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National Institute of Nutrition

ANNUAL REPORT

2007-2008

डॉ.विश्व मोहन कटोच
एम डी, एफ एन एससी, एफ ए एम एस, एफ ए एससी, एफ एन ए
सचिव, भारत सरकार
(स्वास्थ्य अनुसंधान विभाग)
स्वास्थ्य एवं परिवार कल्याण मंत्रालय एवं
महानिदेशक, आई सी एम आर

Dr. Vishwa Mohan Katoch
MD, FNASc, FAMS, FASc, FNA
Secretary to the Government of India
(Department of Health Research)
Ministry of Health & Family Welfare &
Director-General, ICMR

भारतीय आयुर्विज्ञान अनुसंधान परिषद
(स्वास्थ्य अनुसंधान विभाग)
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
वी. रामलिंगस्वामी भवन, अंसारी नगर
नई दिल्ली - 110 029 (भारत)

Indian Council of Medical Research
(Department of Health Research)
Ministry of Health & Family Welfare
V. Ramalingaswami Bhawan, Ansari Nagar
New Delhi - 110 029 (INDIA)

MESSAGE

National Institute of Nutrition (NIN), Hyderabad continues to be our apex Institute addressing all topical questions related to nutritional problems of India. The hallmark of the research activities of this institute has always been an immense diversity in the research questions formulated on a variety of themes of nutritional significance. The vast scope the science of nutrition offers to the researchers and also the presence of other research centres on the same campus – Food / Drug Toxicology and Laboratory Animal Sciences and Community Nutrition provide the necessary impetus to the scientists to indulge in a cross cutting areas of research. The work carried out this year reflects the broad range of scientific investigators, be it in the field of basic research or multi-centric operational research in the community. Use of well designed research protocols with a good mix of quantitative and qualitative research techniques have led to good output of work as seen in this Annual Report. Keeping in view the emergent needs, the Institute is focusing on community based studies that have immediate relevance. Investigations on the problem of overweight and obesity in urban adolescents and on HIV related behavioural sciences and biological issues are few such studies.

The problem of low birth weight infants, health issues relating to osteoporotic hip fractures in adults or those relating to micronutrient malnutrition have also been addressed by NIN researchers this year. A series of interesting studies on cataract and retinal degeneration perhaps need special mention.

I hope that the institute will continue its good work already initiated in the area of pre-clinical toxicology and undertake more such studies impacting on public health, especially of the underprivileged sections of our society. Promotion of nutritional status and enrichment of the quality of life of our population groups should continue to be to the guiding principles of all research endeavours here. The bottom-line should be research in nutrition that is of relevance to large sections of our society.

While conveying my deep appreciations to the entire staff of NIN, I wish them all success in their future endeavours.

(V.M. Katoch)

Tele.: (Off.) +91-11-26588204, 26589620; Fax (Off.): +91-11-26588662, E-mail: secydhr@icmr.org.in; dg@icmr.org.in

CONTENTS

RESEARCH STAFF

DIRECTOR
B.Sesikeran, MD

CLINICAL DIVISION

MATERNAL AND CHILD HEALTH
Veena Shatrugna, MD
(Scientist 'E')
B.A. Ramalakshmi, MBBS, DGO
K. V. Radhakrishna, MBBS, DCH
G. Jagjeevan Babu, MBBS
Bharati Kulkarni, MBBS, DCH
G. Amarendra Reddy, MA, MPhil
Prabhavati Paranjape, BSc

PATHOLOGY
L. Singotamu, MSc, PhD
(Scientist 'F')
P. Uday Kumar, MD
(Scientist 'E')
SSYH. Qadri, MVSc

MICROBIOLOGY
R. Hemalatha, MD
M. Shiva Prakash, M.Sc, PhD
L.A. Ramaraju, M.Sc
S. Anand Rao, M.Sc
B. Narayan Goud, B.Sc
B. Prathibha, M.Sc
G. Krishnaswetha, M.B.B.S

BIOCHEMISTRY DIVISION

A. Vajreswari, MSc, PhD
(Scientist 'F')
V.Vijayalakshmi, MSc, PhD
Ahmed Ibrahim, MSc, PhD
C. Suresh, MSc, PhD
Sanjay Basak, MSc, PhD
P. Sujatha, Msc, PhD, PGDN & DM
S.Hemalatha, MSc, PhD
S. M.Jeyakumar, MSc, Mphil, PhD
A.Prashanth, MSc
G.Sashikiran, MSc
Sheril Alex, MSc
SSV.Prasad, MSc
J.Shashidhar, MSc PhD
D. Dileep, MSc

