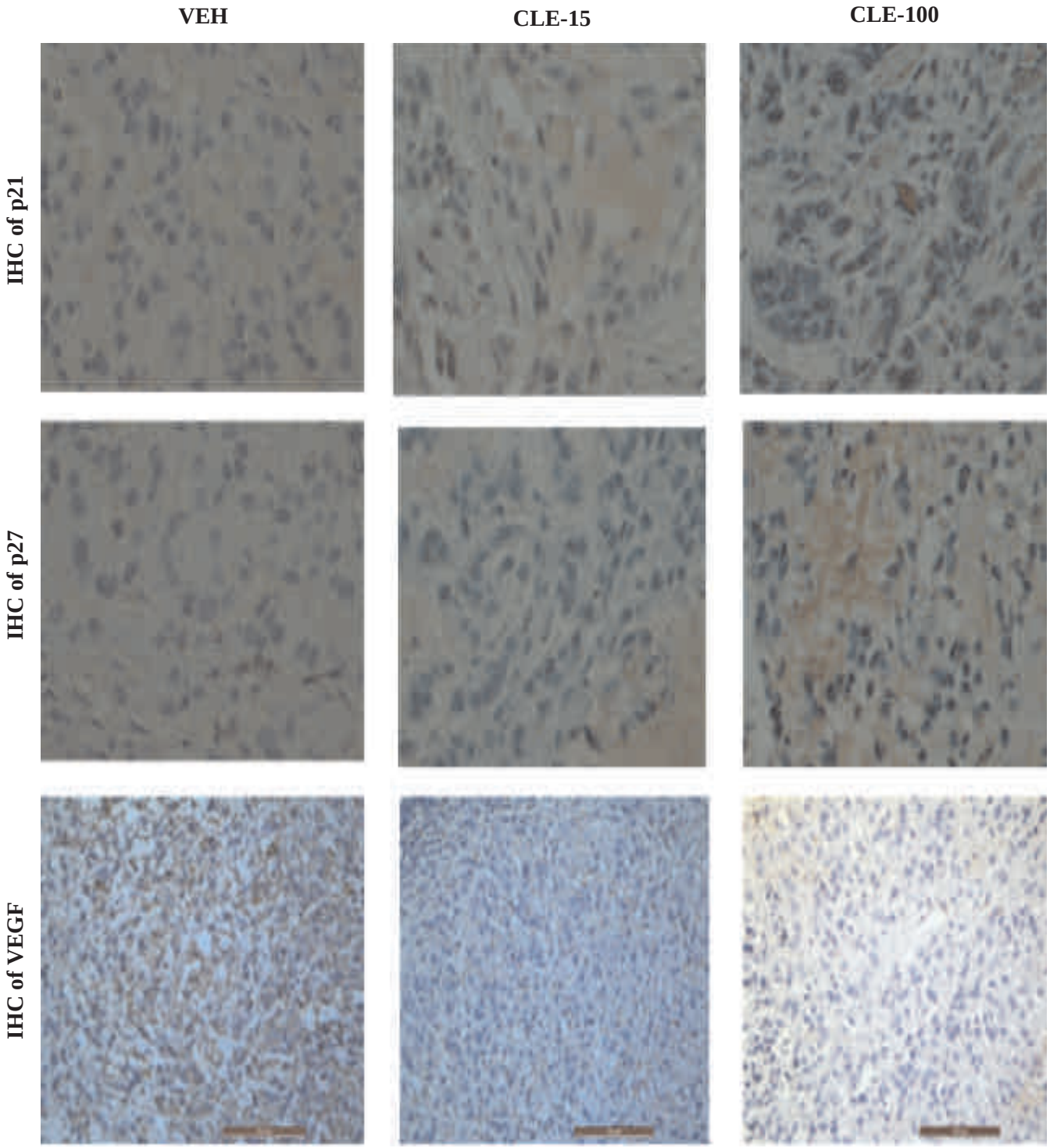


Fig 9. Effect of M. *koenigii* extract on expression of FoxM1, angiogenic, anti-apoptotic gene markers; and expression of PI3k/Akt and p38/MAPK pathway proteins in breast tumor xenografts: Angiogenic gene markers (VEGF & VEFGR), anti-apoptotic gene markers (SURVIVIN & XIAP) and the transcription factor FOXM1 are shown.

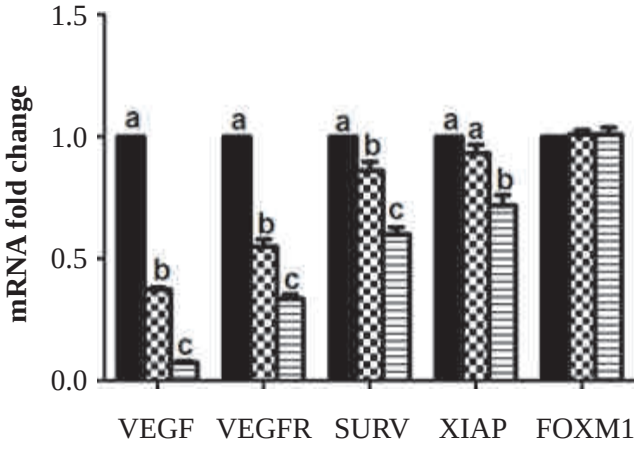
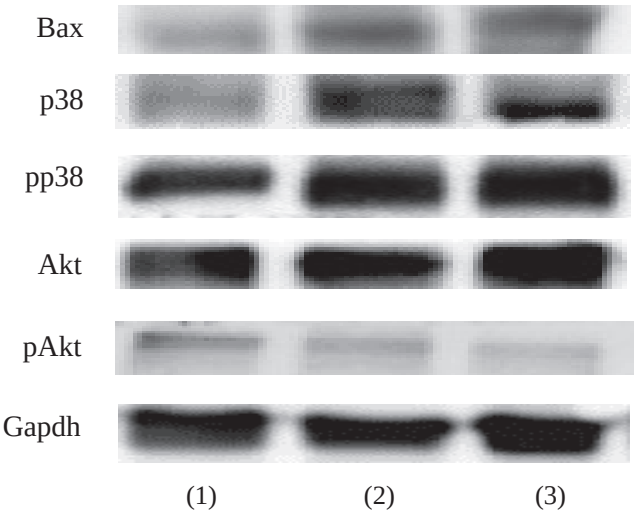


Fig 10. Effect of MLE on PI3k/Akt and p38 MAPK pathways. Tumor extracts were prepared as described in methods and analyzed by 12% SDS-PAGE followed by western blot analysis using specific antibodies.(1) Control treated group; (2) MLE (15mg/100g BW) treated group (3) MLE (100mg/100g BW) treated group.



Data are represented as mean ± SEM of n=4.

CONCLUSION

In summary, our in vitro studies demonstrate that M. koenigii leaf extract is cytotoxic only to cancer cells but not normal cells. Further, the extract inhibited the proteolytic activity of the 26S proteasome only in cancer cells. Our study also provides evidence that MLE acts as a proteasome inhibitor and pro-apoptotic agent *in vivo*, and highlights the potential benefit of M. *koenigii* leaves for the prevention and treatment of breast cancer.

3. REDUCED LONGEVITY IN WNIN/Ob RATS: POSSIBLE ROLE OF CHANGES IN NEURONAL METABOLISM AND NEUROCHEMICAL PROFILE

Increase in the aged population puts a challenge of maintaining a society with healthy aging people. One important characteristic of aging is the decline in cognitive function, but very little is known about the biological basis of the deterioration in cognitive function. This has necessitated the development of an animal model useful for basic geriatric research so that the effects of various interventions can be evaluated. National Institute of Nutrition, Hyderabad has developed an obese rat (WNIN/Ob) from the WNIN rat colony. WNIN/Ob obese rats are the heaviest inbred rat strain in the world. These rats are hyperphagic, hyperinsulinemic, hyperleptinemic, dyslipidemic and show accelerated aging. In general, wild type WNIN rats survive around 36 months but WNIN/Ob obese rats have an average age life span of 15-18 months and thus have decreased longevity. Therefore, the WNIN/Ob obese rat is a good plausible model for obesity and aging related studies.

Glutamate (Glu) and γ-aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters, respectively in the mature mammalian central nervous system. These neurotransmitters are involved in many functions such as motor behaviour, cognition and emotion and are affected during the course of the normal aging.

Several lines of evidence implicate glutamate toxicity as a factor in the pathogenesis of age-related neurological conditions, including Alzheimer's, Parkinson's disease, and Amyotrophic lateral sclerosis. It is well established that a neuronal astrocytic substrate (glutamate-glutamine-GABA) cycle exists in the brain. Dysfunction in these pathways is associated with many neurological and neuropsychiatric disorders. Thus the glutamate-GABA-glutamine axis is of major importance to brain function and cerebral well being. Level of neurometabolites such as N-acetylaspartate (NAA), total creatine (Cr), phosphorylethanoloamine (PE), taurine (Tau), glutamate (Glu), glutamine (Gln), gamma-aminobutyrate (GABA), alanine (Ala) could be reliably measured using *in vivo* and *in vitro* MRS. *In vivo* MRS in humans show that level of neurometabolites get altered with normal aging which acts as a cause or reason of various pathologies and processes of aging.

Available evidence shows a direct correlation between obesity or increased body mass index (BMI) and cognitive decline. Obese subjects are shown to have smaller brains as compared to the age-matched normal weight control. Further, obesity is associated with increased risk for cardiovascular health problems including hypertension, diabetes and stroke. In turn, the cardiovascular burden increases the possibility for cognitive decline and in a few cases is associated with dementia. A recent study has indicated that obesity is associated with detectable brain volume deficits in cognitively normal elderly subjects. In this proposal we would like to evaluate the neurochemical profile and neuro metabolism in different regions of WNIN/Ob rats' brain and compare with age matched control rats.

The key questions of the proposal are, (i) Are there any volumetric changes in the brain of WNIN/Ob obese rats compared to WNIN controls and (ii) whether changes in the neurochemical profile and neuro-metabolism in WNIN/Ob obese rats compared to their age matched controls is associated with their decreased longevity.

Hypothesis: Based on the available literature, we hypothesize that changes in the neurochemical profile and neuro-metabolism in the cerebral cortex of WNIN/Ob obese rats at young age is associated with their decreased longevity. The present study is being conducted in the WNIN/Ob obese rats and their age / sex matched to controls to validate / negate this hypothesis.

OBJECTIVES

- To determine the volumetric changes if any in the brain of WNIN/Ob obese rats as compared to their age matched controls.
- To assess the changes if any in the neurochemical profile in WNIN/Ob obese rats as compared to their age matched controls.
- To explore neuronal glucose oxidation as a marker of neuro-metabolism in the cerebral cortex of WNIN/Ob obese rats and compare it with their age matched controls.

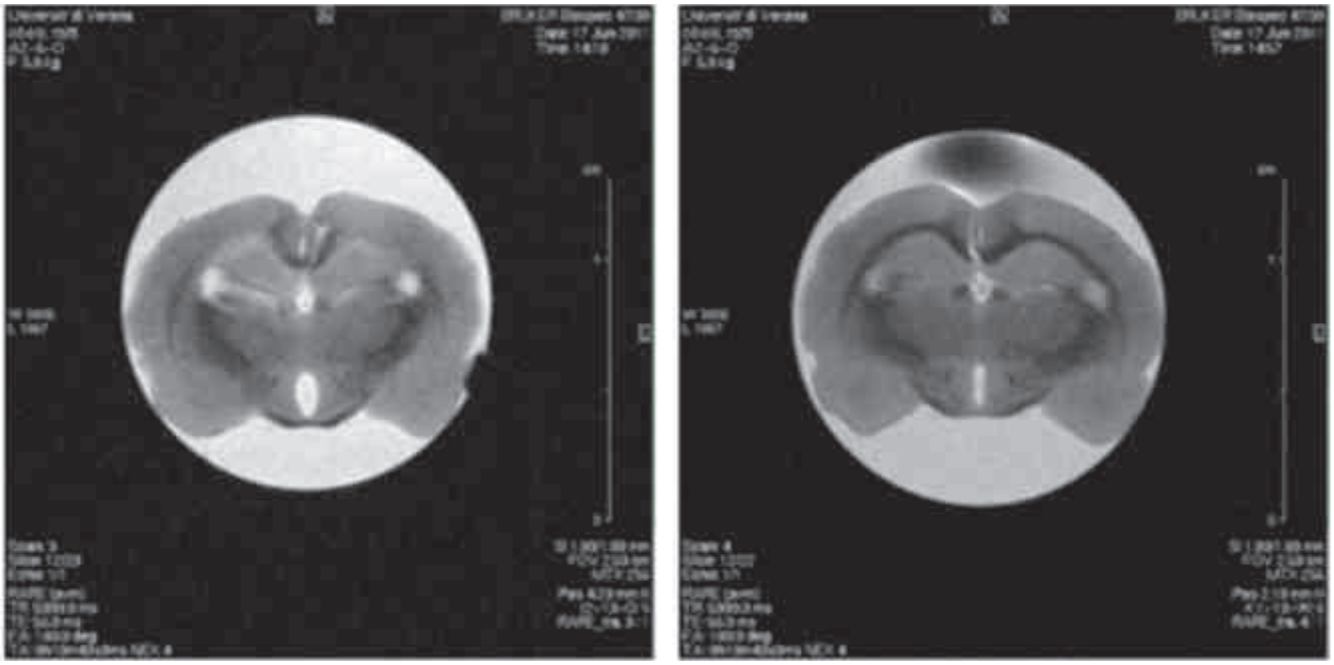
RESULTS

1. *Evaluation of the volumetric changes by MRI in the brain of WNIN/Ob obese rats vs parental WNIN control rats*

Volume changes in the brain were determined in the WNIN/Ob obese and normal WNIN rats of comparable age using Magnetic Resonance Imaging (MRI) as follows. Bruker Biospec Tomograph (Bruker Medical, Ettlingen, Germany) equipped with an Oxford, 33 cm bore, horizontal magnet operating at 4.7 T and a Bruker gradient insert (maximum intensity 20 G/cm) and with T2W Rapid Acquisition Relaxation Enhancement (RARE) imaging was utilized for determining the brain volumes in WNIN/Ob obese and WNIN rats. RARE sequences had the following parameters: field of view (FOV) = 2.50 × 2.50 cm^2; slice thickness: 1.00 mm; interslice distance: 1.00 mm; slice orient: axial; matrix size 256 × 256; echo time (TE) = 56.0 ms; repetition time (TR) = 5000 ms; echoes = 1; averages = 4. Images obtained were analyzed using the software ParaVision® 2.1.1 (Bruker, Germany).

The volumetric analysis changes in the brain of WNIN/Ob obese rats *vs* normal rats by MRI has been completed. The brain regions that were analyzed are cerebral cortex, hippocampus, caudo putamen, hypothalamus and corpus callosum. The volumetric analysis of the brain MRI images did not show any significant differences between the age matched obese and control rats in various brain regions analyzed. This falls in line with our previous observations of wet brain weights in different group of animals. Although the brain wet weights of WNIN/Ob obese rats were lower than those of the WNIN normal control rats,the differences were not statistically significant.

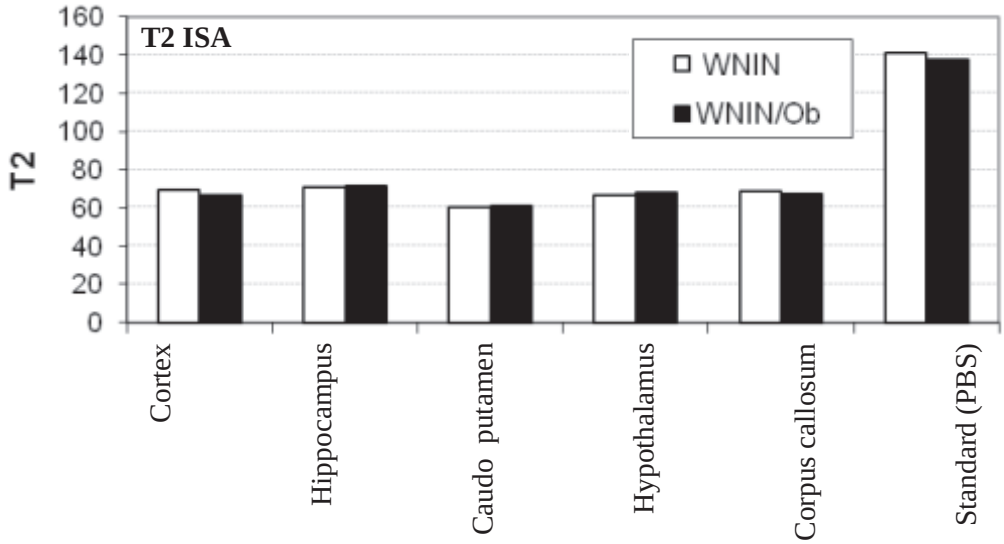
Fig 1. Comparative images showing brain MRI scans of WNIN/Ob obese and WNIN normal rat as captured by Bruker Biospec Tomograph



WNIN/Ob obese rat

WNIN normal rat

Fig 2. Volumetric changes in various brain parts as analyzed from the MRI images



2. *Evaluation of the neuronal metabolism and neurochemical profiling by MRS in the brain of WNIN/Ob obese rats vs controls rats.*

We did the *in vitro* study to find the changes if any in the neurochemical profile in cerebral cortex of WNIN/Ob obese rats as compared to the controls rats. In this study, the concentrations of Glu, NAA and Tau were significantly lower in the cerebral cortex of WNIN/Ob obese rats (than that of controls). The neurochemicals considered as markers of astrogliosis *viz.*, Gln and Inositol (Ino; also shows osmotic stress dysfunction) have been reported to be increased during ageing and in line with these reports we observed increased concentrations of Gln and Ino in the cerebral cortex of these obese rats of 3 months age (compared to the corresponding controls) confirming the imbalance of neurochemicals favouring neurodegeneration and ageing. As these observations were made in the

obese rats at 3 months of age, our findings indicate that at a young age itself the neurochemical profile of these animals is getting altered and resembles the changes seen in the control rat brains at later age (*e.g.* 15 – 18 months), further corroborating the accelerated ageing of the WNIN/Ob obese rats.

Different metabolite concentrations reveal the activity of various metabolic processes. To evaluate, we infused ^{13}C-labeled glucose in these rats and measured amount of ^{13}C being incorporated in the brain metabolites involved in neurotransmitter cycling. We measured the concentrations of ^{13}C labeled $Glu_{C4,}$ $GABA_{C2}$ and Gln_{C4} from the ^{1}H-[^{13}C] NMR spectra of the cortical extract and found these levels to be significantly decreased in the WNIN/Ob obese rats compared to controls, indicating hypoactivity and decreased metabolism.

This indicates insufficient supply of the energy to the obese rat brain even at a young age of three months. That these microenvironmental alterations and metabolic changes normally seen in the ageing brain were observed in the young age in the obese rat brain seems to further strengthen the inference that changes associated with normal ageing appear to be occurring much earlier in the brain of the WNIN/Ob obese rats and could therefore underlie their accelerated ageing/ decreased longevity.

Fig 3. A typical ^{1}H-[^{13}C]-NMR spectrum of cerebral cortex of WNIN/Ob obese rat at 3 months of age obtained after infusion of ^{13}C labeled glucose. The top spectrum (A) was obtained when inversion pulse on ^{13}C channel was 'off' while middle spectrum (B) was obtained when inversion pulse was 'on'. Subtraction of B from A gives difference spectrum (C) that provides information similar to ^{13}C-NMR spectrum with improved sensitivity.

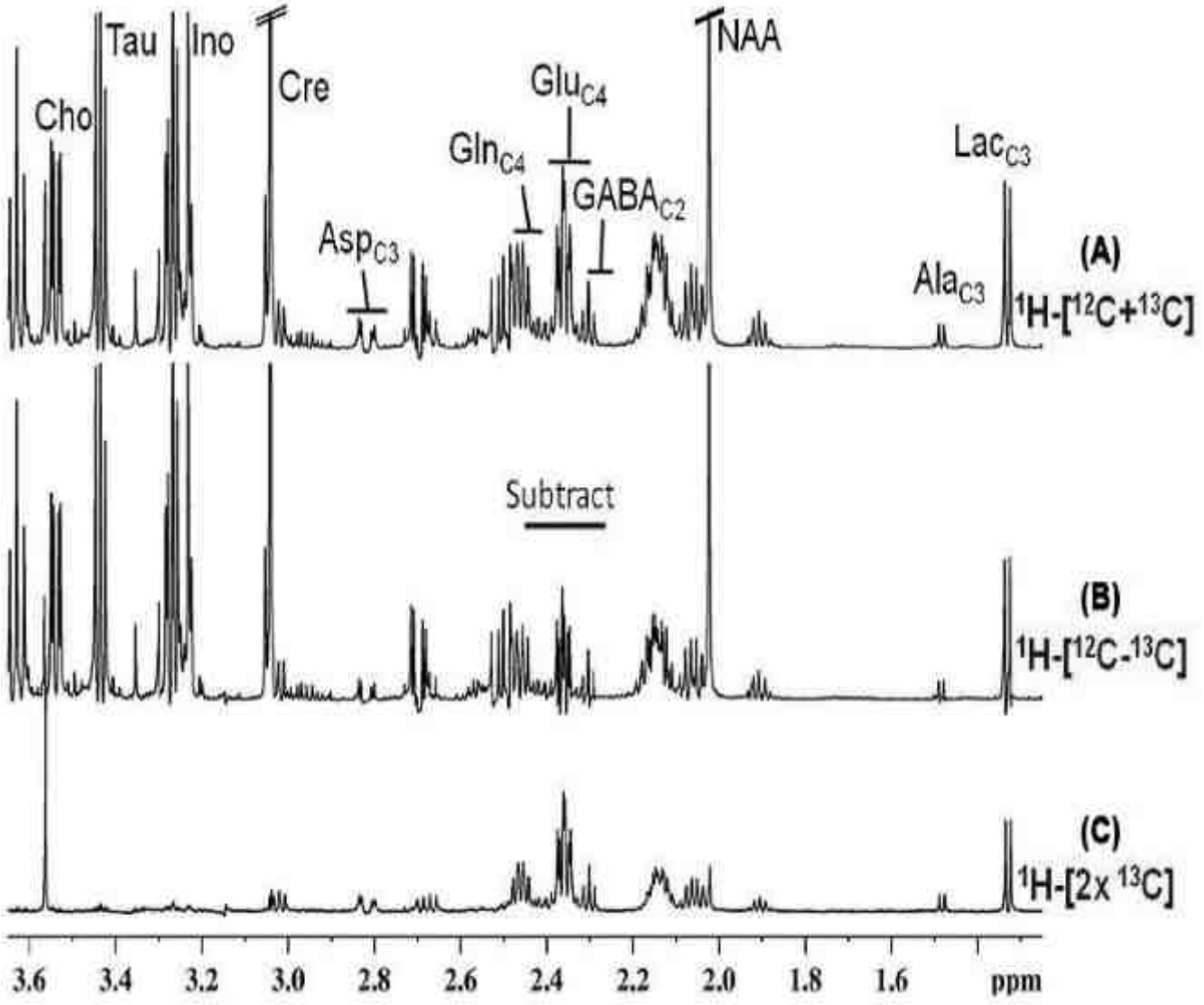


Fig 4. Concentration (µmol/g) of cortical metabolites in 3 month old WNIN, WNIN/Ob lean and WNIN/Ob obese rat

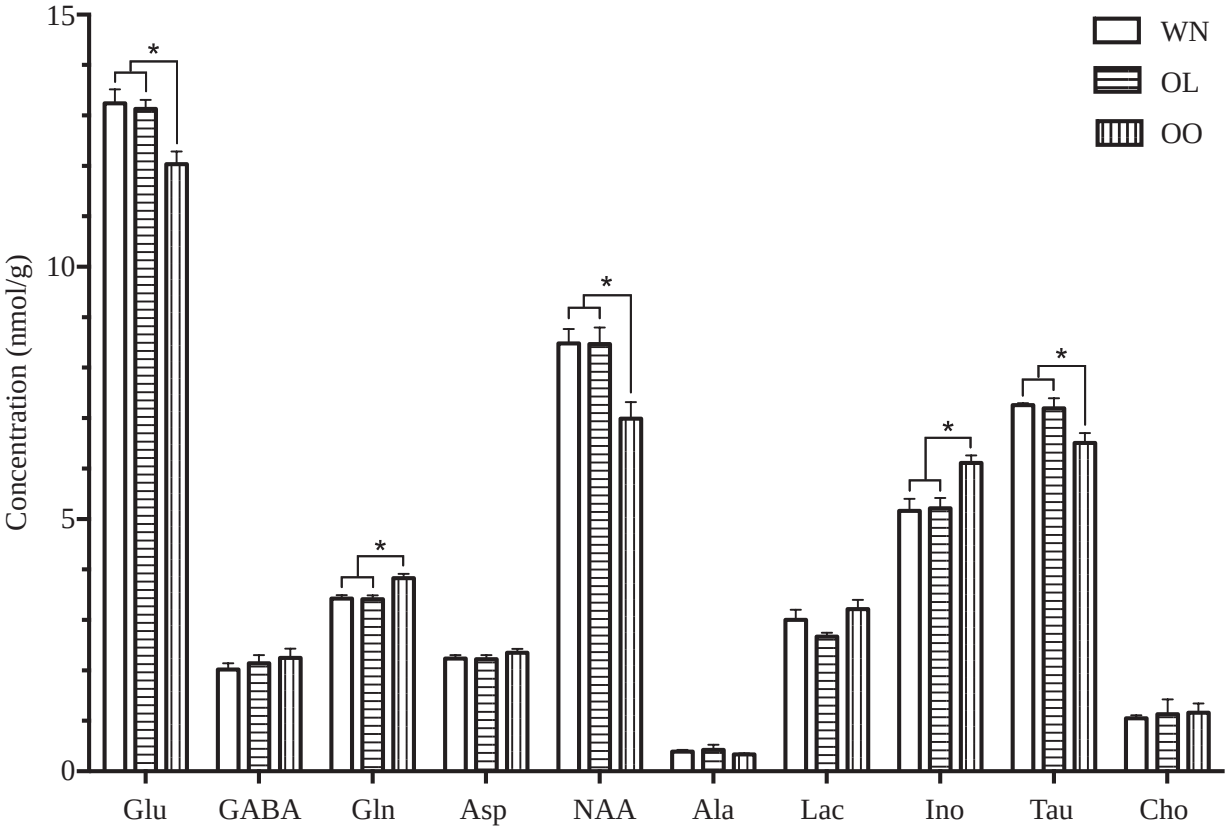


Fig 5. Concentration of ^{13}C labeled cortical amino acids during 15 min infusion of [1-^{13}C]glucose. ^{13}C concentration was calculated from ^{1}H-[^{13}C]-NMR spectra by multiplying ^{13}C enrichment with the total concentration of the respective amino acid

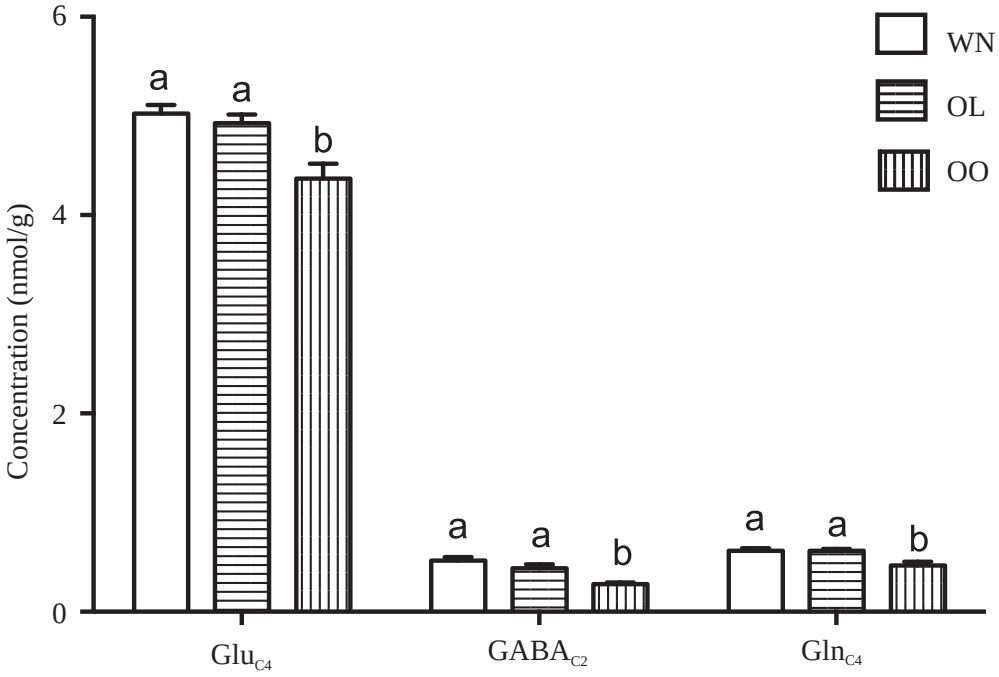
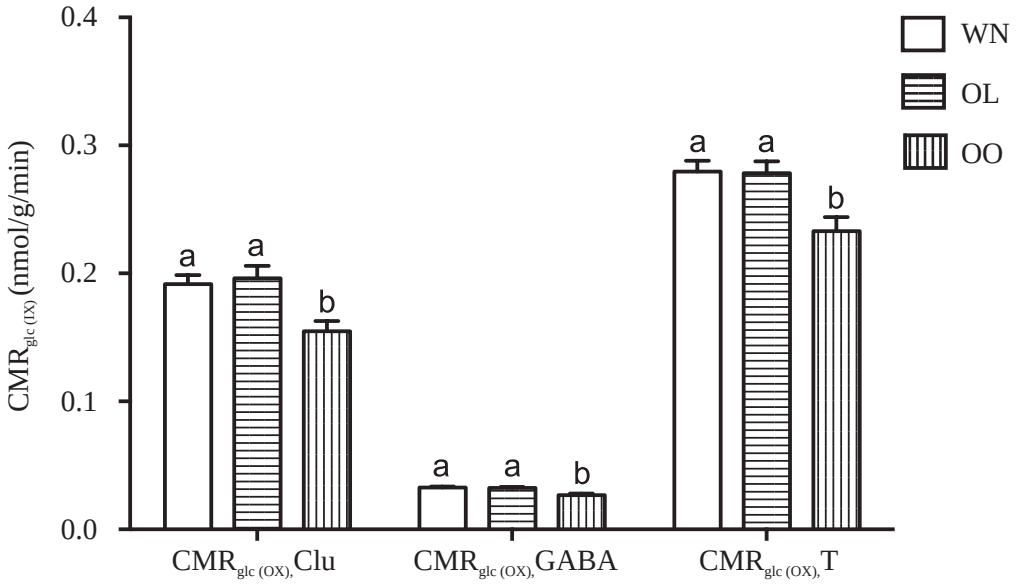


Fig 6. Rate of glucose oxidation associated with glutamatergic neurons, GABAergic neurons and total glucose oxidation in the cerebral cortex of parental WNIN control, WNIN/Ob lean littermate and WNIN/Ob obese rats.



Values are represented as mean±SEM

4. STATUS OF VITAMIN-D IN TYPE 2 DIABETES PATIENTS WITH AND WITHOUT RETINOPATHY

Long-term secondary complications are the main causes of morbidity and mortality in diabetic patients all over the world. One of the most common microvascular complications of diabetes is diabetic retinopathy (DR) which is regarded as a major culprit of blindness worldwide (1). The characteristic feature of DR is the appearance of vascular lesions of increasing severity, ending up in the growth of new vessels. While, the prevalence of DR varied (20-60%) in different studies, DR occurs in 70% of the population having diabetes for more than 15 years. Epidemiological studies suggest that, most of the cases of type 2 diabetes (T2D) could be attributed to modifiable habits and lifestyle factors. Therefore, identification of easily modifiable risk factors is essential to prevent the development of not only diabetes, but also its complications. Recently, there has been increasing evidence from animal and human studies, to suggest that vitamin D (VD) may play a role in modifying the risk of diabetes (2). Since the levels of 25(OH)D3 tend to increase in response to VD intake, they are commonly used as an indicator of VD status. $1,25(OH)_2$ plays a pivotal role in maintaining phosphocalcic homeostasis and bone metabolism, but a variety of biological functions such as cell differentiation, inhibition of cell growth, modulation of immune system and regulation of other hormonal systems were unveiled by research during the past two decades.

Several epidemiological studies have found serum VD deficiency (VDD) in various populations around the world. In spite of abundant sunshine over the Indian subcontinent, accumulated data on VD showed a significantly deficient status in the serum concentrations of normal and different disease states (3). A variety of reasons, either decreased consumption of VD or the body's synthesis of VD from low exposure times under sunlight, have led to the high prevalence of hypovitaminosis D in the Indian context. An association of VDD and increased risk of chronic diseases, such as cancer, T2D, cardiovascular disease, and autoimmune diseases including T1D, multiple sclerosis is shown in epidemiological studies. Though, the effect of hypovitaminosis D on the causation of T1D is well established, the association between VD insufficiency (VDI) and T2D as reported in longitudinal observational studies is not consistent. Several studies in various parts of the world suggest that VDD or insufficiency may increase the risk of metabolic syndrome and its sequelae, T2D, and cardiovascular disease.

Many diabetic patients, but not all, develop one or more microvascular complications is an indication that multiple factors are likely to be involved in predisposing diabetic subjects to complications such as DR. Though, genetic susceptibility of various diabetic complications, including DR has been extensively investigated, the role of environmental factors such as nutritional factors has not been adequately addressed. Nutritional status, micronutrients in particular, may affect the risk of DR by influencing the biochemical mechanisms underlying DR. Recently we reported an association between vitamin-B12 deficiency and hyperhomocysteinemia in DR and alterations in other micronutrients (4,5). In the present study, we have evaluated the status of VD in T2D patients without (DNR) and with diabetic retinopathy (DR) and compared the levels with the apparently normal subjects.

METHODOLOGY

Study design, subjects, and sample collection: A hospital-based, case-control study consisting of 164 T2D patients without retinopathy (DNR; n=82) and with retinopathy (DR; n=82). In addition, and 99 non-diabetic control (CN) subjects. The CN group consisted of asymptomatic subjects of age 50 years and above, free from cardiovascular and renal complications. Type 2 diabetes patients, without and with retinopathy, were matched for duration of diabetes and were recruited from patients attending the Pushpagiri Vitreo Retina Institute, Hyderabad. Normal and diabetes subjects on nutritional supplements for the last six months and a history of nephropathy (based on standard renal function tests) and other complications other than DR were excluded. Clinical as well as biochemical methods were employed to assess the history or presence of diabetic complications other than DR. All the diabetes patients underwent a complete ophthalmic examination comprising of best-corrected visual acuity, indirect ophthalmoscopy, slit-lamp biomicroscopy and fundus fluorescein angiography. Early Treatment Diabetic Retinopathy Study (ETDRS) adaptation of the modified Airlie House classification system was used for Diabetic retinopathy grading and DR was further categorized as Non-proliferative DR (NPDR) and proliferative DR (PDR) (4-6). The study was carried out in accordance with the guidelines of the Helsinki Declaration of 1975 and approved by the Institutional Ethics Committees of Pushpagiri Vitreo Retina Institute and National Institute of Nutrition. A venous blood sample was collected in EDTA tubes after obtaining a written informed consent from all the participants.

Biochemical estimations: Fasting blood glucose, glycosylated hemoglobin (HbA1c), lipid profile (total cholesterol, HDL, triglycerides) and plasma insulin were estimated using kits. Total plasma 25(OH)D3 was estimated by HPLC method. Plasma calcium was estimated by atomic absorption spectroscopy.

RESULTS

- Table 1 depicts the characteristics of the control (CN), diabetes without retinopathy (DNR) and with retinopathy (DR) groups.
- The sex distribution among the groups was approximately the same (male and female were, 55% and 45% in CN, 57% and 43% in DNR, and 58% and 42% in DR, respectively). Further, no significant difference was observed between male and female subjects in all three groups with respect to demographics and measured parameters. Therefore, the data for both men and women were pooled as a whole in the respective groups.
- Mean age, BMI, and hemoglobin levels were comparable among the groups. The duration of diabetes was matched for DNR and DR. Amongst diabetes groups; HbA1c levels were comparable between DR and DNR.
- While plasma levels of total cholesterol, triglyceride and LDL were comparable among the groups, levels of HDL were lower in the diabetes groups compared with the CN group (Table 1). Nevertheless, the levels of HDL were comparable between the DNR and DR groups.
- The mean plasma 25(OH)D3 levels were significantly lower in both the diabetic groups (DNR and DR) compared with the CN group (Fig 1). However, no significant difference was observed between the diabetic groups, irrespective of the presence (DR) or absence (DNR) of retinopathy (Fig 1).
- Nonetheless, the mean levels of VD in all the three groups were below sufficient range (<30 ng/mL) and fall into insufficient (20-30 ng/mL) to deficient (<20 ng/mL) categories. Hence, we determined the prevalence of VDI and VDD in these groups. As can be seen, the prevalence of VDD was 45%, 66% and 63% in CN, DNR and DR groups, respectively (Fig 2) suggesting that, prevalence of VDD was higher in diabetic groups compared with control group.

- The prevalence of VDI was approximately the same in all the three groups: 25%, 23% and 27% in CN, DNR, and DR groups, respectively (Fig 2). Whereas the prevalence of VD sufficiency was 29%, 11% and 10% in CN, DNR, and DR groups, respectively (Fig 2).
- Further, we also compared the VD levels between NPDR and PDR and found no difference between the groups: the mean value of VD was 16.7 ng/mL in the NPDR and 16.9 ng/mL in the PDR group respectively.
- In addition, when duration was controlled in DNR and DR groups, DR patients had comparable levels of VD with DNR patients. Together, these results suggest lowered plasma levels of VD in T2D patients irrespective of the presence or absence of retinopathy .

Table 1. Clinical and demographic profile of control (CN) and diabetes patients without (DNR) and with retinopathy (DR)

Parameter	CN		DNR		DR		F - Value	P - Value
	Mean	SE	Mean	SE	Mean	SE		
Age (years)	54.26a	0.743	53.22a	0.867	57.46b	0.913	6.63	0.002
BMI	25.02a	5.16	24.14a	4.34	24.39a	4.51	1.71	0.221
Hb (g/dL)	14.65a	1.83	13.74a	1.54	14.09a	1.75	1.43	0.244
Duration (years)	-		11.24a	5.34	10.69a	6.10	2.31	0.126
Glucose (mg/dL)	96.35a	3.43	205.28b	2.56	215.51b	8.88	30.73	0.000
HbA1c (%)	5.77a	0.20	10.36b	0.65	10.88b	0.55	27.27	0.000
Insulin (µU/mL)	22.2a	1.76	36.54a	4.34	31.69a	3.50	2.21	0.121
Total Cholesterol (mg/dL)	151.72a	10.90	171.47a	15.24	182.73a	10.77	1.46	0.239
Triglycerides (mg/dL)	153.81a	17.54	185.94a	17.22	169.36a	13.12	0.87	0.426
HDL (mg/dL)	31.76a	2.20	26.02b	1.68	27.99ab	1.29	2.55	0.085
LDL (mg/dL)	104.51a	5.23	121.16a	4.90	120.26a	6.20	2.02	0.141

Values are mean and SE. Mean values across groups were compared by one-way ANOVA 'F' test with least significant difference (LSD). Significant differences (p<0.05) of mean values between the groups are indicated by different superscript letters

Fig 1. Plasma vitamin D levels. Data represent mean and SE in control (CN) and diabetes patients without (DNR) and with retinopathy (DR). Mean values across groups were compared by one-way ANOVA 'F' test with LSD. Different letters on the bars indicate significant differences (p<0.05) of mean values between the groups.

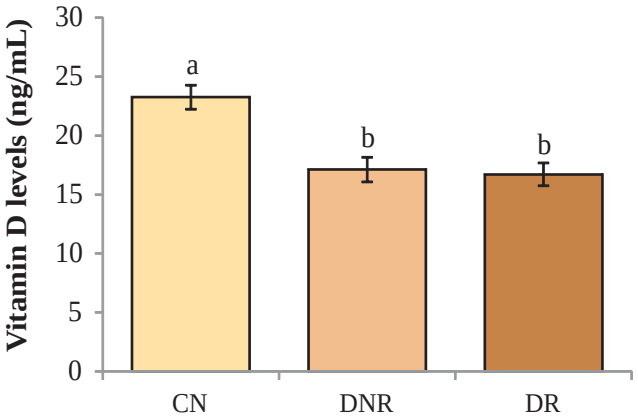
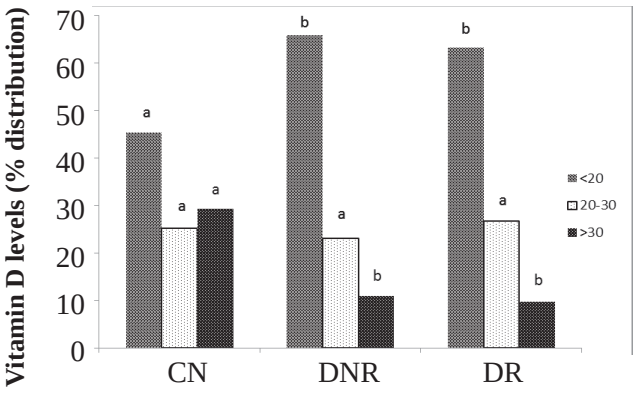
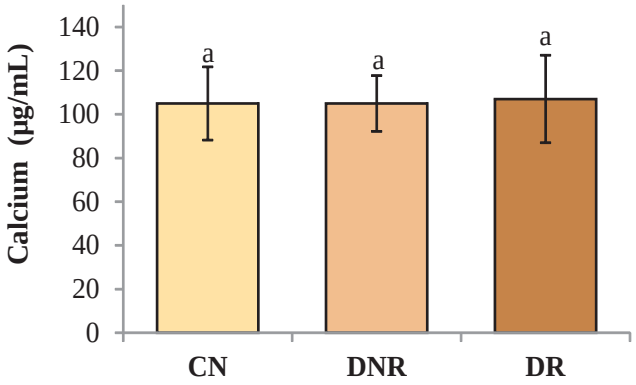


Fig 2. Percentage distribution of vitamin D levels across the groups. Vitamin D levels are distributed into deficient (<20), insufficient (20-30) and sufficient (>30) ng/mL) in each group, respectively. CN- control; DNR- diabetic no retinopathy; DR- diabetic retinopathy. Different letters on the bars indicate significant differences (p<0.05) of mean values between the groups



- The plasma calcium levels were comparable among the groups (Fig 3). In spite of the deficiency of VD in the diabetic groups, no significant difference was observed in the mean levels of calcium between the CN and diabetic groups.
- Interestingly, irrespective of groups, VD levels were significantly associated with glucose (p=0.003), HbA1c levels (p=0.006) and duration of diabetes (p=0.007) but not related to subjects' age, BMI. Even after adjusting the age across the groups, mean VD values were significant among CN, DNR, and DR.

Fig 3. Plasma calcium levels. Data represent mean and SE in control (CN) and diabetes patients without (DNR) and with retinopathy (DR). Mean values across groups were compared by one-way ANOVA 'F' test with LSD. Same superscripts ('a') on the bars indicate no significant differences ($p<0.05$) of mean values between the groups.



SUMMARY

The present study reveals that VD levels are significantly lower in diabetic subjects compared with non-diabetic subjects and more importantly VD levels are below the normal range. However, no significant difference is seen between the levels of DNR and DR subjects. It should be noted that low levels of VD have been recognized in Indians, and recent studies confirm low concentrations of VD and the implications for diabetes and cardiovascular diseases in India. Our study also further substantiates the general prevalence of VDD in India; about 40% adults above 50 years are deficient, and the prevalence is much higher in patients with diabetes. This is the first study to investigate an association between plasma VD status and DR in India and more controlled prospective studies are warranted to confirm the role of VDD in the development and progression of DR. In conclusion, the results of the present study show that lower levels of VD are observed in patients with diabetes irrespective of the presence or absence of retinopathy. However, given previous research indicating possible anti-inflammatory and antiangiogenic properties of VD, the association between VD and DR warrants further investigations.

5. ELLAGIC ACID AMELIORATES DIABETIC RETINOPATHY BY INHIBITION OF AGE FORMATION IN RATS

Chronic hyperglycemia with poor glycemic control is known to cause various secondary complications. The long-term complications of diabetes are thought to be a result of the accumulation of tissue macromolecules that have been progressively modified by non-enzymatic glycation, which subsequently leads to the formation of advanced glycation endproducts (AGE). Formation of AGE plays a key role in the development of several pathophysiologies associated with aging and diabetes (1). Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes. Microvascular lesions, such as microaneurysms, blood barrier dysfunction, and capillary dropout, are key features of diabetic retinopathy. The early stages of DR result from retinal ischemia as a result of nonperfusion of the retina or a decrease in oxygen tension. In the late stages, the ischemia-induced pathologic growth of new blood vessels can cause catastrophic loss of vision. Hyperglycemia induced accumulation of AGE leads to intramural pericyte death and thickening of the basement membrane leading to incompetence of the vascular walls. AGE also stimulate the expression of RAGE (receptor for AGE) leading to activation of VEGF production which in turn cause new blood vessels formation in the retina. In addition, AGE induces apoptotic cell death of pericytes through interaction with RAGE and induction of VEGF.

Studies using retinal vascular cells show that AGE can trigger pathogenic events including loss of pericytes, nerve growth factor (NGF) and increased expression of the glial acidic fibrillary protein (GFAP) and VEGF (2).

Further, injection of AGE into nondiabetic animals induced thickening of the basement membrane of retinal vessels and increased breakdown of the blood-retinal barrier. Animal studies have demonstrated that treatment with AGE inhibitor reduced retinal damage. This raises the possibility that inhibition of AGE formation may prevent the progression of DR. However, designing of molecules with anti-AGE activity is a challenge due to the complexity of reactions involved in the formation of AGE. A number of compounds such as aminoguanidine, pyridoxamine, carnosine, and phenyl thiazolium bromide have been investigated in several *in vitro* and *in vivo* studies. However, most of the compounds have not passed through the clinical trials. Hence, identification and testing of new antiglycating agents with higher levels of efficacy and safety in humans is very much needed.

In the course of identifying and testing new antiglycating agents, we have evaluated a number of traditional and very common dietary sources and found that some spice principles, fruits, and vegetables have the potential to inhibit AGE formation under in vitro conditions (3). Ellagic acid (EA) is one of the commonly found dietary polyphenols. Apart from the greatest sources, such as berries and pomegranate, EA is also present in those dietary sources that we reported to have antiglycating potential (3). Subsequently, we have demonstrated inhibition of AGE formation by EA in various proteins: eye lens protein, hemoglobin, lysozyme and BSA using different glycating agents such as fructose, ribose and methylglyoxal (4). EA also inhibited the formation of glycosylated Hb in human blood under *ex vivo* high-glucose conditions (4).We have also determined the effectiveness of EA against loss of eye lens transparency through inhibition ofAGEs in the lens organ culture system (4). In the present study, we investigated the effect of EA on retinal lesions in STZ induced diabetic rats.

METHODOLOGY

Experimental design and dietary regimen: Two-month-old male WNIN rats with an average bodyweight of 230±14 g were used in the study. The control rats (n=10) received 0.1 M sodium citrate buffer, pH 4.5 as a vehicle whereas the experimental rats received a single intraperitoneal injection of STZ (35 mg/kg) in the same buffer. After 72 h of STZ injection, fasting blood glucose levels were monitored and animals with blood glucose levels >150 mg/dl were considered diabetic. Both control and diabetic animals were fed with AIN-93 diet *ad libitum*. A group of diabetic animals received only AIN-93 diet whereas two groups of diabetic animals received the AIN-93 diet containing 0.2 and 2% EA. Body weight and blood glucose concentration of each animal were measured weekly. Blood was collected once a week from the retro-orbital plexus for glucose estimation, HbA1c formation, and RBC IgG binding analysis. At the end of 12 weeks, animals were sacrificed by CO_2 asphyxiation. The retinas were dissected from the eye, snap frozen and stored at -80ºC for analysis by Western blot and some of the retinas were kept in 4 % paraformaldehyde for subsequent histological studies. All experimental procedures involving animals were approved by the IAEC of National Institute of Nutrition.

Quantitative Real-Time PCR: Total RNA was extracted from retina using Tri-reagent. Isolated RNA was further purified by RNeasy Mini Kit and quantified by measuring the absorbance at 260 and 280 nm. Total RNA was reverse transcribed to cDNA. Real-time PCR was performed with 25ng cDNA templates using SYBR green master mix with gene-specific primers. Normalization and validation of data were carried using β-actin as an internal control and data were compared between control and diabetic samples according to the comparative threshold cycle ($2^{-??ct}$) method.

Whole tissue lysate preparation: Retina was homogenized in a buffer containing 20 mM Tris, 100 mM NaCl, 1 mM EDTA, 1 mM DTT (TNE buffer; pH 7.5) and protease inhibitors.

SDS–PAGE and immunoblotting: Electrophoresis of retinal lysate was performed under reducing conditions on 10% polyacrylamide gels. After electrophoresis, the proteins were transferred onto nitrocellulose membrane and immunoblotting was performed with respective primary antibodies followed by blocking with non-fat milk powder. Later incubated with peroxidase tagged anti-rabbit IgG antibody or anti-mouse secondary antibody conjugated with HRP. β-actin was used as a loading control. The immunoblots were developed using enhanced chemiluminescence detection kit, and digital images were recorded.

Immunohistochemistry: The dissected eyeball was immediately placed in 4% paraformaldehyde in phosphate buffer (pH-7.2), fixed overnight, embedded in paraffin blocks, and cut into 4µm sections. Deparafinized sections were boiled in 0.01M Na-citrate pH 6.0 for 10 minutes at 60°C and blocked with blocking solution (3% goat serum, 3% BSA, 0.3% TritonX in TBS). Slides were incubated in PBS with respective primary antibodies overnight at 4°C. Slides were washed three times with PBS, and the binding of primary antibodies was visualized by Alexafluor-488

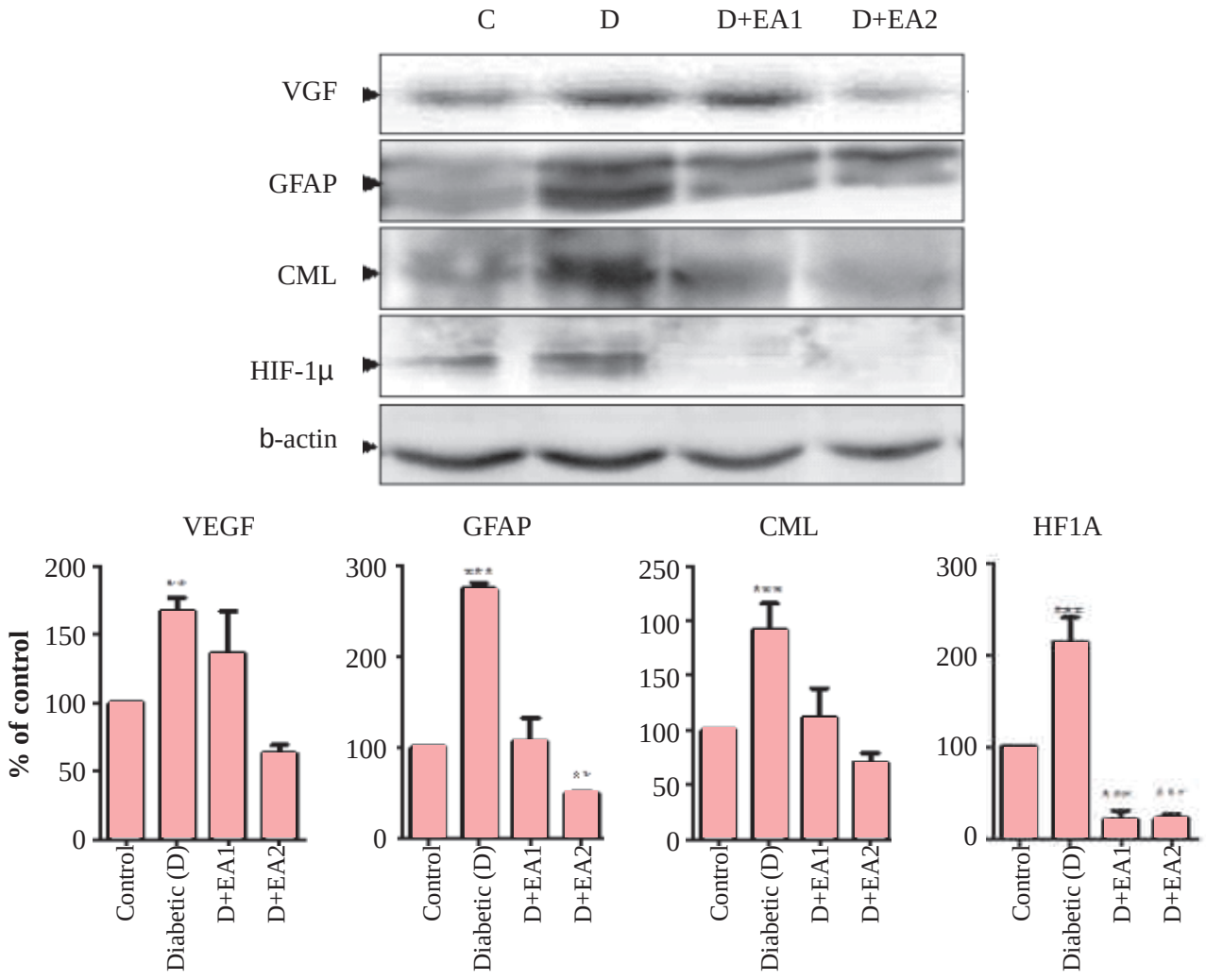
conjugated anti-rabbit and Alexafluor-555 conjugated anti-mouse IgG antibody for 1 hour. Sections were mounted in medium containing DAPI and visualized using a microscope.

Electroretinogram: Electroretinograms (ERG) were recorded from one eye of the anesthetized rat to determine retinal function. Twenty-four hours prior to recording ERG, animals were dark-adapted overnight. Animals were anesthetized, and pupils were dilated with topical 0.1% atropine sulfate and 0.1% phenylephrine. ERGs were recorded from one eye using ERG-JET electrode on the corneas, Scotopic ERG responses were averaged with stimulus intervals of 1 to 180 sec depending on intensity, and 20 to 30 photopic responses were averaged with intervals of 1 sec.

RESULTS

- As reported previously (5) the mean blood glucose levels of diabetic rats were significantly higher when compared with control rats. Further, diabetic rats showed significantly higher levels of HbA1c compared with the controls. Though, blood glucose levels were decreased to a marginal level due to 2% EA feeding to diabetic rats, glucose levels were unaffected by 0.2% EA.
- ***Decreased CML levels and RAGE expression upon ellagic acid feeding:*** To understand the effect of EA on the accumulation of AGE in the retina of diabetic rats, immunoblotting was performed using anti-CML. As expected, there was an increased level of CML in retinal lysates of diabetic rats compared to control rats (Fig 1) indicating increased accumulation of AGE in the retina due to diabetes.

Fig 1. Immunoblot analysis (Panel A) and quantification of immunoblots (Panel B) of VEGF, GFAP, CML, HIF1α and β-actin in retina of control (C), and diabetic(D) rats fed with 0.2% EA (D+EA1) and 2% EA (D+EA2). *Data are mean ± SE (n=3; *p<0.05).*



- Further, there was increased immunostaining for RAGE in diabetic retinal section compared to control rats (Fig 2). Interestingly, in support of our previous in vitro data (4), feeding of EA to diabetic rats inhibited CML formation, and the degree of CML detection was comparable to control group (Fig 1). The effect was more pronounced with 2% EA dose. In accord with CML data, immunostaining showed that RAGE expression was also decreased in retina of EA-fed diabetic rats (Fig 2).
- Alteration in the protein profile due to CML aggregates lead to the insolubilization of protein that have been considered to be the ultimate change that results in retinal cell death. Hence, we determined the expression of proapoptotic protein Bax. While, the expression of Bax was significantly increased in the retina of diabetic rats compared to control rats, feeding of EA to diabetic rats showed significantly lesser staining of Bax in the retina (Fig 3). These results suggest that AGE-mediated cell death in the diabetic retina could be attenuated by EA.
- ***Retinal functional abnormalities:*** Improvement of functional abnormalities in diabetic retina as determined by ERG recording support these findings: there was significant decrease in both scotopic and photopic oscillatory potentials in diabetic rats compared to control rats and feeding of EA improved the OP (Fig 4).
- Neovascularization stimulated by hyperglycemia-mediated induction of vascular endothelial growth factor (VEGF) has been implicated in the pathogenesis of DR. The expression of VEGF, under hyperglycemia, is regulated by hypoxia-inducible factor 1 (HIF-1) due to local hypoxia. Hence, we determined the effect of EA feeding on the expression of HIF-1α, VEGF, and GFAP in the diabetic retina.
- Results of qRT-PCR and immunoblot analysis indicate that there was significant increase in HIF-1α and VEGF in diabetic rats compared to control group and interestingly this increased expression was decreased in the ellagic acid treated groups (Fig1 & 6). Similarly, GFAP was upregulated in diabetic rat and treatment with EA inhibited the GFAP expression as shown by qRT-PCR and immunoblotting (Fig 1 & 5).