MOLECULAR BIOLOGY
Nasreen Zafar Ehtesham, MSc, PhD
(Scientist 'E')
Sudip Ghosh, MSc, PhD
N. Vijaya Banu, MSc, PhD
Abdul Haseeb, MSc
B. Aruna, MSc
Mohd. Nasiruddin, MSc
M.Srivani, MSc
D.Munni Kumar, MSc

BIOPHYSICS DIVISION

K. Madhavan Nair, MSc, PhD
(Scientist 'E')
Y. Venkataramana, MSc, PhD
S. Ranganathan, MSc, PhD
(Radiation Safety Officer)
P. Raghu, MSc, PhD
P. Ravinder, MSc, PhD
B. Satyanarayana, MSc
K. Sreenivasulu, MSc
Vasuprada Iyengar, MSc
Gitanjali, MSc

FOOD CHEMISTRY DIVISION

T. Longvah, MSc
(Scientist 'F')
P. Amrutha Rao, MBBS, DPH
K. Bhaskarachary, MSc, PhD
J.Sreenivasa Rao, MSc
S.Devendra, MSc, Mphil, PhD
R. Ananthan, MSc, PhD
P. Ramulu, MSc, PhD

ENDOCRINOLOGY & METABOLISM DIVISION

M. Raghunath, MSc, PhD
(Scientist 'E')
G. Bhanu Prakash Reddy, MSc, PhD
Rita Saxena, MSc, PhD
P. Suryanarayana, MSc, PhD
K.Rajender Rao, MSc, PhD
C. Vijayakumar Reddy, MSc, PhD
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T. Mrudula, MSc
PNBS Srinivas, MSc
IJN. Padmavathi, MSc
Manisha Ganeshan, MSc
P.B.Sainath, MSc
K.Anand Kumar, MSc
P.Muthenna, MSc

FIELD DIVISION

G.N.V. Brahmam, MBBS, DPH
(Scientist 'F')
Shahnaz Vazir, MA, PhD
(Scientist 'E')
A. Laxmaiah, MBBS, MPH, DPH
(Scientist 'E')
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N. Arlappa, MBBS
Ch. Gal Reddy, MA, MPhil
K. Mallikharjuna Rao, MSc, PhD
I.I.Meshram, MBBS, MD(PSM)
M.S. Radhika, MSc, PhD
Sharad Kumar, MA, MPhil
M. Ravindranath, MA
Little Flower Augustine, MSc

STATISTICS DIVISION

K. Venkaiah, MSc
(Scientist 'E')
T. Prasanna Krishna, MSc, PhD
(Scientist 'E')
M. Vishnuvardhan Rao, MSc, PhD, MTech (IT)
N. Balakrishna, MSc, PhD
Grace Maria Antony, MSc, PGDCA

EXTENSION & TRAINING DIVISION

Krishnakumari Menon, MSc
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T. Vijaya Pushpam, MA, Mphil
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G. M. Subba Rao, MA, PGDJ, PGDT
K. Damayanthi, MSc, PhD
Anilkumar Dube, MA, MCJ, DPM
R. Nageswara Rao, MSc, BJ

LIBRARY

M. Devidas, MA, MLISc

INSTRUMENTATION

M. Raghunath, MSc, PhD
(Scientist 'E')
Ramachander Chowgule
Dip. in Electronics

FOOD & DRUG TOXICOLOGY RESEARCH CENTRE

Director
B.Sesikeran, MD
Kalpagam Polasa, MSc, PhD, MBA
(Scientist 'F')

FOOD TOXICOLOGY

Arjun L Khandare, MSc, PhD
J. Padmaja, MSc, PhD
V. Sudershan Rao, MSc, PhD
S. Vasanthi, MSc, PhD
Priyanka Shankar, MSc

DRUG TOXICOLOGY

M.P. Rajendra Prasad, MBBS, MSc (AN), PhD
(Scientist 'E')
B. Dinesh Kumar, MSc, PhD
Y.Srinivas Reddy, MSc

NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

Director
B.Sesikeran, MD
N.V. Giridharan, MSc, PhD
(Scientist 'F')
S. Kalyanasundaram, MSc
(Scientist 'E')
P. Suresh Babu, MVSc
(Scientist 'E')
N. Hari Shanker, MSc, PhD
B. Kiran Kumar, MSc
B.Pratibha, MSc