Fig 2. Expression of RAGE in the retina of control and diabetic rats fed with ellagic acid

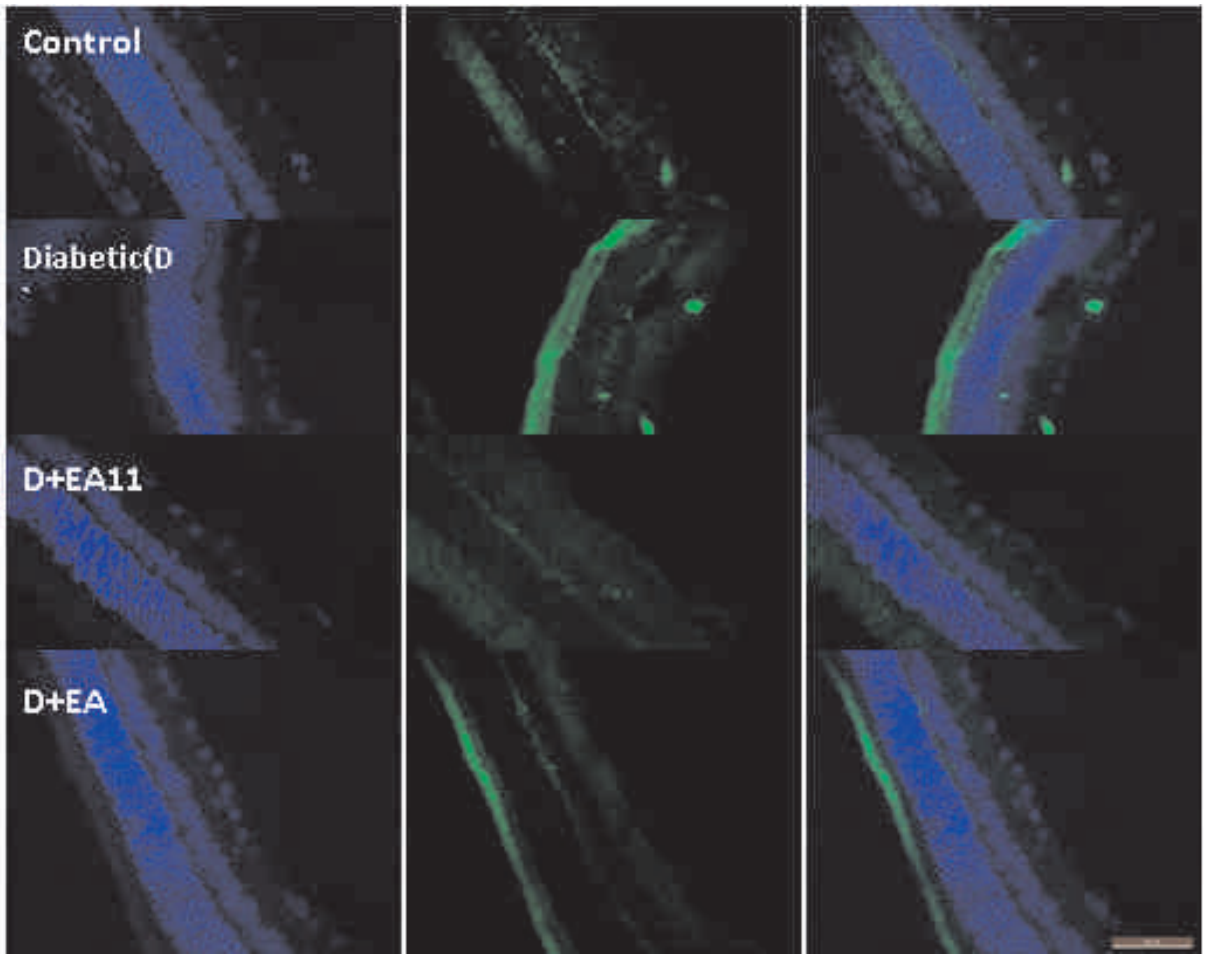


Fig 3. Expression of Bax in the retina of control and diabetic rats fed with ellagic acid

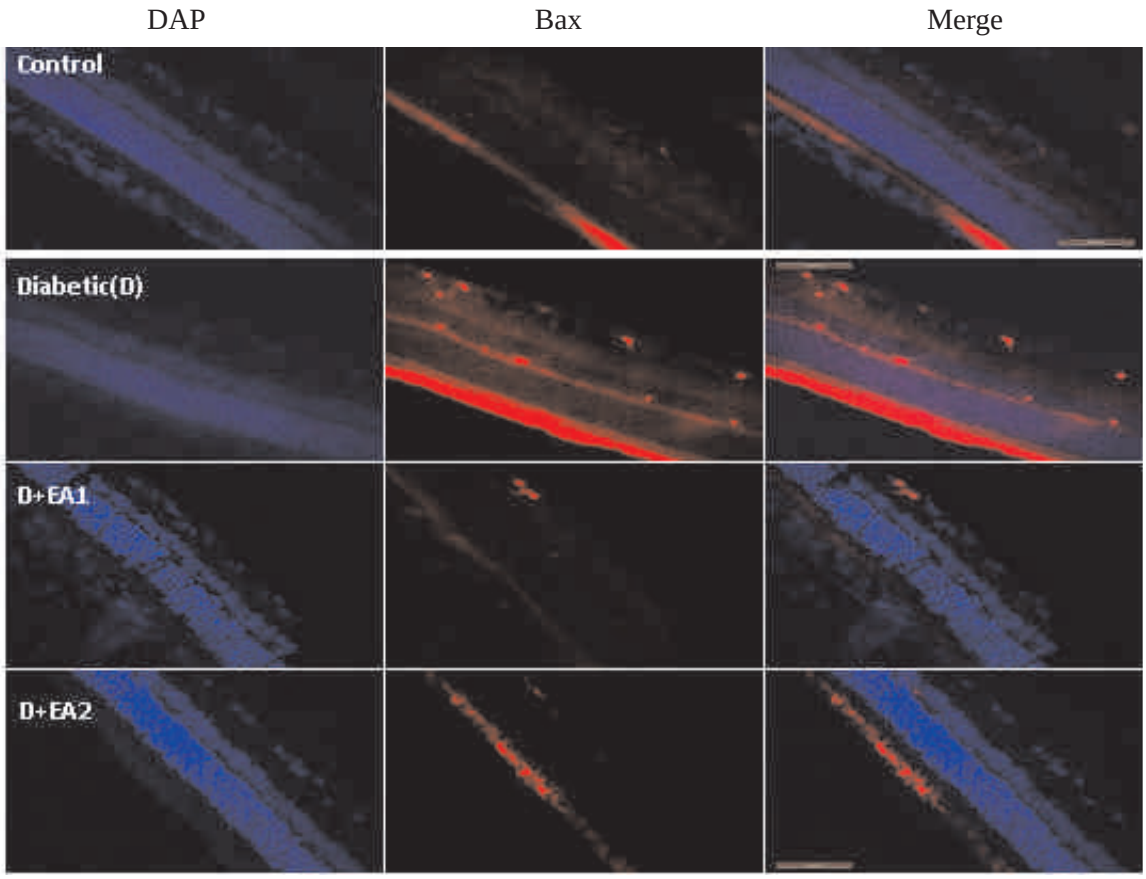


Fig 4. Scotopic and photopic oscillatory potentials of the retina of control and diabetic rats fed with ellagic acid

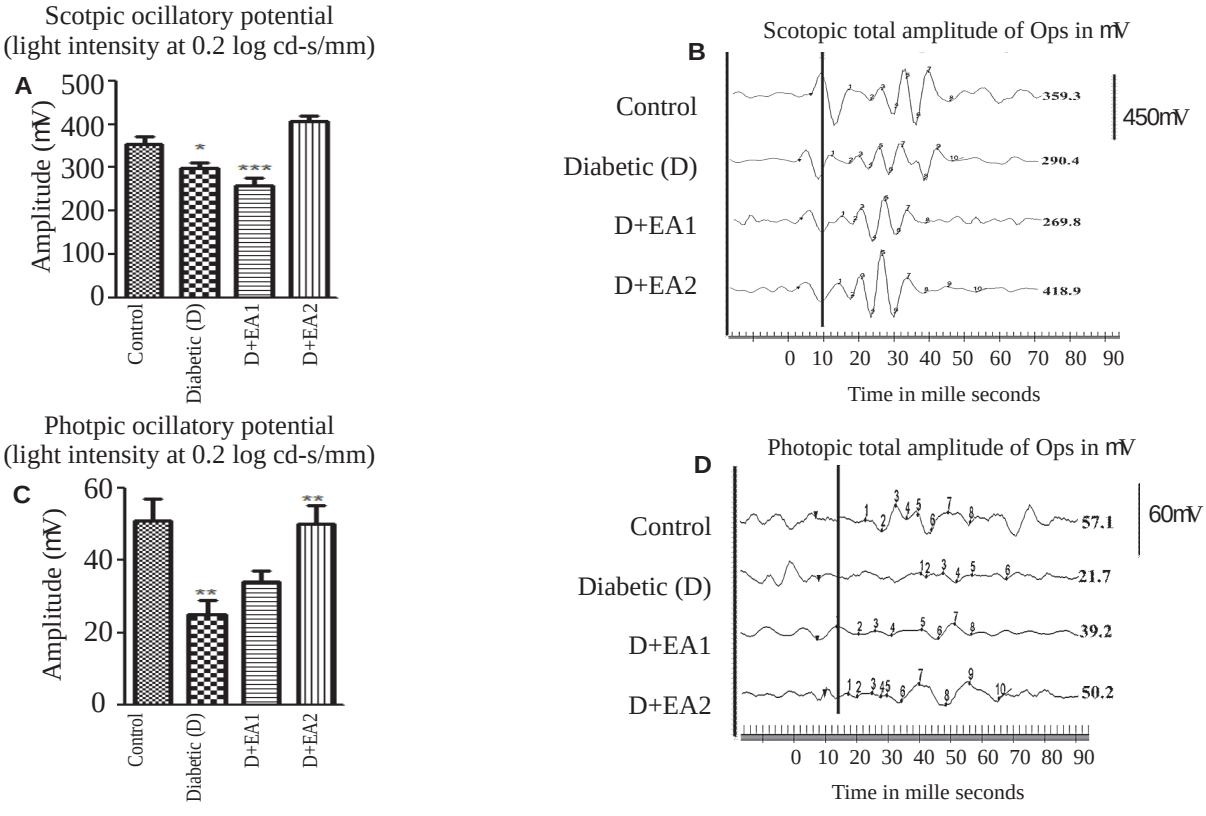
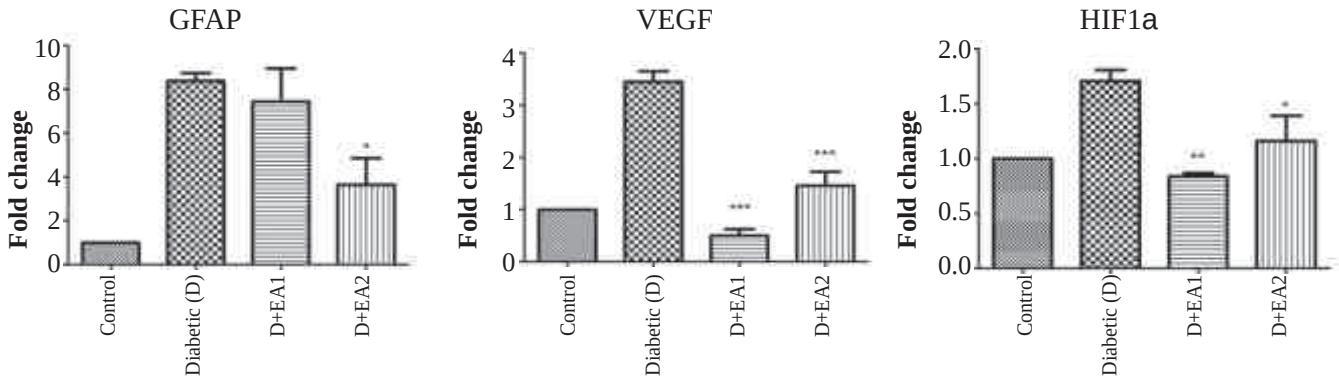


Fig 5. Expression of Gfap, Vegf, and Hif1α in the retina of control and diabetic rats fed with ellagic acid as analyzed by qRT-PCR



SUMMARY

In summary, we describe *in vivo* effect of EA against DR using diabetic rat model and provide the evidence for its role in ameliorating DR. The results indicate that there was an increase in CML levels in STZ-induced diabetic rat retina as compared to control rat retina. Interestingly, feeding of EA to diabetic rats results in considerable inhibition of CML formation. The expression of RAGE was also observed to have reduced upon EA feeding further supports the anti-AGE activity of EA. There was a significant improvement in both scotopic as well as phototopic OP by EA feeding suggesting the protective nature of EA in the retinal function and thereby its physiology. Further increased expression of proapoptotic protein Bax was significantly decreased in the retina of diabetic rats by feeding of EA**.** While EA is known to have various health benefits the results described in the present study provide information regarding potential of EA with respect to DR. Hence EA could be a potential molecule that needs to be explored further for its prospects as preventive and therapeutic for diabetic complications.

6. RESPONSE OF SMALL HEAT SHOCK PROTEINS IN DIABETIC RAT RETINA

Diabetes and its complications are a major cause of morbidity and mortality all over the world. Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes and ranks as a common cause of blindness worldwide. Diabetic retinopathy occurs in 70% of all people with diabetes for more than 15 years. The etiology and pathogenic mechanisms underlying DR is still largely unknown, albeit numerous studies implicate oxidative stress, advanced glycation end products, inflammation, neurodegeneration, etc. The conditions created during diabetes are known to increase oxidative stress as well as down-regulate the cellular antioxidant defense systems. Uncontrolled oxidative stress represents a characteristic feature of diabetes and diabetic complications such as DR. It has been shown that expression of stress proteins is elevated in several pathophysiological conditions to offer protection against tissue damage.

Heat shock proteins (Hsps) also called stress proteins, are elevated in a variety of stress conditions including high temperature (heat shock), hypoxia, ischemia, endotoxins, heavy metals, and reactive oxygen species. A group of low-molecular-weight Hsps, ranging in monomer size from 16 to 27 kDa are referred to as small Hsps (sHsps). sHsps are heterogeneous and are characterized by a conserved C-terminal region, called the alpha-crystallin (α-crystallin) domain. A subfamily of 10 sHsps exists in mammals (1) that include Hsp27/ Hsp25 of rodents (HSPB1), MKBP (myotonic dystrophy protein kinase; HSPB2), HSPB3, αA-crystallin (αAC; HSPB4), αB-crystallin (αBC; HSPB5), Hsp20 (HSPB6), cvHsp (HSPB7), Hsp22/H11/H2IG1 (HSPB8), HSPB9, and ODF (sperm outer dense fiber protein; HSPB10). sHsps characteristically function as molecular chaperones assisting in assembly, stabilization, internal transport of intracellular proteins, maintenance of the cytoskeleton architecture and protection

against programmed cell death. The phenomenon of induction of Hsps is believed to be regulated by a family of heat shock transcription factors (HSFs). There are three known HSFs in mammals: HSF1, HSF2, and HSF4. Among these, HSF1 is considered to be the universal HSF, and it responds to an external stress signal such as high temperature. HSF2 is associated with developmental control whereas HSF4 regulates the postnatal expression of Hsps. Hypoxia-inducible factor-1alpha (HIF-1α) another transcription factor that is specifically activated by decreased tissue oxygen supply regulates rapid induction of Hsp synthesis. The chaperone activity and cytoprotective functions of sHsps are regulated by phosphorylation. αBC is a major sHsp tightly regulated by phosphorylation at three sites; Serines at positions 59 (S59), 45 (S45) and 19 (S19). While p38–mitogen-activated protein kinase (p38MAPK) phosphorylates the S59 of αBC, S45 is phosphorylated by ERK. The kinase responsible for phosphorylation of S19 of αBC is unknown.

Several studies have reported an increased expression of αAC and αBC in various tissues including retina in rodent models of type 1diabetes and implicated their role in preventing retinal cell death (2). But, the expression of remaining members of sHsps, the role of HSFs and more so phosphoregulation of sHsps is still unknown in the diabetic retina. To our knowledge, there are no reports demonstrating the status of phosphorylation and phospho-specific localization of sHsps in diabetic rat retina. Therefore, we comprehensively examined the expression of all sHsps, HSFs, their solubility and regulation of the major sHsp, αBC, by kinase-mediated phosphorylation in the diabetic rat retina.

METHODOLOGY

Experimental design and dietary regimen: Two-month-old male WNIN rats with an average bodyweight of 230±14 g were used in the study. The control rats (n=10) received 0.1 M sodium citrate buffer, pH 4.5 as a vehicle whereas the experimental rats received a single intraperitoneal injection of STZ (35 mg/kg) in the same buffer. After 72 hours STZ injection, fasting blood glucose levels were monitored and animals with blood glucose levels >150 mg/dl were considered for the experiment (n=10). Both control and diabetic animals were fed with AIN-93 diet *ad libitum*. Body weight and blood glucose concentration of each animal were measured weekly. At the end of 12 weeks, rats were fasted overnight and sacrificed by CO2 asphyxiation. Eye balls were dissected out immediately and cut into two hemispheres to remove the lens and the vitreous body. Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the IAEC (institutional animal ethical committee) of National Institute of Nutrition.

Biochemical estimations: Glucose and glycosylated hemoglobin (HbA1c) in blood were measured using commercially available kits.

Quantitative real-time PCR*:* Total RNA was extracted from control and diabetic rat retina using Tri-reagent according to the manufacturer instructions. Isolated RNA was further purified by RNeasy Mini Kit and quantified by measuring the absorbance at 260 and 280 nm. Total RNA was reverse transcribed using High Capacity cDNA Reverse Transcription kit. Real-time PCR was performed in triplicates with 25ng cDNA templates using SYBR green master mix with gene-specific primers. Normalization and validation of data were carried using β-actin as an internal control and data were compared between control and diabetic samples according to the comparative threshold cycle ($2^{-??ct}$) method.

Whole tissue lysate preparation: Retina was homogenized in a buffer containing 20 mM Tris, 100 mM NaCl, 1 mM EDTA, 1 mM DTT (TNE buffer; pH 7.5) and protease inhibitors and the homogenate was centrifuged at 12,000*g at* 4^{o} *C for 20 min.*

SDS-PAGE and immunoblotting: Equal amounts of protein were subjected to 12% SDS-PAGE and transferred to nitrocellulose membranes by Western blot transfer system. Nonspecific binding was blocked with 5% nonfat dry milk powder in PBST and incubated overnight at 4°C with respective primary antibodies diluted in PBST. After washing with PBST, membranes were then incubated with secondary antibodies conjugated to HRP. The immunoblots were developed using ECL detection kit.

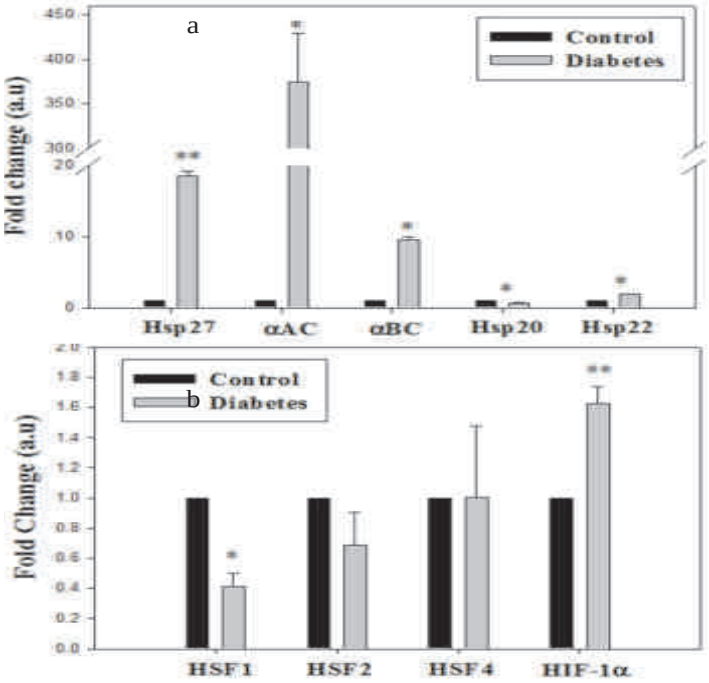
Detergent soluble assay: For analyzing detergent solubility, retina was homogenized in TNE buffer containing 0.5% TritonX. Following centrifugation, the homogenate was separated from a supernatant containing detergent soluble fraction, and the pellet containing insoluble protein fraction and this pellet was washed with PBS, homogenized, sonicated and dissolved in Lammelli buffer.

Immunohistochemistry: The dissected eyeball was immediately placed in 4% paraformaldehyde in phosphate buffer (pH-7.2), fixed overnight, embedded in paraffin blocks, and cut into 4µm sections. Deparafinized sections were blocked with 3% serum, 3% BSA, 0.3% TritonX in TBS and incubated in TBS with respective primary antibodies overnight at 4°C. Slides were washed three times with TBS, and the binding of primary antibodies was visualized by Alexafluor-488 conjugated anti-rabbit and Alexafluor-555 conjugated anti-mouse IgG antibody for 1 hour. Sections were mounted in medium containing DAPI and visualized using a Leica laser microscope.

RESULTS

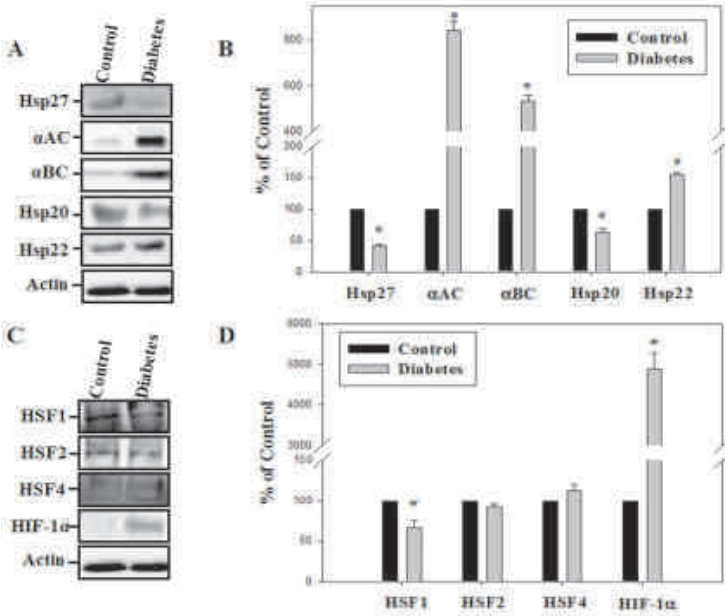
- The mean blood glucose levels of control rats were 91.5±6.17 mg/dl and significantly increased to 390.1±48.7mg/dl in diabetic rats at 12 weeks. Further, HbA1c levels were significantly higher 10.7±0.2% of diabetic rats compared with the controls 6.1±0.14%.
- To determine the response of all sHsps during the chronic hyperglycemia, we analyzed the expression pattern of all sHsps and HSFs in the retina by qRT-PCR and immunoblotting. However, out of 10 sHsps only Hsp27, αAC, αBC, Hsp20 and Hsp22 were detected in the retina.
- Expression of Hsp27 mRNA was significantly increased (18 fold) in diabetic rats (Fig 1A), in contrast its protein levels were downregulated (Fig 2A-B).
- Interestingly, expression of αAC mRNA was remarkably increased (376 folds) in the retina of diabetic rats in comparison with controls (Fig 1A). Correlating with mRNA, the protein levels of αAC were also significantly increased (Fig 2A-B)
- Expression of αBC was also significantly upregulated both at mRNA (27 fold; Fig 1A) and protein level (Fig 2A-B). Although, expression levels of αAC and αBC were higher in the diabetic retina, relatively very low levels were detected in controls.
- Expression of Hsp20 was significantly downregulated both at mRNA (Fig 1A) and protein level (Fig 2A-B) whereas Hsp22 was significantly upregulated both at mRNA (Fig

Fig 1. qRT-PCR analysis of sHsps (*Panel A*) and HSFs (*Panel B*) in the retina of control and diabetic rats. Expression values were represented as fold change over control.



Data are mean ± SEM of three independent experiments.
** p<0.05, ** p<0.01 compared to the control*

Fig 2. Immunoblot analysis of sHsps (*Panel A*) and HSFs (*Panel C*) in the retina of control and diabetic rats. Quantification of sHsp (***Panel B***) and HSF (***Panel D***) immunoblots in the retina of control and diabetic rats; expression was normalized for actin and represented as percent of control.



Data are mean ± SEM (n=3;
**p<0.05 compared to the control)*

1A) and protein level (Fig 2A-B) in diabetic rats as compared to controls.

- To examine the regulation of sHsps, we analyzed the expression of three HSFs as well as HIF-1α in retina under chronic hyperglycemic conditions.
- The expression of HSF1 mRNA was significantly downregulated (Fig 1B); consistent with transcript, the protein levels of HSF1 were also downregulated in diabetic rats (Fig 2C-D). Although, no significant alterations were detected in HSF2 & HSF4 levels in retina of the diabetic rats (Fig 1B & 2C-D), significant upregulation of HIF-1α both at mRNA (Fig 1B) and protein level (Fig 2C-D) was detected in diabetic rats in comparison with controls.
- To determine the soluble properties of sHsps under chronic hyperglycemic conditions, we analyzed the solubility of major sHsps Hsp27, αAC, αBC and pS59-αBC by detergent solubility assay.
- All the four proteins are detected in TritonX insoluble fraction as their levels were significantly translocated from TritonX soluble fraction to insoluble fraction in retina of diabetic rats in comparison with age matched controls (Fig 3).
- Phosphorylation at S59 of αBC was decreased in soluble fraction of diabetic retina with a concomitant increase in insoluble fraction compared to controls (Fig 3). Phosphorylation at S45, S19 of αBC was increased in diabetic retina in comparison to controls (Fig 4A-B).
- Furthermore, the S59 of αBC was phosphorylated by upstream p38MAPK. Therefore we investigated the activation of p38MAPK by performing immunoblotting of total p38MAPK and p-p38MAPK in retina of diabetic rats. Although, there was no significant change in total p38MAPK, the p-p38MAPK levels were significantly increased in retina of diabetic rats in comparison with controls supporting the increased phosphorylation at S59 in diabetes (Fig 4C-D).

Fig 3. Solubility of sHsps in control and diabetic retina. The retinal samples were fractionated into soluble and insoluble fractions and subjected to immunoblottin

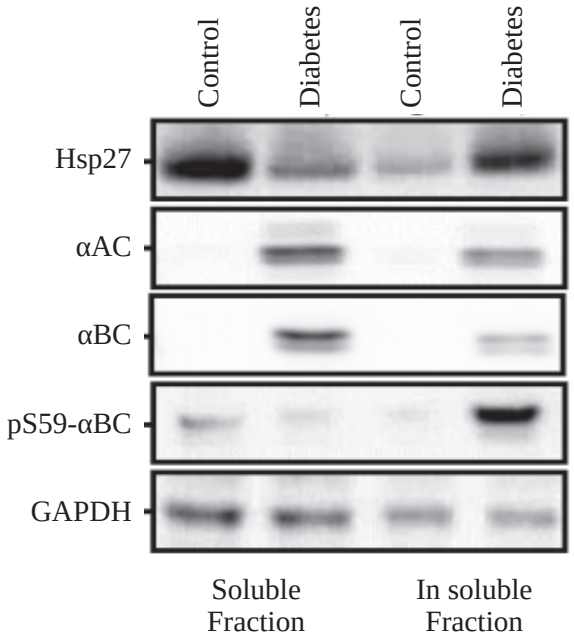
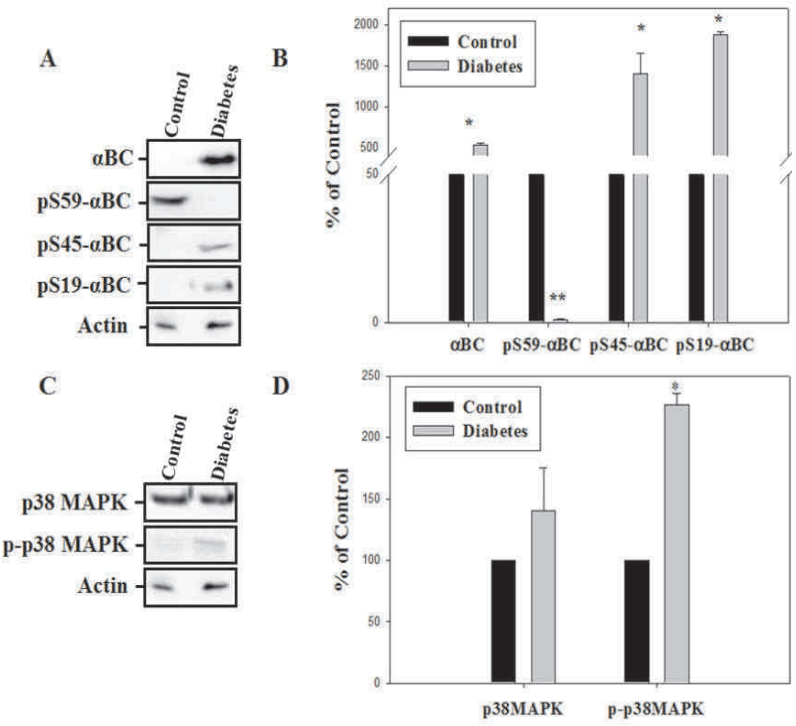


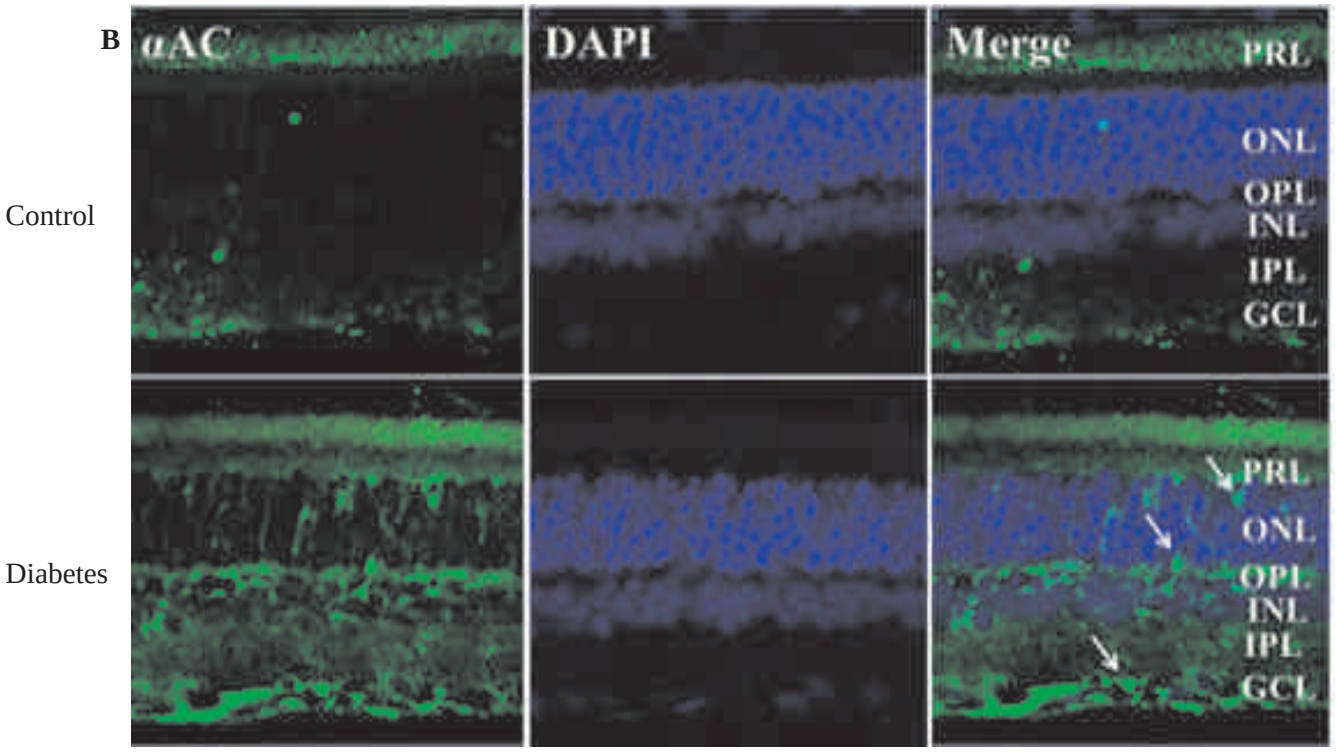
Fig 4. Immunoblot analysis of phosphorylated αBC (*Panel A*) and p38MAPK (*Panel C*) in the retina of control and diabetic rats. Quantification of pS59, pS45, pS19-**αBC (*Panel B*)** and p38MAPK, p-p38MAPK **(*Panel D*)** immunoblots in retina, of control and diabetic rats; expression was normalized for actin and represented as percent of control.



*Data are mean ± SEM (n=3; *p<0.05, ** p<0.01 compared to the control).*

- Immunostaining of αAC was found in photo receptor layer (PRL), inner plexiform layer (IPL), and ganglion cell layer (GCL) in control rats but intense staining was distributed in all retinal layers of diabetic rats (Fig 5).
- Positive immunoreactivity of αBC was predominantly observed in PRL, GCL and to a lesser degree in outer plexiform layer (OPL) with increased staining in diabetic rats compared to those of controls.
- Although, there was a weak signal for pS59, pS45 and pS19-αBC in retina of control rats, intense staining was observed in diabetic rats. The increased immunostaining of pS59-αBC was localized in PRL, OPL and GCL in diabetic rats. While pS45-αBC was localized in PRL, OPL, IPL and GCL of control retina, increased staining was observed in all retinal layers of diabetic rats.

Fig 5. Cellular localization of αAC in the retina of control and diabetic rats. GCL, Ganglion cell layer; IPL, Inner plexiform layer; INL, Inner plexiform layer; OPL, Outer plexiform layer; ONL, Outer nuclear layer; PRL, Photoreceptor layer. Positive signals of αAC in the diabetic retina are shown with arrows.



SUMMARY

In summary, we demonstrated the response of sHsps in retina of STZ induced diabetic rat model. The major findings reported here are: (i) out of 10 sHsp family members, expression of Hsp27, αAC, αBC, Hsp20, Hsp22 was seen in retina, (ii) out of these αAC, αBC and Hsp22 were upregulated in diabetic retina while expression of Hsp20 was down regulated; whereas Hsp27 mRNA levels were increased while protein levels decreased (iii) the expression of HSFs was either decreased or unaltered in diabetic retina and HIF-1α in conjunction with sHsps was upregulated in diabetic retina, (iv) this is also first report of increased expression of pS59, pS45, and pS19-αBC in diabetic retina, (v) demonstrated localization of sHsps & phosphorylated αBCs in diabetic retina and (vi) reported extensive aggregation of Hsp27, pS59-αBC along with αAC and αBC in diabetic retina. Expression of the majority of sHsps in the retina might be regulated in the HIF-1α? dependent manner in diabetic rats as local hypoxia has been shown to stimulate HIF-1α in retina under hyperglycemic conditions. We also demonstrate that the solubility of sHsps including Hsp27, αAC, αBC and pS59-αBC was compromised under hyperglycemic conditions in the retina. Although, further studies are required to clarify the role of sHsps in DR, taken together, these results suggest that sHsp family is crucial for neuronal protection in DR and may aid in developing therapeutic strategies for DR.

7. ALTERED UBIQUITIN-PROTEASOME SYSTEM LEADS TO NEURONAL CELL DEATH IN A SPONTANEOUS OBESE RAT MODEL

Obesity incidence is increasing worldwide, affecting both developed and developing countries. The World Health Organization has reported that obesity has been growing at an alarming rate, accounting for approximately 35% of the population. There is accumulated scientific evidence indicating that obesity is strongly related to a vast number of diseases, including hypertension, hypercholesterolemia, type 2 diabetes, respiratory conditions, arthritis and certain types of cancer. Obesity could have an effect on the brain is a recently emerged concept. Associations between obesity and various neurological disorders have been reported including sleep apnea, anxiety, manic depressive disorders, increased risk of developing cerebrovascular accident and other neurological disorders (1). Additional consideration has been raised that obesity may be linked to various progressive and age-related neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease (AD), and autoimmune nervous system diseases like multiple sclerosis. A more recent association between obesity and neurological function was based upon correlations with biological processes of oxidative stress and inflammation.

Impaired function of the ubiquitin-proteasome system (UPS) has long been implicated as a contributing factor in various neurodegenerative diseases (2). The function of the UPS can be impaired by many factors including the aging process, leading to the formation of ubiquitin protein aggregates resulting ultimately in proteinaceous inclusions detected in non-pathologic aging as well as in many neurodegenerative disorders (2). In fact, the presence of various ubiquitin-decorated protein aggregates is pathonumonic for many such diseases. Recent research has shown that UPS plays a role in various neurodegenerative diseases, such as Alzheimer's disease and Huntington's disease. However, the role of UPS in age-related and obesity-induced neurodegeneration is still to be elucidated. In this context, an animal model that develops neurodegeneration as a consequence of obesity could be ideal to delineate the molecular basis of neurodegeneration due to obesity.

A spontaneously developed obese rat was isolated from the existing WNIN stock of rats, and a colony of WNIN-Obese (WNIN/Ob) rats was established by selective breeding (3). Obese animals have 47 per cent of fat in the body and are hyperphagic, euglycemic, hyperinsulinaemic, hypertriglyceridaemic and leptin resistance (3). WNIN/Ob rats have significantly lower brain weights, and there is up to 50 per cent of the reduction in their organ-to-body weight ratio when compared to their lean litter mates. The average life span of this obese mutant rat strain was found to be 18–24 months as against 30–36 months of their parent WNIN strain. In addition to the obesity trait, these animals were shown to be more prone to the age-related abnormalities such as vision impairment (retinal degeneration and cataract) (4-6), impaired immunity and development of tumors etc.,. Interestingly, preliminary findings do indicate neurodegeneration in WNIN/Ob rat model. Therefore, this study was designed to investigate neurodegeneration in WNIN/Ob rat model that has two crucial causative factors: obesity and accelerated aging. Further, we examined the possible role of UPS in neurodegeneration linked to obesity using WNIN/Ob rat.

METHODOLOGY

Animals: The WNIN/Ob rats of different ages along with their respective lean littermates were used. After the completion of the respective time period, rats were sacrificed by cervical dislocation. The animal was dissected; cerebral cortex of brain tissue was collected and immediately frozen in liquid nitrogen and later kept at -80^{o}C until further analysis. Few brain tissues were collected in 4% w/v paraformaldehyde fixative solution for immunohistochemistry. Animal care and protocols were in accordance with and approved by IAEC.

Transmission electron microscopy (TEM): Prefrontal cerebral cortex of rats was fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) for 24 h at 4C, and washed with PBS for 4 times each 45 minutes, then fixed in 1% aqueous tetroxide for 2 h, later washed with deionized distilled water for 6 times each 45 minutes, dehydrated in series of graded alcohol, infiltrated and embedded in araldite 6005 resin followed by incubation at 80C for 72 h for complete polymerization. Ultrathin (50-70 nm) sections were made with a glass knife on ultra-microtome, mounted on copper grids and stained with saturated aqueous uranyl acetate and counter stained with Reynolds lead acetate. Finally viewed under TEM at required magnifications.

TUNEL assay: To determine apoptosis in the cerebral cortex, TUNEL assay was done using the In Situ Cell Death Detection Kit. Briefly, the sections were deparaffinized, rehydrated, permeablized and incubated with the TUNEL

reaction mixture containing TdT and fluorescein-labeled dUTP for 1 h at 37ºC. DAPI staining was used as the final step in fluorescent staining procedure to label cell nuclei. The apoptotic cells were analyzed using the fluorescent microscope. For negative control, TdT was not included in the reaction mixture.

Quantitative real-time PCR (qRT-PCR): Total RNA was extracted from the cerebral cortex of rats using Tri Reagent. Isolated RNA was further purified by RNeasy Mini Kit (Qiagen) and quantified by measuring the absorbance at 260 and 280 nm. Total RNA was reverse transcribed using High Capacity cDNA Reverse Transcription kit. Real-time PCR was performed in triplicates with cDNA templates using SYBR green master mix with gene-specific primers. Relative expression of genes (relative to lean or control) based on the difference in threshold cycle (Ct) between control versus experimental (lean vs WNIN/Ob and control vs high-fat) groups after normalization to internal control (β-actin) in each group. Data were compared between control and experimental samples according to the comparative threshold cycle ($2^{-??ct}$) method and expressed as fold change over control.

SDS-PAGE and immunoblotting: Tissues were homogenized in a buffer using a glass homogenizer and the homogenate was centrifuged at 12,000xg for 20 min. The supernatant was collected and used for immunoblot analysis. An equal amount of protein from lean and obese tissues was subjected to 12% SDS-PAGE and proteins were transferred onto PVDF membrane. Nonspecific binding was blocked with Blocker reagent in PBST and incubated overnight at 4°C with respective primary antibodies diluted in PBS. After washing with PBST, membranes were then incubated with anti-rabbit IgG (1:3500) or anti-mouse IgG (1:3500) secondary antibodies conjugated to HRP. The immunoblots were developed with enhanced chemiluminescence detection reagents and digital images were recorded.

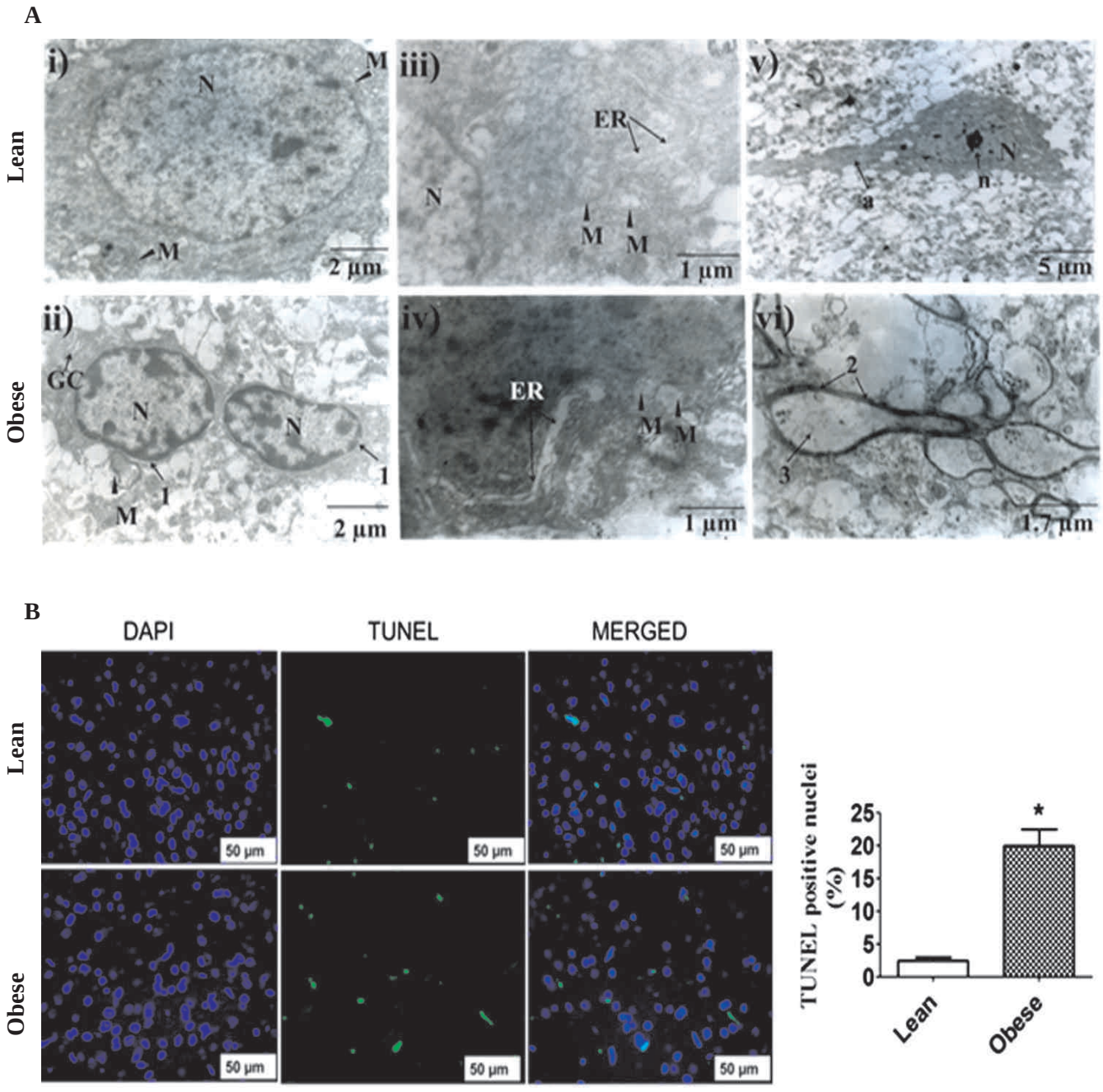
Proteasome activity assay: The enzymatic activity of the proteasome in the cerebral cortex was assayed using a kit. The kit takes advantage of the chymotrypsin-like activity, utilizing an AMC-tagged peptide substrate which releases highly fluorescent free AMC in the presence of proteolytic activity. The kit also includes a specific proteasome inhibitor MG-132, which suppresses all proteolytic activity due to proteasomes. This permits differentiation of proteasome activity from other protease activity that may be present in samples.

Immunohistochemistry: Deparaffinized sections were processed for antigen retrieval. After blocking, the primary antibody was added and allowed to incubate overnight at 4 °C. After incubation, biotinylated secondary antibody solution was added. The sections were stained with DAB. Negative controls were run simultaneously with an omission of primary antibody. The sections were then dehydrated through ethanol and xylene before coverslips with Permount. Immunofluorescence staining was also performed to show BAX and cleaved caspase three proteins in paraffin sections. The procedure was similar to that of immunohistochemistry with slight modifications. The sections were incubated with a primary antibody against BAX and cleaved caspase 3. The binding of primary antibodies was visualized by Alexa Fluor-555 conjugated anti-Mouse IgG for BAX and Alexa Fluor-488 conjugated anti-rabbit IgG for cleaved caspase-3. Fluorescently labeled sections were visualized using a fluorescence microscope.

RESULTS

- TEM analysis of neurons in the prefrontal cerebral cortex of 12-month old obese rats showed distinct signs of neuronal cell abnormalities. The neurons in obese rats showed vacuolated matrix, shrunken cells, dilated nuclear membrane, margination of chromatin, nucleoplasm filled with electron dense material and disappearing ER (Fig. 1A).
- Further, dilated cisternae of ER and vesicular mitochondria were observed in obese rat neurons while such abnormalities were not found in the lean rat. Fig1A (vi) show degenerating neuron in the obese rat with disappeared nucleus, thick neuronal wall, degenerated axon and shrunken cell when compared to normal healthy neuron in the lean brain. Such disintegrating neurons are more often found in the prefrontal cerebral cortex of obese rats.
- Consistent with the results of TEM, TUNEL assay indicated only very few TUNEL- positive cells in lean rats (Fig.1B). In contrast, there was an apparent increase in the number of apoptotic neuronal cells in the cerebral cortex of obese rats.
- When the ratio of brain weight to body weight was calculated, the obese rats were found have lowered ratio of brain weight to body weight compared to their lean counterparts (0.1815 ± 0.08 vs 0.317 ± 0.05). There is up to 58

Fig 1. Ultra structural (TEM) studies and TUNEL assay of neurons in the prefrontal cerebral cortex of 12 months lean and obese rats. Panel A- i) The neurons in lean rats showed intact, normal sized nucleus, mitochondria and Golgi complex; ii) The neurons in obese rats showed dilated nuclear membrane, margination of chromatin, nucleoplasm filled with electron dense material and disappearing ER. The size and shape of the nucleus and mitochondria are altered; iii) Normal structure of ER is observed in lean rats; iv) Dilated cisternae of ER and vesicular mitochondria in obese rat; v) Normal neuron showing nucleolus and axon in lean rats. Scale 5 µm, the original magnification at 3860x; vi) Degenerating neuron in the obese rat with a disappeared nucleus, thick neuronal wall, degenerated axon and shrunken cell. Such neurons were more often found in the cerebral cortex of obese rats. N: nucleus, M: mitochondria, GC: Golgi complex, ER: endoplasmic reticulum, a: axon, n: nucleolus, 1: dilated nuclear membrane, 2: thick neuronal wall, 3: disappeared nucleus. Panel B-Apoptosis by TUNEL assay. Representative fluorescence microscopic images of the cerebral cortex of lean and obese rats. Bar graph shows TUNEL-positive cells.

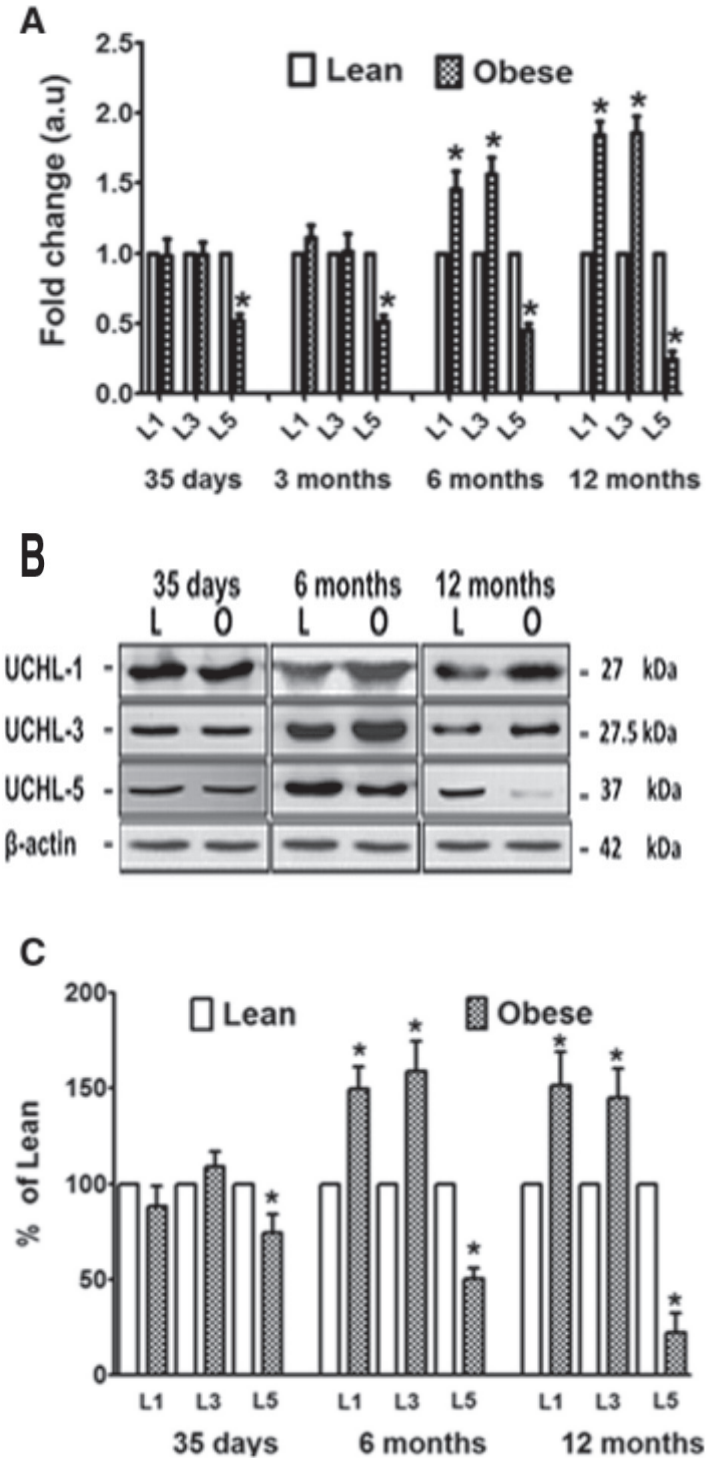


*(mean ± SE; n=6; *p*<0.05).*

per cent of the reduction in their organ-to-body weight ratio in obese rats.

- Since the deubiquitinating enzymes (DUBs) play important regulatory role in the UPS, we examined differential expression of one class of DUBs; UCHs, in the cerebral cortex of obese rats both at transcript and protein level at different ages (35 days, 3, 6 and 12 months). UCHL-1 and UCHL-3 expression was unaltered at 35 days and three months but up-regulated at 6 and 12 months of age in obese rats compared to their respective lean controls (Fig 2A). However, UCHL-5 expression was down-regulated from 35 days onwards in obese rats when compared to their respective lean littermates. Immunodetection data support these observations at protein level (Fig.2B & C).
- Due to down-regulation of proteasome-associated UCH, UCHL-5; we speculated that the activity of proteasome might be affected, and hence we examined chymotrypsin-like activity of proteasome in the cerebral cortex of obese rats at 6 and 12 months of age. The results indicate declined proteasomal activity in the cerebral cortex of obese rats when compared to lean rats at both 6 and 12 months of age (Fig 3A).
- The declined proteasomal activity could also be a result of decreased expression of proteasome sub units or other extrinsic factors. To explore this, expression of three genes (B1, B2, and B5) of 20S catalytic subunit and one gene (Rpn13) from 19S regulatory subunit was investigated. B1 subunit is responsible for chymotrypsin-like proteasomal activity while B2 and B5 are responsible for the trypsin-like, and peptidyl-glutamyl peptide-hydrolyzing activities respectively. qRT-PCR data showed no change in B1, B2, B5 levels whereas there was an up-regulation of Rpn13 in obese rats (Fig 3B).
- Ubiquitin (Ub) as an important component of UPS decides the fate and function of a protein. Immunodetection of Ub in the cerebral cortex of 12 months old obese rats reveals elevated levels of both free Ub and its conjugates in obese rats when compared to respective lean rats (Fig.3C & D).

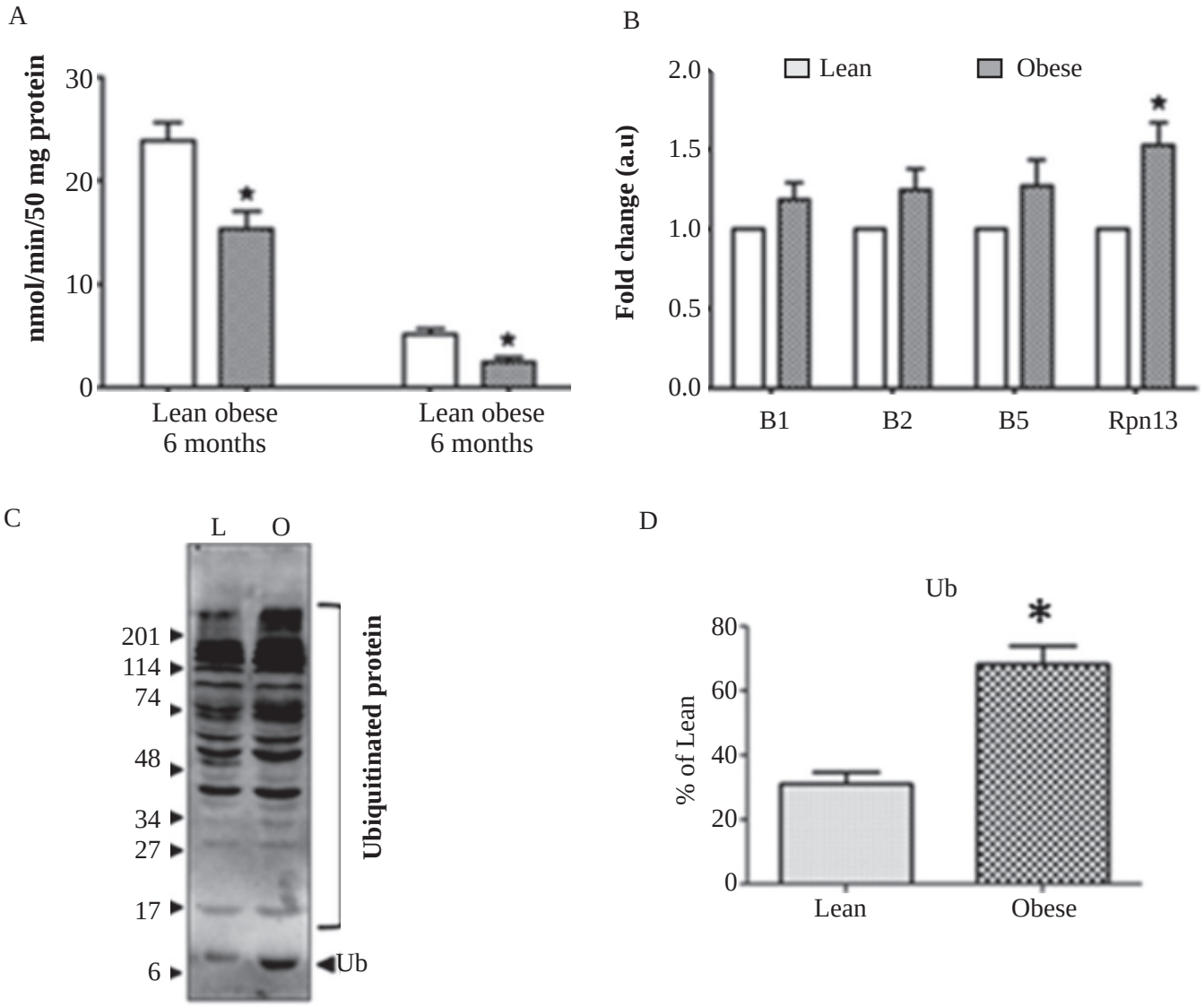
Fig 2. Differential expression of UCHs in the cerebral cortex of rats at different age points. *Panel A*-The expression pattern of UCHs in the cerebral cortex of WNIN/Ob and their lean littermates as analyzed by qRT-PCR. Data are mean ± SEM (n=3; *p<0.05)**. *Panel B*-** Representative immunoblots of UCHs and ***Panel C***-quantification of immunoblots.



*Data are mean ± SEM (n=3; *p<0.05).*

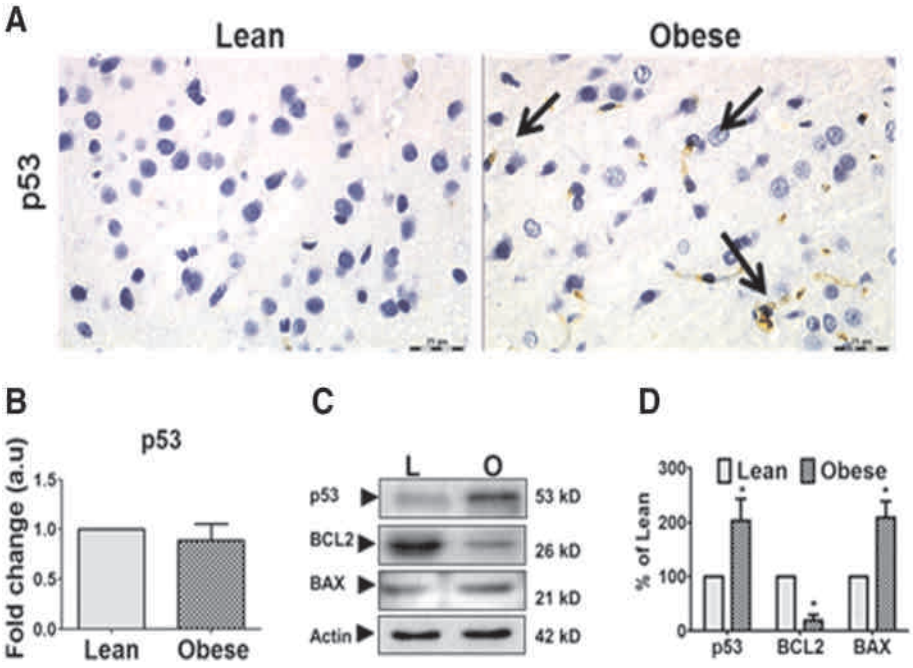
- After observing disturbances in the UPS, we also examined the status of unfolded protein response (UPR), another important protein quality control system located in the endoplasmic reticulum (ER). The data clearly indicated the existence of ER stress.
- Since prolonged ER stress triggers apoptosis, we examined p53 levels by qRT PCR, western blot, and immunohistochemistry. The results showed a significant increase at protein level but not at mRNA level (Fig.4). Further, we analyzed protein levels of BAX and BCL2, two important mediators that regulate apoptosis by western blot. Figure 4C & D revealed increased protein expression of BAX but decreased expression of BCL2 in the cerebral cortex of obese rats.
- Immunofluorescence analysis for BAX and cleaved caspase-3 was also performed in cerebral cortex sections to confirm the apoptotic cell death. The results showed increased immunostaining of BAX and cleaved fragments of caspase-3 in the cerebral cortex of obese rats when compared to lean rats (Fig.5).

Fig 3. *Panel A*- The chymotrypsin-like activity in the cerebral cortex of WNIN/Ob and their lean littermates. *Panel B*- The expression of catalytic subunits B1, B2, B5 and a regulatory subunit Rpn13 of the proteasome in the cerebral cortex of WNIN/Ob and their lean littermates analyzed by qRT-PCR. *Panel C*- Representative immunoblot of ubiquitin and ubiquitinated proteins and *Panel D*- quantification of immunoblot.



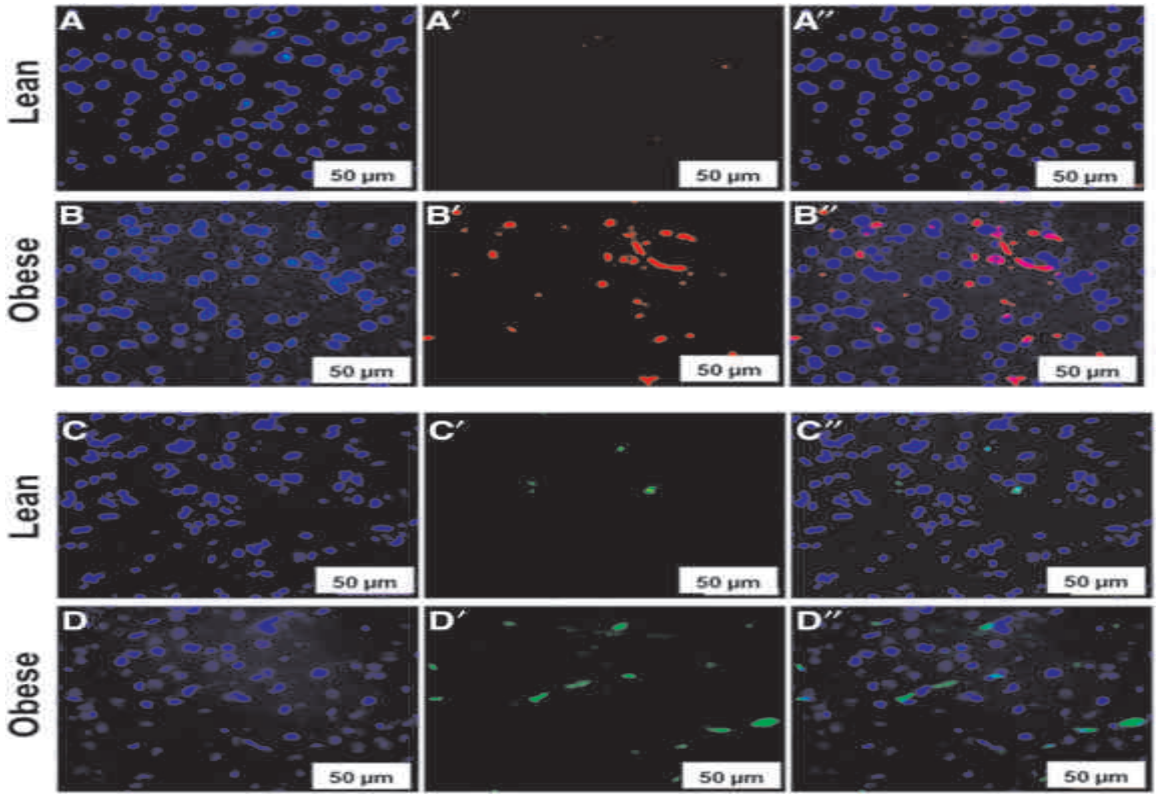
*Data are mean ± SEM (n=3; *p<0.05).*

Fig4. Apoptotic in the brain of 12 months old lean and obese rats. *Panel A*- Expression of p53 in cerebral cortex of WNIN/Ob and lean rats analyzed by immunohistochemistry *Panel B*-The expression of p53 at 12 months in the cerebral cortex of WNIN-Ob and rats analyzed by qRT-PCR. *Panel C*-Representative immunoblots of p53, BAX, BCL2. *Panel D*-quantification of immunoblots for p53, BAX and BCL2 in cerebral cortex of 12 months WNIN/Ob and their lean littermates.