PRE-CLINICAL TOXICOLOGY

Director
B.Sesikeran, MD
Kalpagam Polasa, MSc, PhD, MBA
(Scientist 'F')
N.V. Giridharan, MSc, PhD
(Scientist 'E')
T. Prasanna Krishna, MSc, PhD
(Scientist 'E')
S. Kalyanasundaram, MSc
(Scientist 'E')
P. Uday Kumar, MD
(Scientist 'E')
P. Suresh Babu, MVSc
(Scientist 'E')
R. Hemalatha, MD
B. Dinesh Kumar, MSc, PhD
SSYH. Qadri, MVSc
N. Hari Shanker, MSc, PhD

ADMINISTRATION

G. Krishna Reddy, B.Com
Alexander Verghese
M.J. Radha Bai
A. Subba Reddy, BA
Mohd.Shamsuddin, B.Com
M. Ashok Raj, B.Com

MAINTENANCE

P. Rajamohan Rao, LCE, PGDCPEMM

RESEARCH HIGHLIGHTS

The research activities of the institute this year covered a wide spectrum of projects in the fields of HIV/AIDS, adolescent obesity, osteoporotic hip fractures, diabetes mellitus, spices, fats-cancer interface, effect of pesticide exposure, ayurvedic bhasmas, nutrition in school curriculum etc. The studies conducted had a fine blend of hospital, community and laboratory based research work. Also, studies done in the realms of basic research, molecular biology and laboratory animal sciences revealed some interesting findings. Rich data were generated on health enhancing properties of roots, tubers and vegetables. New information pertaining to protein, iron and zinc content in several rice varieties were collected this year. The new research findings have indeed provided the much needed value addition to the existing research culture of the institute.

1. COMMUNITY STUDIES

1.1 Integrated behavioural and biological assessment among high risk groups for HIV in select districts of Andhra Pradesh revealed the following findings:

Female sex workers (FSWs)

The mean age was about 30 years, average duration of sex work was 5 to 8 years, with mean number of clients during the previous week ranging from 5 to16. Most of them (>90%) had occasional clients also. Consistent condom use ranged from 15 to 85% with regular clients and 36 to 89% with occasional clients. The prevalence of HIV ranged from 8 to 26%, and that of syphilis was 5 to 17%.

Men having sex with Men (MSM)

The mean age ranged from 25 to 30 years. Majority were literate (58% - 82%) and nearly 90% reportedly had non-commercial male/hijra partners. The extent of consistent use of condom was very low and ranged from 1 to 40%. However, condom use during the last sexual intercourse with paying male partners was more than >70% in all the districts. A considerable proportion of MSM (28 to 64%) reportedly had 'paid female partners' and consistent condom use with them ranged from 4 to 37%. The prevalence of HIV ranged from 9 to 26%, and that of syphilis was about 4 to 16%.

Clients of Female sex workers

The mean age ranged from 28 to 31 years, with a majority of them being literate (64% to 90%). Consistent condom use with regular or occasional FSWs, was in general low and ranged from 16% to 38%, while it was negligible with main/steady partner. The prevalence of HIV ranged from 2 to 8%, while that of syphilis ranged from a low 3 to10%.

1.2 Assessment of Prevalence of overweight/ obesity and its determinants among 12-17 year urban adolescents in Andhra Pradesh

Obesity is one of the fast emerging public health problems among adolescents and adults, both in the developed as well as developing countries, leading to high incidence of hypertension, type 2 diabetes, coronary artery diseases, stroke, etc. The present study, was therefore, carried out to assess the prevalence of overweight/obesity and identify its determinants among adolescents of 12 to 17 years old in the urban areas of Andhra Pradesh. A total of 8,142 adolescents of 12–17 years age group were covered in the present study.

The overall prevalence of overweight was about 6%, which was significantly ($p< 0.05$) higher among girls (7.1%) as compared to boys (4.4%) and among the adolescents of high socio-economic status (9.6%) as compared to low socio-economic group (2.3%). The average duration of 'TV watching' was significantly higher ($p< 0.05$) among overweight and obese children (1.4 hrs/day) as compared to controls (1.2 hrs/day) and participation in outdoor games and sports for 6hrs/week was significantly ($p< 0.05$) higher among the non-obese children (31.9%) compared to overweight and obese (17.5%). The proportion of children consuming soft drinks was significantly

higher ($p < 0.05$) among the overweight and obese adolescents (21%) as compared to normal children (16%). The prevalence of hypertension (JNC Criteria VII) was significantly ($p < 0.05$) higher among the overweight and obese children (8.3%) as compared to non-obese children (3.7%). The study highlights the need to educate the adolescents to promote physical activity, healthy food habits and other life style practices to prevent overweight and obesity and the consequent diet-related chronic degenerative diseases.