*Data are mean ± SEM (n=3; *p<0.05).*

Fig 5. Immunofluorescence of BAX and cleaved caspase 3 in the cerebral cortex of 12 months old lean and obese rats. Paraffin sections of cerebral cortex from 12 months lean (A, C) and obese rats (B, D) were labeled with antibodies to BAX (A, B) and cleaved caspase-3 (C, D).



SUMMARY

In this study, we describe the neuronal damage in a spontaneously developed obese rat model, WNIN/Ob rat and provide the molecular basis in the form of altered UPS. This was further substantiated by carrying out studies in a high fat diet rat model. Based on all these evidences it can be concluded that neuronal cell death in WNIN/Ob rat is due to the unbalanced ubiquitin-proteasome system. The up-regulated UCHL-1 may stabilize p53 that drive apoptosis through BAX and caspase-3. The decreased proteasomal activity fails to clear the misfolded and unfolded proteins targeted to ER for degradation and thereby eliciting ER stress. Persistent ER stress induces apoptosis through CHOP. Hence, together the results obtained in two different obese rat models strongly suggests that altered UPS leads to neuronal cell death probably in two independent ways: (i) UCHL-1 mediated apoptosis through stabilizing p53 and (ii) through triggering of ER stress. This is the first report of its kind that provides a role for UPS in neuronal damage or cell death in obesity. However, further studies are warranted to establish the involvement of altered UPS in neuronal damage under obese conditions conclusively.

8. ROLE OF GROWTH HORMONE IN PODOCYTE INJURY AND DEPLETION: IMPLICATIONS IN PROTEINURIA

Diabetic nephropathy (DN), characterized by increased glomerular permeability to serum proteins, is the most common cause of end-stage renal disease (ESRD). The three components; fenestrated endothelium of glomerular capillaries, the glomerular basement membrane (GBM) and the visceral glomerular epithelial cells (podocytes) constitute the glomerular filtration apparatus, which is permeable to water and small dissolved solutes and offers size-selective filtration for the larger molecules. Podocytes are terminally differentiated epithelial cells that regulate glomerular permselectivity, synthesize components of GBM, and counteract the intraglomerular hydrostatic pressure. Podocytes form interdigitating foot processes with bridging slit-diaphragms to regulate renal ultra-filtration and prevent loss of high molecular weight serum proteins into the urine. Normal renal filtration is dependent on the integrity of interaction between podocyte and GBM and an adequate number of podocytes. Podocyte depletion is considered as a hallmark of glomerular diseases including DN. Podocyte depletion could be the result of either apoptosis and podocyte detachment from the GBM. Human biopsy studies indicate that podocytes are injured very early in the pathogenesis of DN. Decreased expression of podocyte proteins such as nephrin and podocin has been documented in experimental models of nephropathy. Although several mechanisms that result in podocyte damage in DN have been proposed, a complete understanding of the molecular and cellular mechanism(s) mediating podocyte loss in DN is lacking.

The pathogenesis of DN is complex, and an array of factors have been suggested as potential contributors to podocyte damage in diabetes mellitus. One of the axes that have been implicated in DN is the GH-IGF1 axis. Progression to ESRD is associated with abnormalities in pituitary growth hormone (GH) secretion and diminished renal clearance of GH. Children with ESRD have an increased number of secretory bursts of GH compared to children with normal renal function. Definite evidence for a causal role of GH in nephropathy has come from acromegalic subjects wherein excess GH levels were associated with significant structural and functional changes in the kidney and albuminuria. In case of mice transgenic for bovine GH, glomeruli was disproportionately enlarged paralleled with glomerulosclerosis and increased albumin: creatinine ratio. Experimental animals with hyperactivity of the GH-axis exhibit podocyte damage and poor renal function. In contrast, GH-deficient dwarf rats had minimal proteinuria, mild renal functional impairment and moderate renal histological scarring. In earlier studies, we demonstrated that podocytes express the GH receptor and respond to GH by inducing the expression of ZEB2 that result in decreased expression of P- and E-cadherins.

One of the abnormalities from patients with diabetes mellitus is loss of podocytes. The urinary sediments from patients with proteinuric diseases including DN contain increased levels of podocyte markers. Interaction between

podocytes and GBM is critical to the normal renal filtration since podocytes anchor to GBM and regulate glomerular permselectivity. Perturbations in various components of extracellular matrix (ECM) are associated with DN. However, the effect of GH on cell-adhesion and ECM components that determine podocyte-GBM integrity is unclear. Gene expression profiling of mouse podocytes by PCR-array identified GH dependent up-regulation of transforming growth factor-beta-induced protein (TGFBIp), a 68-kDa extracellular matrix protein and is postulated to be involved in cell-matrix interaction and cell migration. It is considered that TGFBIp elicits both epithelial-mesenchymal transition (EMT) and apoptosis. In this study, we investigated the effect of GH on the expression of ECM and adhesion molecules of podocytes.

METHODOLOGY

Culturing of podocytes: Conditionally immortalized mouse podocyte (MPC-5) cells were maintained under growth-permissive conditions at 33°C and 5% CO_2. RPMI-1640 medium containing 10% FBS, 100 units/ml of penicillin, 100 µg/ml of streptomycin, and ten units/ml of mouse γ-interferon were used to maintain MPC-5 cells in proliferating condition. To induce differentiation, podocytes were shifted to non-permissive conditions at 37°C without -interferon for ten days. Differentiated podocytes were maintained for 12–16 h in the serum-free medium before treating with hGH or pegvisomant or rTGFBIp.

PCR-Array Analysis: MPC-5 cells were harvested after 48 h of treatment with or without GH (500 ng/ml). Total RNA was isolated, purified using RNeasy kit and subjected to first-strand cDNA synthesis. The expression of 84 genes associated with ECM and adhesion molecules was profiled using cDNA isolated from podocytes employing RT^2 Profiler PCR Array.

Real-time quantitative PCR assay: Total RNA was extracted and purified from MPC-5 cells treated with or without GH (500 ng/ml) or TGFBIp (5 ug/ml). Quantitative PCR was performed using SYBR Green RT-PCR kit. mRNA expression of each gene was normalized using the expression of GAPDH that was used as an internal control.

Estimation of TGFBIp by ELISA: GH dependent secretion of TGFBIp was estimated by stimulating podocytes with GH (500 ng/ml) for varying time periods ranging from 12-48 h. Spent culture media from podocytes exposed to GH was collected at each time point and centrifuged at 200xg for 5 min at 4°C. Supernatants so obtained were used to measure TGFBIp using sandwich-ELISA with mouse monoclonal antibody against TGFBIp as a capture antibody and biotinylated goat antibody against TGFBIp as the detection antibody. Streptavidin-horseradish peroxidases followed by o-phenylenediamine dihydrochloride were added to initiate the color reaction, and the reactions were then stopped with sulfuric acid and read at 490 nm. Recombinant human TGFBIp was used as a standard.

Whole cell lysis and immunoblotting: MPC-5 cells were treated with or without GH or TGFBIp for specified time periods. Cells were washed with ice-cold PBS and lysed using cell lysis buffer supplemented with protease inhibitor mixture. The cell lysate was subjected to centrifugation at 12000x*g* for 15 min to collect the supernatant, which was processed for SDS-PAGE and immunoblotting.

Cell death analysis: For cell death analysis, podocytes were treated with GH for varying period of time ranging from 12-72 h. At each time point, the floating and attached cells were harvested and used for the enumeration of viable and nonviable cells. Cells were reconstituted in fresh media, and an equal volume of trypan blue (0.4%) solution was added. Cells were counted using hemocytometer and light microscope. The number of viable (unstained) cells and nonviable (stained) cells were enumerated and expressed as a percentage of total number of cells (always $\geq$ 500 cells) counted. TUNEL assay was performed to determine the apoptosis of MPC-5 cells treated with GH or TGFBIp. Podocytes were cultured on cover slips and treated with GH or TGFBIp for indicated time periods. Apoptotic nuclei were detected under a fluorescence microscope with the characteristic morphology of nuclear fragmentation and nuclear condensation. Results were expressed as the percentage of apoptotic cells. Detection of caspase-3 activity was performed with Caspase Colorimetric Assay Kit.

Scratch Assay: Mouse podocytes were seeded in 6 well plates and grown under normal growth conditions to reach upto 95% confluence. When the cells are ready, media was aspirated and a scratch was made using a pipette tip. The scratch should be made in the form of '+' in a smooth sweeping motion. Cells were washed twice with pre-warmed media in order to remove any debris from damaged cells. Images of cells were taken before and after treating with GH or TGFBIp at a regular time points to monitor the rate at which the denuded area of cells get colonized by the surrounding migrating cells. The area covered by cells is quantified and represented as the percent area coverage.

Albumin Influx Assay: MPC-5 cells were plated on a 12-well plate and differentiated at nonpermissive conditions as described above by culturing for 10 days. The cells were then treated with either GH (500 ng/ml) or TGFBIp (5 ug/ml) for 24 h. Subsequently, the medium was removed, and cells were washed twice with a 1mM $CaCl_2$ and 1 mM $MgCl_2$ mixture to preserve cadherin junctions. 2 ml of RMPI 1640 medium with 40 mg/ml BSA was then placed in the bottom chamber, and 0.3 ml of RPMI 1640 medium (without albumin) was placed in the top chamber. The cells were incubated at 37°C, aliquots of medium were collected from the top chamber at various (1-, 2-, and 4-h) time points, and albumin concentration was measured in these aliquots using the bicinchoninic acid protein assay kit.

Animal Experiment: Expression of EMT markers was examined in glomeruli isolated from rats injected with GH. Adult female Wistar rats (body weight 200 g) were used in this study. Experimental rats (n=6) received a single ip dose of hGH 1.5 mg/kg/day for two weeks whereas control rats (n=6) have received an equal volume of saline. Prior to sacrificing, the animals were placed in individual metabolic cages for determination of 24-h urine volume, glucose, creatinine, and albumin. For histopathology assessment, kidneys were perfused and fixed with alternating intra-aortic injections of PBS (0.02 M, pH 7.4) and 10% neutral-buffered formalin until blanching of kidneys was achieved. All experimental procedures were approved by IAEC.

Kidney lysate preparation: Following perfusion with PBS, the left kidney was ligated, and the right kidney was perfused with 50 ml of ferric oxide slurry in PBS via the abdominal aorta. The right kidney with iron-containing glomeruli was minced, and glomeruli isolated. Isolated glomeruli were further subjected to homogenization with lysis buffer containing protease inhibitors mixture and used for immunoblotting.

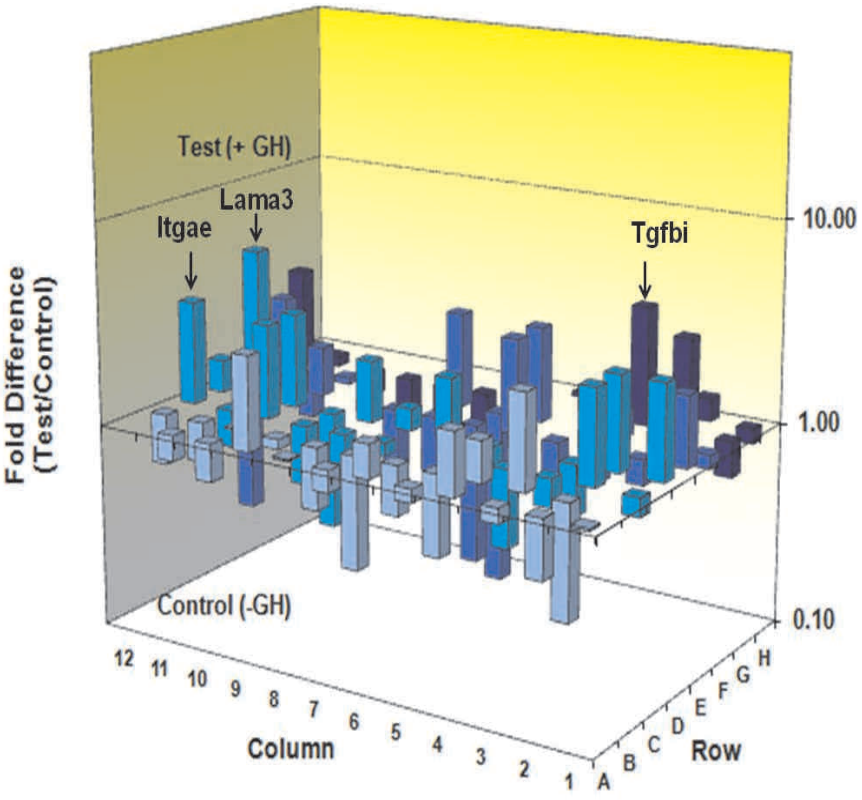
Immunohistochemical analysis: Both paraffin-embedded and cryosections of kidneys from control and experimental rats were prepared. Hemotoxylin and eosin staining was performed to examine the morphology. Immunostaining for WT-1 and synaptopodin was performed in paraffin embedded sections and cryosections, respectively. The number of podocytes per glomerulus was determined by counting the number of WT-1 expressing cells in each glomerulus from paraffin sections.

RESULTS

GH increases TGFBIp expression in podocytes: We used PCR array analysis to investigate the effect of GH on the expression of genes that encode ECM and adhesion molecules in the podocytes. Expression of transforming growth factor-beta-induced gene (*TGFBI*, also known as *BigH3*) was elevated with GH treatment along with other members of ECM and adhesion molecules including collagen type IIα1, integrin E, laminin α3/β3 and MMP-3 (Fig 1). Results obtained from PCR array analysis were confirmed by quantitative real-time RT-PCR measurement of the steady-state abundance of *TGFBI* mRNA which revealed that levels of TGFBIp mRNA were increased 1.5- (12 h), 1.9- (24 h), and 3.1-fold (48 h) following exposure of podocytes to GH.

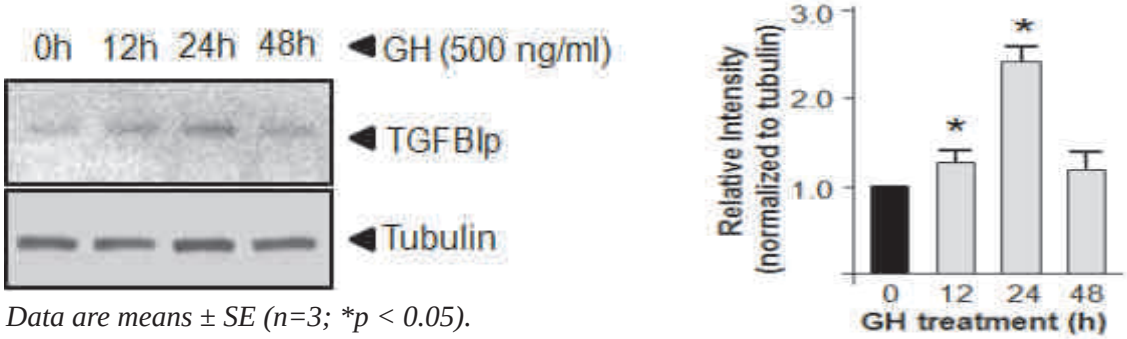
Immunoblotting of lysates from podocytes exposed to GH revealed a time-dependent increase in the levels of TGFBIp protein (Fig.2).

Fig 1. PCR array analysis of GH dependent expression of genes including TGFBIp in glomerular podocytes



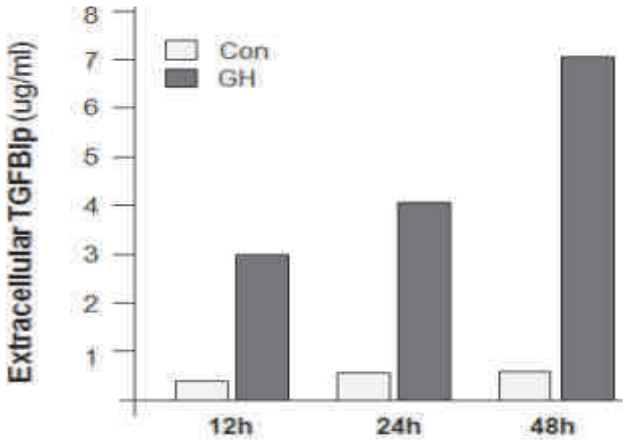
TGFBIp is an extracellular matrix protein and secreted by cells. Therefore, we estimated TGFBIp levels in spent culture media of podocytes that were exposed to GH. Exposure of podocytes to GH resulted in an increased secretion of TGFBIp with increased levels up to 7 folds at 48 hrs of exposure compared with cells that were naïve to GH treatment (Fig 3).

Fig 2. GH dependent expression of TGFBIp in glomerular podocytes. *Left panel:* A representative immunoblot for TGFBIp and tubulin from podocytes treated with GH (500 ng/ml) for the indicated time periods. *Right panel:* Densitometric quantification of TGFBIp expression normalized to tubulin.



*Data are means ± SE (n=3; *p < 0.05).*

Fig 3. GH induces extracellular secretion of TGFBIp. Differentiated mouse podocytes were treated with GH for indicated time periods and spent culture media collected. Clarified supernatant was used to estimate TGFBIp content by sandwich ELISA.



GH induces cell death in podocytes: GH-induced time-dependent cell death in podocytes (Fig.4A). Following 12 h of exposure to GH there was only a marginal decrease in viable cell count, whereas 24, 48 and 72 h exposure to GH the percent of live cells was decreased by 12%, 19%, and 35%, respectively (Fig.4A). By employing TUNEL assay, we have confirmed the mode of cell death in GH-treated podocytes to be apoptosis (Fig.4B). Analogously, podocytes treated with TGFBIp also underwent apoptosis, and it was found that TGFBIp was more effective than GH in inducing apoptosis of podocytes at concentrations tested (Fig.4C). Increased activity of caspase-3 revealed that TGFBIp induced cell death by apoptosis (Fig.4D).

***GH induces epithelial-mesenchymal transition (EMT) in podocytes*:** We assessed the expression of EMT markers in GH treated podocytes and observed induction of ZEB2, which is known to play a key role in EMT process (Fig. 5). In addition, we found that exposure to GH resulted in decreased E-cadherin, increased N-cadherin, and β-catenin expression (Fig. 5).

In similar to GH treatment, exposure of podocytes to TGFBIp also resulted in increased ZEB2, N-cadherin, -catenin and decreased E-cadherin expression in podocytes (Fig.6).

GH and TGFBIp induce podocyte migration and alter filtration barrier function: Both GH and TGFBIp induce migration of cells and TGFBIp is more effective than GH in inducing podocyte migration (Fig. 7). Furthermore, to assess the consequence of podocyte exposure to GH on its filtration barrier function, we employed paracellular permeability assay that measures the rate of albumin flux across the podocyte monolayer. These results reveal that both GH and TGFBIp increase albumin influx across the podocyte monolayer (Fig.7). However, at the concentrations tested, TGFBIp dependent increase in albumin efflux was greater than that observed for GH (Fig.7). Increased permeability of podocytes to albumin suggests reduced podocyte integrity upon treatment with GH or TGFBIp.

Fig 4. GH and TGFBIp induce cell death. A: Differentiated mouse podocytes were treated with GH for indicated time periods. Cells were harvested and stained with trypan blue and cell count was performed, and data presented as % live cells vs. dead cells. Differentiated mouse podocytes were treated with **(B)** either GH or (**C**) TGFBIp, and TUNEL assay was performed. TUNEL positive cells were counted and expressed as % of apoptotic cells. (%) Apoptotic cells=number of TUNEL positive staining cells/number of total cells×100% (at least 100 cells were counted for each sample). **D**: Caspase3 substrate assay was performed in mouse podocytes treated with TGFBIp and its activity was expressed in relation to control. *The data are mean ± SE (*p<0.05; n=5).*

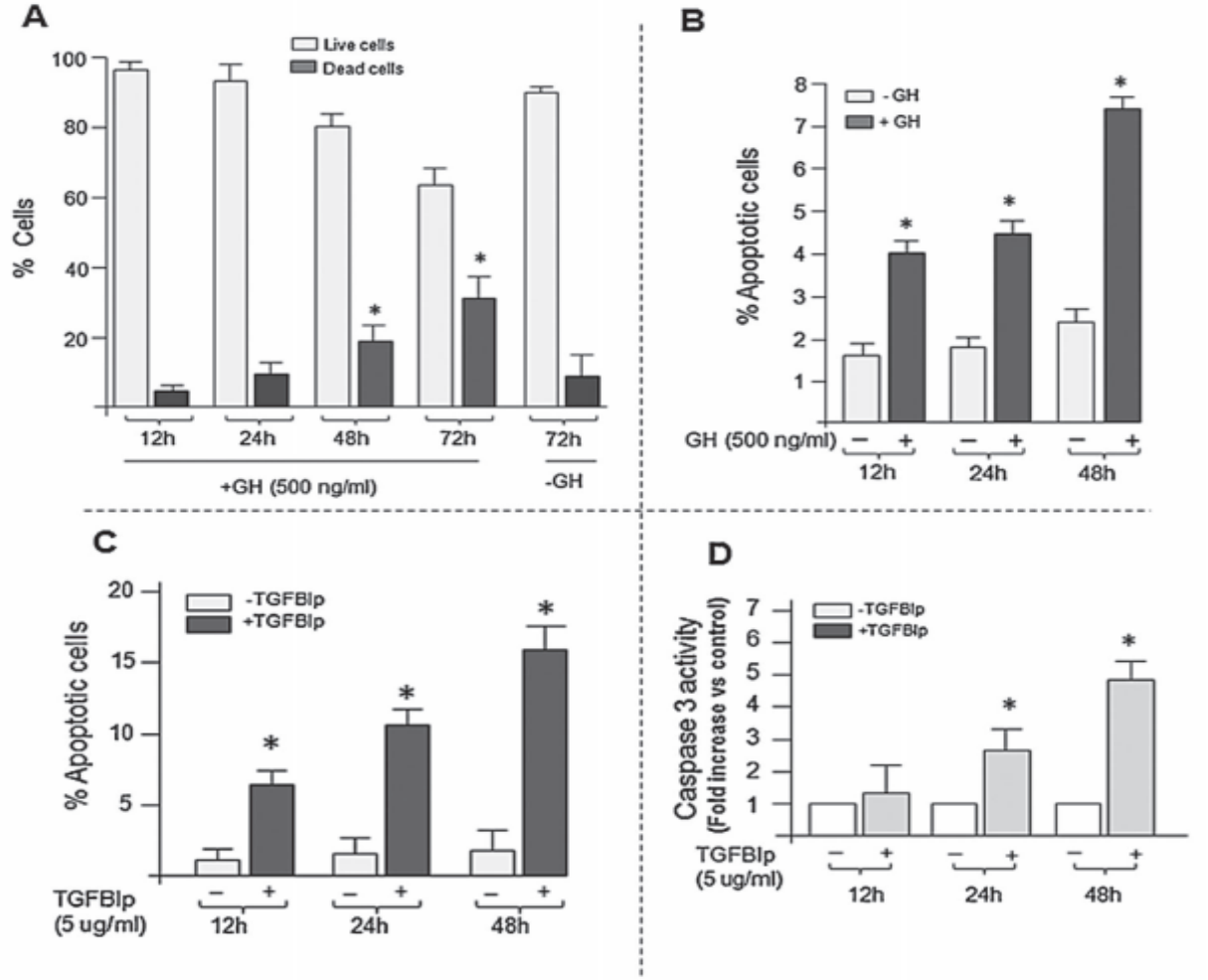


Fig 5. GH induces EMT markers in podocytes. **A**: Representative immunoblots showing the expression of EMT markers in GH treated podocytes. **B**: Densitometric quantification of EMT markers normalized to tubulin. Expression of marker in untreated cells (0 hr) considered as one fold. *Mean ± SE (n=3, *, p< 0.05).*

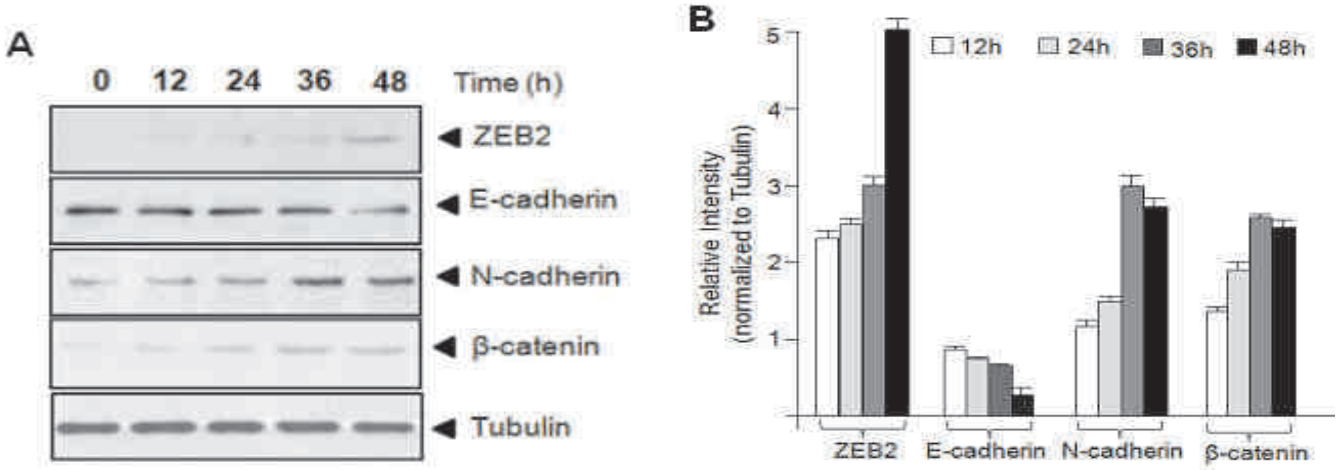


Fig 6. TGFBIp induces EMT markers in podocytes. **A**: Representative immunoblots showing the expression of EMT markers in TGFBIp treated podocytes. **B**: Densitometric quantification of EMT markers normalized to tubulin. Expression of the marker in untreated cells (0 hr) considered as one fold. *Mean ± SE. (n=3, *, p< 0.05)*

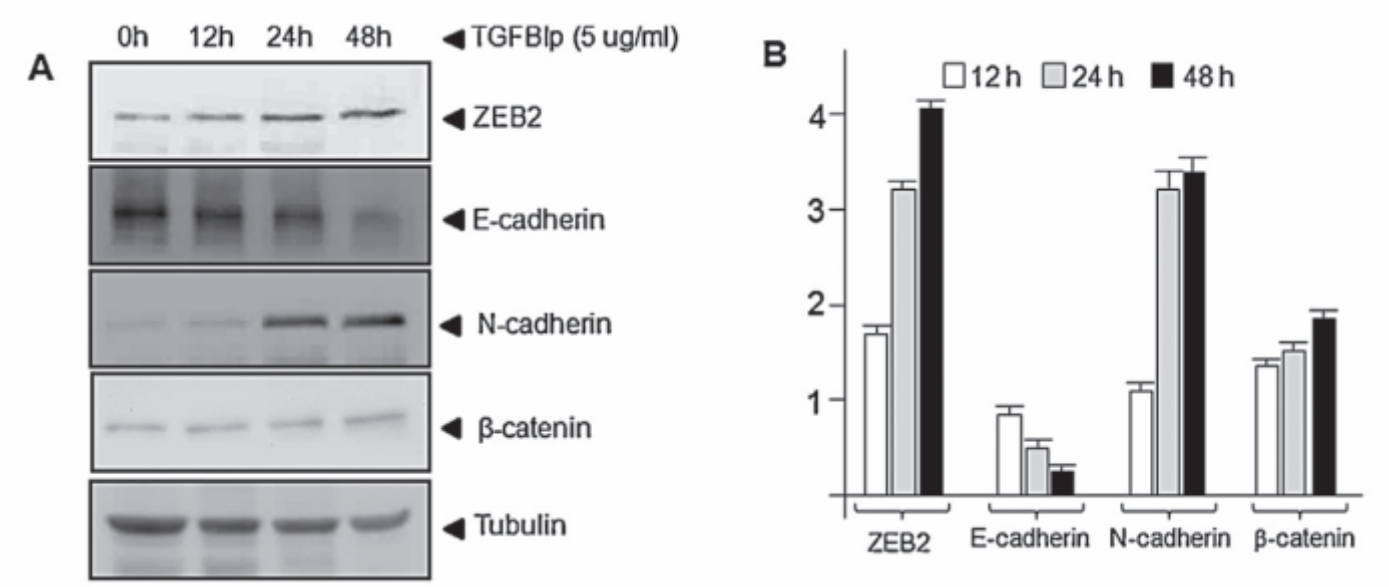
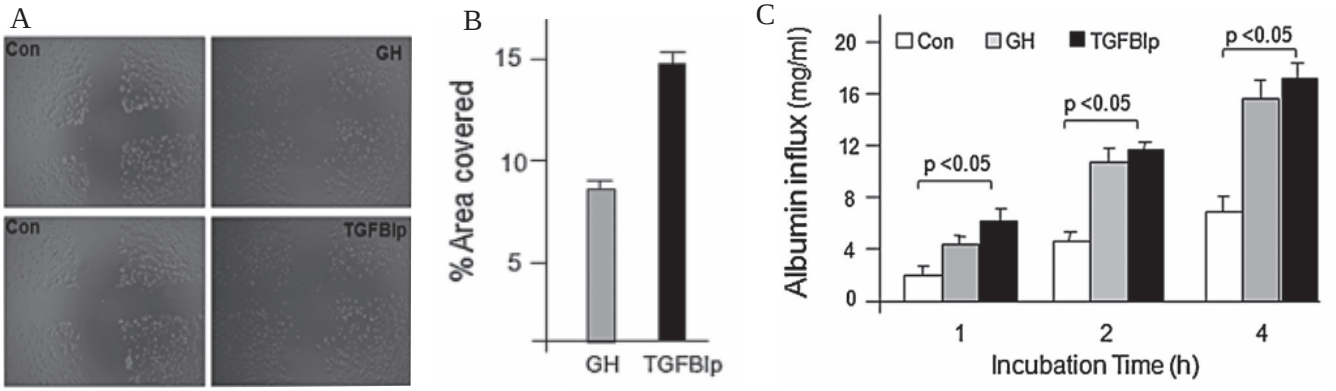
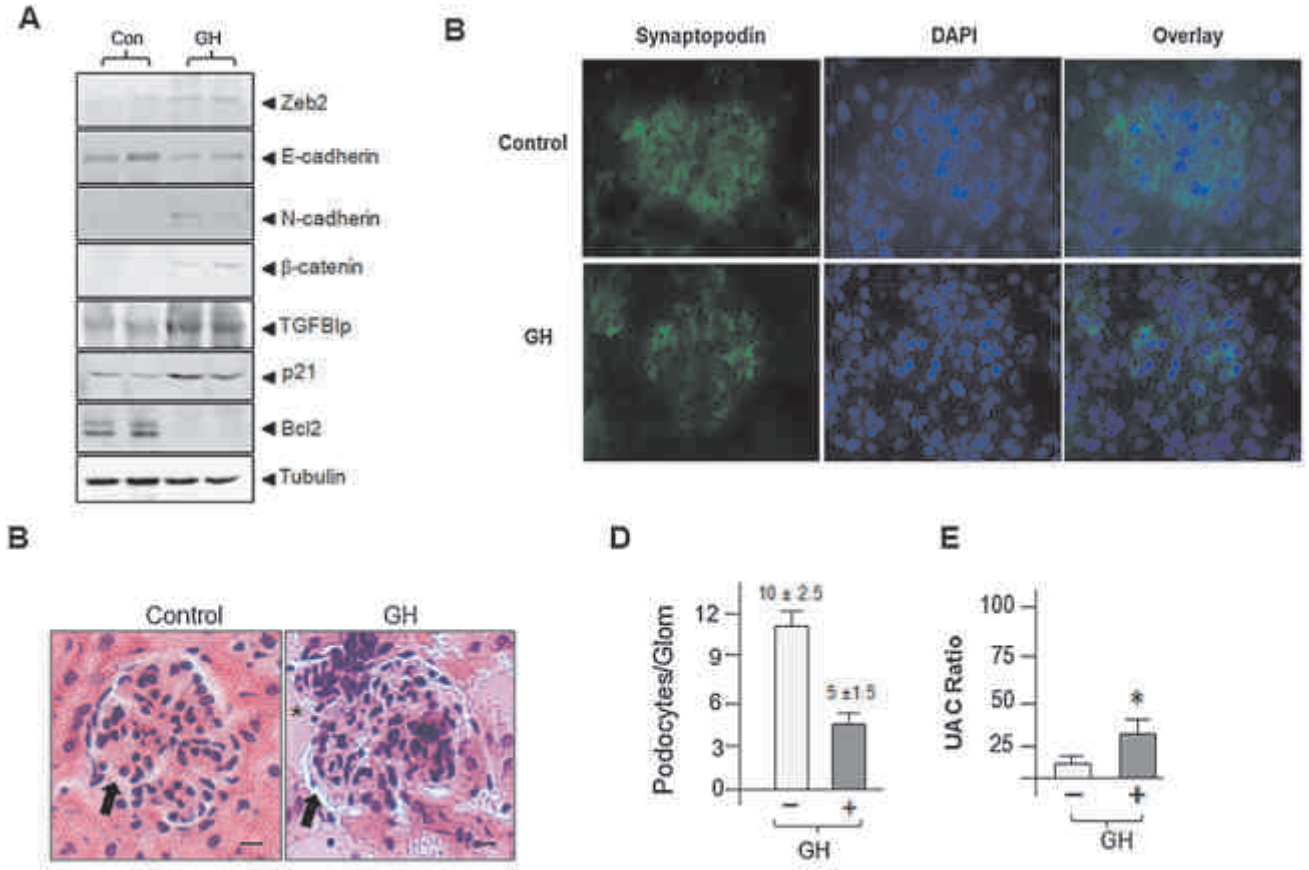


Fig 7. GH and TGFBIp induce podocyte migration and alters permeability: A: Representative images of podocytes treated with or without GH/TGFBIp for 2 hrs. **B**: % area covered with cells after treating damaged cells with GH or TGFBIp for 2 hrs where as an area of the original wound is considered a 100%. **C**: Differentiated mouse podocytes (MPC-5 cells) were grown under nonpermissive conditions as a monolayer and treated with GH (500 ng/ml) or TGFBIp (5 ug/ml) for 24 h, and albumin permeability across the podocyte monolayer was determined at 1, 2, and 4 h following the 24h exposure to GH or TGFBIp. *Data are mean±SE (n=4).*



***GH administration promotes decreased podocyte count in rat kidney*:** To validate the effect of GH on podocyte viability and EMT *in vivo*, we injected rats with GH for two weeks and isolated glomeruli from the kidney. Upon immunoblotting analysis, we found that treatment with GH resulted in increased ZEB2, N-cadherin and β-catenin and decreased E-cadherin expression (Fig.8). Interestingly, we also found elevated expression of TGFBIp in GH-treated rats (Fig.8). We have also noticed increased p21 and decreased BCl2 in a glomerular fraction of GH-treated rats (Fig.8). Decreased staining for synaptopodin in glomeruli from GH-treated rats revealed reduced podocyte density (Fig.8). Hematoxylin and eosin (H&E) reveal altered morphology of glomeruli in GH-treated rats as evidenced by thickening of the basement membrane, mesangial sclerosis and interstitial infiltration (Fig.8). GH-treated rats also showed significantly decreased podocyte count compared with control rats (Fig.8). We collected 24h urine from control and GH-treated rats and estimated albumin and creatinine. We found that albumin: creatinine ratio was increased in rats treated with GH (Fig.8).

Fig 8. ***In vivo*** **administration of GH induces EMT and podocyte depletion. A**: Expression of EMT and apoptotic markers in glomeruli from rats treated with GH for 2 weeks. **B**: Immunofluoresnce detection of synaptopodin in control and GH treated rat glomeruli **C**: H&E staining of control and GH-treated rat glomeruli. **D**: Number of podocytes per glomeruli from control and GH injected rats. **E**: Urinary albumin/creatinine ratio was measured from urine collected for 24 h from control and GH-treated rats. *Data are mean±SE (n=6, *p<0.05).*



CONCLUSION

The results indicate that GH stimulates TGFBIp expression in podocytes. Since the interaction between podocytes and GBM is critical for normal renal function, aberrant expression of TGFBIp is expected to exert a deleterious effect on the podocytes and result in impaired glomerular filtration. Further, our studies reveal that treating podocytes with TGFBIp results in both apoptosis and EMT, thus, results in podocytopathy. Placed in the context of elevated GH levels in type 1 DM, the finding of increased expression of TGFBIp in podocytes argues for a role for increased GH-dependent TGFBIp expression in the pathogenesis of podocyte injury in DM. In summary, the results indicate that GH induced apoptosis and EMT in part mediated by GH-dependent increase in TGFBIp expression. Taken together, the data suggest that the deleterious effect of GH on renal function is independent of IGF-1. The present study adds support for a role for increased circulating levels of GH in the pathogenesis of DN in type 1 DM. We speculate that blockade of TGFBIp could be a novel approach to preventing podocyte damage and disease progression in DN.

9. EFFECT OF REACTIVE OXYGEN SPECIES ON MACROPHAGE SIGNALOSOME: IMPACT ON ANTIGEN PRESENTATION FUNCTION AND T CELL PRIMING RESPONSES

Although reactive oxygen species (ROS) are important for induction of cytotoxic effects against invading pathogens, it is not very clear whether excess ROS production can actually underweigh the beneficial outcome of the innate immunity. Oxidative stress has been implicated in manifesting detrimental effects of various disorders like cancer, aging, diabetes, atherosclerosis and infection (Droge W, 2002). Interestingly, most of these pathophysiological disorders are found to be associated with severe immunosuppression, indicating that excessive production of ROS could be one of the factors responsible for development of immunosuppression in these situations (Land WG, 2004). Although much we know about how ROS induce apoptosis and cytotoxicity to cells especially at toxic concentrations (Buttke and Sandstrom, 1995), mechanisms by which ROS can influence immune system as a second messenger without altering cell viability, is not well understood. ROS may not be the only factor but could be one of the important factors responsible for downregulation of APC function by specifically modulating the components of the array of signaling cascades involved. Evidence that ROS can actually impair the immune responses came for the first time from our study that ROS could downregulate IL-12 induction in macrophages (Khan et al., 2006, Blood, 107:1513-20). Negative regulation of IL-12 could be one of the mechanisms by which ROS can affect T cell responses. T cell priming responses are mainly regulated by the stimuli generated from peptide-MHC and costimulatory-driven signals involving a number of signaling cascades and transcription factors in macrophages. Since ROS are known to modulate many important transcription factors in macrophages that control effector and antigen presentation functions indicate that ROS may have substantial direct/indirect effects on these processes. Therefore, in this proposal we specifically aim to examine whether ROS influence the T cell responses by affecting macrophage signaling important for regulation of its effector and antigen presentation function. This study may shed new lights to understand 'stress induced immunosuppression' and accordingly help us to implement therapeutic interventions to improve the immune status in people undergoing chronic pathophysiological stress.

HYPOTHESIS

Excessive production of ROS causes immunosuppression by affecting macrophage APC function through modulation of signaling cascades.

OBJECTIVES

- Measuring the ability of ROS to affect macrophage-APC function
- Studying ROS-mediated modulations of macrophage signalosome important for APC function
- Examining whether modulation in the macrophage APC function by ROS can subsequently affect T cell responses *in vivo.*

RESULTS

Exogenous ROS inhibit MHC class II presentation of exogenous OVA antigen

We first carried out experiments to understand whether ROS can actually affect antigen processing and presentation mechanisms in macrophages. Antigen presentation assay was carried out *in vitro* using ovalbumin (OVA)-specific I-A^b-restricted T cell line 13.8 (a kind gift from Drs. Satyajit Rath and Vineeta Bal, National Institute of Immunology, India) and thioglycolate elicited peritoneal macrophages from C57Bl/6 mice (H-2^b) as APCs. Peritoneal exudate cells obtained through lavage of the peritoneal cavity of mice injected intraperitoneally three days previously with 1 ml of thioglycolate broth as described (Mukhopadhyay et al., 1999b; 2004) were adhered on tissue culture plates and the attached macrophages were harvested for carrying out various experiments. Macrophages were pre-treated with various concentrations of H_2O_2 for 1 h and pulsed exogenously with titrating concentrations of OVA for a fixed time of 3 h in the presence of H_2O_2. It could be observed that although H_2O_2 did not affect cell viability (Fig. 1A), MHC class II-restricted antigen presentation was inhibited by H_2O_2 in dose dependent manner (Fig. 1B).

b. ROS/ H_2O_2 inhibit antigen processing

Next we allowed the OVA antigen to process for different time points in the presence of 100 µM H_2O_2 and observed whether antigen processing is affected by H_2O_2. The cells from both the groups (control and H_2O_2-treated)

were pulsed with a fixed concentration of 300 µg/ml of OVA for 30 min. It was found that antigen presentation was inhibited by H_2O_2 when added at 100 µM concentration (Fig. 2) indicating that probably ROS /H_2O_2 affects antigen processing to inhibit antigen presentation.

Fig 1. H_2O_2 inhibits MHC Class II-restricted antigen presentation of exogenous OVA antigen: Peritoneal macrophages elicited from C57BL/6 mice were either left untreated or treated with various concentrations of hydrogen peroxide as indicated. (A) Cells were assessed for viability by MTT assay. (B) Cells were fixed and used as APCs for presentation to 13.8 T cells. After 24 h, supernatants were harvested and measured for IL-2 using EIA. Unpulsed APCs were used as negative control.

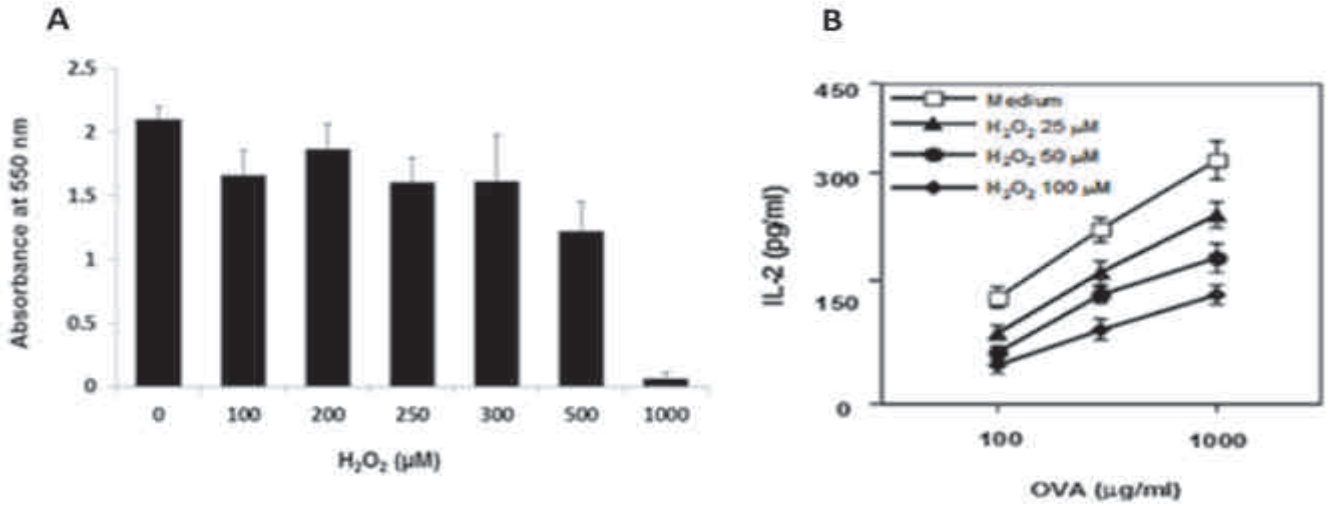
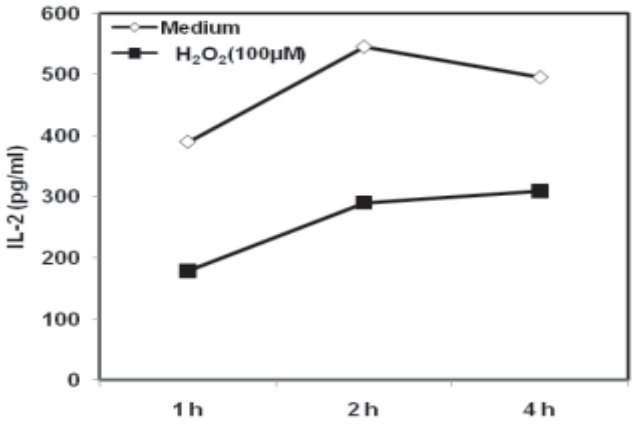


Fig 2. Antigen processing at different time points are inhibited by H_2O_2: Peritoneal macrophages elicited from C57BL/6 were pulsed exogenously with fixed concentrations of OVA (300µg/ml) for 30 min and the cells were allowed process antigens in the presence or absence of H_2O_2 (100µM) for different periods of time. The cells were fixed and used as APCs for 13.8 T cells. IL-2 levels were measured in the culture supernatant after 24 h.



Intracellular ROS/ H_2O_2 inhibit presentation of exogenous OVA antigen

To validate whether endogenously generated ROS/H_2O_2 is also involved in inhibition of MHC class II-restricted antigen presentation, alloxan was used which reacts with intracellular thiols to produce reactive oxygen species. We found that treatment of macrophages with 100 µM alloxan inhibited antigen presentation in a similar way (Fig. 3A) as found with extracellular addition of H_2O_2. Further, it was observed that 100 µM alloxan causes inhibition of OVA presentation even when OVA was added at lower concentration of 100 µg/ml (Fig. 3B). Alloxan causes inhibition of antigen presentation with the increasing concentration of OVA antigen confirming that intracellular ROS, as generated during various pathopysiological concentrations also inhibits antigen presentation.

H_2O_2 at physiological concentration also inhibits MHC class II - restricted antigen presentation and macrophage APC functions

We next examined whether the physiological level of H_2O_2 produced by activated macrophages during respiratory burst can also show a direct inhibitory effect on antigen presentation. The peritoneal macrophages from C57Bl/6 mice were therefore activated with LPS to induce ROS production (used as an endogenous stimulator of

ROS production like H_2O_2) in the absence or presence of 100 µM NAC which is known to be a scavenger of ***ROS***/ H_2O_2. Also, to specifically block the effect of H_2O_2, 100 U/ml of PEG-catalase was used. After 6 h, macrophages were washed and treated for 30 min with OVA antigen and allowed the antigen to process for 3 h. After fixing with 1 % paraformaldehyde, the cells were used as APCs to activate 13.8 T cells. It could be found that LPS treatment causes inhibition of OVA antigen presentation as measured by IL-2 level produced by the 13.8 T cells. Treatment with NAC or PEG-catalase improved antigen presentation of LPS treated macrophages (Fig. 4A). Also, NAC improved antigen presentation by peritoneal macrophages at all the concentrations of OVA antigen used in the study (Fig. 4B). These results suggest that reactive oxygen species particularly the H2O2 is involved in decreasing antigen presentation.

Fig 3. Intracellular ROS inhibits antigen presentation: (A) Peritoneal macrophages elicited from C57BL/6 were pulsed exogenously with fixed concentrations of OVA (300µg/ml) for 30 min and treated with either alloxan or H_2O_2 (100µM). (B) The cells were allowed to process antigens for 3 h. All the cells were fixed and used as APCs for 13.8 T cells. IL-2 levels were measured in the culture supernatant after 24 h.

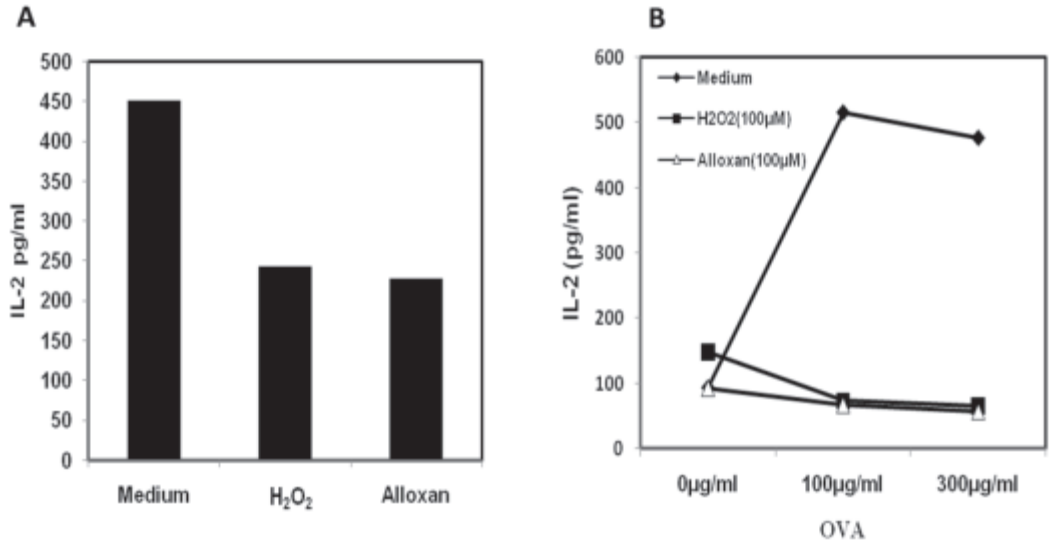
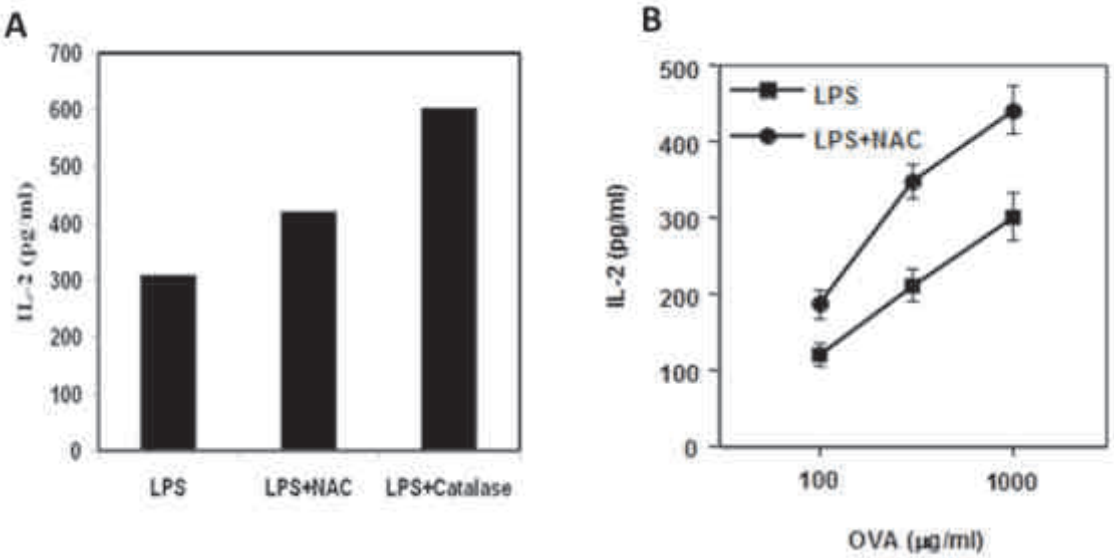
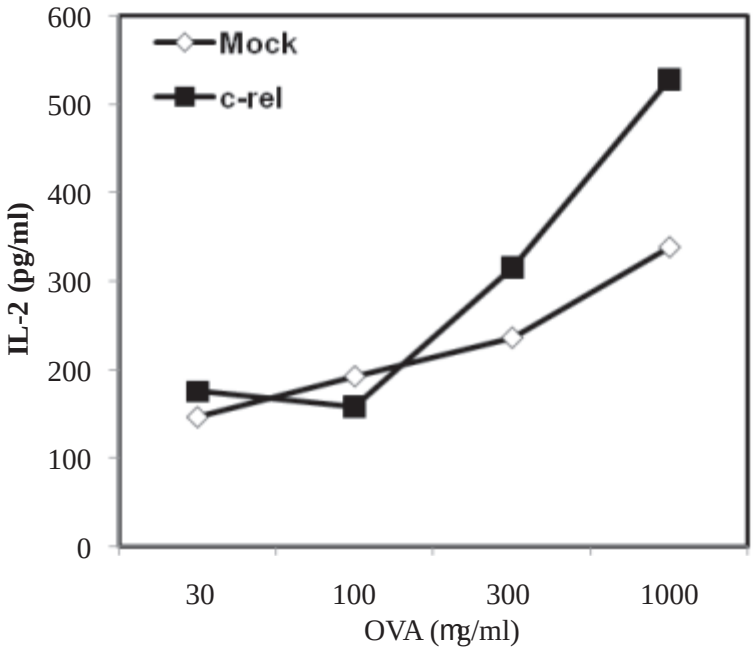


Fig 4. ROS produced by activated macrophages during respiratory burst also inhibit MHC class II-restricted antigen presentation: (A)Peritoneal macrophages elicited from C57BL/6 mice were activated with LPS (3µg/ml) for 6 h in the presence or absence of NAC (100µM) or PEG-catalase (100 U/ml) followed by pulsing with fixed concentration of OVA (300µg/ml). (B) Cells were also pulsed with different concentrations of OVA and allowed to process the antigens in the presence of NAC or LPS+NAC. Cells were then fixed, washed and used as APCs to 13.8 T cells for 24 h and IL-2 was estimated in the supernatants by EIA



ROS/ H_2O_2 target the c-rel transcription factor to inhibit antigen presentation in peritoneal macrophages. H_2O_2 is known to suppresses IL-12 p40 (Th1 cytokine) induction by inhibiting nuclear translocation of c-rel transcription factor. Since antigen presentation and APC functions are dependent on the transactivation of various genes, we checked whether c-rel is one of the central transcription factors regulating various genes that take part in antigen presentation and whether H_2O_2 targets the c-rel transcription factor to inhibit macrophage APC function.

Fig 5. Over expression of c-rel ameliorated H_2O_2-mediated suppression of antigen presentation: Peritoneal macrophages were transfected with either c-rel over expression plasmid or an empty mock vector. Transfected cells were treated with H_2O_2 and pulsed exogenously with titrating concentrations of OVA and allowed the cells to process the antigen for 3 h in the presence of H_2O_2. Cells were then fixed and used as APCs to 13.8 T cells for 24 h and Il-2 level was measured in the supernatant using EIA.



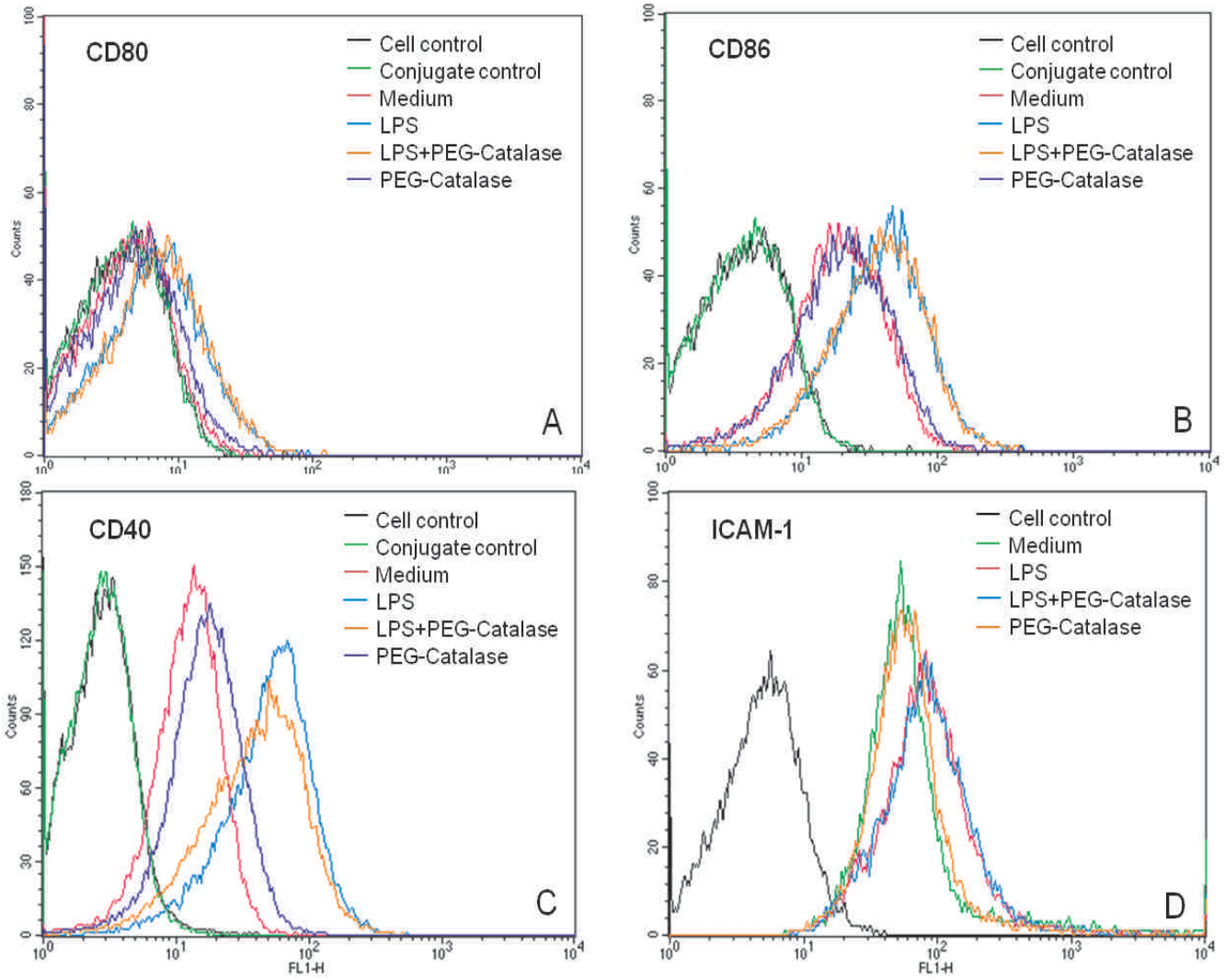
Endogenously generated ROS during respiratory burst does not have significant effect on the surface expression of co-stimulatory molecules

The co-stimulatory molecules like CD80, CD86, CD40, intercellular adhesion molecule-1 (ICAM-1) are known to provide the non-cognate secondary stimulus (signal 2) during antigen presentation, while the signal 1 provides the cognate peptide-MHC-driven stimulus to antigen presenting cells (APC). Many studies indicate that absence of co-stimulatory signaling can lead the T cells to undergo anergy which is marked by the inability of the cells to proliferate in response to peptide-MHC complexes. Thus both the cognate and the non-cognate signals are extremely important for the antigen presenting cells to stimulate T-cells as the presence of these molecules on cell surface is critical for the APC function of these cells.

To understand the effect of endogenous ROS on the surface expression of co-stimulatory molecules, BMC2 macrophages ($H2^b$ haplotype; a kind gift from Dr. Satyajit Rath, NII, New Delhi) were either left untreated or pretreated with LPS (used as an endogenous stimulator of ROS production like H_2O_2) for 24 h in the absence or presence of PEG-Catalase (used as a scavenger of endogenousley generated H_2O_2). Cell were then washed and stained with antibody to CD80, CD86, CD40 and ICAM-1 followed by incubation with FITC conjugated secondary antibody. Cells were washed and fluorescence was measured using flow cytometry (BD FACSVantage SE, Beckton Dickinson, San Jose, CA). We observed that macrophages treated with LPS alone showed upregulation of CD80, CD86, CD40 and ICAM-1 (Fig. 6) as compared to the unstained cell population which was expected. The expression of these molecules remains unchanged in cells treated with both LPS and PEG-Catalase indicating that endogenous H_2O_2 generated by LPS stimulation does not have much effect on surface expression of CD80, CD86, CD40 and

ICAM-1. Our results indicate that inhibition of antigen presentation by ROS might be due to defect in antigen processing rather than weakened co-stimulatory signaling.

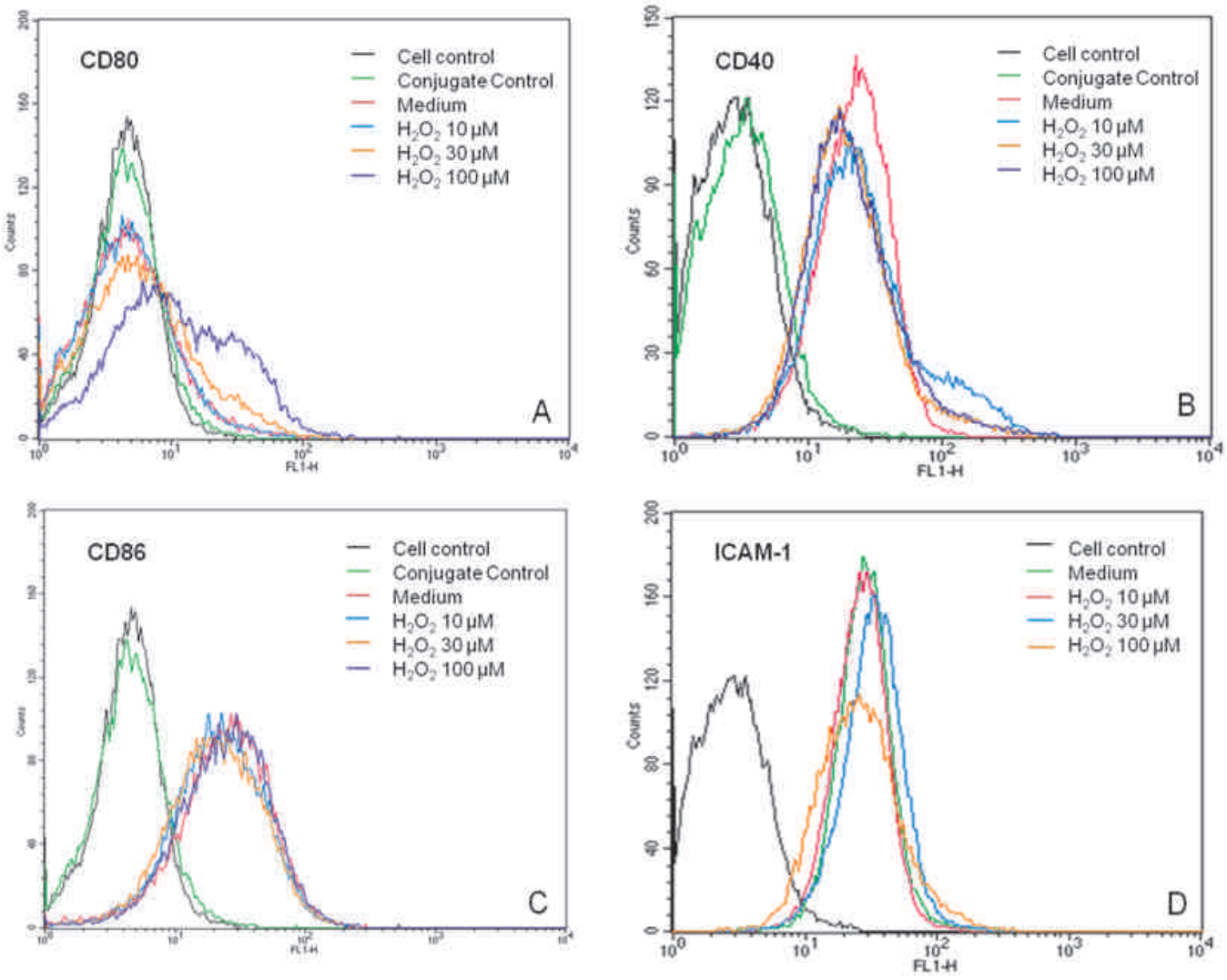
Fig 6. Endogenously generated ROS produced during LPS-mediated activation of macrophages did not significantly changed surface expression of costimulatory molecules: BMC2 macrophages were either left unstimulated or stimulated with LPS (3 µg/ml) in the absence or presence of PEG-Catalase (100 U/ml) for 24 h. The cells were then fixed and incubated with either rat anti-mouse CD80 (A) or CD86 (B) CD40 (C) or ICAM-1 (D) followed by incubation with anti-rat-IgG-FITC conjugate and the surface expression of respective antigens were measured by flow cytometry.



Exogenous ROS (H_2O_2) also did not cause any significant change in surface expression of co-stimulatory molecules:

To examine whether H_2O_2 when administered exogenously has any effect on expression of co-stimulatory molecules on macrophage surface, BMC2 ($H2^b$ haplotype) macrophages were either left untreated or pretreated with different concentrations of H_2O_2 1 h. Cells were then washed and kept at 37°C. After 24 h, cells were stained with antibody to CD80, CD86, CD40 and ICAM-1 followed by incubation with FITC labeled secondary conjugate. Cells were washed and fluorescence was measured using flow cytometry. It was found that H_2O_2 treatment at all the concentrations tested does not alter the surface expression of costimulatory molecules (Fig. 7) indicating that exogenous ROS does not affect the costimulatory signaling to inhibit antigen presentation.

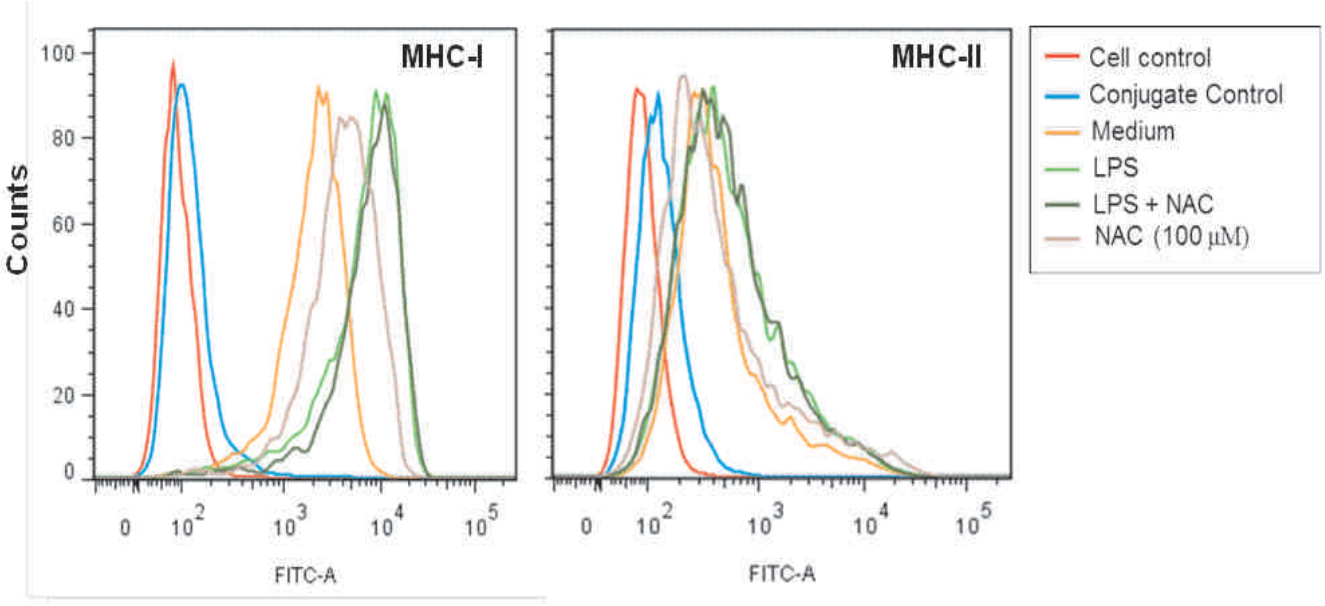
Fig 7. Endogenously generated ROS produced during LPS-mediated activation of macrophages did not significantly changed surface expression of costimulatory molecules: BMC2 macrophages were either left untreated or treated with different concentrations of H_2O_2 for 1 h. The cells were then fixed and incubated with either rat anti-mouse CD80 (A) or CD40 (B) CD86 (C) or ICAM-1 (D) followed by incubation with anti-rat-IgG-FITC conjugate and the surface expression of respective antigens were measured by flow cytometry



Endogenous as well as exogenous ROS (H2O2) does not alter the surface expression of co-stimulatory molecules in peritoneal macrophages

Thioglycolate elicited peritoneal macrophages from C57Bl/6 mice were either left untreated or treated with LPS or LPS+NAC or NAC alone or directly with different concentrations of H_2O_2 for 1 h. Cells were then washed and kept at 37oC. After 24 h, cells were stained with antibody to MHC-I or MHC-II or CD86 followed by incubation with FITC labeled appropriate secondary conjugate. Cells were washed and fluorescence was measured using flow cytometry. Similar to experiments in BMC2 macrophages, we observed that macrophages treated with LPS alone showed upregulation of MHC-I, MHC-II (Fig. 8) as compared to the unstained cell population which was expected. The expression of these molecules remains unchanged in cells treated with both LPS and NAC indicating that endogenous H_2O_2 generated by LPS stimulation does not have much effect on CD86 expression. NAC alone did not show any modulation in expression of these molecules on macrophage surface over the medium control. It was also observed that exogenous H2O2 treatment at all the concentrations did not alter the surface expression of MHC-I, MHC-II (Fig. 8) indicating that exogenous ROS/ H_2O_2 does not significantly affect the co-stimulatory signaling to inhibit antigen presentation.

Fig 8. Endogenously generated ROS did not significantly changed surface expression of MHC-I, MHC-II: Peritoneal macrophages were either left untreated or treated with LPS, NAC or LPS+NAC for 24h and fixed. The cells were then incubated with rat anti-mouse MHC-1 and MHC-II antibodies followed by incubation with anti rat-IgG-FITC conjugate and the surface expression of respective antigens were measured by flow cytometry



***Calmodulin* (CaM) plays a role in H_2O_2 mediated inhibition of antigen presentation**

Calmodulin (CaM) expression is known to be increased during inhibition of IL-12p40 by H_2O_2, where it could bind and sequester c-rel transcription factors in the cytoplasm inhibiting its nuclear translocation to bind to specific. Since earlier we observed that over expression of c-rel transcription factor could ameliorate H_2O_2-mediated suppression of antigen presentation, we hypothesized a role of CaM molecule in this process. Therefore, the direct role of CaM in H_2O_2-mediated inhibition of antigen presentation was next investigated. For this, we used the trifluoperazine (TFP), a known antagonist of CaM. Peritoneal macrophages from C57Bl/6 mice were either left untreated or pre-treated with TFP for 30 min followed by incubation with H_2O_2 (100 µM). The macrophages were then pulsed exogenously with OVA (1 mg/ml) or BSA for 30 min, followed by chasing for 3 h. The cells were then fixed, washed and incubated with 13.8 T cells for 24 h and IL-2 levels were measured by EIA. The results indicated that inhibition of CaM with TFP rescued the inhibition in antigen presentation of OVA by H_2O_2 (Fig. 9) indicating a role of CaM in antigen processing and presentation signaling and H_2O_2 targets antigen presentation and processing by increasing CaM expression.

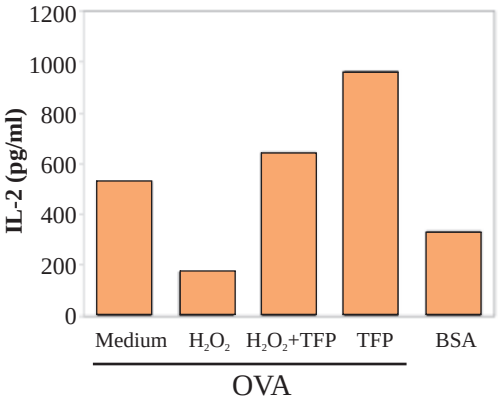


Fig 9. TrifIuoperazine (TFP), an antagonist of CaM, rescued ROS-mediated suppression of antigen presentation: Peritoneal macrophages from elicited C57BL/6 mice were either left untreated or pretreated with TFP (100 µM) for 1 h followed by treatment with H_2O_2 (100 µM) for 1 h. The cells were then pulsed with OVA (1 mg/ml) and allowed to process the antigens for 3h. BSA was used as a control antigen. After fixing the cells were used as APCs to 13.8 T cells for 24 hours and the IL-2 levels were measured in the supernatant by EIA.

SUMMARY

Our results indicate that excessive ROS, produced during various pathophysiological processes, can significantly affect antigen processing to inhibit antigen presentation. We found that intracellular ROS generated by alloxan and ROS generated during respiratory burst inhibit MHC class II-restricted antigen presentation. The inhibition of antigen presentation could be ameliorated by different scavengers of ROS like NAC and PEG-Catalase. Interestingly, expression of

surface co-stimulatory molecules involved in antigen presentation remained largely unaffected, indicating that antigen processing is targeted rather than antigen presentation.

Since, c-rel overexpression in peritoneal macrophages could rescue suppression of MHC-II-mediated antigen presentation, it appears that c-rel is an important target of ROS. TFP, an antagonist of calmodulin (CaM) also rescued ROS-mediated suppression of antigen presentation. CaM is known to inhibit nuclear translocation of c-rel by sequestering with c-rel in the cytoplasm. Therefore, it appears that endogenous or exogenous ROS inhibits antigen processing by increasing expression of CaM, which subsequently inhibits nuclear translocation of c-rel. However, detailed mechanism needs to be studied further.

10. POSSIBLE UTILITY OF HUMAN UMBILICAL CORD BLOOD OR PLACENTA DERIVED MESENCHYMAL STEM CELLS FOR TOXICITY STUDIES

The *in vitro* micronucleus test is a well-known test for the screening of genotoxic compounds. However until now, most studies have been performed on either human peripheral lymphocytes or established cancer cell lines.

This study provides human mesenchymal stem cells as an alternative to the conventional micronucleus test. We grew umbilical cord mesenchymal stem cells (UC-MSCs) on coverslips eliminating the cumbersome technique involving hypotonic treatment, fixation and preparing smears required for suspension culture (lymphocytes).

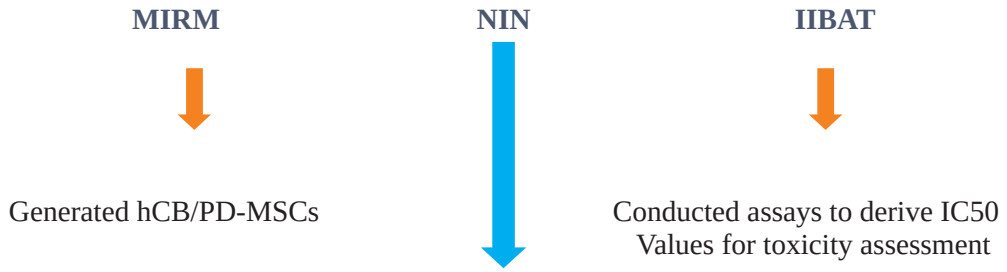
The background frequency of nuclear blebs and micronuclei in UC-MSCs was found to be 7 ± 5, in lymphocytes 16 ± 3.5 and 9 ± 3 and that for A549 cell line was 65 ± 5 and 15 ± 5 per 1000 cells, respectively, suggesting differences in the repair mechanism of normal and cancer cell lines.

We inspected the cytotoxic and genotoxic effects of two known mutagens, mitomycin-C and hydrogen peroxide (H2O2), on UC-MSCs, lymphocytes and A549 cells. Treatment with mitomycin-C and H2O2 demonstrated drastic differences in the degree of cytotoxicity and genotoxicity suggesting a constitutional difference between normal and cancer cells.

In addition we tested two solvents, dimethyl sulfoxide (DMSO) and ethanol, and two drugs, metformin and rapamycin. DMSO above 1% was found to be cytotoxic and genotoxic, whereas ethanol at same concentration was neither cytotoxic nor genotoxic indicating the minimal non-toxic level of the solvents. This study thus offers UC-MSCs as a better substitute to peripheral lymphocytes and cancer cell lines for high throughput screening of compounds and reducing the animal studies.

METHODOLOGY

Experimental design:



Characterization of primary cultures of hPD-MSCs using cellular and molecular markers

Established primary cells (hPD-MSCs) at MIRM, they will be characterized using cellular and molecular markers such as STRO-1, CD90, CD29, and CD31 by Flow cytometer and / or confocal microscopy at NIN-Hyderabad as per established protocols.

Human umbilical cords (n = 5) and blood were collected with the informed consent of the patients who were aged between 26 and 32 years. We followed guidelines of Institutional Ethics Committee (IEC) Manipal Hospital, Bangalore, India, for collecting the samples (UC-MSCs and blood). We followed the methodology previously reported by our group (12) for isolating UC-MSCs.

Briefly, arteries and veins were removed from the cord tissue and washed with PBS (pH 7.2). Cord was cut into 10-cm length and processed for enzymatic digestion by using 1?mg/ml collagenase and dispase (7:1) for 30?min and subsequently adding 0.25% trypsin for 5?min. After neutralisation, the MSCs were centrifuged at 1800?rpm and supernatant was discarded.

Pellet was suspended in minimum essential medium (alpha MEM) (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS). The above steps were repeated thrice. Finally, the plated cells were moved to a 37°C CO2 incubator (Thermo Scientific Inc., OH, USA). Human lung carcinoma cell line (A549) was purchased from National Centre of Cell Science Pune. The cells were cultured in alpha MEM, supplemented with 10% FBS (HyClone) and maintained in a 37°C CO2 incubator (Thermo Scientific Inc.).

Lymphocytes were isolated using LymphoprepTM (AXIS-SHIELD) according to the manufacturer's instructions. Briefly, blood was diluted with an equal volume of 0.9% NaCl and carefully layered over LymphoprepTM in a 50-ml falcon tube and centrifuged at 800?g for 30?min at room temperature (RT). After centrifugation a dis-tinct band of mononuclear cells formed at the interface, which was removed with a pipette. Finally, the harvested fraction was diluted with 0.9% NaCl and centrifuged at 250?g for 10?min at RT. The pel-let was suspended in RPMI medium (Invitrogen) containing 15% FBS (HyClone) (15) and stimulated with 1.5% PHA (Invitrogen) for 48?h according to the manufacturer's instructions.

Immunophenotyping analysis of UC-MSCs using flow cytometry

UC-MSCs from passages 5 were immunophenotypically analysed by flow cytometry for CD surface markers such as CD14, CD19, HLA-DR, CD90, CD105 and CD44 (12). MSCs were trypsinised and fixed in paraformaldehyde (PFA). For CD markers profile analysis, PFA was removed and MSCs were washed with PBS and incubated with mouse anti-human FITC/PE conjugated antibodies against CD14, CD19, HLA-DR, CD90, CD105 and CD44 for 1?h on ice (all anti-bodies was purchased from Becton Dickinson, San Diego, CA, USA). Finally, the cells were identified using a flow cytometry laser 488?nm and analysed for 10 000 gated events using BD Cellquest Pro software.

Proliferation rate determination

Cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (12). Five hundred cells/well were seeded into 96-well plates in 200 µl of growth medium in triplicate. After 2 days mitomycin-C (Sigma Aldrich), hydrogen peroxide, DMSO (Sigma Aldrich), ethanol, rapamycin (Sigma Aldrich) and metformin were added for 48?h. After the treat-ment period, the supernatant was removed and 1?mg/ml final con-centration MTT was added to each well and incubated for 3?h at 37°C in a humidified incubator (Thermo Scientific Inc.). The reaction was terminated by removing MTT and adding 200 µl of DMSO to dissolve the formazan salt. The light absorbance was measured at 570?nm. All the experiments were performed thrice in triplicate.

Multipotent differentiation potential

MSCs were induced to differentiate into the trilineage (adipogenesis, chondrogenesis and osteogenesis) using UC-MSCs published earlier.

***In vitro* micronucleus test**

The MSCs were grown on coverslips in four well and 24-well plates and after 2 days the medium was replaced with treatment medium for another 48?h. After the treatment period, cells were fixed directly by adding 4% PFA for 30?min at RT or at 4°C for overnight to weeks. After fixation cells were treated with triton-X for permeabilisation for 1?h at RT and stained with Giemsa (0.4%) for 30?min. MNs and NBs were analysed blind microscopically (bright field microscope with ×100 objective).

The number of NBs and MNs per 1000 cells was recorded. All the experiments were performed thrice in triplicate. We followed the Organisation for Economic Co-operation and Development (OECD) guidelines 487 and all the

parameters for evaluating NBs and MNs were considered as reported by Fenech (17). Lymphocyte smear were made on glass sides and stained with Giemsa (0.4%) for 30 min. NBs and MNs were analysed as stated above. The experiment was performed thrice in triplicate.

Senescence assay (β-galactosidase staining)

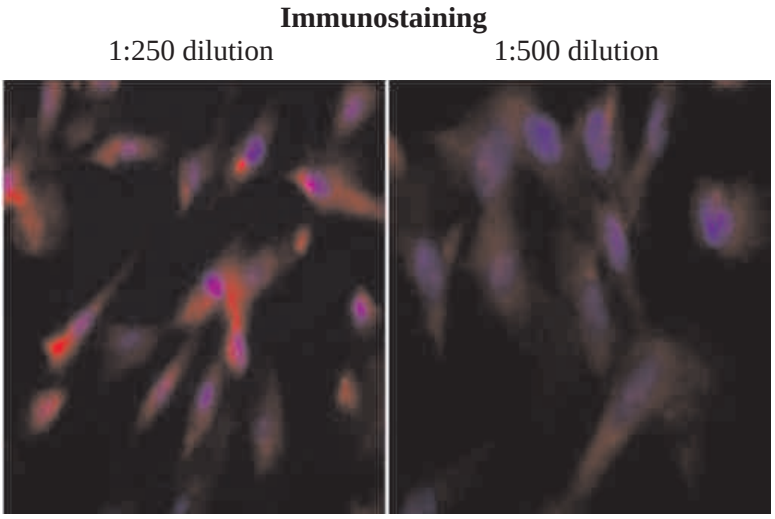
UC-MSCs were cultured in 12-well plates and the senescence assay was done using Senescence β-Galactosidase Staining kit (Cell Signaling Technologies, Danvers, MA, USA) according to the manufacturer's instructions. Experiments were performed three times in triplicate.

The cell cycle time is usually variable among the different donors. According to the OECD guidelines, one cell cycle replication is necessary for defining the treatment duration of the test compound. The population doubling time was calculated for UC-MSCs and found to be 25.24±2.24 h for n = 3 samples and therefore 48 h treatment time was designated for UC-MSCs.

RESULTS

- MSCs were plastic adherent and exhibited fibroblast appearance with abundant cytoplasm and large nuclei as shown in Fig. 1 (C1 and C2). Further we characterised them for their immunophenotypical marker profile by flow cytometry for both mesenchymal and haematopoietic markers.
- UC-MSCs were immunopositive for CD90 (~98%), CD105 (~77.7%), CD44 (~81.45%) and immune-negative for HLA-DR (~99%) and haematopoietic markers CD19 (~99 %) and CD14 (~99 %) as shown in Fig 1B. We further showed that these UC-MSCs could be differentiated towards adipogenic, osteogenic and chondrogenic lineage by specific staining as represented in Fig 1 (A1, A2, and A3).
- We have evaluated the background frequency of NBs and MNs of UC-MSCs (Fig. 1, C1 and C2), lymphocytes (Fig 1, D1, D2 and D3) and A549 cell line (Fig 1, E1 and E2) per 1000 cells by the IVMT. We observed between 7±2.5 NBs and MNs per 1000 cells (n = 5) of UC-MSCs ($P < 0.001$), 16±3.5 NBs and 9±3 MNs per 1000 cells (n= 3) of lymphocytes ($P < 0.001$) and 65±3.5 NBs and 15±3.5 MNs per 1000 cells of A549 cells in 48 h as shown in Fig. 1F. Moreover, we also observed large number of abnormal nuclear shape in A549 cell line (Fig. 1 E3), whereas UC-MSCs exhibited normal nuclear shape. Furthermore we checked the back ground NBs and MNs frequency of these UC-MSCs from passage 4 to 10 and found no increase or decrease in the number of NBs and Mns.
- We have checked the cytotoxic effect of various concentration of mitomycin-C on UC-MSCs, lymphocytes and A549 cell line as shown in Fig 2A. Cytotoxicity was directly proportional to the mitomycin-C concentration. Mitomycin-C treatment (0.1 µg/ml) resulted in 23.3, 31.841 and 36.7% cytotoxicity ($P < 0.001$) and 0.3 µg/ml mitomycin-C resulted in 25.9, 39.16 and 45.24% cytotox-icity ($P < 0.001$) in UC-MSCs, lymphocytes and A549 cells, respectively. We further noticed that mitomycin-C resulted in an increase in the number of NBs and MNs in a dose-dependent manner in UC-MSCs, lymphocytes and A549 cells, as shown in Fig 2B and C. Mitomycin-C (0.1 µg/ml) treatment resulted in ~4, 2 and 1.2-fold increases in the number of NBs ($P < 0.001$) and 4, 3 and 10 fold increases in the number of MNs ($P < 0.001$) in UC-MSCs, lympho-cytes and A549 cells, respectively.
- Observed that 146 µM H_2O_2 resulted in 33.4, 34.2 and 52.5% reduction and 292 µM H_2O_2 resulted in 49.267, 43.19 and 69.2% reduction in viability of UC-MSCs, lymphocytes and A549 cells ($P < 0.001$), respectively, as shown in Fig 2D. We analysed its genotoxic effect and observed that 29.2 µM H_2O_2 was non-geno-toxic whereas 58.4 µM and 146 µM H_2O_2 were slight genotoxic to UC-MSCs, lymphocytes and A549, but the difference was not statistically significant. However 292 µM H_2O_2 showed statistically significant genotoxicity in UC-MSCs ($P = 0.01$) and lymphocytes ($P < 0.0089$) as evident by the increase in the number of NBs and MNs as shown in Fig 2E and F. H_2O_2 at 292 µM was highly cytotoxic to A549 cells so its genotoxic effect was not examined.
- Observed 2 and 3% DMSO to be highly cytotoxic leading to 42.25, 39.41 and 40.65%, and 63.63, 54.43 and 60.93% cell death in UC-MSCs, lymphocytes and A549 cells ($P < 0.001$), respectively, as shown in Fig 3A. DMSO at 0.5 and 1% does not cause genetic damage as evidenced by the lower number of NBs and MNs as shown in Fig 3B and C. However DMSO above 1% showed slight genotoxicity in UC-MSCs ($P = 0.0018$) and lymphocytes ($P = 0.02$), but in A549 cells the difference was not statistically significant.

Fig 1: Characteristic of UC-MSCs. UC-MSCs differentiated to adipocytes detected by oil-O red staining (A1), osteocytes detected by von Kossa staining (A2) and chondrocytes detected by alcian blue staining (A3). Magnification ×200 for all images. CD surface marker profile of UC-MSCs (B). Nuclear blebs and MN observed in UC-MSCs (C1 and C2), lymphocytes (D2 and D3) and A549 cell line (E1 and E2). Abnormal nuclear shape in A549 cell line (E3). Magnification ×1000 for all images. Graph representing the background nuclear blebs and MN frequency of UC-MSCs, lymphocytes and A549 cell line (F). Data represented is from (n=5) repeated thrice (P<0.001).



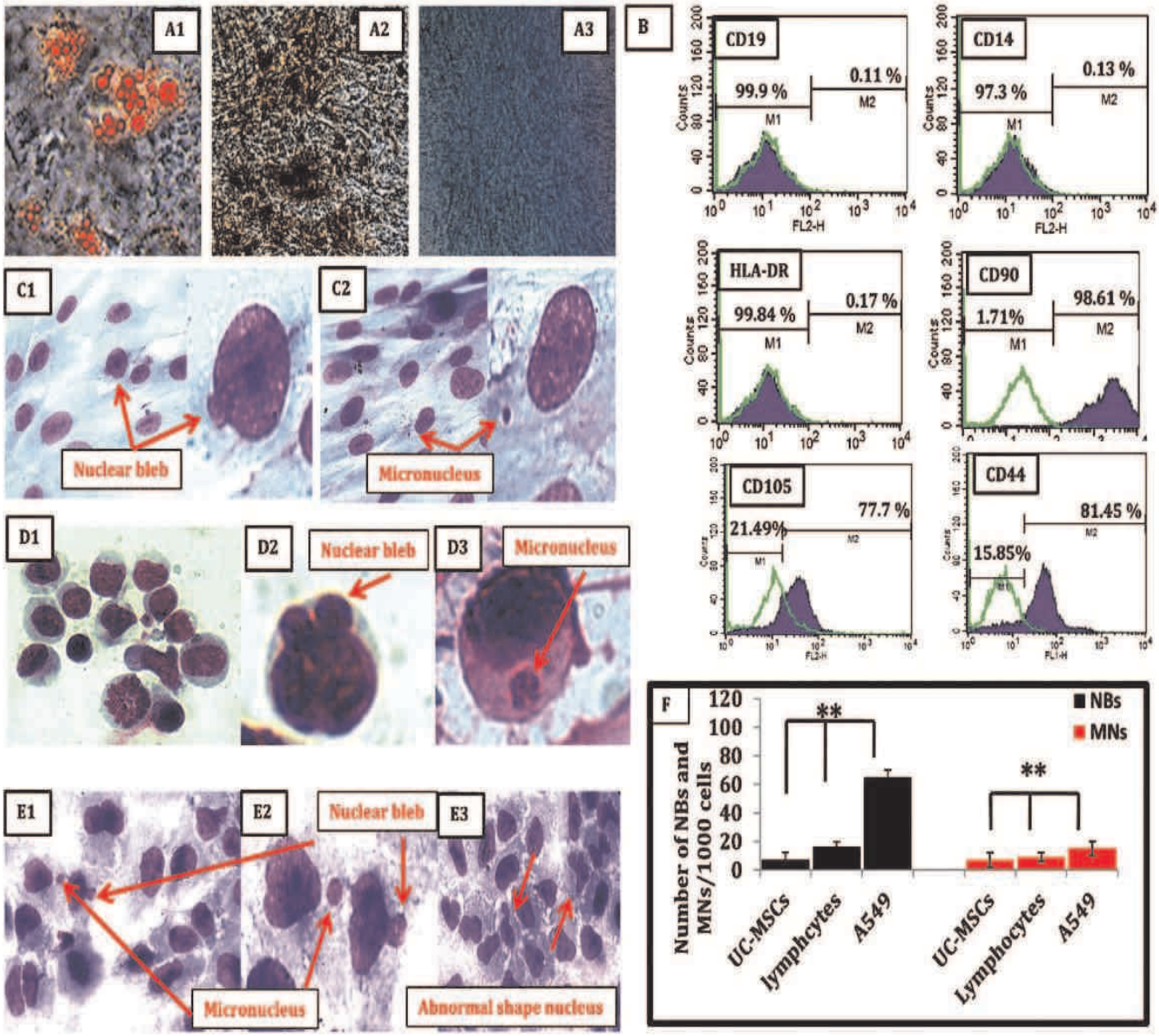
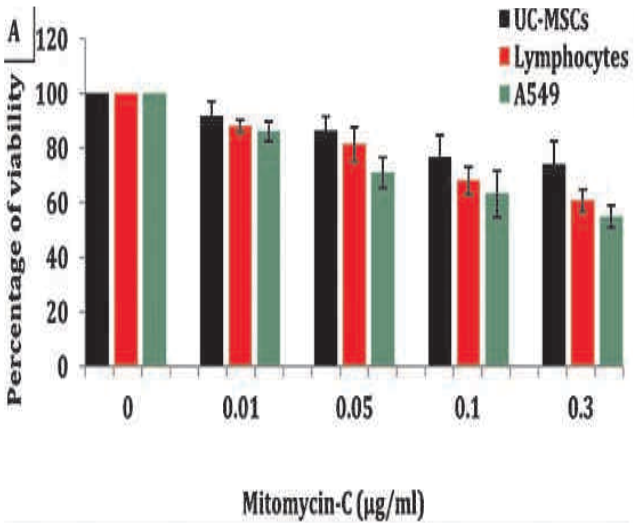
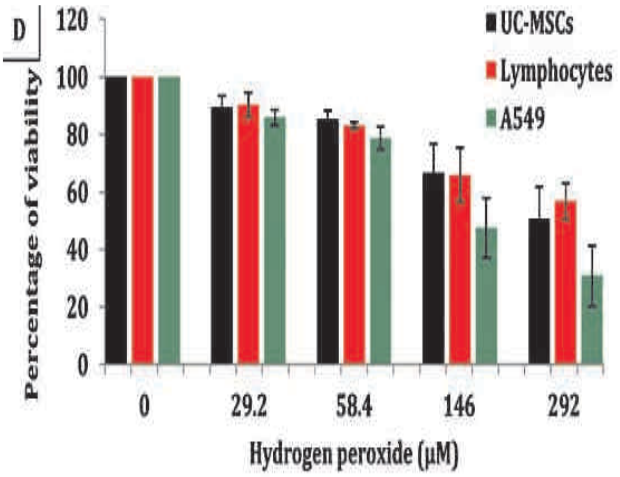
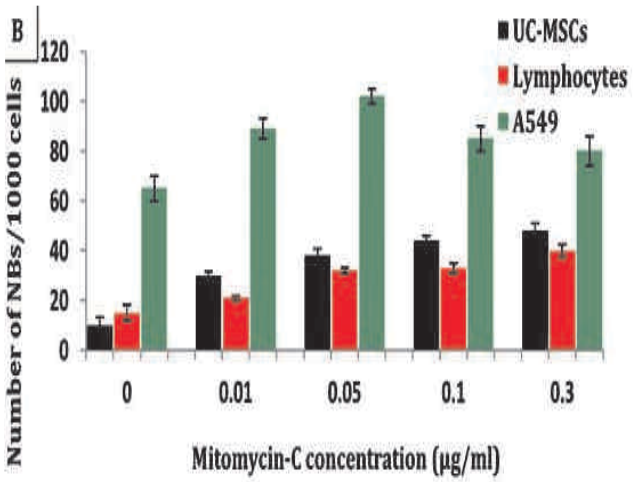
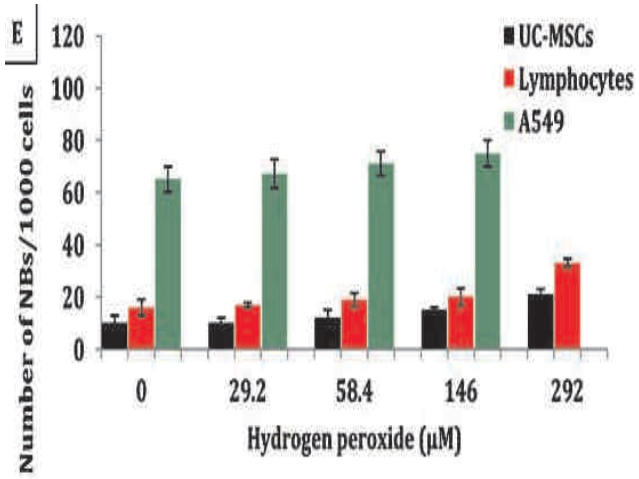


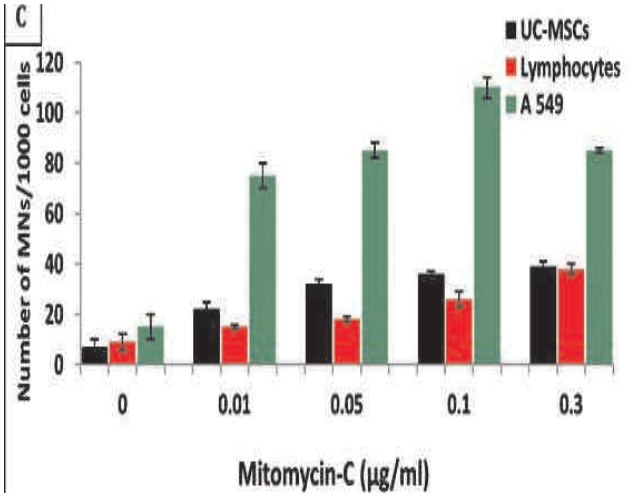
Fig 2. Cytotoxic effect of mitomycin-C and H2O2 on UC-MSCs, lymphocytes and A549 cell line (A, D). Frequency of NBs and MNs induced by mitomycin-C on UC-MSCs, lymphocytes and A549 cell line (B, C). Frequency of NBs and MNs induced by hydrogen peroxide on UC-MSCs, lymphocytes and A549 cell line (E, F).











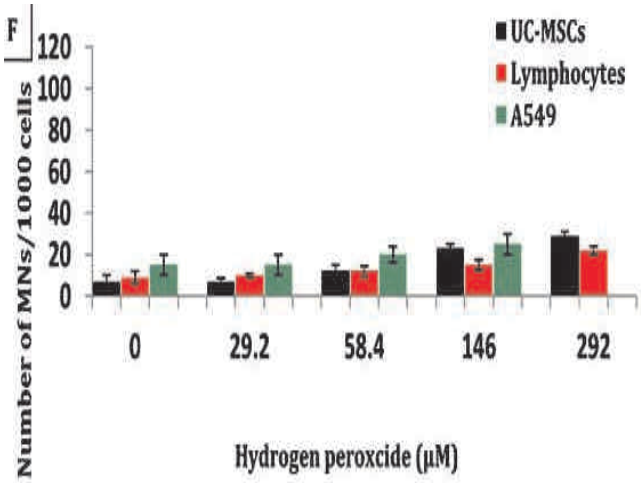
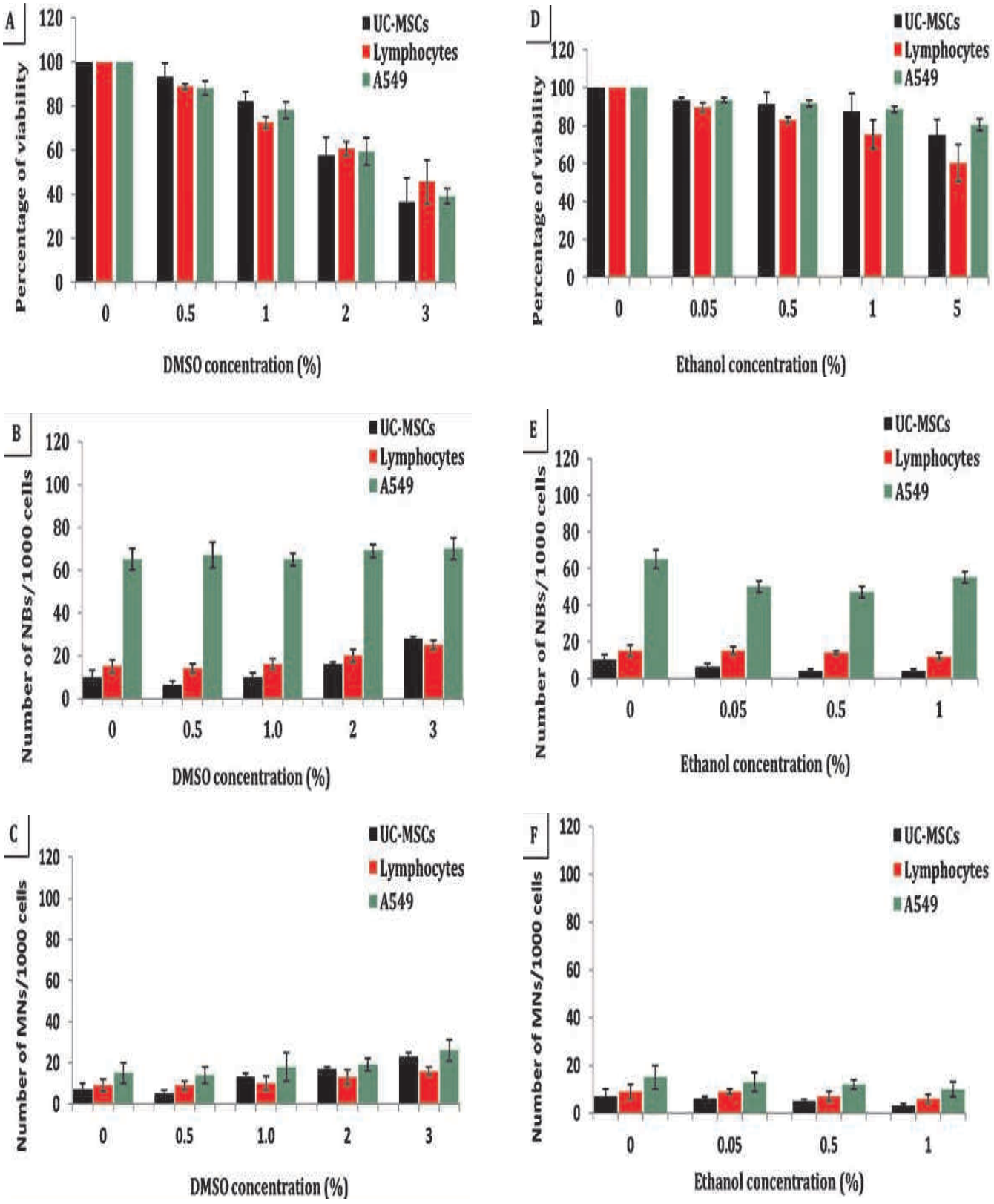
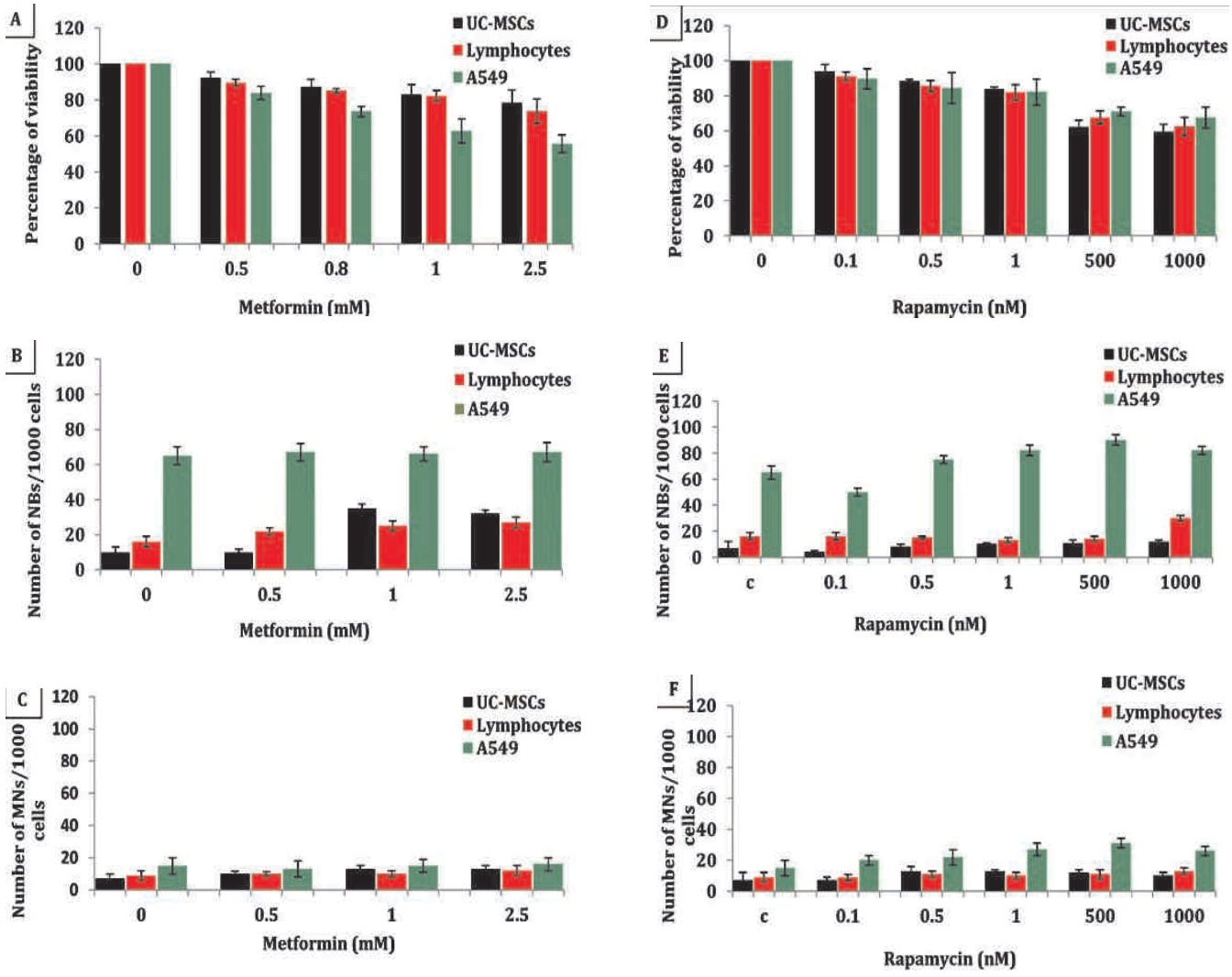


Fig 3. Cytotoxic effect of DMSO and ethanol on UC-MSCs, lymphocytes and A549 cell line (A, D). Dose-dependent effect on the frequency of nuclear blebs induced by DMSO and ethanol on UC-MSCs, lymphocytes and A549 cell line (B, E). Dose-dependent effect of DMSO and ethanol on the generation of MN on UC-MSCs, lymphocytes and A549 cell line (C, F).



- Assessed the cytotoxic and genotoxic effects of ethanol on UC-MSCs. We observed 0.05% (8.565?mM) ethanol to be non-cytotoxic whereas 0.5% (85.65?mM) ethanol was ~8.7, 17.1 and 8.27% cytotoxic and 1% (171.3?mM) was 12.43, 24.62 and 11.45% cytotoxic to UC-MSCs, lymphocytes and A549 cells ($P < 0.001$), respectively. The 5% (856.5?mM) ethanol concentration was ~25, 40 and 20% cytotoxic to the UC-MSCs, lymphocytes and A549 cells ($P < 0.001$) as shown in Figure 3D. Furthermore, we also observed slight decreases in the number of NBs and MNs with the above-mentioned ethanol concentrations with all the three different types of cells as compared to control as shown in Fig 3E and F, but differences were not statistically significant.
- Observed 1?mM metformin resulted in 17% cytotoxicity in UC-MSCs and lymphocytes and 37.4% in A549 cells and 2.5mM metformin led to 22, 26 and 44.5% cytotoxicity in UC-MSCs, lymphocytes and A549 cells ($P<0.001$), respectively, as shown in Figure 4A. Furthermore, we also observed a gradual increase in the number of NBs in UC-MSCs ($P<0.001$) and lymphocytes ($P=0.028$) above 0.5?mM concentration of metformin. However in A549 cells no increase of NBs was observed. Moreover, there was only a slight increase in the number of MNs with the increasing concentration of metformin with all the three different cells used in this study; however the difference was not very significant as shown in Fig 4B and C.
- We observed 500?nM and 1 µm rapamycin was 30-40% cytotoxic to UC-MSCSs, lymphocytes and A549 cells ($P < 0.001$), respectively, as represented in Fig 4D. Furthermore, there was no significant increase in the number of NBs and MNs in UC-MSCs and lymphocytes with the different concentrations of rapamycin (0.1, 0.5, 1 and 500?nM) whereas with A549 there was significant increase ($P < 0.001$) in the number of NBs and MNs as shown in Fig 4E and F. We observed no morphological change in the UC-MSCs when treated with drugs continuously for 7 days (two passages) without any signs of senescence..



- We observed rapamycin at (0.5, 500 and 1000?nM) led to decrease in adipogenesis as shown in supplementary Fig 1, available at Mutagenesis Online (A1, A2, A3, A4, A5, A6 and A7) and chondrogenesis as represented in Supplementary Fig 1, available at Mutagenesis Online (C1, C5, C6 and C7). Furthermore, metformin at 2.5?mM led to cell death in chondrogenesis while 0.5 and 1?mM has shown reduction in chondrogenesis as shown in supplementary Fig 1, available at Mutagenesis Online (C1, C2, C3 and C4). Moreover, osteogenesis was not affected by both the drugs; see Supplementary Fig 1, available at Mutagenesis Online (B1, B2, B3, B4, B5, B6 and B7).

SUMMARY

In summary, we demonstrate for the first time the use of a normal diploid UC-MSCs culture as a platform for simultaneous testing cytotoxicity and genotoxicity of pharmaceuticals as a suitable alternative to peripheral lymphocytes and cancer cell lines. Our investigation, if put into practice would improve the *in vitro* toxicity screening platform leading to a decrease in attrition of drugs in clinical trials.

11. ROLE OF LIPID MEDIATORS IN THE DEVELOPMENT OF DIABETIC RETINOPATHY (DR)

DR is an important secondary complication of diabetes which is characterized by vascular changes in the retinal and sub retinal region, and is the most common cause of blindness in the middle aged working population. The hyperglycemia induced biochemical pathways such as oxidative stress, polyol pathway, non-enzymatic protein glycation are postulated to be responsible for the onset and progression of the DR. It is evident that not all diabetic subjects display the pathophysiology of diabetic retinopathy which in turn suggests the involvement of other predisposing factors in the onset and development of the complication. Sustained inflammation is one of the crucial factors for the onset and progression of diabetes and related complications and, hence it is important to understand how inflammation predisposes a person to the onset and progression of diabetic complication in order to develop therapeutic strategies. The quantity and quality of circulating free fatty acids (FFA) play a very important role throughout the cascade of events such as the genesis of the insulin resistance, onset and development of diabetes and diabetic complications such as DR mainly by altering the inflammatory signals. It has been shown that the level of n6 polyunsaturated fatty acids (n6 PUFA) present in the constituents of plasma, cells and cell membrane are major determinants of pro-inflammatory state. The counter regulatory role of the other class of lipids n3 polyunsaturated fatty acids (n3 PUFA) over the inflammatory process could be beneficial to the living. With the aforesaid background the present study is aimed to understand the role of fatty acids in the pathogenesis of DR with the following objectives:

- To evaluate the association between fatty acids and diabetic retinopathy

RESULTS

A hospital based case control study involving a total of 400 subjects (200 with DR, 100 Diabetic subjects with No Retinopathy (DNR) any complications and 100 normal non-diabetic subjects-CON) with a well-defined inclusion/ exclusion criterion was undertaken with the approval of the Institutional Ethics Committee. Age and sex matched study subject were examined and classified for severity of DR using according to reported methods both by direct and indirect ophthalmoscope into Non-Proliferative DR (NPDR) and Proliferative DR (PDR). Various biochemical analysis were carried out.

The random blood glucose levels are high in the diabetic subjects with and without retinopathy when compared to the control subjects (Table-1). The lipid profiles showed an increase in the levels of triglycerides & decreased levels of high-density lipoprotein cholesterol (HDL) in PDR subjects when compared to the CON while the levels of cholesterol and low density lipoprotein cholesterol (LDL) were not altered.

2. The levels of tumor necrosis factor- (TNF-) and vascular endothelial growth factor (VEGF) were increased in DNR subjects, which further increased in DR subjects. Leptin levels are higher in DNR and NPDR subjects (Table-2). Phospholipase A2 (PLA2) activity is higher in DNR and NPDR subjects while the total antioxidant capacity (TAC) is decreased diabetes groups irrespective of the Presence of DR. The circulating free fatty acids (FFA) levels are higher in DNR while it is lesser in DR subjects shows increased oxidative stress.
3. Total WBC count is higher DNR subjects which is further increased in subject with DR. The data on differential count suggest that the neutrophils levels are higher in DR subjects while lymphocytes counts are lesser (Fig-1).
4. The plasma total fatty acid composition showed that total saturated fatty acids (SFA) levels are higher and total n6PUFA levels were lower in DNR subjects when compared to control and DR groups. In RBC also similar pattern was observed in addition to the increased levels of total n3 PUFA in DNR subjects (Table-3 & 4).

Table 1. Demographic profile and plasma biochemistry

n	224	102	38	63
Demographic profile				
Age	54.7±10.1	57.5±10.9	56.7 ± 8.5	53.3 ± 9.7
BMI	24.2 ±529	25.1±3.6	26.9±5.4	25.8±4.4
Duration of diabetes		9.6± 7	12.5 ±6.7	11.7±6.1
Blood sugar				
Random	108.6±24.1	178.6± 70.3	208.8± 83.8	188.8±87.3
Fasting	102.5±27.7	161± 68	117.3± 59.5	111.4±33.3
Postprandial	119.7±42.1	224.6 ± 76.1	191.6±132.8	145.5±57.4
HBA1C	6.2±1.3	7.5 ± 1.4	8.1±2.3	7.6± 1.79
Lipid profile				
TC	187.9 ±43.4	174.7 ± 50.5	176.4± 68.5	158.7±40.5
HDL	41.3± 11.5	40.8 ± 11.6	40.8 ±18.1	32.6±9.2
LDL	124.5 ± 38.8	108.6± 43.6	105.9± 52.8	97.9±35.3
TG	115.8 ±71.7	130.3±	150.8±102.7	141.2±123.8

Table 2. Circulatory markers of inflammation and oxidative stress

	n	CON	DNR	NPDR	PDR
TNFΔ (pg/ml)	79	5.2 ± 2.2 (n21)	7.7 ± 3.7 (n29)	9.0 ± 4.4 (n14)	14.9 ± 6.1 (n15)
Leptin (pg/ml)	39	21410 ± 12344 (n9)	34227 ± 20771 (n15)	39115 ± 19142 (n7)	24667 ± 9692 (n8)
VEGF (pg/ml)	58	171.8 ± 68.8 (n15)	200.9 ± 79.7 (n20)	280.7 ± 138.7 (n8)	301.6 ± 253.5 (n15)
PLA2 (pg/ml)	72	859.9 ± 931.3 (n16)	1254.7 ± 1109.3 (n20)	1420.0 ± 1144.9 (n19)	776.1 ± 878.1 (n17)
FFA (mM)	122	0.373 ± 0.245 (n37)	0.405 ±0.238 (n34)	0.224 ± 0.149 (n31)	0.217 ± 0.099 (n20)
TAC (ΠM Tro. red.equi	90	0.412 ± 0.149 (n21)	0.317 ±0.115 (n19)	0.342 ±0.134 (n21)	0.304 ±0.130 (n29)
Oxidized LDL (mU/L)	62	5.7 ± 2.4 (n15)	5.2 ± 2.6 (n17)	7.6 ± 2.9 (n14)	6.5 ± 2.8 (n16)

Fig 1. Total and differential white blood cell (WBC) counts

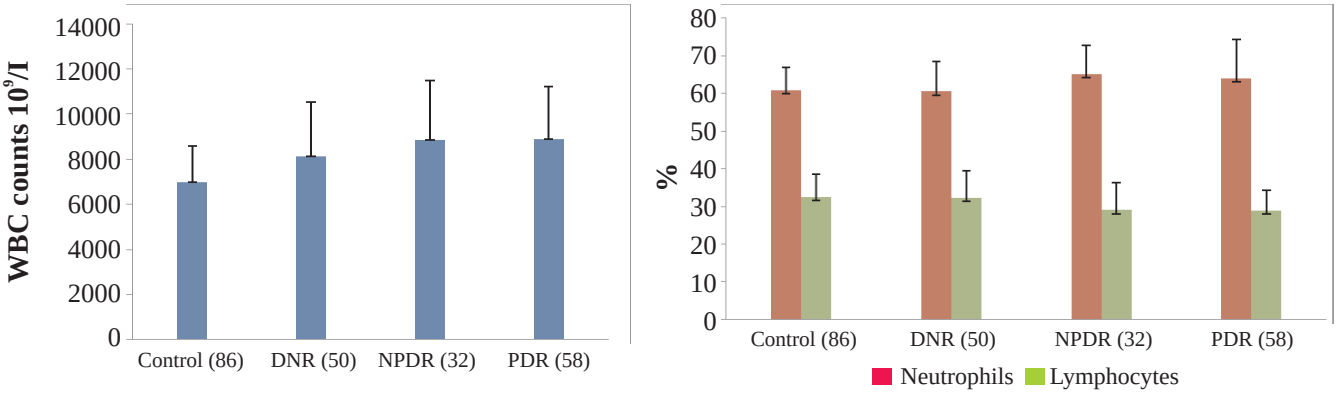


Table 3. Plasma total polyunsaturated fatty acids composition

	CON 29	DNR-14	NPDR-17	PDR-44
16:0	27.8 ± 6.8	31.2 ± 9.7	30.1 ± 6.3	30.5 ± 7.5
16:1	1.5 ± 0.9	0.8 ± 0.5	1.0 ± 1.7	0.8 ± 0.7
18:0	14.3 ± 4.0	18.1 ± 5.1	15.7 ± 5.5	15.8 ± 5.7
18:1c	18.5 ± 3.4	17.5 ± 4.7	17.5 ± 5.1	17.0 ± 3.0
18:2	27.9 ± 7.3	22.5 ±9.4	24.8 ± 8.0	25.7 ± 9.2
20:4	8.0 ± 2.9	7.4 ± 2.8	8.2 ± 2.9	8.4 ± 2.9
20:5	0.89 ± 0.78	1.4 ± 1.28	0.83 ± 0.63	0.64 ± 0.46
22:6	0.76 ± 0.40	0.79 ± 0.46	1.44 ± 1.14	0.87 ± 0.59
6 SFA	42.14 ± 9.9	49.3 ± 14.2	45.8 ± 10.4	46.3 ± 12.6
6n6	36.0 ± 8.8	29.9 ± 11.3	33.1 ± 10.6	34.1 ± 11.4
6n3	1.66 ± 0.91	2.23± 1.46	2.27 ± 1.26	1.52 ± 0.63

Table 4. RBC phospholipid polyunsaturated fatty acids composition

	CON-25	DNR-16	NPDR-15	PDR-30
16:0	44.2 ± 4.3	45.9 ±6.0	44.0 ±4.9	45.0 ±4.1
16:1	0.28 ±0.21	0.22 ±0.34	0.11 ±0.16	0.22 ±0.2
18:0	29.6 ±3.0	30.2 ±3.5	28.3 ±3.0	28.5 ±3.4
18:1c	17.4 ±3.5	14.7 ±2.2	17.1 ±3.3	17.3 ±3.4
18:2	4.3 ±1.2	4.8 ±1.8	5.6 ±2.3	5.0 ±1.5
20:4	2.9 ±1.6	1.9 ±0.58	3.3 ±1.4	2.7 ±1.3
20:5	0.79 ±0.40	1.1 ±1.7	0.73 ±0.59	0.77 ±0.4
22:6	0.46 ±0.38	1.0 ±0.78	0.90 ±1.5	0.53 ±0.44
6 SFA	73.9 ±5.1	76.2 ±4.0	73.3 ±6.1	73.6 ±5.0
6n6	7.2 ±2.4	6.7 ±1.8	8.6 ±2.9	7.7 ±2.3
6n3	1.0 ±0.51	2.1 ±1.9	1.0 ±0.74	1.0 ±0.61

CONCLUSION

The present study showed that the diabetic subjects with or without retinopathy are in a chronic inflammatory state as evidenced by the higher levels of inflammatory markers and increased oxidative stress. Interestingly the lower level of total n6 PUFA (proinflammatory) in DNR subjects might be beneficial in terms of protecting the subjects from developing DR. It is also interesting to note that the higher total n3 PUFA levels in the RBC of DNR subjects might have provided further protection from developing complications.

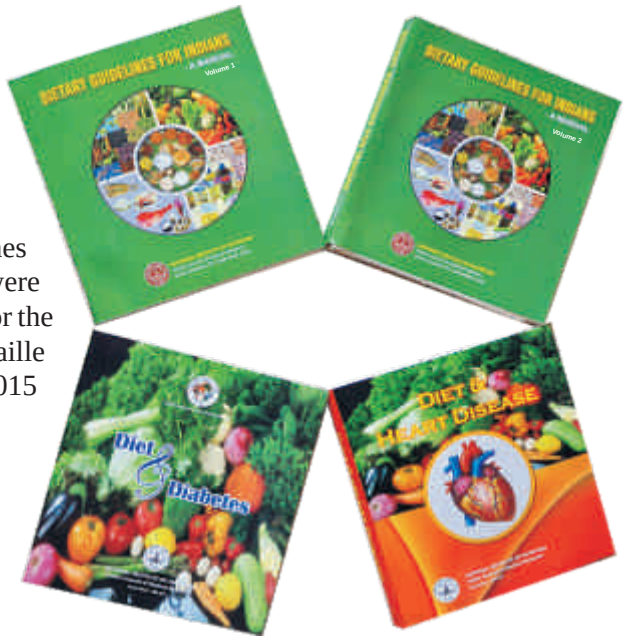
IV PUBLICATION, EXTENSION AND TRAINING DIVISION

A. SERVICE ACTIVITIES

1. PUBLICATIONS

The publications Nutritive Value of Indian Foods, Low Cost Nutritious Supplements (English) and Dietary Tips for the Elderly were reprinted on popular demand.

The three popular publications viz., Dietary Guidelines for Indians, Diet and Diabetes and Diet and Heart Diseases were brought in Braille in coordination with Devnar Foundation for the Blind, Hyderabad. These NIN's popular publications in Braille were released by Director-General, ICMR on 18th February, 2015 at ICMR Headquarters, New Delhi.