2. CLINICAL STUDIES

2.1 Neonatal anthropometric data and body composition in Small for Gestational Age (SGA) babies compared to Appropriate Gestational for Age (AGA) babies

Barker's hypothesis speculates that under-nutrition in intra-uterine period predisposes to increased adiposity in later life. A study was done to compare the body composition of SGA babies with full term normal babies. Contrary to the earlier studies which have shown relatively higher body fat percentage in low birth weight (LBW) babies, this study assessing over 600 newborns has shown that neonatal body fat percent is increasing with increasing birth weight, and fat percent and fat free mass (FFM) were significantly low in LBW babies. No significant differences were observed in trace element levels in either maternal or cord blood.

2.2 Bone parameters of men and women with osteoporotic hip fractures

A study was carried out to measure the bone parameters of men and women with confirmed osteoporotic fractures. The results of the study show that osteoporotic fractures in the study population (Hyderabad, hospital-based study) occurred at least 10-15 years earlier as compared to Western population.

3. BASIC STUDIES

3.1 MICRONUTRIENTS

Micronutrient research group has been involved in developing novel strategies to combat micronutrient malnutrition, especially of iron, zinc and vitamin A and understanding their mechanism(s) of absorption. Towards this, a rapid and sensitive common immuno assay (ELISA) for phytoferritins (plant iron-storage protein) has been developed as a screening tool for identifying high iron in biofortified crops. This was used to quantify phytoferritin levels in pulses which correlated well with their iron density. Zinc bioavailability using Caco-2 cells was developed, standardized and validated by demonstrating modulation of bioavailability in the presence of known absorption enhancers and inhibitors. Caco-2 cells were used to understand the mechanism of negative interactions between iron and zinc during uptake at the enterocyte. Kinetic studies show that zinc inhibition of iron uptake does not occur at DMT-1 (apical iron transporter) and cellular zinc status profoundly affects quantity and pattern of iron uptake.

4. DEGENERATIVE DISEASES

4.1.1 Metabolic programming of insulin resistance: Role of maternal and peri / postnatal chromium status in the offspring. Muscle development and function

Earlier studies demonstrated that chronic maternal micronutrient restriction altered the body composition [body fat %, lean body mass [LBM] and fat free mass [FFM]] in rat offspring and may predispose them to adult onset diseases. Since chromium regulates glucose and insulin metabolism, the effect of maternal Cr restriction (CrR) on muscle development and function in the offspring was studied. In the offspring of WNIN female rats fed a control or CrR diet throughout their phases of growth, pregnancy and lactation or CrR mothers rehabilitated from conception, parturition or weaning indicate that maternal CrR significantly decreased LBM % and FFM % in the male and female offspring suggesting a decreased muscle and / or bone mass. Expression of the myogenic genes : Pax3, MyoD, Myf5 and MyoG, was significantly decreased in their muscle indicating that impaired muscle development could be a contributor to the decreased LBM % and FFM %. Although basal glucose uptake by muscle was higher in CrR than CrC offspring, the fold increase with insulin was comparable suggesting no change in its insulin sensitivity. Interestingly, body composition changes were seen in male offspring only at 18 months of age, whereas, in females they were observed from 12 months onwards.

Rehabilitation from conception but not parturition or weaning partly corrected the changes in expression of myogenic genes but not those in LBM % or FFM % or glucose uptake by the muscle *in vitro*. Thus, maternal Cr restriction in WNIN rats appears to irreversibly impair muscle development and function in the offspring.

4.1.2 Hypoglycemic / insulin like activity in camel milk: Quantification of the effect in animal models of diabetes / insulin resistance

Both the camel milk as well as cow milk (raw, pasteurized or boiled) has no hypoglycemic effect in the streptozotocin induced model of hyperglycemia / type 1 diabetes in WNIN rats at the dosage level tested. However, they have a comparable hypoglycemic effect in high sucrose diet induced model of hyperglycemia / type 2 diabetes in WNIN rats and the effect is heat stable in general in both the milks. Further, the hypoglycemic effect of both these milk samples, appears not to be due to their ability to modulate basal levels of plasma insulin or the secretion to a challenge of oral glucose load. From the results it appears that at the dosage level employed in these studies, the hypoglycemic effect of camel milk does not seem to be greatly different from that of cow milk in both the models of hyperglycemia (i.e., drug or diet induced).