2. TRAINING PROGRAMMES

Regular Training Programmes: This year, a total of thirty candidates have attended the regular training programmes of the Institute viz. (i) MSc (Applied Nutrition) VI Batch 2014-15:16 participants (ii) Annual Training Course on Endocrinological Techniques and their Applications: 4 participants and (iii) Fifty second Post-Graduate Certificate (PGC) Course in Nutrition: 10 participants.

The Mini-Convocation of the 4th Batch of Msc (AN) was held on April 21, 2015 in which certificates were awarded to the successful candidates and also Dr.BK.Nandi Fellowships and Prize weas given to the meritorious students.

Staff and students on the occasion of the valedictory function of 52nd PGC Course in Nutrition

3. EXTENSION ACTIVITIES

3.1 Exhibitions

- III Science Expo 2014 at Solan, Himachal Pradesh, organized by SANSA Foundation, New Delhi (Sept. 18-20, 2014).
- Exhibition on Health, Nutrition and Family Welfare, Medical Research, Technologies developed for scientific & industrial research – Avishkar Expo, organized by SANSA Foundation, New Delhi in Kangra, Himachal Pradesh (Dec. 14-16).
- Represented NIN in the ICMR's poster gallery at the 102nd Indian Science Congress, organized by University of Mumbai in association with Ministry of Science and Technology for Human Development, Govt. of India (Jan.3-7, 2015).
- Participated in the ICMR pavilion organised in the 7th Vibrant Gujarat Summit, held at Gandhinagar, Gujarat (Jan. 11-13, 2015).
- Participated in the Nutrition Stall at the Telangana State Horticulture Expo organized by the Department of Horticulture, Telangana State at People's Plaza Hyderabad (Jan.26-30, 2015).
- ICMR Exhibition focusing Health and Nutrition in the Agricultural Science Congress held at National Dairy Research Institute, Karnal, organised by ICAR, New Delhi (Feb. 3-6, 2015).
- Participated in ICMR Awareness stall at Science and Agriculture Expo in the 6th Vision Rajasthan meeting held at Vidya Bhawan, Krishi Vigyan Kendra, Badgaon village, Udaipur, Rajasthan (March 3-6, 2015).
- Participated in exhibition as part of National Festival of Innovation in Rashtrapathi Bhavan, New Delhi, where NIN's Technology for Double Fortification of Salt (DFS) was displayed as an Innovation in Medical Science (March 11, 2015).

3.2 Popular Lectures/Awareness Camps

1. Delivered three extension lectures on "Nutrition and Health" to the staff of Andhra Bank, Hyderabad (June 23, Oct , Jan.28)
2. Two popular lectures on "Nutrition and Health" and "How to Improve Teaching Skills" were given to the faculty members of SMRK Arts and Fine Arts College, Nashik (23rd and 24th July 2014).
3. Village level community programme was organized in Association with MV Foundation at Durgaguda village (3rd September, 2014).
4. An awareness lecture on "Nutrition" was delivered to the parents of students of Railway Girls High School, Lallaguda, Hyderabad (4th September, 2014).
5. Delivered a popular talk on Dietary Guidelines and Nutrition During Aging at Rajendra Singhji Institute (RSI), Sainikupri, Secunderabad, for the benefit of retired army personnel and their families on 5th Sept, 2014. Over 90 senior citizens took part.
6. Extension lecture on Nutrition during Adolescence at Javid Mission High School in Old City of Hyderabad in Sept. 2015.
7. A series of 10 extension lectures on Nutrition and Food Labels in five study schools – Gowtham Model School, Maredpally; Slate School, Abids; Cambridge School, Saidabad; Oxford Grammar School, Himayatnagar and St. Martin's school, Malkajgiri as part of the ongoing research project on food labels in Sept-Oct. 2014.
8. Extension talk on nutrition during adolescence in Sandeepam Vidyalaya on 17th October 2014.
9. Information Awareness Lecture to the parents of students of CAL Public School, Kapra (8th November, 2014).
10. A Nutrition Awareness programme was organized with NSS volunteers for rural children at a camp organised under community camp of NSS Programme of St.Pious Degree College, Hyderabad at Korremula village in

Rangareddy District of Telangana on 8th December 2014. Over 50 NSS Volunteers, 300 rural children, 3 NSS Programme Officers, 12 teachers and rural women participated.

11. Delivered two extension lectures on the Importance of Healthy Diets and Physical Activity to the Sub-Inspectors on 30th Oct. and 29th Dec. 2014. In the first session, a total number of 66 Civil Sub-Inspectors while in the second session 282 Special Protection Force Sub-Inspectors had participated.
12. Delivered lecture on "Health and Diet Management" to the Bank staff of Andhra Bank Apex College at Gachibowli, Hyderabad (Jan. 28).
13. Nutrition Information Lecture to the NSS volunteers at the winter camp held at Medchal, Ranga Reddy District (28th January, 2015).
14. Organised Nutrition and Personnel Hygiene awareness programme for the Scientists of DRDO, at DRDO, Hyderabad (June 10, 2014 & Feb. 19, 2015).
15. Delivered extension lectures on "Food habits and health management" to the ISW & APSP police personnel of Intelligence Department, Security Wing, Hyderabad (Dec.22, 2014 & Jan. 21, 2015).
16. A popular lecture on Nutrition and Health was delivered to various groups of health functionaries in the Nourish Health and Wellness Conference, organised by Smart Value Products and Services Ltd. Kolkata. About 1000 participants attended the programme (Jan. 20, 2015).
17. Audio Conference on 'Nutritional Aspects' was conducted to the villagers of Vellambi Malai Village located near foothills of Western Ghats, villagers of Aalingi village located at Nagarcoil District of Tamil Nadu and Vizhundamavadi village of Nagapattinam District (Jan.3, Feb.19 & 28, 2015).

4. SPECIAL EVENTS

4.1 World Breast Feeding Week (Aug.1-7, 2014)

An extension programme was organized on this year's theme given by WHO "Breastfeeding – A Winning Goal for Life" (Aug.5).

4.2 National Nutrition Week Celebrations (Sept. 1-7, 2014)

As part of the National Nutrition Week celebrations on this year's theme "Healthy Diet – Foundation for a Stronger Nation", the following programmes were organized.

- Video conference with the students of Annamalai University on "About Balanced Diet" (Sept.1).
- Audio Conference with MS Swaminathan Research Foundation, Chennai on "About Balanced Diet" (Sept. 2).
- Community programmes on "Health and Nutrition" at Duraguda and Adilabad district (Sept.3-4).
- School programme on "Health Diet for School Children" and Extension lecture for Geriatric population(Sept. 5).
- A Press meet was organized emphasizing the National Nutrition week this year's theme on "Healthy Diet – Foundation for a Stronger Nation" (Sept. 5).

4.3 World Food Day celebrations

A one day seminar was organized on "Family Farming: Feeding the World, Caring for the Earth", in association with Association of Food Scientists and Technologists (Hyderabad Chapter) and Oil Technologists Association of India (South Zone).

4.4 Swachh Bharat Abhiyan

On the instructions of ICMR, a workshop on Swachh Bharat was organized and a popular lecture on Role of Sanitation in the prevention of Infectious diseases was delivered by me for school children. A handout on Swachh Bharat Abhiyan-Some Healthy Tips was distributed to all the participated students. The information given in the handout to the school children will promote good health through cleanliness and sanitation to foster the spirit of Swachh Bharat Abhiyan (Oct. 2, 2014).

5. DEVELOPMENT OF COMMUNICATION MATERIAL

- A set of 9 posters, a ready-reckoner booklet and a 4-minute animation video were developed for increasing the food label reading skills of adolescents as part of an ongoing research project.
- Co-ordinated with Devnar Foundation for the Blind, Hyderabad to bring out three of NIN's popular publications (Viz., Dietary Guidelines for Indians; Diet and Diabetes; Diet and Heart Diseases) in Braille.
- Co-ordinated video shooting on anti-cancer, anti-oxidant and anti-mutagenic properties of spices for Beacon Productions – a company that is making a TV series on Indian Spices for DST to be aired on Doordarshan.

B. RESEARCH ACTIVITIES

PROMOTING THE USE OF FOOD LABEL INFORMATION AMONG SCHOOL-GOING ADOLESCENTS

Nutrition transition is underway in India. While under nutrition continues to be a problem in India, there is a rise in proportion of people with overweight/obesity. Obesity among children and adolescents is a growing problem in India not only in the higher socio-economic groups but also among the middle income groups, with as many as 15% of high income group children and over 3% in the low income groups being affected by these. WHO projects that these figures would double by the year 2020 if no corrective measures are taken. There is conclusive evidence to show that obesity in childhood and adolescence is related to the burden of associated chronic diseases in adulthood. High consumption of unhealthy foods is an important risk factor for obesity among children and adolescents. The popularity of these foods has increased over recent years and research says consumption by children has risen dramatically (over 300%) over the last 20 years even in developing countries like India. Governments all over the world have been intensifying their efforts to tackle these growing problems taking a multi-pronged approach. In the present scenario, when there are shifts away from meals to snacks and from at-home to pre-packaged foods, children should be equipped with skills to choose healthy foods.

The production, sale and consumption of pre-packaged foods have witnessed a major surge in India in the recent years. Food labeling is one of the important population-based approaches that can help consumers make healthy food choices by providing the necessary information about the food on the pack. The food label is one of the most important and direct means of communication of product information between buyers and sellers.

Pre-packed foods sold in Indian markets were earlier only labeled with the product name, name and address of the manufacturer, amount of product in the package, the ingredients and date of expiration. Recently, nutrient content declaration has been made mandatory on nearly all pre-packaged foods. Our earlier study carried out with support of WHO- India on 'Current scenario of food labeling in India', indicated that consumers have more nutrition information due to expanded food labeling mandated by the Government. While there is no doubt that food labels will encourage healthy eating, there are hardly any efforts to educate consumers to use the label information or understand the content of the labels. Our earlier studies among adolescents indicated that many of them were not in the habit of reading label information while buying packed foods, even those who read labels were only seeing the quality symbols, price and date of expiry.

Considering that Food labels are potentially powerful tools of communication, which are often not considered when traditional channels are discussed to discourage consumption of unhealthy packed foods, education about food labels at a young age can enhance their knowledge and food label reading skills. Schools provide the best platform to reach a large segment of children and their families and offer the advantage of carrying out such education interventions within the context of the child's natural environment**.** Considering that many of our earlier studies which indicated that school based education related to food and nutrition aspects is preferred and effective mode of learning among children, the onus is on the teachers to impart them with nutrition education. However, an earlier study conducted to assess the food and nutrition related information in India school science textbooks indicated that the current school curricula do not cover these aspects at all.

Given this background, a study will be conducted with the following objectives:

GENERAL OBJECTIVE

Assessing the impact of education intervention on reading of food labels among school-age adolescents.

SPECIFIC OBJECTIVES

- To assess the current knowledge and practices of school-going adolescents on reading the food labels.
- To develop, implement and evaluate an appropriate educational intervention strategy to promote reading and understanding of food labels among them.

METHODOLOGY

Study design: It will be a prospective, cluster (school) randomized school intervention study. The selected schools will be randomized in equal numbers into control and intervention groups.

Study location: The study is proposed to be conducted in Hyderabad- a metro city of South India.

Subjects: All the school children in the age group of 10-15 years (5-9th standard students) from the selected schools will be recruited for the study.

Research tools: A closed-ended pre-coded questionnaire will be developed and pre-tested before using it for knowledge assessment. The same questionnaire will be used to assess the knowledge improvement and retention at two time points.

Ethical considerations: The project proposal was submitted to the Institutional Review Board (IRB) of the National Institute of Nutrition (Indian Council of Medical Research), Hyderabad for ethical clearance. The Institute's scientific advisory committee (SAC) has already accorded approval for the study protocol. Written informed consent was obtained from all the heads of the selected institutions, children and parents.

Sample: Assuming 26% of adolescents read food label and by intervention the percentage will go up-to 40% with 95% CI and 80% power and the sample size for experimental group is decided 344($\approx$ 350) and control size is $\geq$50% of the study population.

Based on the inclusion criteria, middle and high income schools were contacted for inclusion in the study. Total 12 schools agreed to participate in the study among the 17 schools were approached. 6 schools were included in the experimental group and 4 schools were part of control group. 2 schools took part in food pack base survey. In experiment group total 410 students took part and in control 250 students. In food pack survey total 60 students were included.

Data collection: For data collection, we used a self-administered questionnaire which was validated in the earlier phase of the study. The questionnaires were distributed among the participants in their respective classroom settings for self-administration in the presence of one of the investigators and a teacher from each respective school. Filling up the questionnaire took 40-45 minutes on average. After completion of the survey it was collected and was coded on the same day for data entry purpose. After the survey each participants were notified for the next classes schedule for the experiment.

Development of the education material and intervention: Five steps in designing 'Read-B4-U-Eat' intervention material. They were - Item-pool generation, material development, content validity, piloting and efficacy testing. Item-pool was generated based on earlier studies and formative research. Theories of social-cognition and shared-learning were used to develop the module's five components-interactive sessions for guided learning; booklet for self-learning; 9 posters for shared learning; animation film (4 min.) for edutainment; and notes for teachers for reiteration. Content was validated by panel of six experts from diverse fields such as food safety and regulation, communication, nutrition, psychology and education. Each component was piloted among $\geq$10 adolescents. Same day of consecutive weeks were scheduled for the interactive session.

The item-pool had 9 themes: commonly consumed foods; why labels; front-of-pack labeling; mandatory information; quality symbols; nutrient declaration; claims; high-fat-sodium-sugar (HFSS) foods; using labels. Content validity test resulted in modification of messages, visuals and sequencing. Piloting helped modify colors, images and simplify content.

The two interactive sections conducted in each experimental school were conducted in the classroom settings with in a gap of one week. The first session included discussion on macro and micro-nutrient, functions of foods, double burden of malnutrition, different packaged foods in recent diet patterns. The session tried to focus on the food items that are high in fat, sugar, salt and how their consumption can be reduced in daily diet and why it is important to choose healthy foods. The second session dealt exclusively on different packaged food and their labeling format, understanding of nutrient declaration on the food pack and interpretation of them for choosing healthier packaged food. These aspects were explained and practically demonstrated using real food packs. Students were then asked to check different aspects of the labels and compare nutritive values of different foods based on the label information to understand how to use food label information in real shopping environment.

After this session, sets of 9 posters were distributed for display in the classroom and students were given ready-reckoner booklets to take home, read and retain. In addition, a short film was shown in the classrooms using smart board, projector as per availability in the schools.

Post–intervention data collection: After a gap of one –two weeks of the intervention, post-intervention data was collected using the same procedures with the same questionnaire as used for baseline survey. The questionnaire was matched with the code of pre-experiment study and the data were entered in Ms-Excel.

Food-pack study for assessing the food choices based on label information: For this part of study, 60 participants, who were not part of the earlier study, were selected from two schools (@ 30 from each school). They were given two different packs of similar food items along with a questionnaire. They were asked to choose a pack and later fill up the questionnaire which had a set of questions aimed at assessing the aspects considered for selecting the food item. After the baseline data collection, these participants took part in the intervention programme which was similar to the main study group. After a week of completion of the intervention they were given new sets of food packs (2 packs of similar foods from different brands) and were asked to choose the healthy ones and again the reasons for their choice were assessed using a questionnaire.

Retention study: The retention of the educational intervention was checked after 3 months of collection of post-intervention data. For retention, data was collected using the same questionnaire that was used in the baselines and post-intervention phases.

Qualitative analysis: While studying the retention, the participants were asked to share their parents contact number only if they are willing to. Random calls were made to over 50 parents after 2 months of the retention phase and telephonic conversation was focused around a brief theme guide which had few questions on whether their children had shared about the study content at home, have they discussed the idea of using food label information to select food item, have they talked about changing any food that contained more of fat, sugar, or salt. They were also asked to rate the intervention material if they have gone through those.

Data analyses

McNemar Test was used to assesses the differences between pre- and post intervention responses on each of the topics. Moreover, descriptive statistics like frequencies were also used. Chi-square test was used to assess association between various determinants and label use.

RESULTS AND CONCLUSIONS

The section 'Tell us about your favorite packaged food' contained questions on different determinants of food purchasing behavior. The baseline data showed that the adolescents consider brand name or taste to choose a product and rarely look at the label information. Post-intervention results indicated positive changes in using label information for selecting health drinks ($p=0.00$), whereas label information use did not show significant increase for products like snacks or biscuits but frequency of label use was noticeably higher after the intervention. In the post-intervention phase, 25.2% of the adolescents reported that they see the label information while buying biscuits as compared to only 14.1% at baseline. Similarly, for cakes the percentage increased to 14.9% from 7.4%, for breakfast cereals label use increased to 33.6% from its baseline percentage of 18.2% whereas use of label for snacks were it increased to 24.2% from baseline percentage of 17.2%. Though there was a reducing percentage for use of only brand name for selecting a food product we could not identify major changes in consumption frequency.

As regards nutrition knowledge, significant ($p=0.00$/ $p=0.01$) improvement was observed in knowledge related to green Leafy vegetables, trans fats and saturated fats, nutrition during childhood and presence of allergen.

Among major food label information checked on food packs, information like ingredients, fat and nutrient panel was significant whereas use of quality symbols and other parameters of nutrient panel except fat did not significantly change after the intervention as reading this information was already higher at baseline itself.

Specific nutrient information used for different packaged food was also assessed where it was observed that ingredients and fats were more significantly being noticed after the intervention. For snacks, use of veg/ non-veg symbols were also changed positively, for choosing health drinks, health claims seemed to have been guiding the choices of many adolescents after the intervention, which could not be explained by the content of the intervention material.

Positive changes were also noticed in understanding of best before and expiry dates.

In the food packs survey, higher trend of reading label information for choosing a food pack was observed. More importantly, the selection time reduced and selection of healthier food product increased.

The responses from the parents were noted by one investigator instantly while conversing. We could connect 32 parent of them 28 acknowledged that the study was discussed in home. Most of the parent found it very helpful/ or good initiative/ timely approach for adolescent. Few parents pointed out the need of inclusion of parents since the beginning over only using the contact for collecting feedback. 17 of them mentioned there was a change in behavior towards fast or instant food item and they tend to talk about reducing consumption of such items which has high fat/salt. Further detailed analysis is being carried out from the data of the study.

Read-B4-U-Eat' is efficacious nutrition education module to inculcate label reading skills among adolescent consumers.

V PATHOLOGY

1. KNOWLEDGE, ATTITUDE AND PRACTICES [KAP] OF MEDICAL PRACTITIONERS IN HYDERABAD REGARDING FOOD ALLERGY (FA) - A PILOT STUDY

The International Food Safety Authorities Network (INFOSAN) of the World Health Organisation (WHO) recognise food allergy (FA) as a significant public health concern due to the high prevalence and potential severity of the condition and the impact it has on the quality of life and economy.

The extent of the problem of food allergy is not well documented in India as well as the Asian region. The incidence of allergy in India has been documented mostly for fungal/ aeroallergens, which have been shown to play a major role in the pathogenesis of respiratory allergic diseases like rhinitis and asthma in India Diagnostic tests for food allergy includes three levels of diagnostic criteria: (1) Questionnaire-based histories, (2) Specific IgE and/ or skin prick testing (SPT) and (3) Food challenges.

Diagnosis of food allergy is not always easy and mostly physicians are inadequately trained in medical school to deal with subjects with FA. The National Institute of Allergy and Infectious Disease (NIAID), USA, published a guideline for the diagnosis and management of food allergy in the United States, which might be useful in other geographies as well.

OBJECTIVE

To know the extent of basic understanding by clinicians about the FA problem, as they are the first to be approached by patients at the first instance.

METHODOLOGY

A total of 300 medical professionals including gastroenterologists, paediatricians, dermatologists, pulmonologists, general practitioners and homeo-physicians (50 from each category) were contacted / approached and were administered a questionnaire.

RESULTS AND INFERENCE

The study participants were not sufficiently knowledgeable regarding the diagnosis treatment and risk factors for food allergies and related anaphylaxis in Hyderabad. Medical practitioners of all specialities need to be “updated” with regard to their knowledge on the management and treatment of food allergies. Provision or periodic educational programs should be aimed at improving the standard of practice among the participants regarding allergic disorders in general, and food allergies in particular.

VI FOOD CHEMISTRY

1. PREBIOTIC EFFECT OF *LEGUME RAFFINOSE* FAMILY *OLIGOSACCHARIDES*

Legumes are widely grown throughout the world and their dietary and economic importance is globally appreciated and recognized. Legumes not only add variety to human diet, but also serve as an economical source of proteins, dietary fiber and a variety of micronutrients and phytochemicals. Pigeon pea and chickpea are very important pulse crops in India, and together they occupy nearly 45% of the total pulse area and India accounts for about 90% of the world supply of pigeon pea. Carbohydrates constitute the main fraction of grain legumes, accounting for up to 55–65% of the dry matter. Of these, starch and non-starch polysaccharides are the major constituents, with smaller but significant amounts of oligosaccharides. The a-galactosides include raffinose, stachyose, verbascose and higher homologous series. Raffinose family of sugars contain one, two or three galactose units linked to glucose moiety of sucrose by - (1 6) linkage. These sugars cannot be hydrolyzed and absorbed in the intestine, due to the lack of a-galactosidase (E.C. 3.2.1.22) activity in the small intestine but undergo anaerobic fermentation by bacteria in the large intestine, which may result in the production of flatus gases (H_2, CO_2 and small amount of CH_4).

The oligosaccharide fermentation in the caeco-colon by the bacteria lead to a decrease of pH in the colon and consequently, in faeces, due to production of short chain fatty acids (SCFA). It can also lead to nutrient production, such as vitamins of the B complex, (B1, B2, B6, and B12) nicotinic and folic acids, an increase in fecal dry weight excretion and constipation relief due to fecal bulking. Oligosaccharide fermentation can also lead to inhibition of diarrhea, a protective effect against infection a reduction of cancer risk, mainly the gut cancer and proliferation of bifidobacteria. Oligosaccharide fermentation in the coeco-colon has shown an increase in absorption of calcium, and magnesium is due to the binding/sequestering capacity of the oligosaccharides. The hypotheses most frequently proposed to explain this enhancing effect of oligosaccharides on mineral absorption are the osmotic effect, acidification of the colonic content due to fermentation and production of SCFA, formation of calcium and magnesium salts of these acids, hypertrophy of the colon wall. In India, grain legumes are widely consumed in the form of dhal as an economical source of protein. Information on the prebiotic effects of legume dhal with oligosaccharides is scanty. Therefore, this study has been proposed with the following objectives.

HYPOTHESIS

Consumption of legumes will have a positive impact on mineral absorption, bifidobacterial growth and increased short chain fatty acids.

OBJECTIVES

To study the effect of oligosaccharides on mineral absorption, bifidobacterial growth and lipid profiles.

METHODOLOGY

Extraction of oligosaccharides: Raffinose family of sugars can be extracted from legumes.

HPLC Analysis of oligosaccharides:

Analysis of raffinose family of sugars, for normal, routine use HPLC separation on polymeric gel column with ELSD or RI detection is quite satisfactory. The samples will be extracted for oligosaccharides (raffinose, stachyose, verbascose and higher homologous series) and further separated and quantified by using HPLC with ELSD or RI detector.

Processing

Different processing methods such as soaking, cooking, pressure cooking, microwave cooking, germination, soaking followed by cooking for the reduction of raffinose family of sugars was studied.

Animals and diet

Weaning male Wistar /NIN (*n* = 72) rats (4 weeks old) were acclimatized with the basal diet for 7 days in individual cages. Seventy two rats were divided equally into six groups and fed test diets for 3 weeks. The control group (G-I) received the basal diet, which contained (per kg diet) 250 g casein, 50 g corn oil, 35 g AIN-93G mineral mixture, 10 g AIN-93 vitamin mixture, 2.5 g choline bitartrate, 602.5 g of sucrose, and 50 g/kg crystalline cellulose. Raffinose group (G-II) received the basal diet supplement with 30 g/kg raffinose and experimental groups (G-III) received the basal diet supplemented with red gram, (G-IV) green gram, (G-V) black gram and (G-VI) bengal gram flour respectively. Weekly body weights and food intake was recorded. After 18 week on these experimental diets, 5 ml of blood was drawn from these rats from retro-orbital plexus and the rates were sacrificed by CO_2 inhalation. Per day 6 rats were sacrificed with one rat representing each group for 12 consecutive days. After sacrificing the rats, the ceacal content was collected and the following estimation was carried out.

Parameters studied

Estimation of serum glucose and lipid profile

The glucose concentration of blood was determined by glucose oxidase method using multimode detector, cholesterol by oxidase peroxidase method, HDL-C by precipitation method, triglycerides by glycerol phosphate method using standard kit from Biosystems®, Costa Brava, Spain. The LDL-C and VLDL-C was estimated by calculation method.

Serum minerals

Serum minerals were estimated by following the method of Roos and Price (1971). The mineral content of serum was analysed by using AAS (Atomic absorption spectroscopy) method. The individual metal atoms which have been atomized absorb energy specific to its wavelength from the light source (HCL-hollow cathode lamp) and become excited. This absorption by the analyte atoms is measured. The measured absorbance signal constitutes the measure of the concentration of the respective element in the analyzed sample.

Enumeration of the selective gut bacteria: (*Lactobacillus, Bifidobacteria, Enterobacteriaceae and Bacteriods*) by conventional method:

The viable bacteria present in 1 gm of sample (ceacum contents) will be evaluated by the following method. Briefly 0.1 gm of the ceacum content from each rat will be weighed into a sterile container and vortex mixed with 0.9ml of saline. The samples will be further serially diluted from 10^{-1} to 10^{-6} with saline. A 0.1ml aliquot of one particular dilution which will be standardized for a given bacteria will be plated in duplicates on the respective selective medium (given below) for each of the above group of bacteria. The plates will then be incubated at 37^0 C for 24 hrs/ 48 hrs as the case may be depending on the bacteria. All the above processing for the anaerobic bacteria will be carried out in anaerobic jars and for the facultative anaerobes the plates will be incubated in candle jar. The counting of viable bacteria will be done after the required incubation time. The formula that will be used for expressing the cell counts is given below.

Number of cells /gram = number of colonies x dilution factor / weight of the sample

Medium	Organism	Source
Man sharpe Rogosa agar	Lactobacilli	HiMedia, Mumbai, India
Bifidobacterium agar	Bifidobacterium sps	HiMedia, Mumbai, India
Mac Conkey agar	Enterobacteria sps	HiMedia, Mumbai, India

Oral glucose tolerance test (OGTT):

Rats submitted to a fasting period of 12 hours were administered orally with a glucose solution of 2g/Kg body

weight and the tail vein blood glucose levels were measured in samples taken immediately before the oral administration and after 30, 60, 90, and 120 min. using the portable device One Touch® Ultra Easy ® Glucometer (Life Scan, Johnson and Johnson Portugal).

Measurement of body composition by DXA:

The body composition is measured in control and experimental groups (groups 1 to 6) after 18 weeks of study were evaluated using Hologic QDR series discovery model DXA equipment. DXA instrument calibration included an infant step bar phantom (Hologic P/N 010-0757, Rev.004) made up of Plexiglas and used for all scans. The platform was of the same height as the lowest step of the calibration bar, putting the animals in the range of calibration of the machine. The scanner was calibrated using control phantom, and its performance was monitored as per the quality assurance protocol. No sign of scanner drift was observed during the study period. The coefficient of variation was 1-2% for all the measurements. Rats were placed on the platform and were scanned with DXA using Hologic ultra high resolution small animal software. The scans were then analyzed with ultra high resolution analysis software, and values were recorded.

Inflammatory markers

The serum sample was estimated for cytokines like (IL-1β, IL-6, IFNγ, TNF-α) from Invitrogen Bioservices India Pvt. Ltd., by using Custom Luminex Kits.

RESULTS

Standardization and validation of the methodology has been carried out for the determination of raffinose family of sugars in legumes using HPLC-RI. The method was used to determine the raffinose family sugars in commonly consumed legumes (14 varieties). Among the legume tested math bean (5.51%) were shown highest amount of raffinose family sugars followed by white peas (5.43%) and red gram (5.15). The chick peas dhal (2.54%) were shown lowest amount of raffinose family sugars. Fig 1 shows the typical chromatogram of eight different standard sugars and the fig 2 shows the typical chromatogram of sample.

The effect of different processing methods led to decrease in the concentration of raffinose family sugars. Among different processing methods cooking method showed higher reduction of raffinose family of sugars all tested legumes (Table 2). The percent removal of is 96.67%, 96.23%, 81.67%, and 100%, stachyose by 85.45%, 100%, 100%, and 100% and verbascose by 99.36%, 100%, 100% and 100% in black gram, green gram, chick pea and red gram seeds respectively. The lowest reduction of raffinose family of sugars was shown by soaking method (Table 3). The percent removal of is 56.67%, 59.43%, 48.33%, and 67.16%, stachyose by 58.18%, 50%, 53.25%, and 57.38% and verbascose by 67.52%, 50.72%, 48.74% and 63.70% in black gram, green gram, chick pea and red gram seeds respectively. Blood glucose and lipid profile were given in Table 4. From the table it is evident that the blood glucose level of animals fed with standard raffinose, red gram, green gram, black gram and bengal gram oligosaccharide were lowered when compared to control group animals. The serum Cholesterol content in all legume oligosaccharide fed group were decreased when compared to control group similar result were observed in triglycerides, LDL and VLDL cholesterol and HDL cholesterol level is increased in legume oligosaccharide fed animals when compared to control group.

The results of mineral absorption such as iron, zinc, copper, manganese, magnesium and calcium in all animals fed with legume oligosaccharide were promising (Table 5). The results revealed significantly higher net mineral absorption and retention in the rats when supplemented with legume prebiotics than that of control and reference standard (raffinose). The results of legume oligosaccharides on the growth of bifidobacteria and lactobacillus and pathogenic bacteria in cecum content were given in Table 6. The animals fed with legume oligosaccharides cecum content was showing the higher growth of lactobacillus and bifido bacteria at 10^5, 10^6 dilutions (CFU/ gram of ceum sample) when compared to the control and reference standard (raffinose) fed animals. The pathogenic bacteria counts at 10^4, 10^5 dilutions (CFU/ gram of ceum sample) were decrease when compared to control and raffinose fed animals.

After 18 weeks of the study, oral glucose tolerance test was carried to see the effect of legume prebiotics on the amelioration of insulin resistance. The blood glucose concentration from 0 min to 120 min is higher in control and reffinose fed animals when compared with all legume prebiotic fed animals (Table 7).

Table 1. Oligosaccharides contents of commonly consumed legumes

Sl.No	Name	Sucrose	Raffinose	Stachyose	Verbascose	Total (g/100g)
1	Cow pea	1.69	0.71	2.28	0.27	4.95
2	Black gram	1.10	0.10	0.55	1.57	3.31
3	Chick pea dhal	1.07	0.40	1.06	0.01	2.54
4	Green gram	0.96	1.06	0.80	1.38	4.20
5	Green pea	1.52	0.68	1.59	0.78	4.56
6	Hors gram	0.39	0.27	3.66	0.02	4.34
7	Lentil	1.06	0.60	1.24	0.30	3.20
8	Math bean	1.38	0.83	2.86	0.44	5.51
9	Lentil dhal	1.41	0.32	1.26	2.01	5.00
10	Rajmah	1.80	0.45	1.88	0.05	4.17
11	Chickpea whole	1.09	0.60	0.77	0.09	2.56
12	Red gram whole	1.71	0.67	1.22	0.88	4.48
13	Red gram dhal	2.01	0.82	1.40	0.93	5.15
14	White pea	1.83	0.73	2.03	0.83	5.43

Fig 1. Typical chromatogram of standard mixture of eight sugars

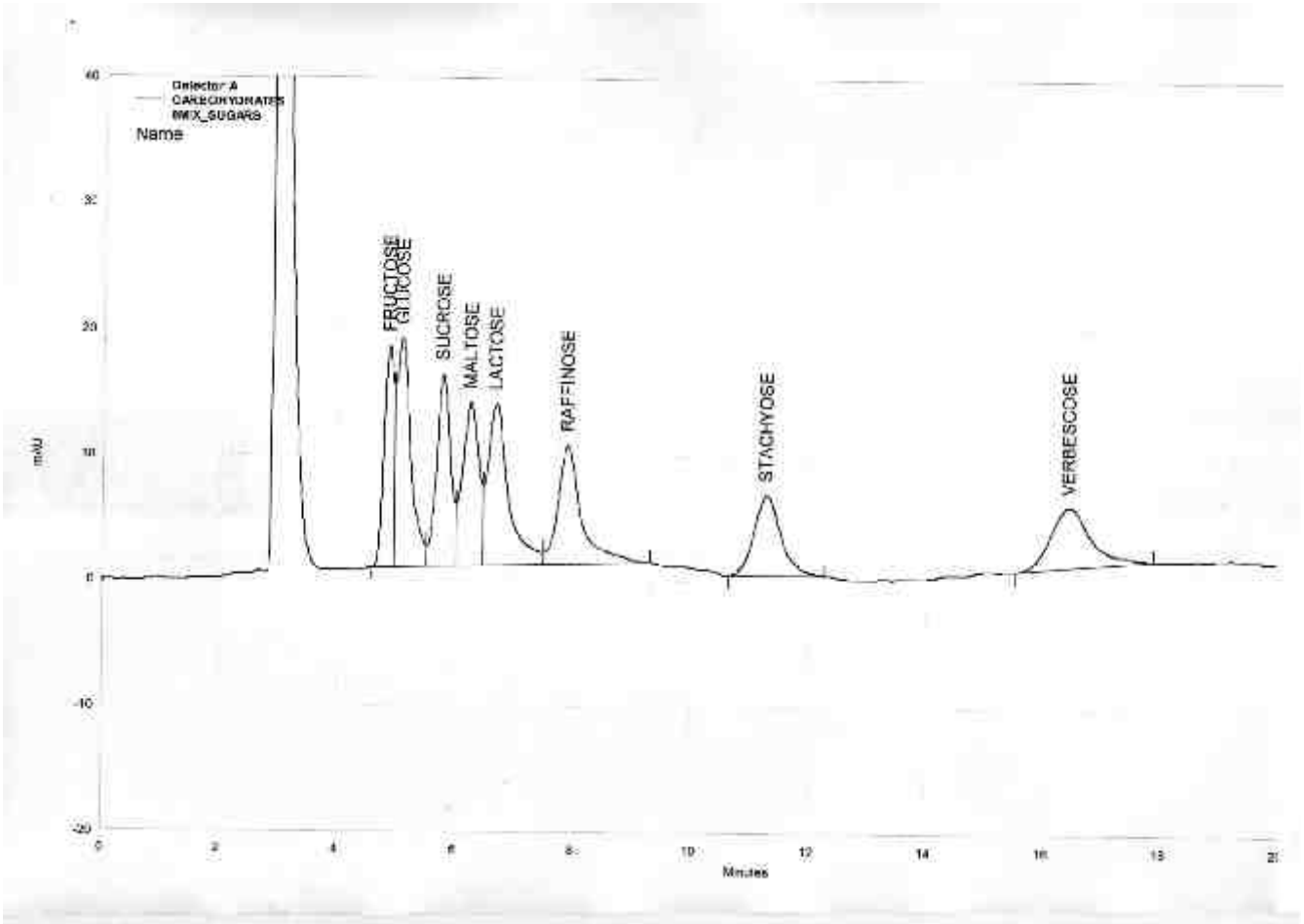


Fig 2. Typical chromatogram of raffinose family sugars from green gram dhal extract

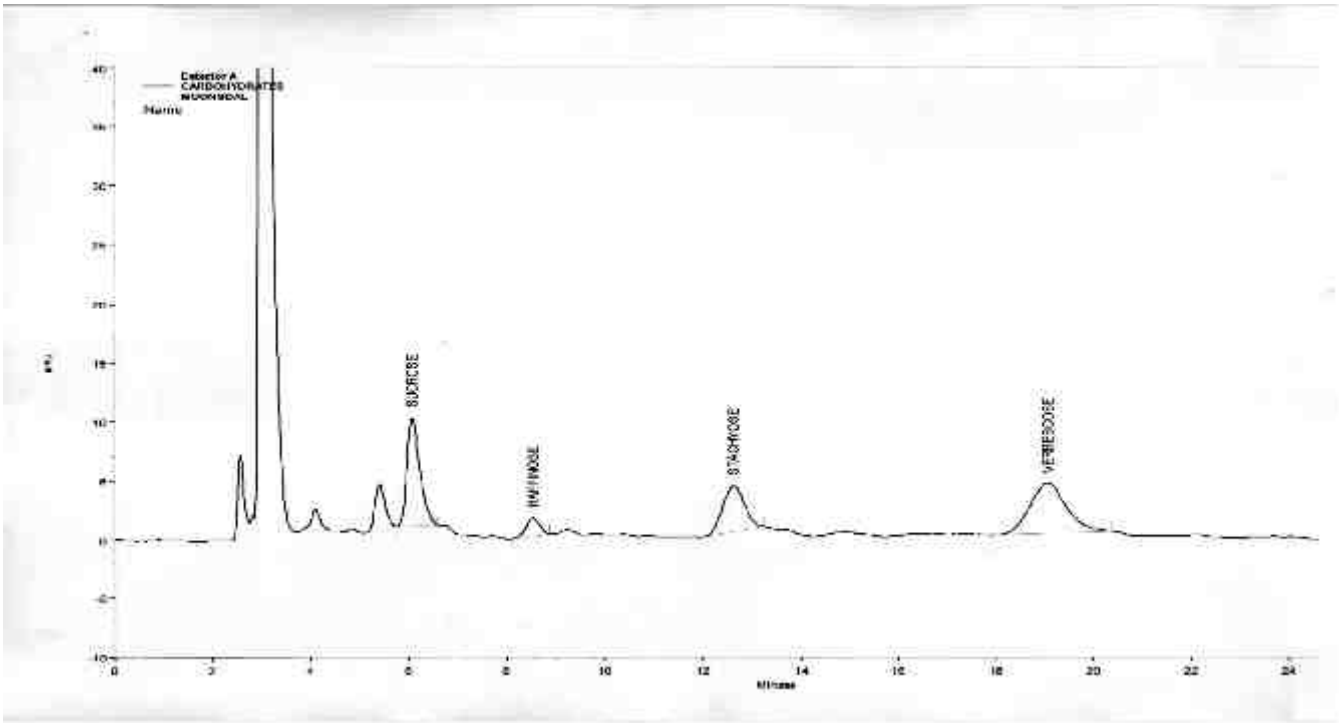


Table 2. Effect of cooking on raffinose family of oligosaccharides (g/100g)

Sample	Sucrose	Raffinose	Stachyose	Verbascose
Black gram -0min	1.10	0.60	0.55	1.57
Black gram -20min	0.52	0.17	0.60	0.13
Black gram -40min	0.14	0.06	0.20	0.09
Black gram -60min	0.02	0.02	0.08	0.01
Green gram -0 min	0.96	1.06	0.80	1.38
Green gram-20min	0.14	0.18	0.06	0.00
Green gram-40min	0.09	0.08	0.02	0.00
Green gram-60min	0.03	0.04	0.00	0.00
Chick pea -0min	1.09	0.60	0.77	1.19
Chick pea-20min	0.05	0.23	0.13	0.11
Chick pea-40min	0.08	0.16	0.05	0.06
Chick pea-60min	0.09	0.11	0.00	0.00
Red gram -0hr	1.71	0.67	1.22	1.88
Red gram-20min	0.18	0.15	0.14	0.18
Red gram-40min	0.09	0.02	0.02	0.03
Red gram-60min	0.00	0.00	0.00	0.00

Table 4. Effect of legume prebiotics on blood glucose and lipid profile (mg/DL)

Groups	Glucose	Cholesterol	HDL-C	Triglycerides	LDL-C	VLDL-C
Group-I Control	78.37	128.99	45.00	153.44	53.30	33.13
Group-II Raffinose	66.67	110.22	48.12	155.49	30.99	31.10
Group-III Red gram	68.08	105.11	48.61	103.17	35.86	20.63
Group-IV Green gram	71.30	114.69	47.60	113.97	46.13	22.79
Group-V Black gram	76.88	118.77	46.18	92.36	46.16	18.47
Group-VI Bengal gram	63.55	95.79	45.69	115.27	51.43	23.05

Table 5. Effect of legume prebiotics on mineral absorption

Analysis of elements from serum sample						
Groups	Fe	Zn	Cu	Mn	Mg	Ca
	µg/100ml	µg/100ml	µg/100ml	µg/100ml	mg/100ml	mg/100ml
Group-I Control	266.0	192.0	165.0	1.0	2.00	11.00
Group-II Raffinose	224.0	223.8	191.9	1.4	2.29	12.25
Group-III Red gram	307.0	196.7	188.2	1.5	2.22	12.06
Group-IV Green gram	283.0	214.1	182.0	1.5	2.40	11.10
Group-V Black gram	273.0	192.0	211.8	1.9	2.69	12.27
Group-VI Bengal gram	343.0	209.7	198.3	1.9	2.97	12.60

Table 6. Effect of legume prebiotics on the growth of bifidobacteria and lactobacillus pathogenic bacteria

Groups	MRS 10^5	MRS 10^6	Bifido 10^5	Bifido 10^6	Mac'Key 10^4	Mac'Key 10^5
Groups-I (n=12)	310.75	33.59	543.41	101.47	30.23	2.66
Groups-II (n=12)	229.95	21.87	835.39	99.35	0.13	0.13
Groups-III (n=12)	840.82	92.17	971.75	128.16	0.83	0.00
Groups-IV (n=12)	374.60	34.58	457.92	102.76	54.15	5.11
Groups-V (n=12)	655.54	50.94	999.64	143.43	54.16	5.10
Groups-VI (n=10)	518.14	49.59	605.18	226.68	30.50	4.31

The effect of legume prebiotics on the body mass composition was determined by using DEXA. The results of body mass composition are promising by decreasing the percentage of fat mass for legume prebiotic fed animals than that of control and raffinose fed animals (Table 8).

The IL-1β is a member of the interleukin 1 family of cytokines. IL-6 is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation. IFNγ, or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral, some bacterial and protozoal infections. Tumor necrosis factor (TNFα, cachexin, or cachectin) is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. The levitated levels of TNF- α, IL-6 and INF-γ were associated with risk of obesity and diabetes.

The effect of legume prebiotics on the inflammatory markers such as IL-1B, IL-6, INFγ and TNF-α result were given in Table 9. From the table it is evident that all the markers tested were decreased in the concentration of legume prebiotic fed animals than that of control and raffinose fed animals.

Table 7. Effect of legume prebiotics on oral glucose tolerance test (mg/DL)

OGTT					
Groups	0 min	30 min	60 min	90 min	120 min
Group-I Control	118	160	167	150	121
Group-II Raffinose	120	158	162	154	129
Group-III Red gram	82	128	130	113	90
Group-IV Green gram	76	137	129	110	96
Group-V Black gram	69	133	131	118	105
Group-VI Bengal gram	82	137	120	108	102

Table 8. Effect of legume prebiotics on body composition by DEXA

Groups	Body Wt	Length (cm)	Fat	Lean + BMC	Total mass	% Fat
Group-I Control	380.00	10.40	127.93	230.03	357.97	35.97
Group-II Raffinose	507.00	10.37	212.63	302.90	515.57	41.23
Group-III Red gram	367.00	10.00	96.07	284.73	380.80	25.20
Group-IV Green gram	365.67	10.33	104.27	261.17	365.60	28.63
Group-V Black gram	261.00	9.73	57.43	202.87	260.30	22.07
Group-VI Bengal gram	322.67	10.10	73.70	254.73	328.47	22.47

Table 9. Effect of legume prebiotics on the inflammatory markers (PPM)

Groups	Sample	IL-1B	IL-06	INF-G	TNF-a
Control	Plasma	29.98	119.23	17.94	3.48
Raffinose	Plasma	19.44	175.14	40.11	14.61
Red gram	Plasma	21.21	185.62	49.86	00.79
Green gram	Plasma	115.16	43.67	07.58	00.00
Black gram	Plasma	111.77	66.90	00.00	00.00
Bengal gram	Plasma	13.60	53.24	11.97	00.00

CONCLUSIONS

The analysis of oligosaccharide content of commonly consumed legumes showed the presence of raffinose family of sugars with varied concentration. These sugars cannot be hydrolyzed and absorbed in the intestine, due to the lack of -galactosidase activity in the small intestine but undergo anaerobic fermentation by bacteria in the large intestine, which may result in the production of flatus gases (H_2, CO_2 and small amount of CH_4). The effect of different house hold processing methods led to decrease in the levels of raffinose family sugars. Oligosaccharide fermentation in the caeco-colon by the bacteria can give many positive health benefits as prebiotics.

The prebiotic potential of legume oligosaccharides in animal model were shown decrease in blood glucose level, improved lipid profile, increased mineral absorption, improved body mass composition and inflammatory markers. The ceacum sample analysis showed that increase in the gut bacterial colonies of lactobacillus, bifidobacteria, enterobacillus and bacteroides by decrease in the pathogenic putrificative bacterial counts and there was reduction in inflammatory cytokines like IL-1ß, IL-6, TNF-α and INF-γ in experimental animals supplemented with legume prebiotics. No such changes were seen in control group.

The further research is in need to understand the role of legume prebiotics to prevent or control diabetes, obesity, cardiovascular diseases, irritable bowel syndrome and other health benefits.

2. STUDIES ON GASTRO-PROTECTIVE EFFECTS OF 'KING CHILLI'

Chillies *(Capsicum sp.)* are one of the most widely cultivated and consumed spices throughout the world. *Capsicum* genus belongs to the Solanaceae plant family comprising more than 200 varieties. The pungent flavor of chillies is due to presence of alkaloid called capsaicinoids found only in the genus *Capsicum*. Capsaicin, dihydrocapsaicin and nordihydrocapsaicin are reported to be the major capsaicinoids in *Capsicum* and these three together accounts for about 90% of pungency. The daily percapita consumption of capsaicinoids from peppers in Europe and the United States in estimated at ~1.5mg and that in India, Mexico and Thailand at ~25-28mg.

Capsaicin has been widely used as a topical analgesic to treat a variety of neuropathic pain conditions including rheumatoid arthritis, diabetic neuropathy, cluster headaches and herpes zoster. The compound has recently attracted significant attention towards certain carcinogenesis and mutagenesis in several cancer cell lines, antimicrobial activity, antimutagenecity effect and a high antioxidant activity.

The patho-physiological basis of gastric protection by chilli (capsaicin) remains unclear. However, capsaicin has been reported to increase gastric mucosal blood flow and this may be related to the release of calcitonin gene-related peptide (CGRP) and nitric oxide (NO). Nitric oxide function as a final transmitter to mediate the capsaicin induced prevention of mucosal injury by potent vasodilator action. Capsaicin-sensitive afferent neurons

stimulations have been reported to participate in gastric mucosal protection against various ulcerogenic factors. Chillies were also found to increase gastric acid secretion in various studies. Gastric acid is essential for releasing trace elements from the food matrix and for solubilisation in the stomach. Essential metal ions are required in the gut not only for nutrition but also for the integrity of the mucosa. The continued secretion of cationic electrolyte elements into gastric juice may be beneficial for the intestinal absorption of dietary trace metals. For example, zinc can facilitate the healing of gastric ulcers.

Capsaicin content in chillies ranges between 0.1- 0.7% where as the world's hottest chilli (Naga King chilli) contains 4.2%. Naga King chilli is cultivated and consumed by the Naga tribes in North-east India. In view of the high capsaicin content and the fact that this is consumed on a daily basis by the Nagas in North-east India, it is of interest to study the effect on gastric mucus protection and mineral absorption.

HYPOTHESIS

The consumption of Naga King chilli/capsaicin protects the gastric ulcer and increase mineral absorption.

OBJECTIVES

- To study the gastro-protective effect of Naga 'King chilli'.
- To study the effect of capsaicin on mineral absorption.

Work done during the year

Studies on effect of gastric protection and mineral absorption of Naga King Chilli was carried out in ethanol induced ulcer model for a period of 7 (acute) and 28 (sub-chronic) through oral intubation and 90 days (chronic) through diet mix using Sprague-Dawley (SD) rats. Two months old rats were housed in individual cages and the experiments were conducted as given in the tables 1a and 1b. All the animals were fed with the normal diet.

Naga king chilli powder and capsaicin standard were dissolved separately in the solvent [10% ethanol; 10% TWEEN 80; 80% saline] and administered daily once through oral gavages. Ten minutes prior to the drug administration, 80% ethanol was administered orally to induce gastric ulcer in the treatment groups. At the end of the experimental period, one ml blood was collected after the overnight starvation to determine mineral content in plasma using Flame-AAS. Then, the rats were euthanized using CO_2 and stomach was removed along with the curvature for mucosal damage examination.

Effect of different red chillies with different pungency (low, medium and high) on gastro protective and mineral absorption were studied up to 90 days. Identification of different pungent chillies was done based on the capsaicinoid content. Guntur chilli, Bird's eye chilli and Naga King chilli were used for low, medium and high capsaicinoid respectively. All the chillies were dried and powdered finely. Two milligram capsaicinoid equivalent of each chilli powder were mixed with the regular diet and fed to the experimental animals for the period of 90 days. Data on food intake and bodyweight of all the animals during the experimentation were observed. All the vital organs were removed and their weights were measured. Stomachs were removed for the various biochemical and pathological examination to study the mechanism of gastroprotection.

Table 1a. Experimental design to study the effect of gastro-protective and mineral absorption of Naga King Chilli through oral intubation upto 7 (acute) and 28 (sub-chronic) days

Group	NC (1)	VC (2)	CAP (3)	KC (4)	CAP+ETH (5)	KC+ETH (6)	ETH (7)	Duration
No. of animals	6	6	6	6	6	6	6	7 days
No. of animals	6	6	6	6	6	6	6	28 days

NC – Normal saline Control; VC – Vehicle Control (solvent [10% ethanol; 10%TWEEN 80; 80% saline]); CAP – Capsaicin standard (15mg/kg bwt. in 1% solvent); KC – King chilli powder (equal to capsaicin); ETH – Ethanol (suitable concentration will be selected based on the pilot study)

Table 1b. Experimental design to study the effect of gastro-protective and mineral absorption of Naga King Chilli through regular diet upto 90 (chronic) days

Groups	NC (1)	CAP (3)	L.CAP (4)	M.CAP (5)	H.CAP (6)
No. of animals	6	6	6	6	6
NC – Normal Diet Control *CAP – Capsaicin standard (2 mg)* *L.CAP – Low Capsaicin Chilli powder (Guntur chilli)* *M.CAP – Medium Capsaicin Chilli powder (Bird's eye chilli)* *H.CAP – High Capsaicin Chilli powder (Naga King chilli)*					

RESULTS

1. Development of ethanol induced gastric ulcer model

Three different concentrations (40, 60 and 80 % v/v) of ethanol were tested to induce gastric ulcer for a period of 28 days. The test drug was daily administered through oral gavages. Average body weight and food intake of animals used in this study were monitored. Body weight of all the experimental animals was increased gradually with an average of ~73 g in group 1 and 3 which is almost double the weight of group 2 and 4. This was in agreement with food intake where group 2 and 4 had lesser than the group 1 and 3. Though the body weight and average food was lesser in group 4, the animals were active up to 24 days. The histo-pathalogical reports of the rats administrated with 80% ethanol showed more rupture of mucinous acini lining in the stomach. Therefore 80% ethanol induced ulcer model was selected for further gastro-protective studies using Naga King chillies.

2. Effect of gastro-protective and mineral absorption of Naga King Chilli

Estimated concentration of Naga king chilli which equal to 10 mg capsaicin/ kg bw was 250 mg of chilli. Therefore 500 mg of fine powdered Naga king chilli was dissolved in 10 mL solvent (10% ethanol; 10%TWEEN 80; 80% saline). All the drugs (control and testing) orally administered were 1 mL/rat/day. Treatment groups were received 1 mL of 80% ethanol 30 min prior to drug administration.

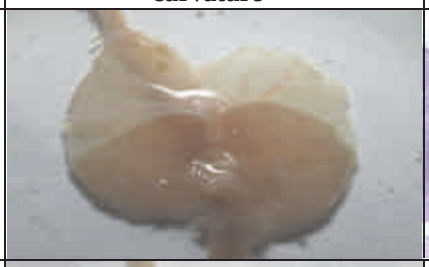
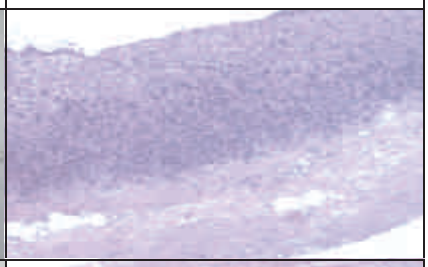
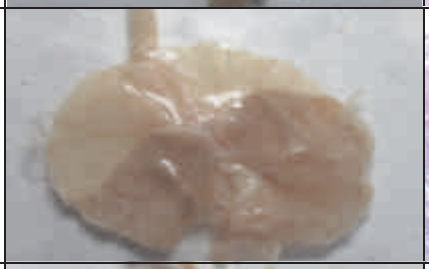
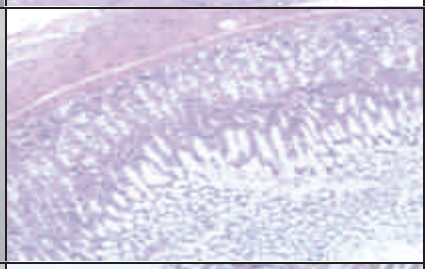
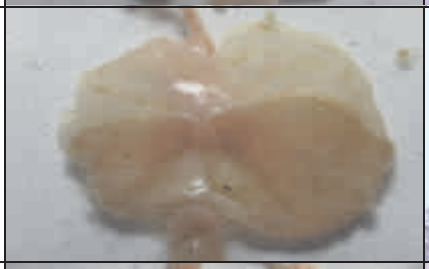
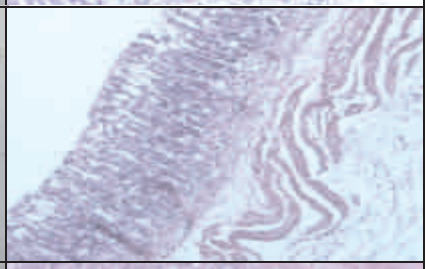
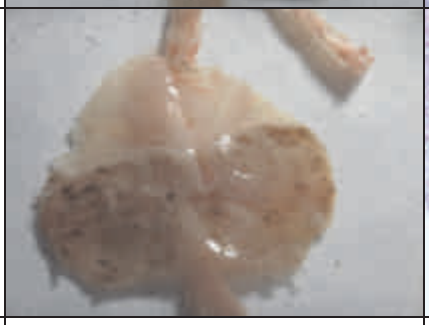
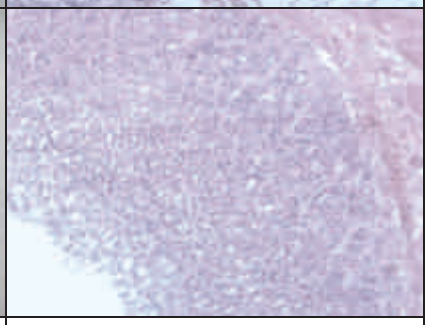
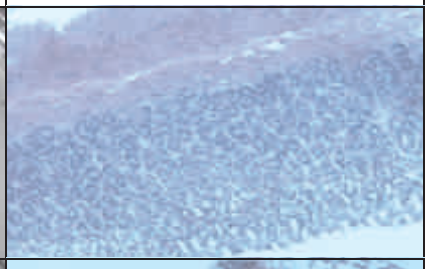
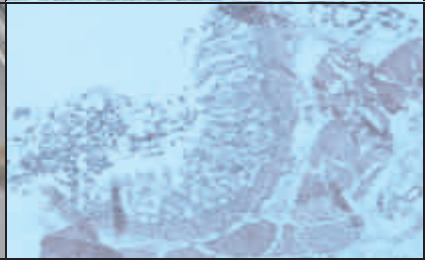
The food intake and body weight of all experimental animals were recorded throughout the study (chronic) and tabulated in Table 2. Body weight of the treatment groups administered with bird eye chilli and Guntur chilli followed by ethanol were slightly reduced. This may be due to the intubation of alcohol (80% ethanol) administration. However, there was no significant difference in food intake is observed between the groups and the average food intake ranged between 14.64±0.74 and 15.64±0.90.

Table 2. Average food intake and body weight during experiment (chronic study)

Group No.	No. of animals	Food intake (g/day/rat)	Body weight gained (g/rat)
1	6	15.64±0.90	254.22±32.38
2	5	14.64±0.74	228.42±17.13
3	5	15.62±0.65	245.20±19.87
4	5	14.97±1.27	210.24±32.28
5	8	15.21±0.96	207.93±31.29
6	7	15.14±0.63	235.03±23.80

Values are mean ± SD

Fig 1. Rat stomachs along the greater curvature and histologic pictures of stomach tissues in different experimental groups

Treatment group	Cut opened along the greater curvature	Histologic pictures of Stomach
Normal Saline Control (6N)		
Ca psaicin		
King Chilli		
Capsaicin + Ethanol		
Treatment group	**Cut opened along the greater curvature**	**Histologic pictures of Stomach**
King chilli + Ethanol		
Ethanol		

Plasma mineral content of experimental rats is tabulated in Table-3. Zinc content was ranged between 0.121±0.011 mg/dL and 0.132±0.012 mg/dL and Mg content was less in medium capsaicin group (1.022±0.109 mg/dL) and more in normal control (1.121±0.069 mg/dL). However, there was no significant different found in Zn, Mg and Ca among the groups. Iron content ranged from 0.211±0.022 mg/dL in group 2 to 0.279±0.070 mg/dL in group 4. Fe content of group 4 (medium capsaicin) were significantly ($P<0.05$) higher than normal control group.

Stomach histo-pathological studies (table 4) shown that, there is no mucosal damage in saline (group 1) and vehicle control (group 2) group (fig 1D). Whereas, moderate sloughing in mucosal layer was observed in capsaicin (group 3) administered group. There was no inflammation signs was noticed in the Naga king chilli (group 4) group. Stomachs of the rats (group 5 and 6) administered either with capsaicin or chilli powder followed by ethanol showed sloughed off mucous lining with enlarged secretary acini. But the submucosa and muscularis mucosa layers were normal (fig. 1F). However, both mucosa and submucosa layers of rats received only 1 mL of 80% ethanol was highly damaged which shows that more ulcers have been created.

Table 3. Mineral content (mg/dL) of plasma samples (chronic study)

Group No.	Group	No. of animals	Zn	Fe	Mg	Ca
1	Normal Control	6	0.132±0.016	0.232±0.042	1.121±0.069	10.366±0.619
2	Capsaicin	5	0.128±0.014	0.211±0.022	1.088±0.189	9.926±0.754
3	Low Capsaicin (Guntur chili; 0.45%)	5	0.123±0.007	0.234±0.034	1.175±0.249	9.569±0.799
4	Medium Capsaicin (Birds eye chili; 0.9%)	5	0.124±0.021	0.279±0.070*	1.022±0.109	9.793±0.453
5	High Capsaicin (Naga King chili; 4.2%)	8	0.121±0.011	0.240±0.065	1.044±0.088	9.493±0.511
6	Ethanol	7	0.132±0.012	0.214±0.021	1.063±0.081	9.453±1.218

Values are mean ± SD

Table 4. Stomach Histo-pathological observation in different treatments (acute)

Group No.	Group	Stomach Histopathology Report
1	Normal Saline Control (6N)	Intact mucosa - No abnormality detected (NAD) in all layers
2	Vehicle Control (6N)	Intact mucosa - No abnormality detected (NAD) in all layers
3	Capsaicin (6N)	Moderate sloughing of mucous layer. Other layers are intact
4	King Chilli (6N)	The mucous layer, the sub-mucous and muscularis mucosa looked normal. No signs of inflammation were noted
5	Capsaicin + Ethanol (6N)	Mucous layer covered in the fibrous tissue cells, secretary acini are shrunken/Mild sloughing of mucous, submucosa and other layers normal
6	King chilli + Ethanol (6N)	Sloughed off mucous lining. Enlarged secretary acini. Submucous and muscularis mucosa – Normal
7	Ethanol (6N)	Sloughed off of mucosa and damaged sub-mucosa with destruction of Goblet cells and acini

Various biochemical markers such as mucosa mucus content, prostaglandin E (PGE) and calcitonin gene-related peptide (CGRP) were studied in the stomach tissue to elucidate the mucosal damage. The results are given in the table-5. Mucosal mucus content in ethanol group was significantly low (0.94 ± 0.241 mg alcian blue/g tissue) compared to all the other groups. PGE concentration was ranged from 3.80 ± 0.22 to 4.98 ± 0.26 pg/ug of protein. There was no significant variation observed between experimental and control groups except the group treated with capsaicin followed by ethanol. Elevated level of CGRP content was observed in all drug administered groups (ranging from 0.0220 to 0.0453 pg/ug of protein) compared to control (ranging from 0.0106 to 0.0192 pg/ug of protein). Antioxidant enzymes namely Superoxide dismutase (SOD) and catalase (CAT) were quantified using ELISA method and tabulated in table 3. SOD levels increased while CAT values showed decreased in the ethanol group.

Table 5. Content of mucosal mucus, Prostaglandin E and antioxidative enzyme levels of gut tissue in the experiment

Group No.	Group	Mucus Content (mg alcian blue/g tissue)	PGE	CGRP	SOD	CAT
			pg/µg of protein		U/µg of protein	nmol/min/µg of protein
1	Saline Control	1.25±0.311^{b}	3.92±0.25^{a}	0.0106±0.0020	0.014±0.0005^{a}	0.023±0.0081^{a}
2	Vehicle Control	1.56±0.841^{c}	4.27±0.17^{a}	0.0192±0.0004	0.014±0.0015^{a}	0.020±0.0027^{a}
3	Capsaicin	1.99±0.841^{d}	3.80±0.22^{a}	0.0453±0.0021	0.017±0.0039^{b}	0.029±0.0117^{d}
4	King Chilli	1.35±0.621^{b}	4.08±0.19^{a}	0.0385±0.0016	0.014±0.0033^{a}	0.027±0.0015^{c}
5	Capsaicin + Ethanol	1.36±0.101^{b}	4.98±0.26^{b}	0.0220±0.0002	0.018±0.0058^{c}	0.025±0.0055^{b}
6	King Chilli + Ethanol	1.54±0.081^{c}	3.99±0.21^{a}	0.0424±0.0002	0.017±0.0006^{b}	0.027±0.0068^{c}
7	Ethanol	0.94±0.241^{a}	4.27±0.15^{a}	0.0332±0.0022	0.022±0.0018^{d}	0.022±0.0048^{a}

Values are mean ± SD (n=3). Means in the same column followed by different letters are significantly different at P<0.05 using one way ANOVA followed by Tukey's multiple range tests.

CONCLUSION

Studies on gastro-protective effect of Naga king chilli was studied using ethanol induced ulcerative model in male SD rats. The experiments were conducted for 7 days (acute); 28 days (sub-chronic) and 90 days (chronic). 80% (v/v) found to be suitable to induce gastric ulcer and the same used for all the experiments. Results of these studies have demonstrated the gastro-protective effect of Naga King chili against gastric mucosal damage induced by ethanol. The observed gastro-protection was possibly mediated to a major extent by the local inflammatory mechanism followed by antioxidant mechanism. Results clearly showed that increased mucosal content and CGRP were higher in the groups treated with capsaicin or Naga king chili followed by ethanol. Elevated levels of antioxidant enzymes in the capsaicin+ethanol and Naga king chili+ethanol groups were clearly shows that the ability of restoration by capsaicin and Naga king chili. Therefore, the combined effect of antioxidant and other complimentary mechanism such as CGRP and PGE are accompanied to protect gastric ulcer induced by ethanol. The present findings of gastro-protection by naga king chili is not only supports the traditional consumption of chili by Naga tribe of Nagaland but also increases the possibility of development of potential therapeutic drugs.

3. BIOACTIVE PHYTOCHEMICALS IN INDIAN FOODS

Epidemiological studies have shown that there is a positive association between intake of vegetables and fruits and reduced cardiovascular diseases and certain cancers. It is generally assumed that the main dietary constituents contributing to these protective effects are the antioxidant components present in fruits and vegetables. To appreciate and to understand important minimal constituents of plant foods to our health, the composition of bioactive phytochemicals in fruits, vegetables, and beverages and their functional properties are very important. Plant foods have been subjected recently to extensive research in order to analyze wide spectrum of biologically active substances they contain. There is a limited data on polyphenols in Indian foods and therefore there is need to generate data in this regard.

AIMS AND OBJECTIVES

- To evaluate certain commonly consumed Indian foods to create database on bioactive phytochemicals.
- To study the effect of processing, cooking and storage on bioactive phytochemicals, present in commonly consumed Indian foods.

1. Determination of total and individual polyphenols in the commonly consumed Indian foods by solid-phase extraction and high performance liquid chromatography with diode array ultraviolet detection

1.1. Samples

The selection of the samples was based on the food consumption data of the epidemiology unit of the National Nutrition Monitoring Bureau, India which is developed on the basis of 24 hr recalls in India. Food samples were collected from different major wholesale markets in twin cities of Hyderabad and Secunderabad. The millets and legumes were cleaned manually to remove any stones and dirt. Then they were finely grounded by using cyclone sample mill (Colorado, USA).The fresh foods were washed and the edible parts were subjected to grinding with liquid nitrogen to homogenize the sample.

1.2. Statistical analyses

All the experiments were performed in triplicate and the results were expressed as mean±SD (standard deviation). Results were compared with one - way analysis of variance (ANOVA). Statistical analyses were performed using SPSS 21.0 at a 5% significant level.

1.3. Determination of Total Polyphenols and Expression of the Results

The extraction procedure and Folin-Ciocalteu assay were done according to a previous methodology. Total polyphenolic content of the commonly consumed foods in India with different equivalents such as gallic, catechin, quercetin and chlorogenic acid were shown in Table 1. Significant differences ($p < 0.05$) were observed between the different polyphenolic standards were used as a reference compounds in phenolic content of the foods after using SPE. In FC method, Molecular weight of the reference compounds and the amount of interferences present in foods will vary the phenolic content of foods. Spectrophotometric method overestimates the phenolic content except in some food samples with high phenolic content. For instance, in green bean samples both spectroscopic and chromatographic methods gave the same total phenols when the interferences were masked. SPE method succeeded in retaining most of the phenols in the cartridge. But the recovery yield from the sorbent material was low. However, researchers achieved a complete extraction of phenols by using N, N-dimethylformamide (DMF) as an extraction solvent in olive oil. Fig 1 represents the total phenolic content and percentage of interferences in different cereals where the interferences were in the range of 51 -84% which is 2 -3 times higher the total phenolic value.

In the total polyphenols assay, it is crucial to identify the appropriate standard for an equivalent chemical instead of certain polyphenols as standard for each food. Food polyphenols exhibit different absorbance towards the F-C reagent. This might result in underestimation of the real total phenolic content of specific polyphenols rich in food samples.

Table 1. Concentration of TPC of commonly consumed Indian Foods (mg/100g)

Foods	Scientific Name	GAE	CE	QE	CAE
Cereals & Millets					
Pearl millet	Pennisetum typhoideum	104.48±1.44[a]	92.18±1.27[b]	60.27±0.83[c]	156.71±2.16[d]
Sorghum	Sorghum vulgare	31.71±1.40[a]	27.98±1.23[b]	18.29±0.81[c]	47.56±2.09[d]
Finger millet	Eleusine coracana	167.83±3.42[a]	148.08±3.02[b]	96.82±1.97[c]	251.75±4.13[d]
Rice, raw	Orysa sativa	14.36±4.28[a]	12.66±3.78[b]	8.28±2.47[c]	21.53±4.42[d]
Wheat	Triticum aestivum	28.57±2.46[a]	25.20±2.17[b]	16.48±1.42[c]	42.84±3.69[d]
Pulses & Legumes					
Bengal Gram dhal	Cicer arietinum	17.82 ± 1.72[a]	15.72 ± 1.52[b]	10.28 ± 0.99[c]	26.72 ± 2.58[d]
Bengal Gram(whole)	Cicer arietinum	130.81±4.92[a]	115.42±4.34[b]	75.47±2.84[c]	196.22±3.38[d]
Green Gram Dhal	Phaseolus aureus Roxb.	20.89±1.99[a]	18.43±1.76[b]	12.05±1.15[c]	31.33±2.99[d]
Red gram Dhal	Cajanus cajan	17.54±4.63[a]	15.47±4.08[b]	10.12±2.67[c]	26.3±3.94[d]
Soya Bean	Glycine max Merr.	113.19±2.58[a]	99.88±2.27[b]	65.30±1.49[c]	169.79±3.87[d]
Vegetables					
Bitter gourd	Momordica charantia	49.48±4.42[a]	43.66±3.90[b]	28.54±2.55[c]	74.22±4.63[d]
Brinjal	Solanum melongena	4.44±1.73[a]	2.15±0.64[b]	3.40±0.02[c]	5.66±1.09[d]
Cucumber	Cucumis sativus	37.23±1.62[a]	32.85±1.43[b]	21.47±0.93[c]	55.84±2.43[d]
French beans	Phaseolus vulgaris	22.89±3.43[a]	17.55±3.03[b]	11.47±1.98[c]	29.83±4.15[d]
Ladies Finger	Abelmoschus esculentus	115.64±3.70[a]	98.50±3.26[b]	64.40±2.13[c]	167.46±4.54[d]
Little gourd	Coccinia cordifolia	40.78±2.07[a]	35.98±1.83[b]	23.52±1.20[c]	61.17±3.11[d]
Leafy Vegetables					
Amaranth, tender	Amaranthus gangeticus	14.64 ± 3.03[a]	12.92 ± 1.03[b]	8.45± 1.02[c]	21.96 ± 1.05[d]
Coriander Leaves	Coriandrum sativum	41.87±2.24[a]	36.95±1.98[b]	24.16±1.29[c]	62.81±3.36[d]
Curry leaves	Murraya koenigii	323.39±4.24[a]	285.34±3.74[b]	186.57±2.45[c]	485.08±4.36[d]
Fenugreek leaves	Trigonella foenum graecum	54.47±4.67[a]	48.06±4.12[b]	31.42±2.70[c]	81.70±4.01[d]
Mint	Mentha spicata	91.99±4.26[a]	81.17±3.76[b]	53.07±2.46[c]	137.99±3.39[d]
Spinach	Spinacia oleracea	18.94±4.44[a]	16.71±1.92[b]	10.92±2.56[c]	28.41±3.66[d]
Roots & Tubers					
Beetroot	Beta vulgaris	104.73±3.08[a]	92.41±2.72[b]	60.42±1.78[c]	157.10±4.62[d]
Carrot	Daucus carota	20.31±4.33[a]	17.92±3.82[b]	11.71±2.50[c]	30.46±4.50[d]
Onion	Allium cepa	46.76±3.70[a]	41.26±3.26[b]	26.98±2.13[c]	70.15±4.55[d]
Potato	Solanum tuberosum	6.41±3.77[a]	4.16±3.33[b]	3.70±0.18[c]	9.62±4.66[d]
Radish	Raphanus sativus	66.78±0.69[a]	58.92±0.61[b]	38.52±0.40[c]	100.17±1.03[d]
Fruits					
Apple	Malus sylvestris	49.54±2.85[a]	43.71±2.52[b]	28.58±1.65[c]	74.32±4.28[d]
Banana, ripe	Musa paradisiaca	8.2±1.05[a]	5.23±0.93[b]	2.73±0.61[c]	12.30±1.57[d]
Guava, country	Psidium guajava	155.93±2.49[a]	137.52±2.11[b]	89.92±1.38[c]	233.79±3.58[d]
Indian Gooseberry	Emblica officinalis	1122.82±1.70[a]	990.72±1.50[b]	647.78±0.98[c]	1684.23±2.54[d]
Lemon	Citrus limon	10.35±2.70[a]	9.13±2.38[b]	5.97±1.56[c]	15.52±4.04[d]
Orange	Citrus aurantium	13.06±2.17[a]	9.76±1.92[b]	4.38±1.25[c]	16.59±3.26[d]
Papaya, ripe	Carcia papaya	28.58±2.95[a]	25.22±2.60[b]	16.49±1.70[c]	42.87±4.43[d]
Pine apple	Ananas cosmosus	23.00±1.74[a]	20.29±1.54[b]	13.27±1.01[c]	34.50±2.62[d]
Pomegranate	Punica granatum	29.06±4.13[a]	25.64±3.65[b]	16.76±2.39[c]	43.59±4.20[d]
Sapota	Achras sapota	6.68±2.16[a]	3.01±1.01[b]	5.28±1.24[c]	9.53±3.24[d]
Tomato, ripe	Lycopersicon esculentum	38.65±3.40[a]	34.10±3.00[b]	22.29±1.96[c]	57.974.10[d]

Table 1. Concentration of TPC of commonly consumed Indian Foods (mg/100g) (contd..)

Foods	Scientific Name	GAE	CE	QE	CAE
Spices & Condiments					
Coriander	Coriandrum sativum	40.15±3.25 [a]	35.43±2.87 [b]	23.16±1.87 [c]	60.23±4.87 [d]
Garlic	Allium sativum	58.11±4.33 [a]	51.28±3.82 [b]	33.52±2.50 [c]	87.17±4.49 [d]
Green Chili	Capsicum annuum	56.46±3.64 [a]	49.81±3.21 [b]	32.57±2.10 [c]	84.69±4.46 [d]
Red Chili	Capsicum annuum	426.63±2.38 [a]	376.44±2.10 [b]	246.13±1.37 [c]	639.94±3.56 [d]
Tamarind pulp	Tamarindus indica	37.61±3.35 [a]	33.19±2.96 [b]	21.70±1.93 [c]	56.42±4.03 [d]
Turmeric	Curcuma domestica	1004.31±4.07 [a]	886.16±3.59 [b]	579.41±2.35 [c]	1506.47±4.11 [d]
Nuts, Oil seeds & Edible Oils					
Groundnut	Arachis hypogaea	104.02±4.77 [a]	91.78±4.21 [b]	60.01±2.75 [c]	156.04±4.15 [d]
Groundnut oil	Arachis hypogaea	2.60±1.53 [a]	2.29±1.35 [b]	1.50±0.88 [c]	3.90±2.29 [d]
Mustard seeds	Brassica nigra	47.87±0.78 [a]	42.24±0.69 [b]	27.62±0.45 [c]	71.81±1.17 [d]
Others					
Cane sugar	Saccharum officinarum	29.652±4.23 [a]	26.16±3.73 [b]	17.10±2.44 [c]	44.47±4.34 [d]
Jaggery	Saccharum offinarum	273.46±4.74 [a]	241.28±4.19 [b]	157.76±2.74 [c]	410.19±3.12 [d]

GAE stands for gallic acid equivalents
CE stands for catechin equivalents
QE stands for quercetin equivalents
CAE stands for chlorogenic acid equivalents
Values expressed in fresh matter.
Results expressed as mean ± SD of triplicates
Rows with different alphabets are statistically significant ($p < 0.05$)

These differences have been reported in plums where chlorogenic acid as an equivalent chemical was recommended to determine the total phenolic content of chlorogenic acid-rich plums to predict more accurate total phenolic content than gallic acid, catechin and tannic acid. This method will not allow to analyses of a large number of samples in a limited period. Thus Folin values can be considered as a rough estimate of the antioxidant content because of possible differences in the response of the various antioxidants present in a food extract. Therefore it is essential to make clear that the FC analysis estimates the total phenolic content, whereas chromatographic techniques are more precise to quantify polyphenols as it was free of interferences which are in accordance with other studies.

Fig 1. Total polyphenols and interferences in the different cereals (mg GAE/100g FW)

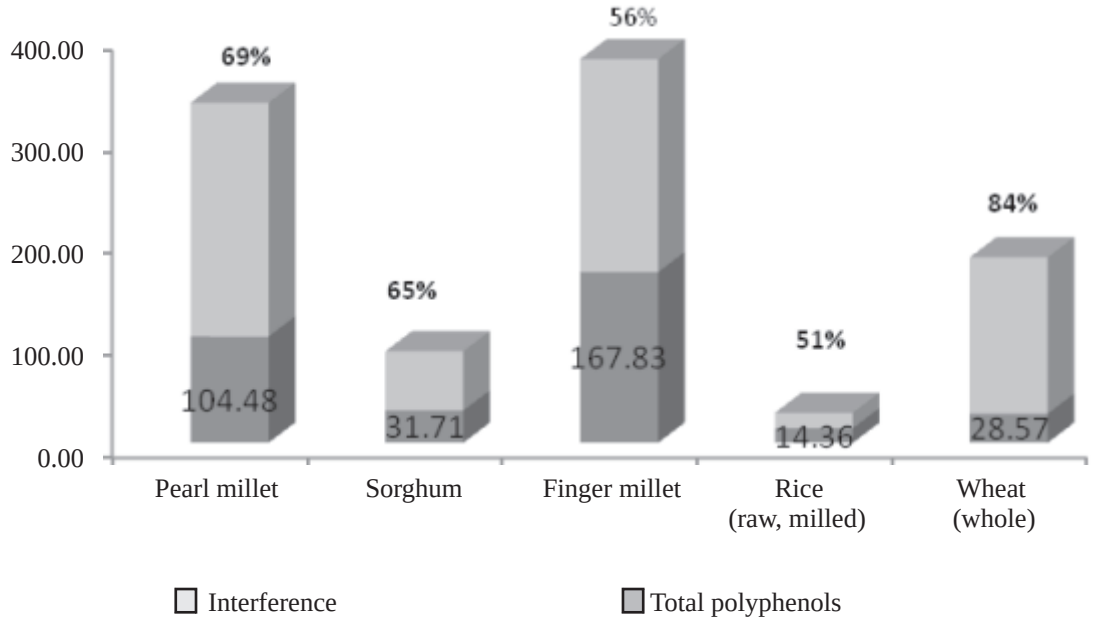
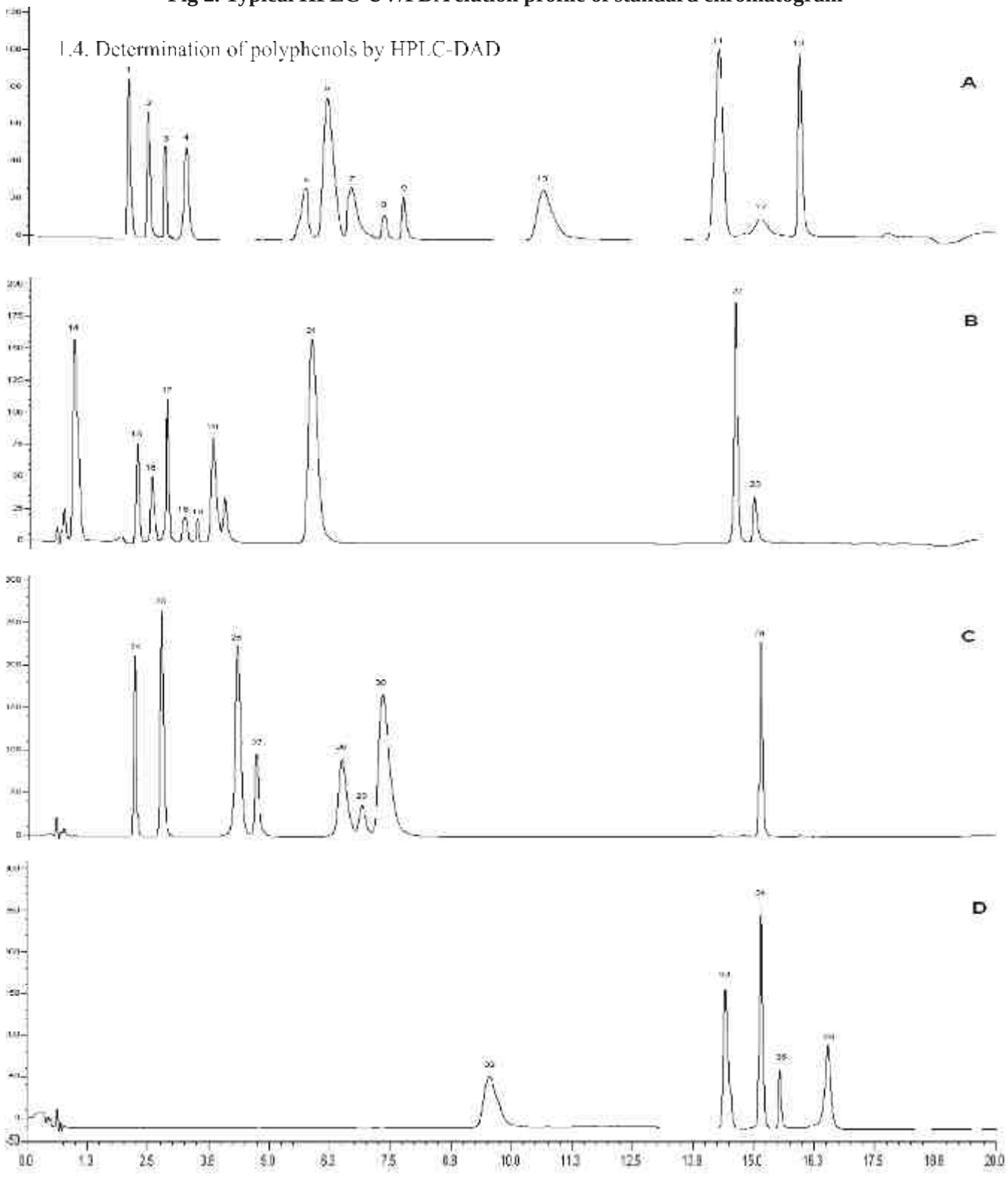


Fig 2. Typical HPLC-UV/PDA elution profile of standard chromatogram



A) 250 nm B) 280 nm C) 320nm D) 370nm. 1.protocatechuic acid 2. 4-OH benzoic acid 3.vanillic acid 4. 3-OH benzaldehyde 5. luteolin-7-O-glucoside 6. quercetin-3-β-D-galactoside 7. quercetin-3-O-rutinoside 8. ellagic acid 9. quercetin-3-O-glucoside 10. daidzein 11. luteolin 12. genistein 13. flavone 14. gallic acid 15. epigallocatechin 16.catechin 17. epigallocatechin gallate 18. syringic acid 19. epicatechin 20. epicatechin gallate 21. O-coumaric acid 22. Naringenin 23. hesperetin 24. chlorogenic acid 25. caffeic acid 26. p-coumaric acid 27. ferulic acid 28. sinapic acid 29. isovitexin 30. resveratrol 31. apigenin 32. myricetin 33. quercetin 34. kaempferol 35. isorhamnetin 36.curcumin

Polyphenols were determined using HPLC. Cereals and millets such as pearl millet, sorghum, finger millet, rice and wheat contained simple polyphenols. Leafy vegetables such as amaranth (tender), coriander leaves, curry leaves, fenugreek leaves, mint and spinach leaves contains glycosides of flavones and flavonols. On the other hand, isoflavones such as genistein, daidzein present only in soya bean, flavanones in citrus, and catechins in apple and pomegranate. Curcumin is present only in turmeric, whereas in mustard seeds, jaggery and cane sugar contain simple polyphenols. This study demonstrates that the simple polyphenols are widespread among the plant foodstuffs and they have a higher antioxidant potency to prevent oxidative damage in cellular level.

Table 2. Individual polyphenolic concentration of commonly consumed foods (mg/100g FW)

Cereals & Millets

	Pearl millet	Sorghum	Finger millet	Rice, raw	Wheat
4-OH benzoic acid	0.31±0.03	nd	0.93±0.46	nd	nd
Apigenin	nd	0.05±0.03	nd	nd	nd
Caffeic acid	0.72±0.09	0.67±0.02	nd	0.44±0.03	0.66±0.02
Chlorogenic acid	nd	0.65±0.03	nd	nd	0.62±0.03
Ferulic acid	0.07±0.02	0.06±0.01	0.43±0.09	0.05±0.03	0.06±0.02
Gallic acid	nd	nd	1.43±0.22	nd	nd
Kaempferol	nd	0.08±0.01	nd	nd	nd
P-Coumaric acid	0.05±0.03	0.04±0.01	nd	nd	nd
Protocatechuic acid	0.14±0.02	0.12±0.01	1.21±0.36	nd	nd
Quercetin	nd	0.06±0.03	nd	nd	nd
Syringic acid	nd	nd	5.65±0.11	nd	nd
Vanillic acid	0.28±0.03	0.26±0.02	0.94±0.28	0.19±0.05	0.11±0.02

Pulses & Legumes

	Bengal Gram dhal	Bengal Gram (whole)	Green Gram Dhal	Red gram Dhal	Soya Bean
3,4, dihydroxy benzoic acid	nd	nd	nd	nd	1.46±0.01
4-OH benzoic acid	nd	nd	nd	0.12±0.03	nd
Apigenin	nd	nd	nd	nd	1.78±0.01
Caffeic acid	nd	nd	nd	nd	5.89±0.01
Catechin	nd	0.82±0.43	nd	nd	nd
Chlorogenic acid	nd	nd	nd	nd	2.53±0.01
Daidzein	nd	nd	nd	nd	1.76±0.01
Epicatechin	nd	3.23±0.56	nd	nd	nd
Epigallocatechin	nd	0.94±0.61	nd	nd	nd
Epigallocatechingallate	nd	4.36±0.69	nd	nd	nd
Ferulic acid	nd	5.87±0.50	nd	nd	nd
Gallic acid	0.51±0.02	nd	0.52±0.04	nd	0.29±0.01
Genistein	nd	nd	nd	nd	2.19±0.01
Kaempferol	nd	nd	nd	nd	1.71±0.01
P-Coumaric acid	nd	nd	0.06±0.03	nd	0.08±0.01
Protocatechuic acid	0.14±0.02	nd	0.15±0.03	0.18±0.05	nd
Syringic acid	nd	5.97±0.28	nd	nd	nd
Quercetin	nd	nd	nd	nd	1.48±0.01
Vanillic acid	0.15±0.04	nd	nd	0.22±0.04	0.42±0.01

Table 2. Individual polyphenolic concentration of commonly consumed foods (mg/100g FW) (contd..)

Vegetables

	Bitter gourd	Brinjal	Cucumber	French beans	Ladies Finger	Little gourd
4-OH benzoic acid	nd	0.16±0.10	nd	nd	nd	nd
Apigenin	nd	nd	0.83±0.01	nd	nd	nd
Caffeic acid	nd	0.61±0.04	nd	nd	nd	0.28±0.04
Catechin	0.63±0.08	nd	nd	0.69±0.07	nd	nd
Chlorogenic acid	0.23±0.05	nd	nd	nd	nd	0.28±0.06
Epicatechin	0.49±0.07	nd	nd	0.50±0.05	nd	nd
Ferulic acid	nd	0.06±0.03	nd	nd	nd	0.08±0.07
Gallic acid	0.19±0.07	0.46±0.09	nd	nd	nd	0.22±0.07
Kaempferol	nd	nd	0.10±0.01	nd	0.09±0.05	nd
Luteolin	nd	nd	0.17±0.01	nd	nd	nd
Myricetin	nd	nd	nd	nd	0.89±0.01	nd
p-coumaric acid	0.08±0.07	0.07±0.05	nd	nd	nd	nd
Protocatechuic acid	0.39±0.32	0.13±0.05	nd	nd	nd	0.11±0.07
Quercetin	nd	nd	0.08±0.01	0.08±0.07	0.05±0.03	0.06±0.04
Quercetin -3 Beta –D Glucoside	nd	nd	nd	nd	5.48±0.04	nd

Leafy vegetables

	Amaranth tender	Coriander Leaves	Curry leaves	Fenugreek leaves	Mint	Spinach
Caffeic acid	0.20±0.01	0.43±0.06	0.37±0.41	0.65±0.06	1.14±0.19	nd
Chlorogenic acid	0.84±0.01	0.39±0.04	0.84±0.07	nd	0.63±0.43	nd
Ferulic acid	0.16±0.03	0.08±0.05	0.12±0.07	0.08±0.04	0.10±0.05	nd
Gallic acid	0.42±0.01	0.28±0.04	0.58±0.03	0.45±0.06	0.79±0.17	0.27±0.06
Kaempferol	0.14±0.01	1.16±0.29	nd	nd	2.31±0.29	nd
Myricetin	nd	nd	1.54±0.15	nd	nd	nd
p-coumaric acid	0.06±0.01	nd	nd	nd	0.08±0.04	nd
Protocatechuic acid	nd	0.14±0.08	0.19±0.05	0.14±0.05	nd	0.11±0.06
Quercetin	0.07±0.01	0.09±0.07	nd	0.08±0.05	0.11±0.07	0.05±0.04
Quercetin -3-Beta-D-Glucoside	nd	nd	8.62±0.25	nd	9.68±0.41	3.66±0.38
Quercetin -3-O-Rutinoside	nd	nd	2.19±0.17	nd	2.47±0.23	nd
Vanillic acid	0.09±0.02	0.19±0.05	nd	nd	nd	nd

Roots & Tubers

	Beetroot	Carrot	Onion	Potato	Radish
4-OH benzoic acid	nd	0.09±0.06	nd	nd	nd
Caffeic acid	0.79±0.05	0.27±0.06	nd	0.60±0.04	nd
Caffeic acid	nd	nd	nd	nd	nd
Chlorogenic acid	0.70±0.05	0.24±0.05	nd	0.58±0.06	0.20±0.04
Ferulic acid	0.08±0.04	nd	nd	nd	0.05±0.04
Gallic acid	0.55±0.08	nd	nd	nd	0.15±0.03
P-Coumaric acid	0.09±0.06	nd	nd	nd	nd
Quercetin	nd	nd	0.06±0.03	nd	nd
Quercetin -3-Beta-D-Glucoside	nd	nd	5.53±0.26	nd	nd

Table 2. Individual polyphenolic concentration of commonly consumed foods (mg/100g FW) (contd..)

Fruits

Polyphenols	Apple	Banana (ripe)	Guava (country)	Indian Gooseberry	Lemon	Orange	Papaya (ripe)	Pine apple	Pomegranate	Sapota	Tomato (ripe)
Caffeic acid	0.21±0.04	nd	nd	0.52±0.12	0.13± 0.11	nd	nd	0.25± 0.08	nd	nd	0.89± 0.37
Catechin	0.56±0.10	1.13± 0.07	0.43± 0.08	nd	nd	nd	0.63± 0.05	0.66± 0.15	1.81± 0.17	1.44± 0.13	nd
Chlorogenic acid	nd	nd	0.17± 0.05	0.46±0.10	nd	nd	0.24± 0.06	0.25± 0.09	1.13± 0.11	0.34± 0.07	0.57± 0.11
Ellagic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.38±0.26
Epicatechin	0.43±0.08	0.84± 0.06	nd	nd	nd	nd	nd	0.48± 0.09	0.72±0.11	nd	nd
Epicatechin gallate	3.74±0.48	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Epigallocatechin	0.47±0.10	0.96± 0.09	0.35± 0.07	nd	nd	nd	nd	nd	nd	nd	nd
Ferulic Acid	nd	nd	0.11± 0.09	nd	nd	nd	nd	0.07± 0.06	nd	nd	nd
Gallic Acid	nd	0.29± 0.05	0.13± 0.05	0.34±0.08	nd	nd	nd	0.18± 0.07	nd	0.37± 0.06	nd
Hesperidin	nd	nd	nd	nd	0.70± 0.22	0.30± 0.08	nd	0.47± 0.12	nd	nd	nd
Kaempferol	nd	nd	nd	nd	0.69± 0.10	0.32± 0.05	nd	nd	nd	nd	0.15±0.10
Myricetin	nd	nd	nd	0.85±0.19	nd	nd	nd	nd	nd	nd	nd
Naringenin	nd	nd	nd	nd	1.34± 0.34	0.56±0.11	nd	nd	nd	nd	nd
P-Coumaric acid	nd	nd	nd	0.08±0.07	nd	nd	nd	nd	nd	0.06± 0.04	nd
Protocatechuic acid	0.07±0.04	nd	nd	0.09±0.03	nd	nd	nd	0.14± 0.11	nd	nd	nd
Quercetin	1.60±0.43	nd	nd	0.12±0.11	nd	nd	nd	nd	nd	nd	0.11±0.09
Quercetin -3-beta-D-glucoside	nd	nd	nd	4.53±0.48	nd	nd	nd	nd	nd	nd	1.39±0.14
Quercetin -3-O-rutinoside	nd	nd	nd	1.26±0.30	nd	nd	nd	nd	nd	1.52±0.35	nd
Resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	30.35± 0.80	nd	nd
Vanillic Acid	nd	nd	0.12± 0.07	nd	nd	nd	nd	0.15± 0.11	nd	nd	nd

Table 2. Individual polyphenolic concentration of commonly consumed foods (mg/100g FW) (contd..)

Spices & Condiments

	Coriander	Garlic	Green Chili	Red Chili	Tamarind pulp	Turmeric
4-OH benzoic acid	0.18±0.06	0.16±0.08	nd	nd	nd	nd
Apigenin	nd	nd	0.41±0.62	nd	nd	nd
Caffeic acid	nd	0.75±0.58	nd	nd	nd	nd
Curcumin	nd	nd	nd	nd	nd	1.45±0.13
Ferulic acid	nd	0.42±0.62	nd	nd	nd	nd
Luteolin	nd	nd	0.43±0.65	0.09±0.05	nd	nd
Naringenin	nd	nd	nd	nd	1.87±0.11	nd
Quercetin	0.09±0.05	nd	0.40±0.62	0.09±0.07	nd	nd

Nut, Oil & Edible oil

	Groundnut	Groundnut oil	Mustard seeds
4-OH benzoic acid	0.10±0.06	0.01±0.02	12.49±0.04
Caffeic acid	0.44±0.08	0.05±0.02	4.2±0.05
Chlorogenic acid	nd	0.09±0.02	nd
Ferulic acid	0.10±0.08	nd	0.14±0.01
Gallic acid	nd	0.07±0.01	11.31±0.08
Naringenin	1.54±0.26	nd	nd
P-Coumaric acid	0.06±0.05	nd	0.71±0.06
Protocatechuic acid	nd	0.02±0.01	0.19±0.04
Sinapic acid	nd	nd	0.42±0.01
Vanillic acid	0.18±0.05	0.06±0.01	2.96±0.03

Miscellaneous foods

	Jaggery	Cane sugar
4-OH benzoic acid	0.09±0.01	nd
Ferulic acid	nd	0.04±0.01
Gallic acid	nd	3.7±0.02
Protocatechuic acid	0.09±0.01	2.3±0.01
Sinapic acid	nd	0.07±0.01
Vanillic acid	0.03±0.01	nd

nd: not detectable
Values expressed in fresh matter.
Results expressed as mean±SD of triplicates

2. Processing and storage effects on total and individual polyphenolic content of selected foods

I. Effects of soaking and germination on total polyphenols content in the commonly consumed millets and legumes

Germination is a natural process occurring during growth period of seeds in which they meet the minimum condition for growth and development. During this period, reserve materials are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo. Germination induces an increase in free limiting amino acids with modified functional properties of seed components. The commonly consumed millets and legumes were washed in distilled water. They were then germinated for 0, 24, 48 and 72 h.

Table 3 shows zero hour total polyphenolic content of commonly consumed millets and legumes. Among these red gram whole contains high phenolic content (431.32±2.91) followed by Green gram whole (395.76±2.16), Black gram whole (372.01±2.84), Rajmah (305.87±2.72), Horse gram whole (297.76±1.51), Bengal gram whole (251.52±2.77), Bajra (235.15±1.42) and the lesser content was observed in Ragi (161.84±1.37).Overnight soaking (0-hr germination) of legumes and millets in water brought about a significant reduction in total polyphenols content. In addition increasing the germination period to 24, 48, and 72 hrs had further decreasing effect on the total polyphenols content (Fig 3).

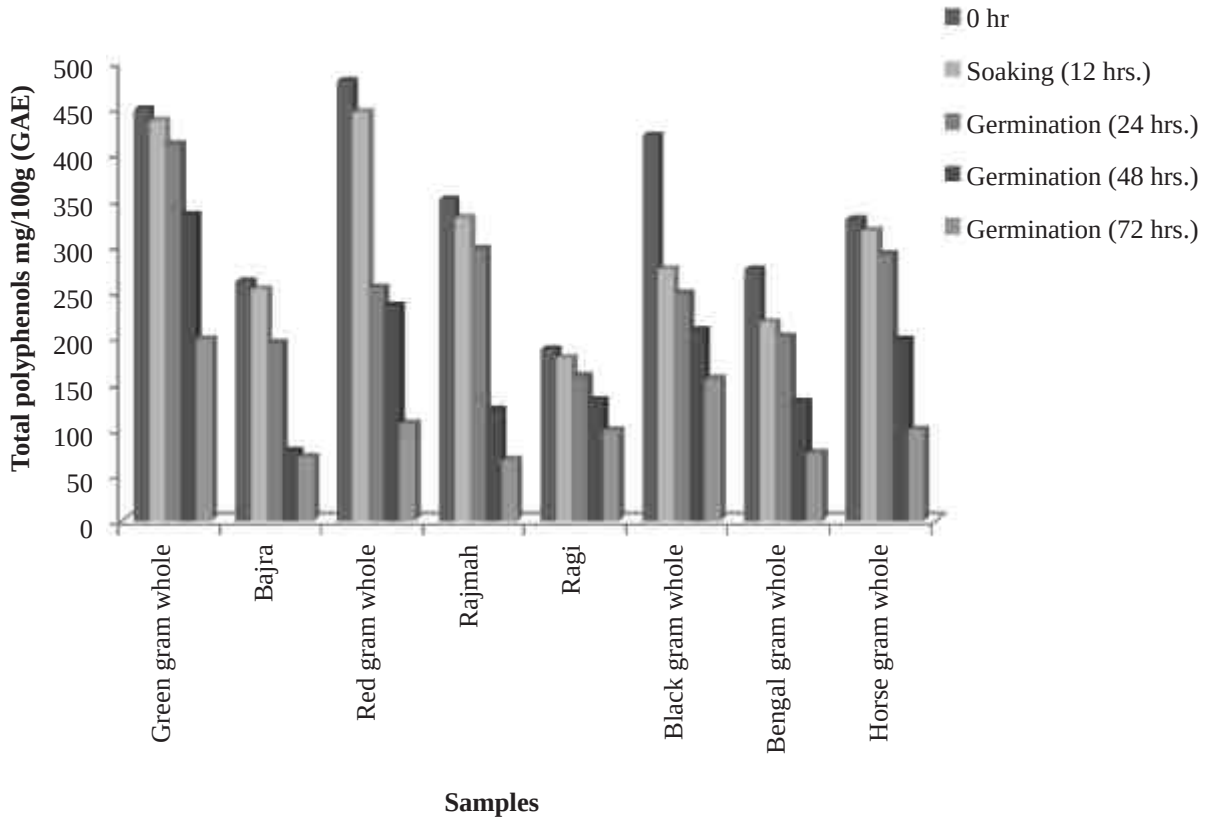
It can be observed from the above table that the soaking (12 hrs) process can reduce the total polyphenolic content from 20 - 50% among the millets and legumes were studied. The maximum reduction (50%) of total polyphenols was observed in green gram whole which is 395.76±2.16 to 205.4±3.57. The minimum reduction of polyphenolic contents were observed in bajra (22%) and ragi (20%) as 235.15±1.42 to 184.43±3.44, 161.84±1.37 to 128.97±1.73 respectively.

Table 3. Effects of soaking and germination on total polyphenols content in the commonly consumed millets and legumes

Samples	0 hr	Soaking (12 hrs)	Germination (24 hrs)	Germination (48hrs)	Germination (72 hrs)
Green Gram whole *(phaseolus aureus Roxb.)*	395.76±2.16 (447.99) [11.66]	205.4±3.57 (435.72) [52.86]	170.14±2.80 (410.17) [58.52]	121.86±4.38 (332.76) [63.38]	72.67±2.99 (197.84) [63.27]
Bajra *(Pennisetum tyhoideum)*	235.15±1.42 (260.78) [9.83]	184.43±3.44 (252.19) [26.87]	122.25±1.92 (193.46) [36.81]	41.23±4.19 (75.84) [45.64]	38.65±2.44 (69.66) [44.52]
Red Gram whole *(Cajanus cajan)*	431.32±2.91 (479.45) [10.04]	258.21±2.09 (445.49) [42.04]	166.73±2.11 (253.85) [34.32]	132.64±3.05 (234.67) [43.48]	43.58±4.21 (106.05) [58.91]
Rajmah *(Phaseolus vulgaris)*	305.87±2.72 (349.92) [2.59]	184.06±2.26 (329.91) [44.21]	126.92±2.03 (296.05) [57.13]	53.65±1.87 (120.77) [55.58]	30.57±3.81 (66.12)[53.77]
Ragi *(Eleusine Coracana)*	161.84±1.37 (186.99) [13.45]	128.97±1.73 (177.20) [27.22]	101.36±2.54 (157.70) [35.73]	81.92±1.51 (131.66) [37.78]	57.8±4.59 (98.60)[41.38]
Black Gram whole *(Phaseolus mungo Roxb.)*	372.01±2.84 (419.35) [11.29]	152.93±3.38 (274.41) [44.27]	121.95±1.36 (247.51) [50.73]	97.64±1.24 (207.56) [50.96]	66.69±4.72 (154.69) [56.89]
Bengal Gram whole *(Cicer aritinum)*	251.52±2.77 (273.65) [8.09]	107.61±3.42 (216.21) [50.23]	90.68±3.01 (200.48) [54.77]	54.43±3.62 (128.88) [57.57]	31.68±4.12 (73.64)[56.98]
Horse Gram whole *(Dolichos biflorus)*	297.76±1.51 (328.58) [9.38]	223.35±2.09 (315.95) [29.31]	158.01±1.76 (290.45) [45.60]	81.49±2.79 (196.78) [58.59]	40.02±2.91 (99.47)[59.77]

Values are mean ± SD of five determinations on fresh weight basis
Values in parenthesis ( ) are in dry weight basis
Values in parenthesis [ ] are moisture content of the sample

Fig 3. Total Polyphenols Content changes during Soaking & Germination of Millets and Legumes (GAE mg/100g) (DW)



In the 24 h germination process, red gram whole was decreased with its total polyphenolic content from its initial zero hr total phenolic content (431.32 ± 2.91) to (166.73 ± 2.11). And further decreasing was observed in 48 hrs and 72 hrs germination as (132.64 ± 3.05), (43.58 ± 4.21) respectively. In the case of 24, 48 and 72hr germination process in the green gram whole, the total phenolic content was decreased to (170.14 ± 2.80), 48 h (121.86 ± 4.38), 72 h (72.67 ± 2.99) from the zero hour non germinated phenolic content (395.76 ± 2.16). From the above table, it is evident that the total phenolic content has been decreased significantly from its zero hr to 72 hrs during the germination process in all of the millets and legumes.

The observed reduction in the polyphenols particularly after germination was a result of formation of hydrophobic association of tannins with seed proteins and enzymes. In addition, loss of tannins during germination also may be due to the leaching of tannins into the water and binding of polyphenols with other organic substances such as carbohydrate or protein. Apart from that, during the period of soaking prior to germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols. Overall this study presented here that the soaking and germination process were shown to reduce the total polyphenols content in the millets and legumes. However, it is interesting to see the individual polyphenols retention during soaking and germination period.

Individual polyphenols in commonly consumed foods

Polyphenol identification was determined and quantified using RP-HPLC analysis comparing the retention time of reference standards. The reference standard mixture is shown in [Fig.2.Chromatograph]. Comparing the retention times of the peaks obtained from the selected soaked, germinated, non germinated 70% acetone extracts with the peaks obtained from the standard mixture, it was possible to identify 22 components: Benzoic acids such as protocatechiuc acid, vanillic acid, gallic acid, 4-OH benzoic acid and cinnamic acids such as p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, sinapic acid and Flavones such as apigenin , luteolin and Flavonols such as kaempferol, quercetin, rutin, myricetin and Isoflavones daidzein, and catechins such as (+)-catechin, (-) gallo catechin, (-) epicatechin, (-) epigallocatechin, (-)epicatechin gallate and epigallo catechin gallate (Table 4).

The amounts of each identified polyphenol were determined by RP - HPLC analysis using standard peak area values obtained by serial dilutions of the polyphenols standard mixture. Data reported in Table 4 shows the presence of phenolic components concentrations in the selected soaked, germinated and non germinated samples. Phenolic compounds such as ferulic acid, protocatechuic acid, p-coumaric acid were commonly present in the selected millets and legumes. Millets such as bajra and ragi were rich in gallic acid and protocatechuic acid. The individual polyphenolic contents are decreasing from non germinated to 72 hr germinated grains. Epigallocatechin is in high in bengal gram and the phenolic compounds concentrations also increasing from 12 hr to 24 hr. Later in 48 and 72 hr phenolic compounds reduction has been observed. Similar trends were observed in the horse gram whole and rajmah. Other than this, whole legumes such as black gram, green gram and red gram whole individual phenolic content also ranges from 0 hr >12 hr <24hr >48 hrs < 72 hr.

Table 4. HPLC quantification of phenolic compounds in soaked, germinated and non-germinated millets and legumes (mg/100g)

Samples	Individual Polyphenols	0 hr	12 hr	24 hr	48 hr	72 hr
Bajra	Caffeic acid	0.81 ±0.09	0.20±0.01	0.17±0.02	0.14±0.03	0.13±0.02
	Ferulic acid	0.92±0.10	0.23±0.01	0.24±0.04	0.16±0.04	0.15±0.02
	P-coumaric acid	0.35±0.04	0.09±0.01	0.09±0.02	0.06±0.01	0.06±0.01
	Protocatechuic acid	2.25±0.24	0.56±0.04	0.59±0.11	0.39±0.09	0.37±0.04
	Vanilic acid	1.81±0.20	0.45±0.03	0.28±0.36	0.31±0.07	0.30±0.04
	4-OH-Benzoic acid	1.44±0.16	0.36±0.02	0.38±0.07	0.25±0.06	0.24±0.03
Ragi	Syringic acid	5.56±0.60	3.76±0.04	3.49±0.15	2.55±0.10	2.41±0.07
	Ferulic acid	0.34±0.04	0.23±0.01	0.21±0.01	0.16±0.01	0.15±0.01
	Gallic acid	1.24±0.13	0.84±0.01	0.78±0.03	0.57±0.02	0.53±0.02
	Protocatechuic acid	0.84±0.09	0.57±0.01	0.53±0.02	0.38±0.02	0.36±0.01
	Vanillic acid	0.68±0.07	0.46±0.02	0.43±0.02	0.31±0.01	0.29±0.01
	4-OH-Benzoic acid	0.54±0.06	0.36±0.04	0.34±0.01	0.25±0.01	0.23±0.01
Bengal gram whole	Epigallo catechin	20.86±0.64	14.26±0.74	14.85±1.73	9.48±1.59	8.11±1.74
	Catechin	0.38±0.01	0.26±0.01	0.27±0.03	0.17±0.03	0.14±0.03
	Epicatechin	2.81±0.09	1.92±0.10	2.00±0.23	1.27±0.21	1.09±0.23
	Syringic acid	5.68±0.17	3.88±0.20	4.05±0.47	2.58±0.43	2.21±0.47
	Epigallo catechin gallate	3.74±0.11	2.56±0.13	2.66±0.31	1.70±0.29	1.45±0.31
	Ferulic acid	5.38±0.16	3.68±0.19	3.83±0.45	2.44±0.41	2.09±0.45
	Epigallo catechin	0.35±0.01	0.24±0.01	0.25±0.03	0.16±0.03	0.13±0.03
Black gram whole	Chlorogenic acid	1.14±0.08	1.23±0.18	1.35±0.22	0.69±0.02	0.82±0.19
	Ferulic acid	0.23±0.02	0.25±0.04	0.28±0.05	0.14±0.02	0.17±0.04
	Sinapic acid	0.07±0.01	0.07±0.01	0.08±0.01	0.04±0.01	0.05±0.01
	Kaempferol	0.41±0.03	0.45±0.07	0.49±0.08	0.25±0.01	0.30±0.07
	Daidzein	0.35±0.03	0.38±0.06	0.41±0.07	0.21±0.02	0.25±0.06
Horse gram whole	Epigallo catechin	13.34±0.26	14.81±0.71	14.63±1.48	8.96±0.43	8.31±0.24
	Syringic acid	3.65±0.09	4.03±0.19	3.99±0.40	2.44±0.12	2.26±0.06
	Catechin	0.29±0.08	0.27±0.01	0.26±0.03	0.16±0.01	0.15±0.01
	Epicatechin	1.83±0.09	1.99±0.10	1.97±0.20	1.20±0.06	1.12±0.03
	Chlorogenic acid	1.11±0.05	1.21±0.06	1.20±0.12	0.73±0.04	0.68±0.02
	Caffeic acid	0.22±0.04	0.22±0.01	0.22±0.02	0.13±0.01	0.12±0.01
	P-coumaric acid	0.10±0.02	0.07±0.01	0.09±0.01	0.05±0.01	0.05±0.01
	Ferulic acid	0.23±0.02	0.25±0.01	0.25±0.03	0.15±0.01	0.14±0.01
	Gallic acid	0.83±0.04	0.90±0.04	0.89±0.09	0.54±0.03	0.50±0.01
	Protocatechuic acid	0.56±0.03	0.61±0.03	0.60±0.06	0.37±0.02	0.34±0.01
	Vanillic acid	0.46±0.03	0.49±0.02	0.49±0.05	0.30±0.01	0.27±0.01

Table 4. HPLC quantification of phenolic compounds in soaked, germinated and non-germinated millets and legumes (mg/100g) (contd..)

Samples	Individual Polyphenols	0 hr	12 hr	24 hr	48 hr	72 hr
Green gram whole	P-coumaric acid	0.08±0.02	0.08±0.01	0.09±0.01	0.05±0.02	0.07±0.01
	Luteolin	0.73±0.16	0.78±0.02	0.89±0.04	0.49±0.15	0.68±0.11
	Myricetin	1.87±0.41	1.98±0.05	2.26±0.09	1.26±0.37	1.74±0.27
	Quercetin	0.05±0.01	0.06±0.01	0.06±0.01	0.03±0.01	0.05±0.01
	Kaempferol	0.36±0.08	0.38±0.01	0.44±0.02	0.24±0.07	0.34±0.05
	Gallic acid	0.74±0.17	0.79±0.02	0.90±0.04	0.50±0.15	0.69±0.11
	Protocatechuic acid	0.50±.11	0.54±0.01	0.61±0.02	0.34±0.10	0.47±0.07
Rajmah	Epigallo catechin	16.21±0.29	15.48±3.73	15.48±1.71	10.10±0.46	9.03±1.79
	Catechin	0.32±0.05	0.28±0.07	0.28±0.03	0.18±0.01	0.16±0.03
	Epicatechin	2.30±0.21	2.08±0.50	2.08±0.23	1.36±0.06	1.21±0.24
	Epigallo catechin gallate	2.91±0.05	2.77±0.67	2.78±0.31	1.81±0.08	1.62±0.32
	Ferulic acid	0.29±0.03	0.26±0.06	0.26±0.03	0.17±0.01	0.15±0.03
	P-coumaric acid	0.13±0.04	0.10±0.02	0.10±0.01	0.06±0.01	0.05±0.01
	Quercetin	0.08±0.02	0.07±0.02	0.07±0.01	0.04±0.01	0.04±0.01
	Gallic acid	0.99±0.03	0.94±0.23	0.94±0.10	0.61±0.03	0.55±0.11
	Protocatechuic acid	0.67±0.02	0.64±0.15	0.64±0.07	0.41±0.02	0.37±0.07
Red gram whole	Epigallo catechin	15.83±0.76	13.82±0.69	16.48±2.81	7.30±0.67	8.74±1.49
	Catechin	0.29±0.01	0.25±0.01	0.30±0.05	0.13±0.01	0.16±0.03
	Epicatechin	2.13±0.10	1.86±0.09	2.22±0.38	0.98±0.09	1.17±0.20
	Apigenin	1.47±0.07	1.28±0.06	1.53±0.26	0.67±0.06	0.81±0.14
	Rutin	5.33±0.26	4.65±0.23	5.55±0.95	2.46±0.23	2.94±0.50
	Gallic acid	0.96±0.05	0.84±0.04	1.00±0.17	0.44±0.04	0.53±0.09
	Protocatechuic acid	0.65±0.03	0.57±0.03	0.68±0.12	0.30±0.03	0.36±0.06
	Vanillic acid	0.53±0.03	0.46±0.02	0.55±0.09	0.24±0.02	0.29±0.05
	4-OH - Benzoic acid	0.42±0.02	0.36±0.02	0.44±0.07	0.19±0.02	0.23±0.04

Values are expressed as mean ± SD of five determinations on fresh weight basis

Some studies quoted that germination also a convenient process to enhance polyphenolic contents and related antioxidant activity. Overall, different legume seeds have different responses towards germination. Although some legume seeds belong to the same species, they still have different responses towards germination. Different methods of processing such as dry heating, cooking, roasting, germination and fermentation need not necessarily reduce or completely eliminate polyphenols and phytic acid. The increase in the tannins to catechins ratio could be attributed to the imbibing of seed during the beginning of the germination process.

II. Effect of drying and storage effects of selected green leafy vegetables

Identification and quantification of the polyphenols in the commonly consumed Green leafy vegetables (GLV), using different drying methods helps to improve the processing techniques and limit the loss during the course of industrial processing as well domestic cooking. These techniques may be used to control and optimize the polyphenol content in the regular diet. This study reports the effects of drying methods on polyphenols retention during different storage periods (0, 7, 14, 21, 30, 45, 60 and 90 days) in four different GLVs. The samples namely fenugreek leaves, coriander leaves, mint leaves and curry leaves were selected for the study and dried in different conditions such as shade, solar cabinet and sun, then stored in the air tight containers for the period of 90 days.

Table 5 shows the total polyphenolic content of the different green leafy vegetables during shade, solar cabinet and sun drying from 0 to 90 days. Among the selected green leafy vegetables solar cabinet dried leafy vegetables

shows higher total polyphenolic content followed by shade and sun drying. The first day (0 day) seems to be higher polyphenolic content and it is further reduced from 7th day to 90th days. Among the selected green leafy vegetables, curry leaves polyphenols content was high (989.57 GAE mg/100g) followed by mint leaves (842.40 GAE mg/100g), fenugreek leaves (534.02 GAE mg/100g) and coriander leaves (337.12 GAE mg/100g) on dry weight basis. It has been observed that the total polyphenolic content was reduced significantly from 0 day to 7th day in curry leaves, whereas there was less polyphenol content loss in coriander leaves.

Table 5. Total Phenolic content of different green leafy vegetables during shade, solar cabinet and sun drying from 0, 7, 14, 21, 30, 45, 60 and 90th days (GAE mg/100g) (DW)

Foods	Drying Methods	0	7	14	21	30	45	60	90
Fenugreek leaves	Shade Drying	534.02	310.01	222.92	165.55	123.97	118.63	73.78	57.99
	Solar Cabinet Drying	534.02	327.72	279.20	185.70	170.25	136.44	93.17	71.33
	Sun Drying	534.02	122.65	98.28	81.44	76.59	69.29	56.96	50.75
Coriander leaves	Shade Drying	337.12	263.30	221.35	210.23	173.61	158.00	123.89	71.64
	Solar Cabinet Drying	337.12	320.14	249.50	219.44	195.41	176.65	150.77	127.45
	Sun Drying	337.12	256.18	147.11	139.96	120.93	94.11	68.11	49.33
Mint leaves	Shade Drying	842.4	405.89	244.16	216.83	119.28	104.34	76.10	57.56
	Solar Cabinet Drying	842.4	552.34	406.83	375.79	156.49	124.16	113.96	79.71
	Sun Drying	842.4	245.90	198.95	188.44	44.68	35.14	23.42	13.14
Curry leaves	Shade Drying	989.57	282.27	212.09	169.20	136.46	114.64	91.84	73.20
	Solar Cabinet Drying	989.57	617.01	491.11	380.67	332.11	315.30	270.95	216.59
	Sun Drying	989.57	262.91	149.94	130.73	96.73	72.44	57.09	25.67

Table 6 shows the individual polyphenolic content of the fenugreek leaves during different methods of drying. Individual polyphenols such as caffeic acid, ferulic acid, quercetin, gallic acid and protocatechuic acid were found in fenugreek leaves. Caffeic acid was found to be high in all types of drying followed by gallic acid and protocatechiuc acid. Similar trends were observed in individual phenolic content of coriander, mint and curry leaves during 90 days of storage periods.

Dried Leafy vegetables had an increased shelf life and significantly greater phenol content compared to fresh leaves. It appears that some of the polyphenols in the fresh leaves were destroyed or converted to non-antioxidant forms during the drying process. This study clearly explains that the drying process may result in high or low levels of individual polyphenols depending on the type of phenolic compounds present in the plant material and their location in the plant cell. Dried Leafy vegetables have a greater phenolic content, increased shelf life compared to fresh leaves.

III. Storage Effects of selected Green Leafy Vegetables and Fruits

Fig 4, shows the total polyphenolic content of the five different green leafy vegetables namely spinach, amaranth, coriander, mint and curry leaves. In room temperature the total polyphenolic content was reduced from 0 hr to 48th hr on dry weight basis. Similar trend were observed in refrigerator (4ºC) stored green leafy vegetables. Among these selected vegetables curry leaves were found to be high in total polyphenolic content followed by mint, coriander, spinach and amaranth. In the room temperature, there is 40-50 % of total polyphenolic content from 0 to 24 hrs and further loss were observed in 48 hrs (50 - 60%). In case of refrigerator temperature, it has been observed that there is 30 – 40% of total polyphenolic content loss from 0 hr to 24 hrs and further loss of 40 % in (48hrs) and 50% in (72 hrs).

Table 6. Individual phenolic content of fenugreek leaves during shade, solar cabinet and sun drying from 0,7,14,21,30,45,60 and 90th days (mg/100g) (DW)

Individual Polyphenols	0 day	7th day	14th day	21st day	30th day	45th day	60th day	90th day
Shade drying								
Caffeic Acid	5.78	2.38	2.30	2.23	2.20	2.21	2.17	2.17
Ferulic Acid	0.29	0.15	0.15	0.14	0.14	0.14	0.13	0.13
Quercetin	0.20	0.11	0.11	0.10	0.10	0.10	0.10	0.10
Gallic Acid	3.73	1.55	1.54	1.45	1.45	1.44	1.41	1.41
Protocatechuic Acid	0.88	0.38	0.38	0.35	0.35	0.35	0.34	0.34
Solar Cabinet Drying								
Caffeic Acid	5.78	2.35	2.33	2.31	2.15	2.06	1.97	1.94
Ferulic Acid	0.29	0.14	0.14	0.14	0.13	0.13	0.12	0.12
Quercetin	0.20	0.11	0.11	0.10	0.10	0.09	0.09	0.09
Gallic Acid	3.73	1.53	1.51	1.50	1.40	1.34	1.28	1.26
Protocatechuic Acid	0.88	0.37	0.37	0.36	0.34	0.33	0.31	0.31
Sun Drying								
Caffeic Acid	5.78	2.37	2.32	2.28	2.15	2.07	2.06	1.86
Ferulic Acid	0.29	0.15	0.15	0.14	0.13	0.13	0.13	0.11
Quercetin	0.20	0.11	0.11	0.10	0.10	0.09	0.09	0.08
Gallic Acid	3.73	1.53	1.51	1.48	1.39	1.35	1.34	1.20
Protocatechuic Acid	0.88	0.38	0.37	0.36	0.34	0.33	0.33	0.29

Individual polyphenolic content of the green leafy vegetables shows simple polyphenols such as gallic acid, protocatechuic acid, chlorogenic acid and caffeic acid were found to be common. Gallic acid was found to be high in spinach followed by myricetin. Chlorogenic acid and kaempferol were found to be high in amaranth leaves. Chlorogenic and protocatechuic acid were found to be high in coriander leaves. Luteolin, chlorogenic acid and gallic acid were found to be high in mint leaves and Myricetin and chlorogenic acid was found to be high in curry leaves in 0 hr.

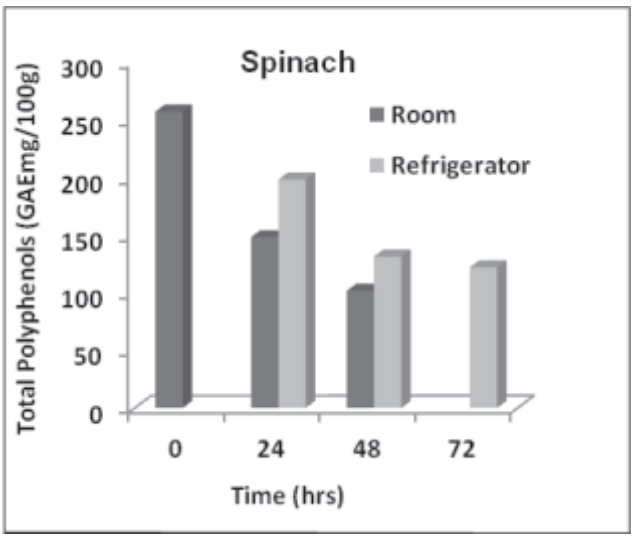
Total polyphenolic content of the selected fruits such as apple, orange, grapes, papaya and pomegranate in room (0, 24, 48 and 72 hr) and refrigerator temperature (0, 24, 48, 72, and 96 hrs) were analyzed. Among the selected fruits grapes was found to be high (206.36 GAE mg/100g) in total polyphenolic content followed by pomegranate (82.41 GAE mg/100g), apple (33.58 GAE mg/100g), orange (25.38 GAE mg/100g) and papaya (17.17 GAE mg/100g).

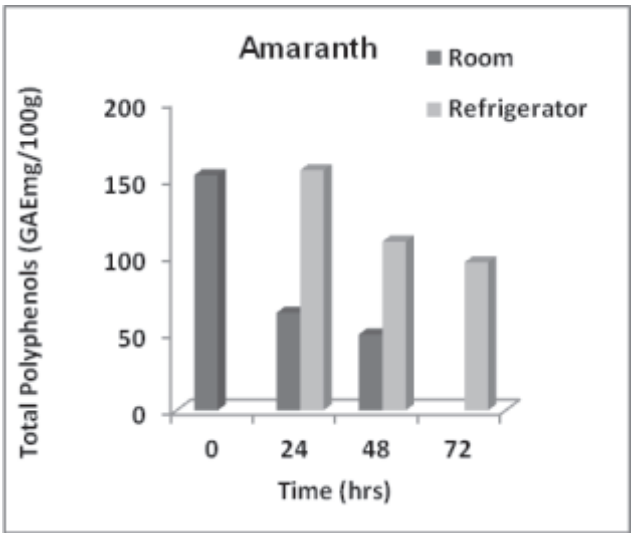
There was very limited loss 2-3 % from 0 to 24, 48 and 72 hr stored fruits in room temperature. In refrigerator temperature the loss were found to be very less (<1%) from 0 to 24 hr. Further 1 -2 % loss were observed from 24 to 48, 72 and 96 hrs.

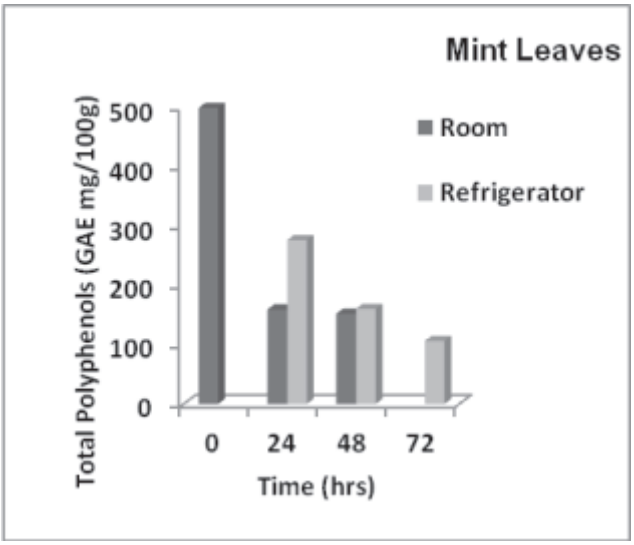
The individual polyphenolic content of the selected fruits in room and refrigerator temperature in different storage points were analyzed. Catechin, epigallocatechin and epicatechin were found to be high in apple. Hespertin and naringenin were found to be high in orange. Catechin, epigallo catechin, epicatechin, myricetin and vanilic acid were found to be high in grapes. Very limited quantity of individual polyphenol namely chlorogenic and ferulic acid were present in papaya. Catechin, epigallo catechin, epicatechin were found to be higher in pomegranate.

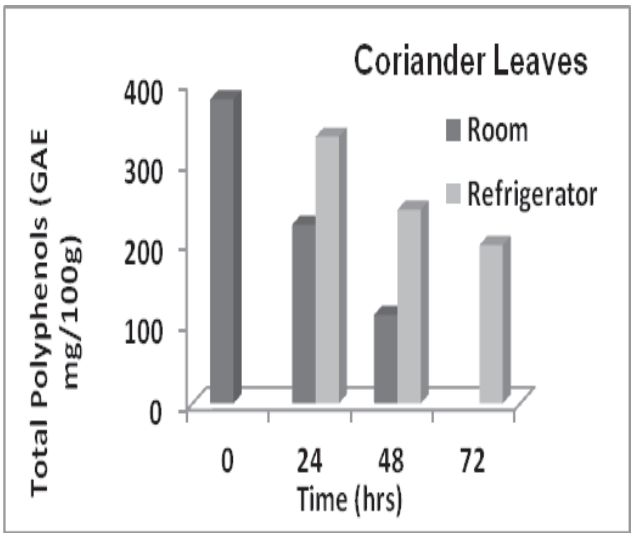
Overall there is no significant loss of individual polyphenols in different storage points either in room or refrigerator temperature. But there is significant difference of individual polyphenolic content, among room and refrigerator temperature stored fruits.

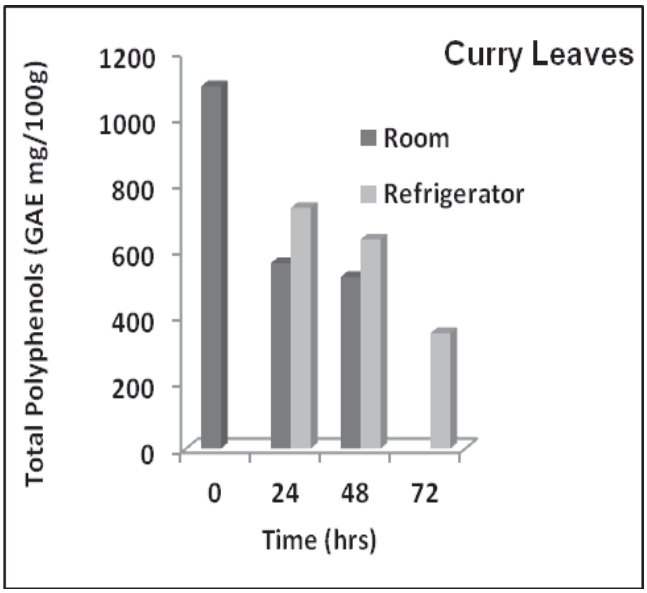
Fig 4. Effect of temperature and storage time on total polyphenols in Commonly consumed green leafy vegetables (GAE mg/100g) (DW)











CONCLUSIONS

This study reports the content of individual phenolic acids and flavonoids in 49 commonly consumed foods. This study proposes RPHPLC with DAD as a suitable method to quantify each polyphenolic compound rather than using Folin- Ciocalteu reagent to determine the total polyphenolic content. Effect of cooking, storage and different drying methods have a great impact on total and individual polyphenolic content of foods. Soaking and germination of the selected millets and legumes reduced the total phenolic content and the individual phenolic content though initially increases (on 24 and 48 hrs) but gradually decreases on 72 hrs germination. The findings from this study suggest that millets and legumes, green leafy vegetables and fruits are rich sources of polyphenols which have been shown as powerful antioxidants. This data is very much useful in assessing the dietary intakes of various polyphenols in Indian diet. It is necessary to look into the bioaccessibility and bioavailability of these molecules in humans to understand the biological functions of polyphenols present in the commonly consumed foods against degenerative diseases.

VII FOOD & DRUG TOXICOLOGY RESEARCH CENTRE

1. SEROTYPING RNA VIRUS TO STUDY MOLECULAR EPIDEMIOLOGY OF DENGUE SUPPLEMENTING EMERGENCY PREPAREDNESS AND CAPACITY BUILDING IN METRO CITIES OF KARNATAKA, INDIA

AIMS AND OBJECTIVES

Spatio-Temporal phylogeny: To understand the speed and direction of viral propagation in DENV outbreak

(i) To study the spread of distinct lineages of genetic diversity and to construct a phylogenetic history of dengue viruses using isolation time as calibration points and to establish its origin, causes, date of introduction, rate evolution pattern and their relation with the pathological indices in densely populated urban settings of the cities of Bangalore and Mangalore in Karnataka, India.

Case Management and clinical outcome

(ii) To study changing transmission pattern with sero-surveillance, structural differences of dengue virus DE lineages (Serotype 2: Asian and American) and their relation to pathogenesis/disease incidence.

Work done during the year

METHODS

This includes: [i] Selection of study sites with demography, [ii] sample collection and Geo-coding [iii] *Pert-chart* compliance and [iv] monitoring and evaluation indicators (as per IHA, WHO: 2005). The satellite coordinates of Geo-coding data vis-à-vis respective residential address, names, PIN, hospital codes were incorporated in blood sample collections sheet of individual patients as they all are reflecting the endemic phenomena of an epidemic infestation.

In Bangalore, out of 67 blood samples collected during active phase of infestation, 15 discarded, 31 samples showed +ve with NSI test but these samples did not yield any result in PCR test. Rest 21 samples were again undertaken for PCR with conventional primers whereas 10 samples exhibited DENV serotypes S1- 3, DENV serotype S2- 5, DENV serotype S3- 2, with type-specific primers whereas in other samples yielded non-specific presence of serotype mixtures with 11 collections. These were DENV serotype S1 + S3- 2, S2 + S3- 2, S2 + S4- 7.

Virus strains and serum samples: Virus seeds for the four dengue serotypes (DENV-1, DENV-2, DENV-3, andDENV-4) were obtained from the virus stock of National Institute of Virology, Bangalore. Serum samples from 75 patients were received from National Institute of Nutrition, Hyderabad for serotyping by employing a conventional reverse transcriptase-polymerase chain reaction (RT-PCR).

RNA Extraction: The seeds samples were subjected to RNA extraction by using the Qiagen RNA extraction kit (cat#52906) by using 140 micro liters of the samples as per the kit manufacture's protocol.

Detection of dengue infection: The detection of dengue virus infection was carried by using the extracted RNA from the serum samples in a RT-PCR assay. The RNA extracted from the virus strins was used as positive controls for the assay. The RNA extracts served as templates in a one-step RT-PCR kit protocol from life Technologies (cat#12574-026). The oliginucleotide priners sequences D1 and D2 shown in table1were used for the detection. The PCR involved the following steps: Denaturation (94^{o}C, 30sec), primer annealing (55^{o}C,1min) and extension (72^{o}C, 2min). The amplified products were electrophoresed on a 2% agarose gel with ethidium bromide with 100bp ladder

(Bangalore Genie) as a molecular weight marker. Samples that were positive for dengue virus showed a DNA 511bp product.

Typing of dengue virus: Serotyping by the one-step RT-PCR was performed for all the samples that were positive by the dengue virus detection PCR. The components and PCR conditions were similar to the above mentioned PCR except that the D2 primer was replaced by the serotyping-specific primers TS1,TS2,TS3andTS4 that are shown in the table 1.the serotypes were identified based on the size of the final DNA product that is different and specific for each DENV serotype in a 2% agarose gel with ethidium bromide. The final DNA product sizes for each serotypes are shown in Table 1.

Table 1. Primers used to detect and type dengue virus from serum samples

Primer	Sequence	Genome position	Size in BP of DNA product
D1	5'-TCAATATGCTGAAACGCGCGAGAAACCG - 3'	134-161	511
D2	5'-TTGCACCAACAGTCAATGTCTTCAGGTTC -3'	614-644	511
TS1	5'-CGTCTCAGTGATCCGGGGG -3'	568-586	482(D1+TS1)
TS2	5'-CGCCACAAGGGCCATGAACAG -3'	232-252	119(D1+TS2)
TS3	5'-TAACATCATCATGAGACAGAGC -3'	400-421	290(D1+TS3)
TS4	5'-CTCTGTTGTCTTAAACAAGAGA -3'	506-527	392(D1+TS4)

Sequencing of Dengue virus serotypes: Three of the serum samples W6, B2 and M11 that were positive for DENV-1, DENV-2 and DENV-3 respectively were subjected to sequencing (Amnion Biosciences) and the results are summarized in Table 2.

Table 2. Sequencing results for three DENV Serotypes

Samples Tested	Sequence	Accession Number and subtype match
W6	AATCGTAGTACGCAGCAGCGGGGCCACACCGGG GGAAGCTGTACCTTG GTGGTAAGGAATACAATGGCCCCCAGGCACCTA ACACAGGACATTAGG ACCCGGAGGCAACAACCCGGGGGG	Dengue virus 1 Isolate D1/IN/RGCB294/2007 Sequence ID:gb|JN903578.1|
B2	GGCTGCTGCGCGCACATGCGGGGCGCAGGCGAG ATGAAGCTGCAGTCTC GCTGGAAGGACTAGAGGTCAGAGGAGACCCCCC CGAACCAAAAGACGC ATATTGACGCGGGGAAACCCCAAAAATCCTGCTG TCTCCCCACCATCATT CCAGGCACAAAAGGCCAGCACGCAATGGGGCGC ATCTGCGGTTCCTTTTT ACCGAGCGGTGTATTGCGCACGCA	Dengue virus 2 StrainThNH-P12/93 Sequence ID: gb|AF022438.1|
M11	CTAACCGGGCAGTAGACAGCGGGGCCGAGCACT GAGGGAGCTGTACCTC CTTGCAAAGGACTAGAGGTTAGAGGAGACCCCC CGCAAACAAAAACAGCA TATTGAAGCTGGGAGAGACCAGAGATCCTGCTGT CTCCTCAGCATCATTCC AGGCACAGAACGCAA	Dengue virus 3 Isolate 13GDZDVS30E Sequence ID: gb|KF954949.1|

RESULTS AND DISCUSSIONS

The genus flavivirus includes 53 arthropods borne viruses that cause severe encephalitis fever and fibroid illness in humans. Dengue virus (DENV), belongs to this genus are important public health problem in tropical and sub tropical countries including India. Nearly half a million cases of dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS) are estimated to be hospitalized throughout tropical and sub tropical regions.

In the present study, we collected serum samples from several (n=75) suspected dengue cases reported from Bangalore, India and the samples were serotyped using an RT-PCR-based method. In the present study we were able to characterize a very low number of suspected dengue samples (14.6%; 11 out of 75 samples) at the molecular level. This could be probably explained by the fact that the majority of samples were collected from suspected dengue virus infected patients in post-viremic phase. In the post-viremic phase viral load is considerably lower, leading to lower rate of viral isolation and subsequent conversion into c-DNA by reverse transcription.

It appears that, there is a certain pattern of evolution of dengue serotypes across India, where Northern and Eastern regions are dominated serovar DENV-2 whereas Sothern regions are dominated by serotype DENV-3. This can be attributed by the evolutionary pressure due to differences in its vector genotypes as well as MHC haplotype distribution in divergent ethnic groups in these regions. More detailed data regarding the dengue serotype distribution pattern is required to understand evolutionary patterns and virulence genotypes of the dengue virus in India.

CONCLUSION

As per our epidemiological records it is revealed that because of the absence of susceptible host, the incidence started late. In urban/metro areas of eastern part of Bangalore there was lack of dengue virus cases. This is because of the lack of susceptible host subjects in the area for the considerable period of time. Therefore it is a paramount importance to address in greater detail whether differences in viral dispersion is associated precisely with the viral fitness, strain competition and ultimately associated with the increase of disease severity using Geographic Information System (GIS) an urban and heterogeneous metro cities of Bangalore and Manglore. Present pilot study helped use correlate preseily the areas where the infections took place and to understand the particularities of outbreak and also the factor helped developing transmission.

2. EMPOWERMENT OF FARM WOMEN IN MITIGATING THE PESTICIDE RESIDUES AT THE FARM AND HOUSEHOLD LEVELS

AIMS AND OBJECTIVES

I) To assess the extent of contamination in three selected vegetables (most commonly consumed) viz., brinjal, tomato and okra (where in the incidence of pesticides residues is very high) and three selected fruits viz., grapes, guava and pomegranate (for which extensive pesticide spraying is in operation) from IPM & non-IPM farms.

ii) To identify and promote the ideal household processing methods to minimize the residues in the selected vegetables and fruits without altering the quality/quantity of content of nutrients.

iii) To educate the farm women/home makers on the Good Household Practices (GHPs).

iv) To assess the impact of education on farm women/home makers on the reduction/minimization of residues levels in selected vegetables and fruits.

Work done during the year

Nine villages (5 – IPM and 4 – Non-IPM) which were identified in Ranga Reddy district where in major cultivation was vegetables and fruits. About 300 samples of vegetables viz., brinjal, tomato, okra and fruits such as

guava, pomegranate, and grapes were collected from these identified villages to assess the extent of contamination with pesticide residues and also to assess after subjecting to different methods of household processing and effect of processing if any on micro-nutrients. The samples were subjected for different household methods of processing viz., 1) Raw, 2) After soaking in distilled water, 3) After rubbing and soaking in distilled water and 4) After soaking in 2% sodium chloride solution. Samples were analyzed for pesticide residues using approved (QuEChERS Quick, Easy, Cheap, Effective, Rugged and Safe - AOAC, 2007)) method which were standardized and validated in the laboratory using GC/GC-MS/LC-MS/MS. They were analysed for the extent of contamination with pesticide residues viz., pesticide residues viz., Dimethoate, Chloropyriphos, Ethion, Paraquat-di-chloride, Carbendazim, Benomyl, Quinalphos and Cypermethrin and the quantity of micro nutrients viz., calcium, magnesium, potassium, phosphorous, sodium, copper, manganese and zinc. Micro-nutrient levels in the samples were analyzed using ICP-MS. The analysis revealed that a significant reduction was observed in the pesticide residue concentrations when the vegetable/fruit samples were rubbed and soaked in distilled water. No such change was seen in the micronutrient levels.

Data analysed for the identified farm women on KAP studies in these villages revealed that 25.2% farm women of IPM and 28.6% of Non-IPM said that they were aware about the pesticide residues while 55.7% of IPM and 66.1% of Non-IPM said that they were not aware about the residues. 10% farm women of IPM and 3.6% of Non-IPM have mentioned that they have undergone training for the agricultural/farming activities in Krishi Vignan Kendra. 90% IPM farm women and 96.4% Non-IPM farm women said that they have not taken any such training. Only 2.9% farm women of IPM who have undergone training have received IEC Material.

As a part of educational activity carried among the farmers and farm women in these villages, a video film was developed which dealt with the precautionary measures that are to be adopted while engaged in the farming activities and also the good household practices which are to be followed while cleaning/cooking the vegetables to reduce the residues and other contaminants in vegetables/fruits. Further, a cost effective, re-usable protective device (apron/coat, head gear, gloves and shoes) using the available material such as empty urea bags was developed so as to protect themselves from the adverse health effects due to exposure.

3. QUANTITATIVE DETECTION OF HEAVY METALS AND PHTHALATES IN TOYS

The history of toys is as old as the history of human civilization. Toy is an object for children to play and learn about themselves and their world. In the recent past, a number of reports on the presence of heavy metals and phthalates in toy samples had appeared from all over the globe. In India too, an organization called "Toxics Link" had published a report entitled "Toying with toxics – an investigation of lead and cadmium in soft toys in three cities in India" in August, 2006. This study had stated that the toy samples collected from Mumbai, Delhi and Chennai had the presence of Lead and Cadmium.

Origin of proposal*:* In 2008, The Honorable Bombay High Court had received a public interest litigation filed by Consumer Welfare Association (PIL No. 79/2007) alleging high levels of heavy metals found in toys sold in India and seeking action against the sale of toxic toys in India. Based on this, Ministry of Health and Family Welfare, Government of India [vide No.C.30011/10/08-HR, dated 12th January, 2009] had constituted an expert committee to conduct a study to assess the harmful level of certain chemicals used in the manufacture of toys. The expert committee deliberated on the issue in detail in its meeting held at ICMR head quarters in New Delhi. It then identified two national institutes namely National Institute of Nutrition (NIN), Hyderabad and National Institute of Occupational Health (NIOH), Ahmedabad possessing the requisite infrastructure and capabilities along with proven record to undertake a national level study in estimating the harmful chemicals and heavy metals in the toys sold in different party of the country . In order to validate and maintain quality control, All India Institute of Medical Sciences (AIIMS), New Delhi (a non-ICMR institution) was given the responsibility of monitoring and coordination.

OBJECTIVES

- To determine the levels of heavy metals namely Lead(Pb), Cadmium(Cd), Chromium(Cr), Arsenic(As) and Mercury(Hg) in toy samples obtained from four different geographical regions (North, South, East and West) of India.
- To estimate the phthalates Di butyl phthalate (DBP), Benzyl butyl phthalate (BBP), Di-ethyl hexyl phthalate (DEHP), Di–n– octyl phthalate (DNOP), and Di-isononyl phthalate (DINP) content in the sampled toys.
- To compare the level of heavy metals and phthalates in toy samples collected from rural as well as urban areas and between the three different categories of toys -local, unbranded and branded.
- To provide recommendations based on the study findings.

METHODOLOGY

The methodology for the study was finalized acknowledging the limitations and caveats as mentioned in the annexure. A standard methodology was developed following international norms to analyze the toy samples for metals and phthalates using Atomic Absorption Spectrophotometer (AAS), Inductively Coupled Plasma-Mass Spectrophotometer (ICP-MS) and Gas Chromatography-Mass Spectrophotometer (GC-MS). The analytical methodology for estimation of Lead and Cadmium in coded samples had been validated with inter-laboratory variations of 30% between seven laboratories (five Government and two private laboratories).

STUDY DESIGN

Sample size: The sample size was calculated as 2560 (160 in each category) based on thirty percent of toys had heavy metals (Pb and Cd) more than the prescribed toxic limits, with 95 percent confidence interval, 10 percent error and a design effect of 2. The samples were collected from urban and rural areas and were grouped under branded, unbranded and local categories based on specifically framed 12point criteria.

Estimation of heavy metals and phthalates: The accessories (clothes, ornaments, hair *etc*.) from the toy samples were removed. The toys were cut into small pieces and grinded using cutting mill (Retsch SM100C) to get the particle size of 0.5 mm or smaller. The grinded samples were stored in labeled bottles for analysis. For estimation of heavy metals, the finely grinded toy samples were mixed thoroughly and a uniform quantity of powdered sample was digested using microwave digestion technique. The supernatant was taken for analysis of heavy metals using AAS-GF/ ICP–MS. For estimation of phthalates, viz., DBP, BBP, DEHP, DNOP and DINP was carried out in the representative number of samples at NIOH using Gas Chromatography/ Mass spectrophotometer (GC–MS).

Leaching Study*:* The leaching studies were conducted following migration and wiping methods: *Migration study was carried out in* accordance with American Society for Testing and Materials (ASTM F963-08 and EN71 Part 3). Wiping study was carried out in accordance with Consumer Product Safety Commission – D.C. 20207, 1997 using Whatman filter paper wrapped over swab.

Inter Laboratory correlations*:* The intra class correlation was computed for lead and cadmium to assess the agreement between the laboratories (NIN/ NIOH with AIIMS), and for phthalates (NIOH and TUV South Asia, Bangalore).

Important Findings

- 95% of toy samples were found to have lead level within permissible limits (90 mg/kg)**.**
- 98% of toy samples were found to have Cadmium level within the permissible limits (75 mg/kg).
- Chromium level was detected below 60 mg/kg (permissible limit) in 96.5% of the samples.
- Arsenic level was within 25 mg/kg (permissible limit) in 97.8% of the samples. Most of the samples exceeding this limit were found in toy samples from south zone.
- Mercury was within the permissible limit (60 mg/kg) in all the samples.
- The detected phthalates viz., DBP, BBP, DEHP, DNOP, DINP were above the prescribed limit in 4.6%, 0.2%, 15.8%, 0.6% and 21% of toys respectively.

- DEHP and DINP were found to be more than 10 times above the prescribed limit (0.1%) in 3.4% and 12% of the total toy samples respectively.
- In total toy samples, 32.6% had any one phthalate above 0.1% and more than half of these toys were teethers (toys kept in the mouth).
- The country wise distribution of any phthalate above 0.1% showed that 16.9% of the samples were made in China followed by 13% in countries about which identify could not be ascertained which and 1.9% were made in India.
- Higher levels of heavy metals as well as phthalates were detected in local category of samples.
- Leaching (Migration and Wiping) studies did not show any significant migration of Lead and Cadmium.
- The randomly selected sample in this study revealed that 51.3 % of the sampled toys were labeled as 'Made in China' (however, it was not possible to authenticate the origin) as compared to 8.8 % labeled as 'Made in India' and 1.9 % from other countries.

RECOMMENDATIONS

- Phthalates levels need to be controlled and monitored since teethers were found to contain phthalates in higher than the permissible limits.
- Toys particularly from the eastern zone need to be investigated for the source of high phthalate content and appropriate remedial action taken.
- The source of high Arsenic in the locally made toys from the south zone need to be investigated to identify the additive/chemical being used and necessary measures should be taken to prohibit its usage.
- The methodology for heavy metals and phthalates estimation in toy sample as standardized and validated may be adopted at the national level.
- Development of infrastructure and human resources for monitoring the quality of toys needs to be undertaken to empower proper monitoring by the concerned agencies.

4. EVALUATION OF THE IMPACT OF GENETIC POLYMORPHISM ON PHARMACODYNAMIC ACTIVITY OF COMMONLY PRESCRIBED ANTIHYPERTENSIVE DRUGS (THIAZIDE DIURETICS, ACE INHIBITORS, CCBS AND B-BLOCKERS)

Hypertension is a growing concern all over the world and is expected to increase up to 29% by the year 2025. The guidelines are updated for treatment of hypertension both bynational and international agencies as per the indigenous needs and which is based on therapeutic outcome. In India various factors which includes altered socio-economic status of the population, nutritional profile and genetic variations impact on pharmaco-dynamic activity. Therefore, monitoring the prescription profile of the anti-hypertensive drugs is one of the primary objectives followed by assessment of impact of genetic polymorphism on the therapeutic outcome of the drug.

METHODOLOGY

A survey has been conducted at General Medicine ward at Tertiary care Hospital and a total of 2169 prescriptions in a period of one year were screened. As per the procedure at least 60 prescriptions were collected per day for two days in a week and four weeks in a month to ensure representations from all seasons in a year in a pretested schedule. The prescriptions confined to the treatment of hypertension were around 25% (550). The data was compiled for obtaining the information on anti-hypertensive prescription profile.

RESULTS

- Among the subjects attending to the General Medicine ward for various complaints, 25% of them were prescribed with antihypertensive drugs. The distribution of gender was 68.4% with mean age±S.E of 51.9±0.61 in males whereas 31.6% with mean age±S.E of 52.6±0.89 females.
- The majority of patients were on monotherapy (69.45%), followed by two class combination therapy (26.54%). The proportion of subjects prescribed 3 or more classes of antihypertensive drugs was (3.81%).
- The antihypertensive drugs prescribed are CCBs (28.27%), BBs (26.7%), ACEIs (22.7%), ARBs (21.73%), thiazide diuretics (0.52%). In monotherapy, the order of antihypertensive drugs was Atenolol (20.42%), Amlodipine (18.06%), Ramipril (17.8%), Telmisartan (12.57%).
- The most common prescription for two-drug therapy consists of BBs-CCBs (23.97%).
- The antihypertensive effect recorded based on blood pressure was in 30-40% among majorily prescribed monotherapy drugs.

CONCLUSIONS

The percentage of men with hypertension is twice that of women. The prescription profile demonstrates that Monotherapy is chosen over the combination therapy. The categories of drugs prescribed are as per the JNC VII except for the poor prescription of Diuretics. In view of the antihypertensive potential recorded in only 30-40%of the subjects, we are in a process to evaluate the impact of genetic variations and nutrigenomics profile.

VIII NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

A. SERVICES ACTIVITIES

BREEDING AND SUPPLY OF ANIMALS

During the period a total 27,164 animals were bred and out of which 21,432 animals were supplied to various outside institutions and 2666 animals supplied within the institute. An amount of ` 54,61,420/- (Rupees Fifty Four Lakhs Sixty One Thousand Four Hundred and Twenty only) has been generated. Details of individual strains of animals bred and supplied are shown in Tables 1- 3.

SUPPLY OF ANIMAL FEED

1. Stock Animal Feed

The stock feed of 63160 Kgs (Rat & Mouse feed 53700Kgs + Guinea pigs & Rabbit feed 9460 Kgs) was prepared during the period. Out of this, a total of 24377 Kgs feed (Rat & Mouse feed 19001 Kgs + Guinea Pigs & Rabbit feed 5376Kgs) was supplied to outside institutions generating an amount of ` 36,15,833/- (Rupees thirty six lakhs fifteen thousand eight hundred and thirty three only). An additional 42211 Kgs of feed (Rat & Mouse feed 36016 Kgs + Guinea Pigs & Rabbit feed 6195 Kgs) was also supplied within the institute. The details of stock feed supplied are shown in Tables 4 & 5.

2. Experimental Animal Feed

In addition, Centre also prepared 933Kgsof custom made experimental animal feed. Paddy Husk supplied to 121 kgs outside institutions. An amount of ` 3,98,601/- (Rupees Three Lakhs Ninety Eight Thousand Six Hundred and One only) was generated are shown in Table-6.

3. Blood and Blood Products

During the period, a total of 702ml of blood and blood products have been supplied to 9 different institutions an amount of `1,56,850/- (Rupees one lakh fifty six thousand eight hundred and fifty only) has been generated are shown in Table-7.

4. Human Resource Development

During this period in the junior level Laboratory Animal Technicians Training Course (LATTC), there were 11 participants underwent training in Laboratory Animal Sciences. In the senior level Laboratory Animal Supervisors Training Course (LASTC) 6 candidates were trained. In the Ad-hoc training course 27 candidates from different organizations were trained for a period varying from one week to 4 weeks.

The Centre celebrated World Laboratory Animal Day on 24th April 2014in association with ICMR and Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA). On this day, a one day seminar on Humane end points and implementation of three hours on Animal Experimentation was conducted. There were more than 180 delegates of private and government organizations have participated including CPCSEA nominees of IAEC from various institutions. During these celebrations some of the retired staff of the NCLAS were also felicitated. Apart from these members from local animal welfare organizations have been felicitated.

Details of training courses conducted by NCLAS
(April 2014 to March 2015)

Sl. No	Type of course and current number	Qualification & eligibility criteria	Duration	Fee `		No. of seats every year
				Govt.	Pvt	
1	46th Laboratory Animal Technicians Training Course (LATTC) 16th June - 31st July 2014	Undergraduate with knowledge of English read and write	6 weeks	4000	8000	11
2	34rd Laboratory Animal Supervisors Training Course (LASTC) 1st Sept --30th Nov 2014	Graduation in Life Sciences, Medical & Veterinary sciences	3 months	8000	16000	6
3	Ad-hoc or Modular Training Course-Open throughout the year for National, International & WHO Sponsored candidates	Sponsorship from the Head of the Institution	1-3 weeks	1000 per weeks	5000 per weeks	27

B. RESEARCH ACTIVITIES

1. EFFECT OF ENVIRONMENT AND MICROORGANISMS IN THE DEVELOPMENT OF OBESITY IN WNIN OBESE RATS

Obesity being a connoisseur's prediction earlier has become a metabolic disease of epidemic proportion in developed as well as developing countries. The development of this syndrome has been attributed to life style changes, lack of exercise, and the nutritional practices followed. An important contributory factor that has been observed so far is the impact of indwelling as well as surrounding microorganisms from outside. There are number of studied which show the involvement of viruses in the development of diabetes. But in recent times even viruses have been implicated in the manifestation of obesity.

Recent studies showed that immunity is impaired under obese conditions and once the the immunity is impaired the subjects are liable for opportunistic infections, degenerative diseases like cancer, diabetes, osteoporosis, cataract etc. Obesity can be directly attributed to the plane of nutrition and lack of exercise and the microorganism under these conditions are likely to be different in normal and obese individuals, but whether it is the cause or effect is not yet known. Recent evidences indicate different gut micro flora in obese and lean subjects both man and laboratory animals. Gut flora seems to increase fatness in lean animals showing that there is a definite role of microorganisms in the manifestation of obesity.

At our institute, we have isolated and established two obese mutant strains.WNIN/OB and GR-OB, which, apart from being obese are euglycemic and glucose intolerant respectively. The animals are hyperphagic, hyperinsulinemic, lipimic and leptin resistant. Their average life span is 1 and ½ year, and by the time they cross one year, they develop opportunistic infections, tumors, cataract, osteoporosis etc.

In the present proposal the microbial load (both bacterial and viral) in the animals maintained in conventional colonies from 0 day to 35,90,180, and 1 year and above 1 year along with other parameters of obesity.

Table 1. Details of Different Species and Strains of Laboratory Animals Bred and Supplied from NCLAS (APRIL- 2014 TO MARCH- 2015)

Sl. No	Species	Strain or Breed	Stock as on	Total number of animals							Balance as on
				Bred during the period	Available	Supplied to NIN	Supplied to other institutions	Supplied Total	Died	Disp.	
1	Mouse	BALB/c (in bred)	526	7352	7878	359	6776	7135	…	180	563
		C57BL/6J (in bred)	284	5770	6054	757	3634	4391	146	…	1517
		NIH (S) Nude (in bred)	166	229	395	…	115	115	9	…	271
		NCr. Nude	147	96	243	…	8	8	52	…	183
		FVB/N (in bred)	150	40	190	…	…	…	155	…	35
		Swiss (in bred)	1234	2480	3714	32	2528	2560	262	…	892
2	Gerbils	…	424	238	662	30	…	30	54	248	330
3	G.Pig	Dunkin Hartley (white)	232	1328	1560	60	1026	1086	69	73	332
		NIH Colour	46	343	389	1	264	265	31	28	65
4	Rabbit	New Zealand (white)	86	162	248	1	123	124	19	18	87
Total			3295	18038	21333	1240	14474	15714	797	547	4275

Table 2. Details of Different Species and Strains of Laboratory Animals Bred and Supplied from NCLAS (APRIL- 2014 TO MARCH- 2015)

Sl. No	Species	Strain or Breed	Stock as on	Total number of animals							Balance as on
				Bred during the period	Available	Supplied to NIN	Supplied to other institutions	Supplied Total	Died	Disp.	
1	Rat	CFY/NIN (inbred)	78	32	110	…	…	…	29	5	76
		Fischer 344 N (inbred)	323	95	418	8	131	139	6	50	223
		Holtzman (inbred)	61	298	359	…	60	60	61	20	218
		SD (Sprague Dawley) Out bred	1108	2255	3363	558	1824	2382	10	220	751
		Wkyoto (inbred)	72	98	170	…	30	30	34	17	89
		WNIN (inbred)	2004	3861	5865	703	3587	4290	69	184	1322
2		WNIN / Gr-Ob	782	387	1169	33	…	33	32	316	788
3		WNIN / Ob-Ob (inbred)	843	424	1267	61	…	61	88	321	797
4		SD NIN Nude	183	172	355	…	…	…	60	75	220
5	Hamster	Golden (inbred)	294	1504	1798	63	1326	1389	62	106	241
6	Monkey	Rhesus	24	…	24	…	…	…	…	…	24
Total			5772	9126	14898	1426	6958	8384	451	1314	4749
TABLE-1 (TOTAL)			3295	18038	21333	1240	14474	15714	797	547	4275
TABLE-2 (TOTAL)			5772	9126	14898	1426	6958	8384	451	1314	4749
GRAND TOTAL			9067	27164	36231	2666	21432	24098	1248	1861	9024

Table 3. Sale of animals from NCLAS (April -2014 to March-2015)

Month	Animals																			Transport Boxes charges	Handling Charges	Transportation		Animal Sale Amount in Rs	Total Amount
	MICE														G. Pig										
	BALB/c An.N	C57BL/6J	NIH Nude	NCr. Nude	FVB/N	Swiss	Fischer 344 N	Holtzman	Wkyoto	Sprague Dawley	WNIN	WNIN/Gr-Ob	WNIN / Ob-Ob	FVB	SD NIN Nude	D. Hartley (white)	NIH (Colour)	Hamster -Golden	RABBIT- New Zealand (white)			Road Transport	Air fright charges		
April 2014	80					78				50	320					25		15		1650				155860	157510
May 2014	645	3				63					405					184		50	30	9100	1500	5900	20638	422820	460232
June 2014	514	127				85					200					20		120	1	6300	1500	6200	18446	225470	257916
July 2014	516	70				354			30	102	234					122		218	22	37100	3000	14700	43449	446430	544679
Aug. 2014	600	145				180					325					40		48	6	9500	1250	5900	17420	302050	336120
Sept. 2014	508	193	20			152	105			105	313					142		124		27400	5750	20250	63654	411620	528674
Oct. 2014	345	125				150		30		156	66					195		140	2	20300	1750	7000	22066	361350	412466
Nov. 2014	635	93	10			56		30		144	348					105			33	7000	1250	5000	15996	434280	463026
Dec. 2014	670	627				314				450	518					195		105		32100	5000	20000	60211	693640	810951
Jan. 2015	725	46		12		90				560	148					150		100	8	26400	1750	7900	30775	488940	555265
Feb. 2015	1058	514		30		275				168	619					90		186		27840	4000	16000	47083	589630	684553
Mar. 2015	963	1005	14	12		422	20			322	441					165		240	26	33400	3750	15900	50007	929330	1005857
Grand Total	7259	2948	44	54		2219	125	60	30	2057	3937					1433		1346	128	238090	30500	124750	4E+05	5461420	6217249

Table 4. Stock feed supplied from NCLAS
(APRIL-2015 TO MARCH–2015)

Month	(A) Diet formulated protein		(B) Govt. Supply Protein		(C) Private supply protein		(D) Internal Supply Protein	
	20 %	**14 %**	**20 %**	**14 %**	**20 %**	**14 %**	**20 %**	**14 %**
April-2014	4650	600	860	220	150	-	3570	635
May 2014	4600	360	1625	280	105	-	3140	470
June 2014	3900	1080	720	27	225	-	2685	540
July 2014	4400	600	1537	20	500	160	3628	485
August 2014	4250	700	810	125	430	-	3043	520
September 2014	4700	600	1081	300	150	10	3595	520
October 2014	5050	600	1720	198	101	-	2960	480
November 2014	3750	720	900	122	200	200	2580	470
December 2014	4350	600	1050	350	125	-	3325	755
January 2015	4450	900	1341	130	100	5	2740	510
February 2015	4200	1560	1085	-	190	1000	2650	340
March 2015	5400	1140	1650	2850	525	1200	2100	470
Total in Kgs	53700	9460	14379	4622	2801	2575	36016	6195
Grand Total	**63160**		**19001**		**5376**		**42211**	

Tables 5. Sale of feed from NCLAS (April - 2014 To March- 2015)

Sl. No	Month	Feed			Paddy husk	Transport Charges `	Handling Charges `	Sale of feed Amount in `	Total Amount in `
		Rat, Mouse & Hamster feed in Kg (20 % Protein)	Rabbit, G. Pig& Monkey feed in Kgs (14 % Protein)	High fat diet (Special) - Kgs					
1	April - 2014	1180	150	5	--	--	--	177975	177975
2	May- 2014	1665	105	24	--	27949	2250	243990	274189
3	June - 2014	762	181	--	--	2282	1075	118475	121832
4	July - 2014	2017	710	10	--	49446	2250	326410	378106
5	August - 2014	1830	430	--	15	32491	1000	283750	317241
6	September - 2014	1385	160	25	--	7413	1500	229355	238268
7	October - 2014	1918	101	11	--	23901	1000	282916	307817
8	November - 2014	1032	450	6	--	13080	500	190600	204180
9	December - 2014	1600	125	12	--	12808	750	239060	252618
10	January - 2015	1471	105	10	--	17375	1500	212430	231305
11	February - 2015	1605	740	200	100	25015	1500	345300	371815
12	March - 2015	4625	1525	108	6	51961	3250	965572	1002796
Grand Total		**21090**	**4782**	**411**	**121**	**263721**	**16575**	**3615833**	**3878142**

Table 6. Special feeds / diets formulated and supplied from NCLAS (APRIL 2014- MARCH 2015)

S. No	Date	Bill No	PARTY	Type of Diet	Quantity in Kgs	Amount in `
1	17.04.2014	4389	M/S.Malayaja. Acharaya & BM Reddy Coll Pharmac, Saldevanhalli, B'luru	HFD	5	3175
2	23.04.2014		Animal House NIN	28% Protein	50	14250
3	01.05.2014	4390	M/S. Sanzyme Ltd.	Cholesterol diet	5	5800
4	20.05.2014		PCT. NIN	Spl. Diet	22	0
5	21.05.2014	4394	M/S. Sanzyme Ltd.	Cholesterol Diet	5	5800
6	28.05.2014	4500	Natl. Brain Res. Centre, Manesar	HFD	10	11590
7	28.05.2014	4499	Dr.Md.Akhtar. Dept. Pharmac. Hamdard Univ, New Delhi	HFD	4	4050
8	20.06.2014	4524	M/S Teena Biolabs Pvt. Ltd.	Spl. Diet	71	10950
9	05.06.2014		Animal House NIN	28% Protein	10	2850
10	20.06.2014		Animal House NIN	28% Protein	50	14250
11	04.07.2014	4556	Dr. Medha Murthy. I.I.Sc., B'luru	HFD	10	10400
12	18.07.2014		Animal House NIN	28% Protein	50	14250
13	16.08.2014		Animal House NIN	28% Protein	50	14250
14	08.09.2014	4653	CCMB	Spl. Diet	4	2000
15	11.09.2014		Dr.M.Raghunath. NIN	Spl. Diet	60	0
16	15.09.2014		Dr.M.Raghunath. NIN	Spl. Diet	40	0
17	23.09.2014		Animal House NIN	28% Protein	50	14250
18	30.09.2014	4674	Natl. Dairy Res. Inst. Karnal	HFD	25	25875
19	16.10.2014	4689	Univ. of Hyd	Protein Def.	6	19516
				Control Diet	5	
20	21.10.2014		Animal House NIN	28% Protein	50	14250

Table 6. Special feeds / diets formulated and supplied from NCLAS (APRIL 2014- MARCH 2015) (contd..)

S. No	Date	Bill No	PARTY	Type of Diet	Quantity in Kgs	Amount in `
21	30.10.2014	4703	Dr.I. Patro. Jiwaji Univ. Gwalior	8% Protein	10	3250
22	17.11.2014	4724	Natl. Brain Res. Centre, Manesar	Protein Def.	3	5050
				Contol Diet	3	
23	18.11.2014		Animal House NIN	28% Protein	50	14250
24	04.12.2014	4740	Natl. Brain Res. Centre, Manesar	Iron Def.	2	11810
				Control Diet	10	
25	05.01.2015	4781	Natl. Brain Res. Centre, Manesar	Protein Def.	5	8250
				Contol Diet	5	
26	02.02.2015	4820	Dr.I.Patro. Jiwaji Univ. Gwalior	8% Protein	100	30000
27	03.02.2015	4822	Dr.N.Pipil, BRD Med.Coll. Gorakhpur	HFD	20	14700
28	02.03.2015	4909	Natl. Brain Res. Centre, Manesar	Protein Def.	5	8000
				Control Diet	5	
29	02.03.2015	4910	Indian Inst. Intergrative Med. Jammu	HFD	20	24900
30	02.03.2015	4911	BRD Medical College. Gorakhpur	HFD	20	14700
31	11.03.2015	4856	Mr.K.Naresh. Bhaskar Coll. Of Pharmacy. Moinabad	HFD	3	2565
32	12.03.2015	4930	IICT	Cholestrol Diet	60	48720
33	20.03.2015	4945	Indian Inst. Intergrative Med. Jammu	HFD	20	24900
				Total	**923**	**398601**

NOTE: 28% Protein Diet for Animal House@ Rs.285/Kg for sale: Rs.480/Kg

Table 7. Sale of blood & blood products from NCLAS
(April - 2014 To March- 2015)

Sl No	Bill No	Name of the Institute		Quantity in ml	Handling charges & Packing charges	Amount `	Total
APRIL-2014							
7	4471	Prof.K.P.Sharma, University of Hyd	Rat Plasma	200	1000	20000	21000
9	4475	Dr.MVVN Reddy, Aurigene Discovery, Hyd	Monkey Blood	10	250	2000	2250
11	4477	Dabur Research Foundation, Ghaziabad	BALB/c Plasma	15	1000	3600	4600
MAY-2014							
21	4493	Bharat biotech International ltd, Hyd	Rabbit Blood	30	250	6000	6250
JUNE-2014							
11	4552	Biological -E, Ltd, Hyd	Rabbit Serum	5	550	2000	2550
13	4553	Mylan Laboratories, Hyd	Rat Plasma	15	550	3000	3550
JULY-2014							
2	4555	Mylan Laboratories, Hyd	Rat Plasma	15	550	3000	3550
AUGUST-2014							
5	4619	Nektar Therapeutics India (P) Ltd, Hyd	Monkey Blood	70		14000	14000
SEPTEMBER- 2014							
Nil							
OCTOBER- 2014							
13	4686	Nektar Therapeutics Ltd, Hyd	Monkey Serum	22		8800	8800
NOVEMBER- 2014							
5	4716	Nektar Therapeutics Ltd, Hyd	Monkey Serum	20	250	8000	8250
DECEMBER- 2014							
16	4769	Delta Laboratories, Bangalore	C57Bl/6j Plasma	100	250	30000	50500
			SD Rat Plasma	100	250	20000	
JANUARY-2015							
Nil							
FEBRUARY-2015							
Nil							
MARCH-2015							
31	4962	TCG Life Sciences Ltd, Kolkata	Mouse Plasma	100	1550	30000	31550
Total				**702**	**6450**	**150400**	**156850**

STUDY DESIGN

Animal Selection: 24 rats were used for the study. 12 lean rats and 12 obese rats.

METHODOLOGY

Collection of Samples: Rats were given free access to the diets. They were fasted 18 hours before sacrifice. After being deprived of food, they were then sacrificed by placing in a CO_2 chamber. Then a ventral midline incision was made, and the small intestine cecum was removed and sent for microbiological enumeration.

The ratio of Bacteroidetes and Firmicutes was done by Pour plate Method.

STATISTICAL ANALYSIS

The 't test' was performed to compare the mean differences between the CFU of Bacteroidetes and Firmicutes at 10^{-2} dilution factors in both groups of lean and Obese rats using Graphpad prims 5.0 software. The t test p value <0.05 (two sided test) was considered as significant statistically.

The identification of bacteroidetes and firmicutes was done by gas chromatographic analysis using fatty acids methyl esters (GC-Fame).

RESULTS

The species of Firmicutes detected by FAME Analysis in Obese rats are as follows:\

1. Staphylococcus-cohnii and 2. Staphylococcus epidermidis.

Of these two species identified Staphylococcus-cohnii was detected in more number of samples when compared to Staphylococcus-epidermidi.

The species of Firmicutes detected by FAME Analysis in Lean rats are as follows:-

1. Bacillus-subtilis, 2. Bacillus-GC group 22, 3. Paenibacillus-validus.

Of these three species identified Bacillus-subtilis was found in more number of samples followed by Paenibacillus-validus and Bacillus-GC group 22.

The species of Bacteroidetes detected by FAME analysis Obese rats are as follows:-

1. Prevotella-melaninogenica, 2. Eikenella-corrodens.

Prevotella-melaninogenica was found more in obese rats when compared to Eikenella-corrodens.

The species of Bacteroidetes detected by FAME analysis Lean rats are as follows:-

1. Prevotella-melaninogenica 2. Eikenella-corrodens.

Eikenella-corrodens was found more in Lean rats.

CONCLUSION

The data support the key idea that the gut microbiota can contribute to the patho-physiology of obesity. This could be considered when developing strategies to control obesity and its associated diseases by modifying the gut microbiota. With additional experiments and studies, this work could lead to identification of microbial markers that make up a kind of obesity or leanness profile in animals a vital-stats sheet of the gut world that would help understanding how people are likely to respond to microbes. Beyond that, the knowledge gained from this study can be applied to agriculture science and we could grow foods that are specifically designed to provide the optimal balance of nutrients and energy for various life stages.

IX PRE-CLINICAL TOXICOLOGY RESEARCH CENTRE

1. PRE-CLINICAL SAFETY EVALUATION OF VEGETABLES CULTIVATED USING DEOILED KARANJA SEED CAKE

Karanja (*Pongamiapinnata*) has been identified as an alternative source of biodiesel with release of lot of waste material which may become eco-concern. However, this waste material is a rich source of N, P, K. along with Karanjin which is an anti-nutritional component. The Indian Institute of Chemical Technology (IICT) have taken a program wherein they have prepared Expelled cake (EC/T_5) and De-oiled cake (DC/T_{10}) from this waste to produce an organic-fertilizer. Their pilot experiments in growing vegetables at IICT were productive. However, the safety issues with consumption of vegetables which are grown in new category of organic-fertilizers have to be established.

The present proposal is undertaken as Public-Public Partnership (PPP) program between three Government Organizations viz., Indian institute of Chemical Technology (CSIR), Andhra Pradesh Horticulture University (APHU) and National Institute of Nutrition (ICMR) based on expertise so as to fulfill the following objectives.

- Development of De-oiled Cake (DC) and Expelled Cake (EC) prepared from Karanjin Seed.
- Monitoring the productivity of common vegetables (Tomato, onions, Amaranthus) grown in regular (RF/T_1), Expelled cake (EC/T_5) & Deoiled cake(DC/T_{10}) fertilizers along with pre and post experimental soil testing.
- Assessing the compositional equivalence and to evaluate pre market safety of Tomato, onions, Amaranthus grown in regular, DC & EC fertilizers.

METHODOLOGY

The study involves preparation of EC & DC from karanja seed cake by IICT followed by cultivation of crops viz. Amaranthus, Onion and Tomato in RF/T_1, EC/T_5 & DC/T_{10} at APHU. The following investigations have been carried out by NIN.

COMPOSITIONAL ANALYSIS

The Amaranthus, onion and tomato grown in RF/T_1, EC/T_5& DC/T_{10} at APHU have been analyzed for i. Proximates, ii. Fibre, iii. Minerals iv. Vitamins by standard procedures of AOAC.

PRE-CLINICAL EVALUATION

The preclinical safety evaluation includes i) Acute and ii). Long term toxicity studies using SD Rats at Centre for Advanced Research for Preclinical Toxicology, NIN (ICMR) as per regulatory guidelines of Food safety and standards Authority of India (FSSAI).

***I. Acute Toxicity Study*:** This test was carried out in *Sprague dawley* rats (30M + 30F),aged 6–8 weeks, weighing 180 – 200g. The animals were grouped (5M + 5F) to receive lyophilized test material of vegetables (Amaranthus / Onion/Tomato) grown in EC & DC. The animals were exposed orally to test material more than three times of Daily Dietary Intake (3XDDI) in divided doses of 2 - 3 administrations within 24 hours. Similarly, an acute toxicity test with purified karanjin (60%) has been conducted by exposing more than 50 & 100 folds higher concentration than the karajnin expressed in the vegetables grown in EC & DC. This is followed by daily observations for mortality and bi–weekly monitoring of live phase, cage side and physical activities till 15th day of post exposure. At the end of experiment all animals were euthanized and gross necropsy of vital organs was conducted.

***ii. Sub Chronic Toxicity Study*:** This test had carried out in *Sprague dawley* rats(60M+60F), aged 6–8 weeks, weighing 180-200g. The animals were randomly divided into ten groups (6M+6F) to receive lyophilized test material of amaranthus / Onion/ Tomato grown in RF/T_1 & EC/T_5 & DC/ T_{10} fertilizer. The test material has been prepared by mixing individual vegetable uniformly in NIN powder diet following SOP's and fed orally for 90days daily before providing NIN pellet diet as *ad-libitum*. The control group of animals has received NIN regular diet.

The animals were observed daily for morbidity and mortality. Live phase, cage side, routine physical, physiological, neurological activity was observed bi-weekly till the end of the experiment. Daily feed and test material intake was quantified and recorded. Bi weekly Body weights were recorded. Pre and post urine examination was conducted. The clinical chemistry profile, hematology profile was assessed by standard procedures on last day of experimental phase. The animals were euthanized and gross necropsy of all organs has been conducted within 48hrs of last exposure. All organs are collected for histopathological evaluation. Data is being compiled and analyzed for significant differences between individual vegetable and control group of animals.

RESULTS

There were marginal differences in compositional analysis (i. Proximates, ii. Fibre, iii. Minerals iv. Vitamins) of Amaranthus, onion and tomato grown in regular, DC & EC fertilizers.

Acute

- No pre–terminal deaths were recorded in any group of animals received vegetables (Amaranthus, Onion & Tomato) grown in karanja seed cake.
- All animals were found active with no abnormal clinical signs in the test groups.
- Feed intake, body weight gain was normal.
- No abnormal changes in gross necropsy and Organ weights.
- There was no mortality and morbidity in the animals received pure Karanjin, a 100 folds higher concentration than the Karanjin likely to be expressed in vegetables intended for human intake as per RDI.

Sub-chronic

- There was no mortality in animals fed with vegetables (Amaranthus, Onion and Tomato) daily for 90 days.
- All animals were found active with no abnormal clinical signs in the test groups.
- Feed intake, body weight gain was normal.
- No abnormal changes in gross necropsy and Organ weights were observed.

CONCLUSIONS

- The compositional analysis includes i. Proximates, ii. Fibre, iii. Minerals iv. Vitamins in vegetables (Amaranthus, onion and tomato) grown using DC & EC fertilizers were comparable with vegetables grown in regular fertilizer.
- There was no mortality in rats exposed to vegetables (Amaranthus, Onion and Tomato) cultivated in EC, DC fertilizers more than three times of recommended Daily Dietary Intake (DDI) once orally without any morbidity.
- In acute toxicity study, the rats exposed to oral dose of 100 folds higher concentration of pure Karanjin than the karanjin likely to be expressed in vegetables grown using EC, DC fertilizers were also found to be safe.
- The daily feeding for 90days of vegetables (Amaranthus, Onion and Tomato) cultivated in regular, Expelled Cake and De-oiled Cake fertilizers, did not produced any significant adverse effects on biochemical and hematological parameters in rats under experimental condition.

2. PRE-CLINICAL EVALUATION OF IRON FILINGS IN CTC-TEA AND ITS BREW

Tea, the second most widely consumed beverage in the world, is categorised in to Cut Tear Curl (CTC) tea and Orthodox tea based on processing method as follows:

i. Cut Tear Curl (CTC) tea is prepared by Crushing green tea leaves between two sharp toothed rollers to make a thick paste followed by drying

ii. Orthodox tea prepared by drying the leaves in dryers.

Presence of iron filings in CTC tea is inevitable during crushing process mainly due to wear and tear of machinery. Since, it is considered as 'staple beverage' in India and other neighbouring countries, fixing permissible limits of iron fillings became essential to address the safety concern.

In view of this, permissible limit of iron filings in tea was fixed by regulatory agencies of various countries viz., Kenya-40mg/kg, Srilanka-500mg/kg, Egypt-150mg/kg, Poland-100mg/kg, British standards-120mg/kg. In India, Core Committee constituted by the Ministry of Commerce and Industry, Government of India has fixed iron filing in tea with a limit of 250mg/kg.

The present investigation is therefore undertaken with the following objectives:

- Assessing the Iron levels in Tea & Brew samples collected from dealer.
- Risk assessment study with Tea and its brew.

METHODOLOGY

Phase – I – Analysis of Iron levels and Heavy metals:

- Monitoring Iron filings: The tea powder samples (CTC & Orthodox variety) have been collected from the dealers in southern parts (Valparai, Tamilnadu) and the local vendors of Hyderabad. The Iron filings have been analyzed by BIS standard method of IS3633:2003, which includes powdering of 25gm of Tea sample and subject for rolling of magnet (supplied by tea board) over the tea powder several times. This is followed by weighing the material attached to magnet and expressed as mg iron filing/g tea powder.
- The Iron levels have been analyzed by microwave digestion of 0.2 to 0.5 g of tea powder and brew mixed with 5mL of HNO_3 and 2mL of 30% H_2O_2 and subjected to flame Atomic Absorption Spectrophotometer.
- The other metals like Lead, Chromium, Mercury, and Arsenic have also been analyzed in CTC, Orthodox, Brew by Graphite Furnace - AAS.

Phase – II (Risk assessment study):

- The preparation of Tea in India varies from other Countries. Therefore, the investigation has been undertaken in CTC, Orthodox, Brew samples which are prepared following the Indigenous and standard British procedures.
- ***Acute Toxicity test:*** Acute toxicity test was carried out in *Sprague dawley* rats by exposing the test material viz., CTC & iron filings (4.5mg) once orally in a constant volume of 1ml by oral gavage. A total of 20 (10M+10F) rats aged 4–6 weeks weighing 150–180 gms have been received from National Center For Laboratory Animal Sciences, for conditioning. They are equally divided into two groups viz., 1) CTC (5M+5F) 2) Iron filings (5M+5F) by randomization. The CTC group has received 22.5mg/kg rat and iron filing group received 22.5mg/kg rat which is hundred times higher than the maximum permissible limit. Both groups of animals were observed daily for 14 days after exposure to the test material. At the end of the experiment, all animals were euthanized and their organs were collected for gross necropsy. In case of pre-terminal death, an autopsy was conducted to collect the vital organs for histo-pathological examination.
- ***Subchronic study*** **: This study involves preparation of brew, it's exposure in various concentration to the SD rats.**
- ***Preparation of Brew (liquor):*** The brew was prepared by boiling individual samples (Orthodox/CTC/CTC with

Iron filing) in drinking water at 60°C for five or thirty minutes as per SOP. The brew was cooled at room temperature for approximate 20 mints before feeding (2ml) orally to experimental animals of the respective groups. The iron filing sample was powdered and mixed in water (0.5mg in 2ml) for feeding to animals.

- **Exposure Profile in experimental animals:** Since the study is on safety evaluation of iron filings, the exposure level of test material has been calculated based on the permissible limit of iron particles in CTC-tea is 250mg/kg a statutory Advise of FSSAI. The daily adult human consumption of tea brew is from approximately 10 gm/ day (i.e. divided in 5 times/day) tea powder. Therefore maximum, permissible limit of iron filling content should not be more than 2.5mg/10gm.

The present preclinical investigation is planned to evaluate safety profile of iron filing as per the regulatory requirement. The SD rats have been selected as the experimental model and the exposure levels have been calculated based on the body surface area so as to mimic the clinical exposure level.

The safety evaluation was undertaken in the animals with recommended exposure level which was 10 times higher than the recommended levels of iron filing as well by feeding direct Iron filing.

All SD rats used for experiment were acclimatized for 14 days and randomized into seven groups viz., (i) Control (6M+6F), (ii) Orthodox (6M+6F), (iii) CTC-5 – boiled for five minutes (6M+6F), (iv) CTC - 30 (6M+6F), (v) CTC+IF-5 (6M+6F) (vi) CTC+IF-30 (6M+6F) (vii) IF(6M+6F) to receive the respective test material prepared as brew daily for 28days. As a part of routine examination all animals were subjected to qualitative urine analysis followed by monitoring various study parameters. The animals were observed daily for mortality, live phase, cage side, physical, physiological and neurological activity was observed bi-weekly till the end of the experiment. The feed intake, Body weights were recorded Bi weekly. The urine analysis (qualitatively) was monitored pre and post exposure to the test material in all groups of animals. The clinical chemistry as well as serum ferritin and hematology have been undertaken in all groups within 24 hrs after last exposure. All animals were euthanized for gross necropsy of all organs followed by histopathological examination after 24hrs of last exposure. In addition plasma ferritin was also monitored using Elisa Kit in randomly selected animals from each group.

RESULTS

a. Phase – I

- The Iron filings in CTC Tea samples collected from local dealers at Valparai were above 250ppm as estimated by BIS magnet method.
- The Iron levels in tea samples after removing the Iron filings were below 250 ppm in 60% of the samples as measured by AAS.
- In the branded and orthodox varieties iron filings were much below the permissible limits of 250 ppm. Similarly the Iron levels measured by AAS was also found to be in traces.

The Iron levels in Brew prepared from all CTC, Orthodox Tea samples were in negligible amount.

b. Phase - II

In Acute exposure of CTC and Iron filings to Rats, no pre-terminal deaths were recorded. All animals were found to be active with steady weight gain and food intake till 14th day of post exposure.No gross necropsy changes were found.

The mortality in chronic exposed group of animals with orthodox (10%), CTC-5 group (10%) and CTC+IF-5 group (20%). In all survived animals no abnormal clinical signs, behavioral activity etc., Feed intake, body weight gain were comparable among group. The clinical chemistry, clinical hematology parameters were within normal range.No gross changes were observed in any of the organs examined during necropsy. No significant histopathology changes were observed due to test compound exposure.

CONCLUSION

The Iron filings in CTC Tea samples were found to be above permissible limits and after magnet rolling they were below the limits. The Iron levels estimated by AAS in 60% of CTC Tea samples were below the permissible limits. Whereas, in branded, orthodox and tea brew samples, these levels were negligible.

The rats were exposed to Iron filings more than 100 times of permissible limits once orally, and observed for14 days under experimental conditions.

There was no mortality in groups of animals fed IF directly and CTC with Iron filling (10mints) which is 10 times more than permissible limits daily for 28 days. The early pre-terminal mortality was recorded in orthodox, CTC (5mints) and CTC+IF (5mints) tea brew perhaps due to technical errors in feeding process. The post exposure study results of live phase activity, clinical observations, chemistry and hematology and various histopathology observations did not appear to be due to exposure to test material, serum and liver ferritin, gross necropsy investigation of vital organs didn't suggest any abnormal finding in spite of exposure to CTC with iron filings more than 10times of permissible limit (250ppm) in experimental conditions.

LIBRARY AND DOCUMENTATION SERVICES

Library continued to cater to the documentation and information needs of the Institute and other Research Organizations, Home Science and Medical Colleges. The library has played a key role in reference activities by offering information dissemination services like MEDLINE Searches, Proquest Medical Library Full Text Database of journals and other online retrieval activities using the LAN Network of the Institute. Library continued to participate in exchange of data, journals and information using the URL <http://Groups.yahoo.com/group/ICMR Librarians>.

Resource Sharing and User Education Programmes etc are continuously being undertaken by the Library. Institute's Scientific papers going in for publication in Scientific Journals etc., are being routed through the Library and a data-base of the published papers is also made accessible through on-line services using NIN Website (www.ninindia.org).

The Library services are being further strengthened through confirmed support from Indian Council of Medical Research for accessing E-journals from JCCC@ICMR and J-Gate database. The Library is also a member of ERMED Consortia of National Medical Library, New Delhi provided by ICMR for accessing E-journals Online Subscription of 4 Core Journals such as LANCET, NATURE, NEJM, SCIENCE has been renewed by ICMR is also accessible.

Excellent Photostat support to the Scientists, technical as well as to the administrative staff was provided at all times.

The following library services were expanded as detailed below:

1. NEW ADDITIONS

Books	....	3
Reports	....	115
Thesis / Dissertations	....	4
CDROMS	....	27
PC Quest CD's	...	12
General CD's	...	15

2. JOURNALS DELETED

Indian Journals

1 CMIE Monthly Review of A P Economy (State of India)
2 Computers Today
3 Food and Nutrition World
4 Food Digest
5 Food Technology Abstracts
6 Hotel and Food Service Review
7 Indian Medical Tribune
8 Indian Psychological Abstract & Reviews
9 JAMA (India)
10 Nutrition, Immunity and Health
11 Pediatrics (India)

Foreign Journals

1 Cereal Research Communication
2 Gerontology
3 Scanning
4 Spectrum

3. OTHER ACTIVITIES

Journals Bound	. . . .	870
Visitors using the Library	. . . .	2,785
Circulation of Books/Journals etc	. . . .	865
No. of E-mails sent outside	. . . .	792
No. of E-mails received	. . . .	4,752
Photocopying (No. of pages)	. . . . .	4,16,141
Number of Annual Reports mailed	. . . .	472
No. of INTERNET Searches provided	. . . .	120
No. of Reprints sent	. . . .	70

4. TOTAL LIBRARY COLLECTIONS

Books	. . . .	18,100
E – Books	……	36
Journals (Bound Volumes)	. . . .	39,156
Journals subscribed for 2014	. . . .	194
E – Journals subscribed for 2014	……	4
Journals received (Gratis/Exchange) for 2014	. . . .	120
E – Journals received (Gratis) for 2014	…..	28
Microforms (Microfiche)	. . . .	1,080
Slides	. . . .	280
Reports	. . . .	13,619
Theses & Dissertations	. . . .	406
MEDLINE CDROMS Discs	. . . .	383
Current Contents on Diskettes with abstracts	. . . .	664
Proquest (Full Text E-Journals) on CD ROMS	. . . .	495
General CD's	. . . .	281

Ph.D PROGRAMMES

PhD AWARDEES

S. No	Research Scholar	Title of the thesis	Supervisor	University
1.	Mr. Jitendra K. Sinha (2015)	Accelerated ageing/ reduced longevity of WNIN/ Ob obese rats: Role of altered neurochemical profile, oxidative damage and trophic support in the brain	Dr. M. Raghunath	Osmania
2.	Mr. Mehrajud-din Bhat (2015)	Role of UPP in vit-D deficiency induced muscle atrophy and hypo-insulinemia	Dr. Ayesha Ismail	Osmania
3.	Mr.V.Sudhakar Reddy (2015)	Role of small heat shock proteins in diabetic complications: modulation by nutritional and dietary factors	Dr.G. Bhanuprakash Reddy	Osmania
4.	Mr.Ramesh Athe (2014)	Meta-Analysis Approach on "Micro-nutrients Food Fortification and its Effect on Health, Social and Economic Factors"-A Statistical Model Building	Dr. Vishnuvardhan Rao	Osmania
5.	Mr.M.A. Patil (2014)	Studies on Diabetic Complications: Evaluation of Animal models and Role of Dietary agents	Dr. P.Suryanarayana	Osmania
6	Ms. Madira Soudarya Lakshmi (2014)	Establishment of propayable cell lines from Adipose tissue of adult WNIN Obese Mutant Rat (WNIN/Ob and WNIN/ GR-OAB)	Dr. Vijayalakshmi Venkatesan	Osmania
7	Ms.Anupama Tyagi (2014-Thesis submitted)	Anti inflammable potential & n-3 PVFA in experimental, Biochemical and molecular mechanism.	Dr.S.Ahmed Ibrahim	Osmania

RESEARCH SCHOLARS REGISTERED FOR PhD

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
1	Swarnim Gupta (2008 - completed)	Dietary diversification of Indian diets to improve iron bioavailability	Dr. K. Madhavan Nair	Osmania
2	Deethu Sara Varghese (2008 - completed)	Assessment of body composition in Indian females using different techniques	Dr.Y. Venkata Ramana	Osmania
3	B.Shankar Anand (2009)	Understanding the role of T cells and secreted cytokines in the Development of insulin resistance during obesity	Dr.Sudip Ghosh	Osmania
4	Anju E. Thomas (2010 - completed)	Foetal programming for neuro musculoskeletal development in the rat offspring: Role of antenatal and perinatal magnesium deficiency	Dr. M. Raghunath	Osmania
5	Bindu (2010 - completed)	Polyphenol rich dietary ingredients as novel sources of proteasome inhibitors and their role as anti cancer agents	Dr. Ayesha Ismail	Osmania
6	N. Pallavi (2010)	Regulatory role of zinc in hepcidin mediated iron metabolism	Dr. K. Madhavan Nair	Osmania
7	Y.Sravanthy (2010)	Effects of prenatal iron supplementation on iron-zinc homeostasis and placental zinc transporters: Studies in pregnant women and in BeWo cell lines	Dr. K. Madhavan Nair	Osmania
8	Sarin Sara Jose (2010)	Studies on Nutritional Assessment of Diabetic Complications	Dr. G Bhanuprakash Reddy	Osmania
9	Himadri Singh (2010)	Establishment of propayable cell lines from Pancreas (Ducal Epithelial Cells) of Adult WNIN Obese Mutant Rats (WNIN/Ob and WNIN/GR-Ob)	Dr. Vijayalakshmi Venkatesan	Osmania
10	Anil Sakumari (2010)	Modulation of adipose tissue inflammation and function of dietary n-3 pvfa: Potential role in metabolic syndrome.	S. Ahmed Ibrahim	Osmania
11	Golla Venkateswarlu (2010)	Role of dietary fatty acids in inducing endoplasmic reticulum stress in stromal vascular cells: implications in the development of obesity associated insulin resistance	Dr.Sudip Ghosh	Osmania
12	K. Nagabhushan Reddy (2011)	Dietary phytate-Zinc ons: “Role in suppressing colon cancer”	Dr. M. Raghunath	Osmania
13	Shampa Ghosh (2011)	Biochemical molecular and epigenetic changes associated with maternal vit-B12 restrictions induced alterations in C57BL/ 6 mice offspring	Dr. M. Raghunath	Osmania
14	P. Ravindranadh (2011)	Purification, characterization and primary structure elucidation of human milk factor that enhances iron absorption	Dr.P.Raghu	Osmania

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
15	Vijayendra charyA (2011)	Regulatory T cell function. And vitamin D regulating enzymes expression in pregnant women with vitamin D deficiency	Dr. R. Hemalatha	Osmania
16	M. Ankur (2011)	Effect of excess nitric oxide in the patho-physiology of motor neuron degeneration in neurolathyrism	Dr. Arjun L. Khandare	Osmania
17	Sowmya Sharma (2011)	Modelling the developmental Origins of Health Disease using mouse embryonic stem cells - Cellular, Molecular and Epigenetic approaches.	Dr. Vijayalakshmi Venkatesan	Osmania
18	V. Varsha (2011)	Evaluation of the impact of genetic polymorphism on pharmacodynamic activity of commonly prescribed antihypertensive drugs (thiazide diuretics, ace inhibitors, CCBs and ß-blockers)	Dr. B.Dinesh Kumar	JNTUH
19	Anuradha Challa (2011)	Impact of dietary facts rich in n-6 and n-3 poly unsatured fatty acids on adiposity and insulin resistance in diet induction obese rat model: a missing molecular link with vit. A metabolism	Dr.SM.Jeya Kumar	Osmania
20	V.Anantha Krishna (2011)	Impact of nutritionally superior varieties of mustard oil on lipid metabolism.	Dr.SM.Jeya Kumar	Osmania
21	Naga muralidhar (2011)	Genetic and epigenetic approach towards obesogenicity in a rat	Dr. K. Rajender Rao	Osmania
22	K.Sandeep kumar (2011)	Role of miRNA in the development of obesity and diabetes	Dr.Sudip Ghosh	Osmania
23	J. Vahini (2011)	Studies on assessment, identification and modification of glycemic index in diets commonly consumed by people	Dr.K. Bhaskarachary	Osmania
24	N. Naveena (2011)	Studies on Polyphenols in some plant foods as a source of antioxidants	Dr.K. Bhaskarachary	Osmania
25	A. Kiran Kumar (2012)	Metabolic response of zinc depletion and excess in contrasting cells: Studies in osteoblasts, myocytes and enterocytes.	Dr. K. Madhavan Nair	Osmania
26	M. Purna Chandra (2012)	Manipulation of dietary fat to enhance carotenoid bioavailability and bioconversion to vitamin A: Development of mechanism based strategies.	Dr.P.Raghu	Osmania
27	N. Himaja (2012)	Effects of Fos coated probiotics on fetal immune-programming and other health benefits	Dr. R. Hemalatha	Dr.NTRUHS
28	S Vishwaraj (2012)	Role of Molecular Chaperones in Chronic Tissue Remodeling Diseases	Dr.G. Bhanuprakash Reddy	Osmania

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
29	Sneha Jakhotia (2012)	Role of Small Heat Shock Proteins in Diabetic Nephropathy	Dr. G Bhanuprakash Reddy	Osmania
30	K Shruthi (2012)	Role of Ubiquitin Proteosome System In Diabetic Complications	Dr. G Bhanuprakash Reddy	Osmania
31	T Shalini (2012)	Assessment of Nutritional Status of Geriatric Population	Dr. G Bhanuprakash Reddy	Osmania
32	J Sugeetha (2012)	Impact of dietary saturated fatty acids on the progression of Nonalcoholic fatty liver disease in fructose induced model of Steatosis- Role of adipose tissue insulin sensitivity and secretory function.	S. Ahmed Ibrahim	Osmania
33	Daniella Chyne (2012)	Studies on the biodiversity of food resources in Meghalaya	Dr. R. Ananthan	Osmania
34	K. Mangthya Naik (2012)	Studies gastro protective effects of Naga King chili	Dr. R. Ananthan	Osmania
35	S.Alekhya (2012)	Identifying microbiological and hygienic factors affecting safety of stress foods and addressing them through vendor education.	Dr. V.Sudershan Rao	Osmania
36	Prashanthi PS (2012)	Studies on Xanthophylls: Dietary sources, processing, bioavailability and biological effects	Dr.K. Bhaskarachary	Osmania
37	J.Sreenivas Rao (2012)	Effect of Cooking/ Processing on the Bioavailability of Provitamin A carotenoids in Indian foods	Dr.K. Bhaskarachary	Osmania
38	M. Srujana (2012)	Effect of pesticide exposure among the farm children and their mothers on the various biochemical parameters associated with reproduction, neurotoxic enzymes, oxidative stress and impact on the micronutrient status.	Dr. J Padmaja Rambabu	Osmania
39	Venkat Reddy.B (2012)	Monitoring of organophosphate pesticide metabolites in commonly used fruits, juices, vegetables and urine samples of urban children and its toxic effect	Dr.S.N.Sinha	Osmania
40	P. Sushma (2012)	Post Transcriptional Variations due to miRNA and Risk in Oral Squamous Cell Carcinoma	Dr.P.Udaykumar	Dr.NTRUHS
41	Archana Konapur (2013)	Targeted nutrition communication for promoting consumption of variety of foods for improving micronutrient status of rural families	Dr. K. Madhavan Nair	Osmania

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
42	Dripta Roy Choudhury (2013)	Functional benefits of inclusion of fruits in supplementary nutrition programme (SNP): A randomized community trial among ICDS preschool beneficiaries on micronutrient status ,gut health, growth and development	Dr. K. Madhavan Nair	Osmania
43	M Siva Prasad (2013)	Status of Micronutrients and its influence on Molecular Mechanisms in Diabetic Nephropathy	Dr.G. Bhanuprakash Reddy	Osmania
44	Padmanav Behera (2013)	Studies on the potential of Islet-like Cell-Aggregates (ICAs) generated from Mesenchymal Stem Cells of Human Placenta for treating Type 1 Diabetes in NOD mice.	Dr. Vijayalakshmi Venkatesan	NTRUHS
45	Raja Gopal Reddy (2013)	Role of vitamin A metabolic pathway on the development of non-alcoholic fatty liver disease: A study on nutrient-nutrient interactions	Dr.SM.Jeya Kumar	Osmania
46	Sivakesavarao Kommula (2013)	Effect of long-term pre-diabetes on risk of renal, retinal and lens abnormalities: Biochemical, molecular mechanisms and role of dietary agents	Dr. P. Suryanarayana	Andhra Univ
47	MVS Prasad (2013)	Biochemical and Molecular studies on role of diet in the induction of obesity: Rat as a model system	Dr. K. Rajender Rao	Osmania
48	D M Dinesh Yadav (2013)	Studies on identification of candidate gene(s) associated with obesity in WNIN/Ob rat	Dr. K. Rajender Rao	Osmania
49	Keren Susan Cherian (2013)	A study on body composition and energy balance in selected groups of junior athletes	Dr.Y. Venkata Ramana	Osmania
50	Vilasagaram Srinivas (2013)	Role of maternal long chain fatty acids on angiogenic factors in first trimester placenta and their invasive properties: Implication to feto-placental growth	Dr.Sanjay Basak	Osmania
51	Rishika Jadai (2013)	Effect of Cowpea isoflavones as a natural source for treatment of osteoporosis in MG-63 human osteosarcoma cells and to assess its synergetic role with Vitamin D in bone formation.	Dr.C.Suresh	Osmania
52	G. Srividya (2014)	Anticancer and proteasome inhibitory potential of cinnamon in prostate cancer: *In vitro* and *In vivo* studies	Dr. Ayesha Ismail	Yet to be registered

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
53	P.Kondaiah (2014)	Effect of zinc supplementation prior to iron on iron absorption, and iron status in deficient rats: in vitro and in vivo studies	Dr.P.Raghu	Osmania (Yet to be registered)
54	K.Narendra Babu (2014)	Probiotic potential and other beneficial effect of ocimum, ginger and piper nigum on immune-inflammatory disease conditions	Dr. R. Hemalatha	Dr.NTRUHS
55	D.Vasundhara (2014)	Effect of probiotic supplementation on bacterial vaginosis (BV) in pregnant women	Dr. R. Hemalatha	Dr.NTRUHS
56	Rajeshkumar K (2014)	Role of advanced glycation end products in chronic tissue remodeling diseases	Dr. G Bhanuprakash Reddy	Osmania
57	P Swathi Chitra (2014)	Role of growth hormone in colon Carcinoma; Intervention with polyunsaturated fatty acids	Dr. G Bhanuprakash Reddy	Osmania
58	Nivedita Dubey (2014)	Nutritional composition bioavailability and allergenicity profile of nutritionally enriched GM food crops.	Dr.B. Dinesh Kumar	Osmania
59	Anita Singh (2014)	Development of herbals (Asparagus racemosus, Bacopa Monnieri, Withania Somnifera, Convolvulus pluricaulis, Tribulusterrestris, Phyllanthusa-marus) and their combinations as potential immune-modulators and anti-inflammatory products	Dr. B.Dinesh Kumar	Yet to be registered
60	A. Kiranmayee (2014)	Impact of Statins in vitamin D deficiency and Genetic polymorphism in Indian population	Dr.B. Dinesh Kumar	Yet to be registered
61	Bidyalakshmi Loukrakpam (2014)	Studies on the food system of the Meitei community of Manipur and its nutritional implications	Dr. R. Ananthan	Osmania
62	Kondeti Suresh (2014)	Studies on regulation of FGF21 in obese and prediabetic rat models	Dr. K. Rajender Rao	Yet to be registered
63	S G D N Lakshmi Reddy (2014)	Development and validation of an index for assessing food safety at household level	Dr. V.Sudershan Rao	Yet to be registered
64	Arnab Chatterjee (2014)	Transcriptomic Analyses of Functionally Contrasting Tissues involved in Zinc Homeostasis	Dr.Sudip Ghosh	Osmania
65	U.V. Rama Krishna (2014)	Isolation, characterization and anti cancerous activity of bio active molecules from camellia sinensis	Dr.S.N.Sinha	Osmania (Yet to be registered)
66	Neelima AS (2014)	Intracellular mechanism of naturally available neuroprotective compounds in mitigating the combined toxicity generated by Lead and beta amyloid peptides in human brain cells	Dr.C.Suresh	Osmania

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
67	Talari Aruna (2014)	Nutritional quality, prebiotic potential and other health benefits of Raffinose family oligosaccharides of Pigeon Pea (Cajanus Cajan, L)	Dr.S.Devindra	Osmania
68	S.Kiruthika (2014)	Agricultural interventions for improving nutritional status among <5 year old rural Indian children.	Dr.Bharati Kulkarni	Osmania (Yet to be registered)
69	G.Sumalatha (2014)	Isolation and identification of Vit. B12 producing probiotic strains from dairy products	Dr.M.Shiva Prakash	
70	Mohd. Shujauddin (Yet to be registered)	Dynamics of intrauterine inflammation in relation to malnutrition during pregnancy-foetal outcome and metabolic changes in adulthood	Dr. R. Hemalatha	
71	Ajumeera Rajanna (Yet to be registered)	Embryonic Stem Cells as Model System to Study the Developmental Origin of Health with Micronutrient Deficiency- Obesity/Type 2 Diabetes	Dr. Vijayalakshmi Venkatesan	
72	Dr.Srinivas Rao (Yet to be registered)	In vivo and In vitro evaluation of novel bone substitute made of ECM (Extra-cellular matrix) and nHA (nano Hydroxyapatite)	Dr.P.Udaykumar	

New Enrollment 2015

S.No	Research Scholar	Title of the thesis	Supervisor
1.	Mr.V.Sudershan Reddy	Iron homeostasis in adolescent girls with iron deficiency anemia –Role of genetic varients and gut microbiome	Dr. R. Hemalatha
2.	Ms.Divya Singh	Not yet assigned	Dr.Sudip Ghosh

AWARDS / HONOURS CONFERRED ON SCIENTISTS

Name of the Scientist	Awards/ Honour
Dr.K.Yadamma, Technical Officer 'B'	Best Poster Award for Paper entitled “Protective effective of curcumin on cyclophasphomide induced chromosomal aberrations in germ cells of mice” at the 4th World Congress on Cancer Science & therapy, held in Chicago, USA (Oct.20-22).
Dr.C.Suresh, Scientist 'D'	ICMR Young Bio-medical Scientist fellowship for the years 2014-2015 by Indian Council of Medical Research for a period of six months from January 2015 to June 2015 to get Biomedical training in the areas of “Combined toxicities of Lead and Alzheimer's disease on Brain cells, its progression and protection by Various Naturally available Bioactive compounds" at Savannah State University, USA.
Dr.K.Madhavan Nair, Scientist ‘F’	elected as Fellow of the National Academy of Agricultural Sciences with effect from Jan.01, 2015

PARTICIPATION OF SCIENTISTS AT INTERNATIONAL MEETINGS/ WORKSHOPS/ CONFERENCES/ TRAINING PROGRAMMES

S. No	Name of the Scientist	Meeting/ Conference attended	Date
1	Dr.S.Vasanthi	8th session of the Codex Committee on Contaminants in Foods, at The Hague, The Netherlands	Mar.31-Apr 4, 2014
2	Dr.Sylvia Fernandez Rao	4th Annual Regional meeting of the South Asia Infant Feeding Research Network, at Islamabad, Pakistan. Presented a paper on "Mapping of current policies that support counselling for infant and young child feeding (IYCF) in India (Uttar Pradesh, Maharashtra and Andhra Pradesh) suing the Net-Map methodology".	Apr.7-11, 2014
3	Dr.V.Sudershan Rao	Resource person for SATNET Asia National Training Programme on Food Safety and Quality Assurance for Agriculture Trade Facilitation, at Dhaka, Bangladesh	June 1-2, 2014
4	Dr.K.Madhavan Nair	Micronutrient Forum Global Conference, at Addis Ababa, Ethiopia. Invited speaker of the session on Scaling up micronutrient interventions – Bridging the gaps between evidence and implementation: Integrating iron guidelines to support programs – Integration to implementation	June 2-6, 2014
5	Dr.Bharathi Kulkarni, Dr. N.Balakrishna and Dr.KV.Radhakrishna	Sponsored symposium of Project Grow Smart: An integrated micronutrient and early learning intervention among preschoolers in rural India" at Micronutrient Forum 2014 "Bridging Discovery and Delivery, at Addis Ababa, Ethiopia	June 2-6, 2014
6	Dr.G.Bhanu Prakash Reddy	Final meeting of DBT-EU FUNCFOOD project on "Impact of agents with potential use in functional food on induction of biomarkers of age-related diseases", at Stockholm, Sweden.	June 25-27, 2014
7	Dr.R.Ananthan	Third International Conference on Nutrition and Food Science 2014 (ICNFS-2014), held at Copenhagen, Denmark. Presented a paper on "Assessment of nutrient composition and capsaicinoid content of some red chillies	June 18-20, 2014
8	Dr.B.Dinesh Kumar	17th World Congress of Basic and Clinical Pharmacology (WCP2014), "Pharmacology at the cutting edge", at Cape Town, South Africa. Presented a paper on "Innovative approaches in non-clinical safety evaluation of oral recombinant anti human papilloma virus vaccine (Hpv 16 and 18) in mice, rats".	July 13-18, 2014
9	Mr.T.Longvah	29th International Horticulture Congress, at Brisbane, Australia. Presented a paper on "Indigenous vegetables and its role to the nutritional well-being of the indigenous population in Northeast India"	Aug.17-22, 2014

S.No	Name of the Scientist	Meeting/ Conference attended	Date
10	Dr.K.Bhaskarachary	6th Asian Congress of Dietetics on the theme "Advancing health through innovating dietetic practice across Asia", held at Taipei, Taiwan. Presented papers on "Professional dietetics: Credentials, competency and international outreach drives" and "Socio-demographic perspective of school nutrition in India: Paradigms and impact of educational and interventional initiatives".	Aug.21-24, 2014
11	Dr.Sudip Ghosh	Phase II Workshop on "Safety risk assessment of foods derived from genetically engineered plants", held at DuPont Stine Haskell Research Center, Located in Newark, Delaware, USA.	Sept.15-19, 2014
12	Dr.G.Bhanuprakash Reddy	DHR Short-term Fellow at the Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA	Oct. 8 – Dec. 5, 2014
13	Dr.K.Yadamma	4th World Congress on Cancer Science and Therapy, at Chicago, USA. Presented poster on "Protective effect of curcumin on cyclophosphamide induced chromosomal aberration in germ cells of mice".	Oct.20-22, 2014
14	Dr.AL.Khandare	32nd Conference of International Society for Fluoride Research, held at Chiang Mai, Thailand. Presented a paper on "Prevalence and severity of fluorosis in Doda district (hilly area of Jammu and Kashmir)	Nov.25-29, 2014
15	Mr.T.Longvah	International symposium on "Biodiversity, Food and Nutrition", at Columbo, Srilanka. Delivered keynote address on "Rice biodiversity for human nutrition".	Dec.7-9, 2014
16	Dr.K.Bhaskarachary	International seminar on "Protein nutrition and novel protein ingredients in 21st century – tackling the protein problem", at University of Manchester, UK. Presented a paper on "Changing pattern of food consumption in India and its impact".	Jan.20-22, 2015
17	T.Longvah	Preparatory meeting for studying the agrobiodiversity, food system and the nutritional wellbeing of the matriarchal society in Meghalaya and Patriarchal society in Nagaland, at Biodiversity International, Rome, Italy	Feb.9-10, 2015
18	Dr.K.Madhavan Nair	Invited as a Government representative for the 3rd SEAChange workshop held at Singapore	Feb.11-12, 2015

WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN

1. World Laboratory Animal Day: Seminar on "Humane Endpoints and Implementations of 3R's in Animal Experimentation", organized by NCLAS in association with Committee for the Purpose of Control and Supervision of Experiments on Animals and Humane Society International India, Hyderabad (April 25).
2. Meeting on "Fortification Technical Exchange" to discuss on fortification related issues with a team of FFI (Food Fortification Imitative) from USA and India (April 28).
3. 46th Laboratory Animal Technicians Training Course (LATTC) (June 16- July 31, 2014). Eleven candidates participated in the course.
4. Thirty fourth Laboratory Animal Supervisors Training Course (LASTC) (Sept.1 – Nov.30, 2014). Six candidates participated in the course.
5. Symposium on "New Trends in Dietary and Therapeutic Lifestyle Management for Heartcare", organized by Indian Dietetic Association, AP Chapter in association with National Institute of Nutrition, Hyderabad (Aug. 21)
6. In connection with the World Food Day celebrations, a one day seminar was organized on "Family Farming: Feeding the World, Caring for the Earth", in association with Association of Food Scientists and Technologists (Hyderabad Chapter) and Oil Technologists Association of India (South Zone) (Oct. 16).
7. Annual Training Course on Endocrinological Techniques and their Applications (Oct.8- Nov.21)
8. Brain Storming Session on "Foods for Health", A joint initiative of Ministry of Food Processing Industries (Govt. of India) and NIN, Hyderabad (Nov.14)
9. National Consultation for Revision of National List of Essential Medicines (NLEM-2011), organized by Ministry of Health and Family Welfare, Government of India (Nov.16-17)
10. Fifty second Post Graduate Certificate Course in Nutrition. Ten participants attended the course (Jan.06 – March 20, 2015).

SERVICES RENDERED TOWARDS INCOME GENERATION

1. PATHOLOGY SERVICES

• During the year, a total income of ` 98,500/- was generated from various projects of Institute's preclinical toxicology and surgical pathology and cytology samples analysation.

2. TRAINING PROGRAMMES

- An amount of ` 6,40,000/- was generated from the tuition fee collected from the first and second year participants of 2 year MSc (Applied Nutrition) course (1st year – 16 and 2nd year – 16 candidates).
- An amount of ` 1,32,000/- was generated from eight private candidates admitted to the regular training programme viz., Post Graduate Certificate Course in Nutrition including two participants from Bangladesh.

SCIENTIFIC PUBLICATIONS

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Ahamed Ibrahim S : Scientific basis of fat requirement for Indian and recent trends in CVD. Indian J Comm Health 26:S1:54-58,2014.
2. Akileshwari C, Raghu G, Muthenna P, Mueller NH, Suryanarayana P, Mark Petrash J, Bhanuprakash Reddy G : Bioflavonoid ellagic acid inhibits aldose reductase: implications for prevention of diabetic complications. J Functional Foods. 6: 374-383,2014.
3. Alekhya Sabbithi, Naveen Kumar R, Kashinath L, Bhaskar V, Sudershan Rao V : Microbiological quality of salads served along with street foods of Hyderabad, India. Int J Microbiol. ID 932191,2014
4. Anand Kumar K, Lalitha A, Umakar Reddy, Chandak GR, Sengupta S, Raghunath M : Chronic maternal vitamin B12 restriction induced changes in body composition & glucose metabolism in the wistar rat offspring are partly correctable by rehabilitation. PLOS One9:e112991, 2014.
5. Anwar MJ , Radhakrishna KV, Sharma A, Vohora D: Raloxifene preserves phenytoin and sodium valproate induced bone loss by modulating serum estradiol and TGF-β3 content in bone of female mice: Eur J Pharm Sci 62:219-226,2014.
6. Anwar MJ, Radhakrishna KV, Vohora D :Phenytoin and sodium valproate but not levetiracetam induce bone alterations in female mice . Can J Physiol Pharm 92: 507-511, 2014.
7. Archana K, Subba Rao MG, Swarnim Gupta, Madhavan Nair K : Millets in meeting nutrition security: issues and way forward for India. Indian J Nutr Dietet 51:306, 2014.
8. Arlappa N, Laxmaiah A, Balakrishna N, Harikumar R, Mallikharjuna Rao K, Brahmam GNV: Prevalance of hypertension and its relationship with adiposity among rural elderly population in India. Int J Clin Cardiol 1.003,2014.
9. Arlappa N, Meshram II, Balakrishna N, Harikumar R, Rao KM,Laxmaiah A. Prevalence of anaemia among different physiological groups in the rural areas of Maharashtra. Indian J.Comm Health : 26:278-284,2014.
10. Ashrafunnisa Begum, Venkateshwari A, Balakrishna N, Jyothy A : Association of GSTM1 & HMOX-1 Gene Polymorphisms in COPD: A study from South Indian Population. Int J Sci Res 3 :318-324,2014
11. Babu Geddam JJ, Thimma Reddy Y, Balakrishna N, Sunil Kumar Reddy, Ajay Kumar P , Radhakrishna KV : Osteoporotic hip fractures in low-income group population, hospital based case control study from India. Int J Med Public Health 4:336-342,2014
12. Basak S, Ehtesham NZ, Sesikean B, Sudip Ghosh : Detection and identification of transgenic elements by fluorescent-PCR-Based capillary gel electrophoresis in genetically modified cotton and soybean. J AOAC Int 97:159-165,2014.
13. Betsy A, Sudershan Rao V, Sinha,SN, Vishnu Vardhana Rao M, Kalpagam Polasa : Assessment of dietary intakes of nineteen pesticide residues among five socioeconomic sections of Hyderabad- A total diet study approach. Environ Monit Assess . 186:217-228, 2014
14. Bharati Kulkarni , Hills AP, Byrne NM: Nutritional influences over the life course on lean body mass of individuals in developing countries. Nutr Rev. 72:190-204,2014.
15. Bharati Kulkarni , Kuper H, Radhakrishna KV , Hills AP, Byrne NM, Taylor A, Ruth Sullivan, Liza Bowen, Wells JC, Yoav Ben-Shlomo, Smith GD, Shah Ebrahim, Sanjay Kinra: The Association of early

lifesupplemental nutrition with lean body mass and grip strength in adulthood: evidence from APCAPS. Am J Epidemiol 179:700-709,2014.

16. Bharati Kulkarni , Kuper H, Taylor A, Wells JC, Radhakrishna KV, Sanjay Kinra, Yoav Ben-Shlomo, Smith GD, Shah Ebrahim, Kurpad AV, Byrne NM, HillsAP: Assessment of body composition in Indian adults: comparison between dual-energy X-ray absorptiometry and isotope dilution technique. Br J Nutr 112:1147-1155, 2014.
17. Bharati Kulkarni, Mamidi RS, Balakrishna N, Radhakrishna KV: Body composition assessment in infancy and early childhood : comparison of anthropometry with dual-energy x-ray absorptiometry in low-income group children from India. Eur J Clin Nutr 68:658-663,2014.
18. Bhaskarachary K, Betsy A, Sudarshen Rao V, Vishnu Vardhan Raa M, Kalpagam Polasa : Dietary exposure assessment of lead and cadmium in rural population of Andhra Pradesh-India-A total diet study approach. Int J Food Nutr Sci 3: 45-52,2014.
19. Bhaskarachary,K; Ramulu P, Udayasekhararao P, Bapurao S, Kamala Krishnaswamy, Syed Q, Udaykumar P, Sesikeran B: Chemical composition, nutritional and toxicological evaluation of peanut (Arachis hypogea) grown in fly ash amended soils. Indian J Nutr Dietet 51:11-22,2014.
20. Chalamaiah M, Hemalatha R, Jyothirmayi T, Diwan PV, Uday Kumar P, Nimgulkar C, Dinesh Kumar B : Immunomodulatory effects of protein hydrolysates from rohu (Labeo rohita) egg (roe) in BALB/c mice. Food Res Int 62 :1054–1061,2014.
21. Duncan JL, Biswas P, Kozak I, Navani M, Syed R, Soudry S, Menghini M, Caruso RC, Jeffrey BG, Heckenlively JR, Bhanuprakash Reddy G , Lee P, Roorda A, Radha A: Ocular phenotype of a family with FAM161A-associated retinal degeneration Ophthalmic Genet Doi.10.3109/138168.10.2014.929716, 2014
22. Hemalatha R, Manoj Kumar, Nilita Das, Nishanth Kumar S, Hanumantha Reddy Ch, Ravinder N : Effect of probiotic lactobacillus salivarius UBL S22 and prebiotic fructo-oligosaccharide on serum lipids, inflammatory markers, insulin sensitivity, and gut bacteria in healthy young volunteers: a randomized controlled single-blind pilot study. J Cardiovasc Pharmacol Ther doi:10:1177/1074248414555004, 2014.
23. Hemalatha R, Naseha Mahmood, Manoj Kumar, Sudarshan Reddy V, Hanumantha Reddy Ch, Shiva Prakash M: Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized controlled trial. Mediators Inflamm Article Id:348959, 2014.
24. Hemalatha R, Ouwehand AC, Forssten SD, Babu Geddam JJ, Raja Sriswan M, Bhaskar V, Radhakrishna KV: A Community-based randomized double blind controlled trial of lactobacillus paracasei and bifidobacterium lactis on reducing risk for diarrhea and fever in preschool children in an urban slum in India. Eur J Nut Food Safety 4:325-341,2014.
25. Jose SS, Radhika MS, Balakrishna N, Brahmam GNV, Bhanuprakash Reddy G: Development of raw food based quantitative food frequency questionnaire for its reproducibility and validity in urban individuals of Hyderabad, India. Int. J Food Nutr. Sci.3:180-187,2014.
26. Khaleel Pasha Md, Qamar Ayesha, Lakshmi Narasu M, Pandu Ranga Rao K, Prabhakar B. Suseela, K. Balakrishna N, Srinivasulu M, Gopal Reddy M : Study of Magnesium, Copper, Zinc, Iron and Ceruloplasmin in Liver Cirrhosis and Ovarian Cancer Ascitic Patients. Br J Med Res 4: 3902-3911, 2014.
27. Khandare AL, Babu JJ, Ankulu M, Aparna N, Amol Shirfule, Shankar Rao G: Grass pea consumption & present scenario of neurolathyrism in Maharashtra State of India. Indian J Med Res 140:96-101, 2014.
28. Kinra S, Johnson M, Kulkarni B, Rameshwar Sarma KV,Yoav Ben-Shlomo, Smith GD: Socio-economic clash position and cardiovascular risk in rural Indian adolescents: evidence from the Andhra Pradesh children and parents study (APCAPS). Public Health 128:852-859, 2014
29. Kowshik J, Giri H, Kishore TK, Kesavan R, Naik Vankudavath R, Bhanuprakash Reddy G, Dixit M, Nagini S: Ellagic acid inhibits VEGF/VEGFR2, PI3K/Akt and MAPK signaling cascades in the hamster cheek pouch carcinogenesis model. Anticancer Agents Med Chem 14:1249-60,2014.
30. Kuper H, Taylor A, Radhakrishna KV, Ben-Shlomo Y, Gupta R, Kulkarni B, Prabhakaran D, Smith GD, Wells J,

Ebrahim S, Sanjay Kinra: Is vulnerability to cardiometabolic disease in Indians mediated by abdominal adiposity or higher body adiposity. BMC Public Health 14:1239, 2014.

31. Little Flower Augustine, Madhavan Nair K, Sylvia Fernandez Rao, Vishnuvardhana Rao M, Ravinder P, Balakrishna N, Laxmaiah A, Shahnaz Vazir : Adolescent life-event stress in boys in associated with elevated IL-6 and hepcidin but not hypoferremia. J Am College Nutr 33:354-362,2014.
32. Maheshwar M, Subba Rao GM, Venkaiah M, Raghunatha Rao D: The quality of nutrition research reporting by leading daily newspapers in India. J Media Commun Stud 6:92-98,2014.
33. Maheshwar M, Vijayapushpam T, Sylvia Fernandez Rao: A Current appraisal of health and nutrition related claims in Indian children television food advertisements. J Social Sci Studies. 1:125-135, 2014.
34. Manish Kumar J, Shrivastava S, Nair K, Sreenivasa Reddy S, Uday Kumar P, Kumar Talluri MVN, Naidu VGM, Ramakrishna S: Improvement of bioavailability and anti-inflammatory potential of curcumin in combination with emu oil. Inflammation 37:2139-55,2014
35. Mansoori T, Balakrishna N, Viswanath K: Influence of disc area on retinal nerve fiber layer thickness measurement by spectral domain optical coherence tomography. Indian J Ophthalmol 62: 615-8, 2014.
36. Matsuzaki M, Kuper H, Bharati Kulkarni, Radhakrishna KV, Viljakainen H, Taylor AE, Ruth Sullivan, Liza Bowen, Tobias JH, Ploubidis GB, Wells JC, Dorairaj Prabhakaran, Smith GD, Shah Ebrahim, Yoav Ben-shlomo, Sanjay Kinra: Life-course determinants of bone mass in young adults from a transitional rural community in India: the Andhra Pradesh children and parents sudy (APCAPS)[1-3.] Am J Clin Nutr 99:1450-1459,2014.
37. Mehrajuddin Bhat M, Bindu Noolu, Qadri SSYH, Ayesha Ismail : Vitamin D deficiency decreases adiposity in rats and causes altered expression of uncoupling proteins and steroid receptor co-activator 3. J Steroid Biochem Biol 144:304-312,2014.
38. Muthenna P, Raghu G, Anil Kumar P, Surekha MV, Bhanuprakash Reddy G: Effect of cinnamon and its procyanidin-B2 enriched fraction on diabetic nephropathy in rats . Chem-Biol Interact. 222:68-76,2014.
39. Nimgulkar C, Sravani E, Venu R, Chalamaiah M, Shyam P, Ravindranath A, Dinesh Kumar B: Role of in-silico COX2 molecular docking bioinformatics tool and in-vivo carrageenan induced various inflammatory phase in mouse and rat models, in evaluation of anti-inflammatory activity of molecules. Int J Pharma Res Rev 3:57-65, 2014.
40. Ovais S, Pushpalatha H, Bhanuprakash Reddy G, Rathore P, Bashir R, Yaseen S, Dheyaa A, Yaseen R, Tanwar O, Akthar M, Samim M, Javed K: Synthesis and biological evaluation of some new pyrazoline substituted benzenesulfonylurea/ thiourea derivatives as anti-hyperglycaemic agents and aldose reductase inhibitors. Eur J Med Chem 80:209-217, 2014.
41. Patil MA, Suryanarayana P, Uday Kumar P, Srinivas M, Bhanuprakash Reddy G: Evaluation of neonatal streptozotocin induced diabetic rat model for the development of cataract. Oxid Med Cell Longev. Article ID; 463264, 2014.
42. Pavithra N, Sathish L Babu N, Venkatarathanamma V, Pushpalatha H, Bhanuprakash Reddy G, Ananda K: Evaluation of α-amylase, α-glucosidase and aldose reductase inhibitors in ethyl acetate extracts of endophytic fungi isolated from anti-diabetic medicinal plants. Int. J Pharmaceut Sci Res 5:5334-5341,2014.
43. Prashanth A, Jeyakumar SM, Giridharan NV, Vajreswari A: Vitamin A-enriched diet modulates reverse cholesterol transport in hypercholesterolemic obese rats of the WNIN/Ob strain. J Atheroscler Thromb 21:1197-1207, 2014
44. Prashanth A, Jeyakumar SM, Singotamu L, Harishankar N, Giridharan NV, Vajreswari A. Mitochondrio-genesis and apoptosis: pPossible cause of vitamin A-mediated adipose loss in WNIN/Ob-obese rats. Nutr Metab (Lond) 11:45 doi:10-.1186/1743-7075-11-45,2014
45. Qureshi IA, Arlappa N, Qureshi MA : Prevalence of malaria and anemia among pregnant women residing in malaria-endemic forest villages in India. Int J Gynaecol Obstet 127:93, 2014

46. Raghu P, Ravindranadh P, Sudershan Rao V, Kalpagam Polasa, Sesikeran B: Effect of oxytocin injection to milching buffaloes on its content & stability in milk. Indian J Med Res. 139:933-939, 2014.

47. Rajender Rao K, Padmavathi I, Anju JN, Sugeetha KT, Giridharan NV, Raghunath M: Leptin gene promoter DNA methylation in WNIN obese mutant rats. Lipids in Health and Disease (Published online 5, Feb 2014).

48. Rajesh M, Rajanna A, Vijayalakshmi V, Balakrishna Murthy P: Proliferation and TH1/TH2 cytokine production in human peripheral blood mononuclear cells after treatment with cypermethrin and mancozeb in vitro. J Toxicol. ID 308286, 2014.

49. Ramakrishna UV, Sinha SN, Neelam Kumari, Bhatnagar V: A Review on pharmacognistic, phytochemical, chemical profile and apoptosis induction in yeast cells of Guazuma Ulmifolia. PHARMANEST. Int J Adv Pharmac Sci 5:2130-2141,2014.

50. Sinha JK, Ghosh S, Raghunath M: A review on Progeria: a rare genetic premature aging disorder. Indian J Med Res 139:114-121,2014.

51. Sharma S, Vijayalakshmi V, Balakrishna Murthy P, Ramesh B: Human mesenchymal stem cells as a novel platform for simultaneous evaluation of cytotoxicity and genotoxicity of pharmaceuticals. Mutagenesis. Doi:10,:geu086 [Epub], 2014.

52. Shrivastava S, Kulkarni P, Dinesh T, Manish Kumar J, Naidu VGM, Mallika A, Bhanuprakash Reddy G, Ramakrishna S: Piperlongumine, an alkaloid causes inhibition of PI3 K/akt/m TOR signaling axis to induce caspase-dependent apoptosis in human Triple-negative breast cancer cells. Apoptosis. 19:1148-64, 2014.

53. Sinha JK, Ghosh S, Swain U, Giridharan NV, Raghunath M: Increased macromolecular damage due to oxidative stress in the neocortex and hippocampus of WNIN/OB, a novel rat model of premature aging. Neuroscience. 269:256-264,2014.

54. Sinha SN, Venkat Reddy B, Vasudev K, Vishnu Vardhana Rao M, Noor Ahmed M, Shaik Ashu, Kumari A, Bhatnagar V: Analysis of dialkyl urine metabolites of organophosphate pesticides by a liquid chromatography mass spectrometry technique.. Anal Meth 6:1825-1834,2014.

55. Sreenivasa Reddy S, Shruthi K, Sudhakar Reddy V, Raghu G, Suryanarayana P, Giridharan NV, Bhanupraksh Reddy G: Altered ubiquitin-proteasome system leads to neuronal cell death in a spontaneous obese rat model. B B A General Subjects. 1840:2924-2934,2014.

56. Srinivasa Rao J, Bhaskarachary K, Longvah T: Evaluation of trace metal content by ICP-MS using closed vessel microwave digestion in fresh water fish. J Environ Public Health. Article id: 201506, 2014.

57. Srinivasa Rao M, Suryanaryana Murty U, Madhusudhan Rao K, Kartik N, Preeyantee G, Balakrishna N: Assessment of malaria incidence using the Richards model in Arunachal Pradesh, India. Epidemiol Infect 142:2227-36,2014.

58. Sudershan RV, Naveen Kumar R, Bharathi Kulkarni, Kashinath L, Bhaskar V, Polasa K: E. coli pathotypes and their antibiotic resistance in young children with diarrhea in Hyderabad , India. Int J Curr Microbiol Appl Sci 3:647-654,2014.

59. Sudershan RV, Naveen Kumar R, Kashinath L, Bhaskar V, Polasa K: Foodborne infections and intoxications in Hyderabad India. Epidemiol Res Int Article ID. 942961, 2014.

60. Sudhakar Reddy V, Uday Kumar Ch, Bhanuprakash Reddy G: Effect of chronic hyperglycemia on crystallin levels in rat lens. Biochem Biophys Res. Commun. 446:602-607,2014.

61. Sudhakar Reddy V, Uday Kumar Ch, Raghu G, Bhanuprakash Reddy G: Expression and induction of small heat shock proteins in rat heart under chronic hyperglycemic conditions. Arch Biochem Biophys 558:1-9, 2014.

62. Suguna D, Madhavi LH, Nagaiah G, Balakrishna N: Prevalence of Tobacco use among In-Patients at Sangareddy, India. Indian J Forensic Med Toxicol 8 :78-83.2014 .

63. Sunitha K, Bhaskarachary K, Manohar Rao D: Identification and quantification of major carotenoids in Coleus forskohlii. Indo Amer J Pharm Res 4:2407-2413, 2014.

64. Swarnim Gupta, Nair KM, Ravinder P, Ananthan Rajendran, Raghu P: Herbs as a dietary source of iron screening for iron bioavailability and absorption-promoting activity in selected herbs. Nutr Food Sci 44:443-454,2014.

65. Swetha B, Sudershan RV, Balakrishna N, Snehasree Saha, Subba Rao GM: Food risk perceptions of women in rural and urban households- A study in India. Eur J Nutr Food Safety 4: 380-391,2014.

66. Sylvia Fernandez Rao, Hurley KM, Madhavan Nair K, Balakrishna N, Radhakrishna KV, Ravinder P, Nicholas T, Harding KB, Reinhart GA , Black MM: Integrating nutrition and early child-development interventions among infants and preschoolers in rural India. Ann N Y. Acad.Sci.1308:218-231,2014.

67. Sylvia Fernandez Rao, Vijayapushpam T, Balakrishna N: Comparison of Nutrition Knowledge and Snacking Habits of Children from Schools Affiliated to Sate Education Board and Central Board of Secondary Education. Indian J Pub Health Res.Develop 5:153-157,2014 .

68. Tyagi A, Uday Kumar , Sai Santosh V, Suryam Reddy, Saazida Bhanu Md, Ahamed Ibrahim: Partial replacement of dietary linoleic acid with long chain n-3 polyunsaturated fatty acids protects against dextran sulfate sodium-induced colitis in rats. Prostaglandins Leukot Essential Fatty Acids 91:289-297,2014.

69. Venkaiah K, Hari Kumar R, Hemalatha R, Vara Prasad SP, Srinivasan K, Brahmam GNV: Impact of intervention program on sexual behavior, HIV and sexually transmitted infections among self-identified men who have sex with men in select districts of Andhra Pradesh, India. World J AIDS 4:458-469,2014.

70. Venkanna A, Siva B, Poornima B, Vadaparthi PR, Rajendra Prasad K, Ashok Reddy K, Bhanuprakash Reddy G, Suresh Babu K: Phytochemical investigation of sesquiterpenes from the fruits of schisandra chinensis and their cytotoxic activity. Fitoterapia. 95:102-108,2014.

71. Venkat Reddy B, Rajendra Prasad B, Sinha SN, Noor Ahmed: New mathematical derivations for calculation of ATP yield due to the complete oxidation of different types of fatty acids. Indian J Biochem Biophys 51:52-57,2014.

72. Vijayalakshmi V, Somasundaram I, Behera P, Bohara R: Non-obese diabetic mice and diabetes. Med J DY Patil Univ VIII:18-21,2014.

73. Vijayalakshmi V, Soundarya Lakshmi M: Promise(s) of using mesenchymal stem cells in reproductive disorders. Indian J Med Res 140 (S):72-79,2014.

74. Vijayapushpam T, Maheshwar M, Raghunatha Rao D: A Comparative analysis of television food advertisements aimed at adults and children in India. Int J Innov Res Sci Eng .2:476-483,2014.

B PAPERS PUBLISHED IN PROCEEDINGS/ BOOKS/CONFERENCES/ABSTRACTS

1. Archana K, Subba Rao GM, Balakrishna N, Harikumar R, Nair KM: Developing dietary diversity scale for designing a targeted communication to promote consumption of micronutrient rich foods among rural households. In "46th Annual National Conference of Nutrition Society of India", NSI conference, Ludhiana, 6-8th Nov., P.131, 2014.

2 Arlappa N, Balakrishna N, Harikumar R, Laxmaiah A: Ocular manifestation of vitamin A deficiency and its association with nutritional status of rural children in India. Proceedings of 2nd International conference on Nutrition and Growth held at Barcelona, Spain, 20th Jan to 1st Feb, 2014

3 Arlappa N, Balakrishna N, Harikumar R, Meshram II, Kodavanti M, Madhari R, Chitti G, Sharad K, Manchala R,Laxmaiah A: Nutritional status and its association with the protein and energy intakes among rural adolescent girls in India.. Proceedings of 2nd International conference on Nutrition and Growth held at Barcelona, Spain, 20th Jan to 1st Feb, 2014.

4 Arlappa N, Kokku SB: Drought, food security and micronutrient malnutrition. In "Handbook of Public Health in Natural Disasters", 337-365pp, 2014.

5 Arlappa N, Ravikumar BP, .Balakrishna N, Laxmaiah A: Prevalence of Type-II Diabetes Mellitus and metabolic

syndrome and their determinants among urban geriatric population - A community-based study. 3rd World Congress of Gerontology and Geriatrics: Held at Bengaluru, 17-19 Nov 2014.

6 Augustine, LF, Nair KM, Sylvia Fernandez-Rao, Balakrishna N, Vishnuvardhana Rao M, Ravinder P, Laxmaiah A: Linking the Bio-Behavioural pathway of stress, allostatic load and hepcidin: a cross sectional study among adolescent boys In "APAS Golden Jubilee Science Congress", Nov.13-15,2014, p.76.

7 Bnanuprakash Reddy G: Nutrigenomic studies onl complications of diabetes and obesity. At the National seminar on "Nutrignomics: a promising too for combating chronic diseases", at the P G Dept of Home Science, Sardar Patel Univ. Vallabh Vidyanagar, Feb.3-4,2014.

8 Bhanuprakash Reddy G, Raghu G, Muthenna P, Akileshwari C, Suryanarayana P, Petrash JM: Inhibition of ALR2 and AGE formation by ellagic acid: prevention of treatment of diabetic cataract and etinopathy. Annual meeting of association for research in vision and ophthalmology, Orlando (FL, USA) May 4-8, 2014. Abstract . Invest Ophthalmol. Vis Sci. 55:E1056, 2014.

9 Bhanuprakash Reddy G, Reddy SS, Reddy VS, Raghu G, Giridharan NV: Dysregulated ubiquitin-proteasome system leads to neuro degeneration in a spontaneous obese rat model. At the annual meeting of experimental biology-2014, San Diego, April 26-30, 2014. Abstract published in FASEB J 28 (sup.1):591,2014.

10 Bhat M, Syed Qadri S.Y.H, Ismail A: Abstract entitled "Vitamin D deficiency induced muscle wasting occurs through the Ubiquitin Proteasome pathway", presented at the Vitamin D and Human Health Conference held in London, UK during April 2014- published in' Nutrients 6: 2759-2919, 2014.

11 Deshpande J, Bhanuprakash Reddy G, Shankaranarayananan J, Bhattacharya A, Juturu V: Lutein improves visual function in early-stage streptozotocin-induced diabetic rats. The 17th International Carotenoid symposium, Park City (Utah USA), June 29-July 4, 2014.

12 Dripta Roy Choudhury, Sylvia-Fernandez Rao, Balakrishna N, Nair KM: Describing and assessing contextual factors for testing functional benefits of regular consumption of snp meal diversified with guava among ICDS preschool beneficiaries. In 46th Annual National Conference of Nutrition Society of India, NSI conf., Ludhiana, 6-8th Nov., P.109, 2014.

13 Hemalatha R, Sesikeran B: Role of probiotics as functional food in lifestyle diseases by Probiotics in Prevention of Lifestyle disorders, Ed. by Prof. BS Ramakrishna, 62-74pp, 2014.

14 Jakhotia S, Reddy VS, Bhanuprakash Reddy G: Implications of small heat shock proteins in diabetic nephropathy, presented at the 83rd annual meeting of the Society of Biological Chemists (India) in Bhubaneswar, 17-21 December 2014. Abstract published in SBCI proceedings, Abstract # P-06-007, 2014.

15 Kiruthika S, Balakrishna N, Laxmaiah A, Nair KM: A Multivariate analysis on the national nutrition monitoring bureau (NNMB, 2006) data base to assess the determinants of anemia among rural Indian men and women of age >20 years. In "46th Annual National Conference of Nutrition Society of India", NSI CON, Ludhiana, 6-8th Nov., P.84, 2014.

16 Maheshwar M: Deforestation-a threat to nutrition In "National Conf. on Conservation of Eastern Ghats", 4th & 5th Dec.2014, S V University Tirupathi. P80. 2014.

17 Nair KM:Scaling-up of interventions to control anemia-bridging the gap between evidence and implementation. In "APAS Golden jubilee science congress", Nov.13-15,p.111, 2014.

18 Nair KM, Archana K: Opportunities for millets as functional foods. "Proceedings of Global consultation on millets promotion for health and nutritional security ", Dec 2013, pp. 145-153, 2014

19 Nair KM, Little Flower Augustine: National nutrition agenda: current status and way forward, in" APAS Golden jubilee science congress"; Nov.13-15, p.69, 2014 20 Raghunath M: Brain storming on "Food to Food Fortification for the Addressal of Micronutrient Deficiencies" , by Dept of Biotechnology and CFTRI, at CFTRI, Mysore, Sept, 9, 2014. Presented a paper " Micronutrient deficiencies, developmental programming and the diabesity epidemic in India: Food fortification as a strategy for its better management". 2014.

21 Raghunath M: Maternal g deficiency induced increase in adiposity in WNIN rat offspring: Role of stress,

inflammation and epigenetics", SNEHA- MRC International workshop, Fariyas Resorts, Lonavala, Pune 26-28, Sept 2014.

22 Raghunath M: National Consultation cum Brain storming Session on "Fortification of Edible Salt with Micronutrients", at AIIMS, New Delhi, January 20, 2015, Presented a paper ,"NIN DFS : Technology used in its development, factory production and supply for different programmes".2014.

23 Raghunath M: National level Workshop on "Obesity: A life style challenge", at Dr V.S.K.Govt Degree and P G College, Visakhapatanam, A.P. Feb 3-4, 3015. Presented the keynote address entitled, "Epidemic of Obesity and Diabetes in India: Role of Maternal micronutrient deficiencies and developmental programming". 2014.

24 Raghunath M: National Seminar on "Endocrine system – Disorders and stress response" at Govt College for Women (UG &PG) ,Osmania University, Secunderabad, Dec 16-17, 2014. Presented a paper entitled," Diabesity epidemic in India : Role of developmental programming and maternal micronutrient deficiencies". 2014.

25 Reddy VS, Bhanuprakash Reddy G: Role of small heat shock proteins in muscle under chronic hyperglycemia, at the annual meeting of experimental biology, San Diego, April 26-30, Abstract, FASEB J 28 (1 Supp): 1102. 42,2014.

26 Shruthi K, Reddy SS, Redy PY, Sivalingam P, Harishankar N, Bhanuprakash Reddy G: Amelioration of neuronal cell death in a spontaneous obese rt model by calorie restriction through modulation of ubiquitin proteasome system. Presented at the 83rd annual meeting of the society of biological chemists (India) in SBCI proceedings, Abstracaat #P-13-005, 2014.

27 Sivaprasad M, Shalini T, Balakrishna N, Radhika MS, Arlappa N, Suryanarayana P, Bhanuprakash Reddy G: Presented at the 83rd annual meeting of the society of biological chemists (India) in SBCI proceedings, Abstract #P-01-010, 2014.

28 Sylvia Fernadez Rao, Balakrishna N, Engle PL, Bentley M, Shahnaz Vazir : Role of maternal locus of control in infant and young child feeding intervention in rural India. Maternal Health in India: Contemporary Issues and Challenges. Ed by Mohammad Akram. Rawat Publications New Delhi. 241-254pp, 2014

29 Sylvia Fernandez-Rao, Nair KM, Balakrishna N, Radhakrishna KV, Ravinder P, Hurley K, Nick Tilton, Greg Reinhart, Kim Harding, Black M: Multiple micronutrient nutrient fortification of anganwadi meal improves iron status and cognitive development of preschoolers in the anganwadi centres, in 46th Annual National Conference of Nutrition Society of India, NSI, Ludhiana, 6-8th Nov., P.78, 2014.

30 Syed SYH Qadri, Christian E.Newcomer: Laws, regulations, and guidelines shaping research animal care and use in India. Laboratory Animals ed. Javier Guillen, AP , 2014.

31 Swarnim Gupta, Nair KM, Ravinder P: A High throughput screening tool for iron dialyzability in food samples: screening of food combinations for improving iron bioavailability, in "46th Annual National Conference of Nutrition Society of India", NSI conf., Ludhiana, 6-8th Nov., P.57, 2014.

32 Qureshi IA, Arlappa N, Qureshi MA: Prevalence of malaria and anaemia among pregnant women residing in malaria endemic forest villages in India. Int J Gynaecol Obstet. 127:93,2014.

PART-C

1. Bhanuprakash Reddy G: Nutrition and cataract. In Touch : 16 (issue 1 April-June):2-7, 2014.
2. Desai C, Dinish Kumar B: Four and half decades of Indian journal of pharmacology (Editorial). Indian J Pharmacol 46:473-474,2014.
3. Gupta YK, Dinesh Kumar B: Clinical trials and evolving regulatory science kin India. Editorial. Indian J Pharmacol 46:575,2014.

SCIENTIFIC ADVISORY COMMITTEE

S.No.	Name & Address	Email ID / FAX / Contact No.
1	**Dr. Sandip K. Basu** JC Bose Chair Professor National Academy of Sciences India 782, Sagar Presidency, F-8, Sector-50, Noida – 201301, UP	Tel: 011-26863702 Cell : 09891498821 sandip@niscair.res.in sandipkbasu@gmail.com
2	**Prof. P.B. Seshagiri** Professor Dept. of Molecular Reproduction, Development & Genetics, Indian Institute of Science Bangalore – 560 012	Tel: 080-22932687 Fax:080-23600999 polani@mrdg.iisc.ernet.in
3	**Dr. Subeer S. Majumdar** Incharge, Cellular Endocrinology and Embryo Biotechnology, NII, Aruna Asaf Ali Marg New Delhi - 110 067	Tel: 011-26703751/ 011-26703638 Fax: 011-26742125/ 011-26742626 EMAIL: subeer@nii.res.in
4	**Dr. T.S. Rao** Adviser Department of Biotechnology, Block-2, 8th Floor CGO Complex, Lodi Road New Delhi – 110 003	Tel : 011-24364065 Mobile : 09873483538 Email: tsrao@dbt.nic.in
5	**Dr. P. Prakash Babu** Professor & HoD, Dept. of Biotechnology School of Life Sciences, University of Hyderabad Hyderabad-500 046	Tel: 040-23134584; 23013336 Mobile: 9441583525 Email:prakashbabuphanithi@gmail.com
6	**Dr. Arun Kumar KP** Scientist &Head-Molecular Genetics Department Bldg. 7, Gruhakalpa, 5-4-399 / B, Nampally Hyderabad-500 001,	Tel: 040-24749345 9666694000; Fax: 24749448 Email. arun@cdfd.org.in
7	**Dr. D. Swarup** Former Director-CIRG and Head Division of Medicine, IVRI, B-5, 102, Elita Promenade JP Nagar Phase 7, Bangalore-560078	Mobile: : +91-782-968-0777 devendra.swarup@gmail.com
8	**Dr. V.V. Kulkarni** Director, National Research Centre on Meat, Chengicherla, P.O.Box No.19, Boduppal Hyderabad 500092	Office: 040-29801672 Mobile: 08008007931 Fax: 040-29804259 Email: nrcmeat_director@yahoo.co.in
9	**Dr. K. Nachimuthu** Former Director of Research, Tamilnadu Veterinary and Animal Sciences University, No.4, Damodaran Street Panchavadi, Chetpet Chennai – 600 031	Mobile: 094441 66181 Email: nachimuthu@hotmail.com
10	**Dr. R.J. Yadav** Scientist-G, National Institute of Medical Statistics, ICMR, New Delhi – 110 029	Tel: 011-26588636 Mobile: 9868637736 Email: rjyadav@hotmail.com