4.1.3 Generation of database on health beneficial effects of plant foods commonly consumed in India: Roots, Tubers and other vegetables

As a part of efforts to generate the database on the phenolic content and antioxidant activity (AOA) of plant foods commonly consumed in India, data has been generated this year on roots, tubers and vegetables. In general there was a wide variation in the phenolic content and AOA of the foods analysed. Among the roots and tubers, beetroot and carrot had the highest and the lowest phenolic content and AOA as determined by the DPPH scavenging and FRAP methods. Among the vegetables, red cabbage and ridge gourd had the highest and the least phenolic content. DPPH scavenging activity was the highest in beet root followed by red cabbage in a close second place while ridge gourd had the least activity. On the other hand, FRAP was highest in red cabbage followed by a distant second in raw mangoes while pumpkin had the lowest activity. In general, there was a significant correlation between the phenolic content and AOA (both methods) in the foods studied.

4.2 RESEARCH ON CATARACT AND RETINAL DEGENERATION

4.2.1 Erythrocyte aldose reductase activity and sorbitol and diabetic retinopathy

Activation of polyol pathway due to increased aldose reductase (ALR2) activity has been implicated in the development of diabetic complications including diabetic retinopathy (DR), a leading cause of adult blindness which is also the most common complication of diabetes. However, the relationship between hyperglycemia-induced activation of polyol pathway in retina and DR is still uncertain. The relationship between ALR2 levels and human DR in a case-control study was investigated. Type-2 diabetes (T2D) patients with DR showed significantly higher specific activity of ALR2 as compared to T2D patients without DR. Elevated levels of sorbitol in T2D patients with DR substantiated the increased ALR2 activity in erythrocytes of DR patients. Thus, levels of ALR2 activity and/or sorbitol in erythrocytes may have value as a quantitative trait to be included among other markers to establish a risk profile for development of DR.

4.2.2 Effect of curcumin on hyperglycemia-induced vascular endothelial growth factor expression in streptozotocin-induced diabetic rat retina

Diabetic retinopathy is one of the most devastating microvascular complications of diabetes. Neovascularization stimulated by hyperglycemia mediated induction of vascular endothelial growth factor (VEGF) has been implicated in the pathogenesis. Various small molecules have been investigated for their ability to inhibit angiogenesis. In this study, it was demonstrated that feeding of curcumin and turmeric to diabetic rats inhibited expression of VEGF. This study highlighted the importance of biologically active compounds derived from dietary agents that could be explored further for the prevention and/or treatment of diabetic retinopathy.

4.2.3 Effect of turmeric and curcumin on oxidative stress and antioxidant enzymes in streptozotocin-induced diabetic rat tissues

There is increasing evidence that complications related to diabetes are associated with increased oxidative stress. Curcumin, an active principle of turmeric, has several biological properties including antioxidant activity. The protective effect of curcumin and turmeric on streptozotocin (STZ)-induced oxidative stress in various tissues of rats was studied. Results of this study indicate that curcumin and turmeric controlled diabetes-mediated oxidative stress by inhibiting the lipid and protein oxidation and reversing altered antioxidant enzyme activities without altering hyperglycemic state in most of the tissues. Hence, turmeric and curcumin might be beneficial in preventing the diabetes-induced oxidative stress.

4.2.4 Anticataractogenic effect of ginger against streptozotocin - induced diabetic cataract in rats.

Formation of advance glycation end products (AGE) through non-enzymatic glycation of proteins is one of the mechanisms that is implicated in the development of diabetic complications. Therefore, inhibition of AGE formation is of considerable value in ameliorating the complications of diabetes like cataract. A number of dietary sources for their antiglycating activity was evaluated and ginger, one of the agents, has shown antiglycating activity in the *in vitro* studies. In this study, it was demonstrated that ginger is effective in delaying the onset and progression of diabetic cataract in rats. These results, thus, provide a basis for the antiglycating effect of ginger that may have pharmacological implications in the treatment of diabetic complications.

4.2.5 Inhibition of aldose reductase by rutin

Inhibition of ALR2 represents one of the means for the treatment of diabetic complications. In the course of investigations on the evaluation of aldose reductase inhibitory activity from natural sources, a significant inhibition with some dietary sources was found. Based on the data base search it was found that rutin is a common flavonoid in these sources. Further, rutin is one of the flavonols, which is abundantly present in many fruits and vegetables. Studies conducted at NIN indicate that rutin inhibited ALR2 with an IC_{50} value 16 µM. It is specific towards ALR2 (over ALR1) and prevented the accumulation of sorbitol in RBC. These results suggest the significance of rutin as a specific and potent ALR2 inhibitor, which could be explored further for preventing or delaying of diabetic complications.

4.3 MOLECULAR BIOLOGY

4.3.1 Polymorphisms in adiponectin and TNF alpha and its association with insulin resistance, obesity and hypertension

The etiology of T2DM has a strong genetic component and variations in several candidate genes have been widely implicated. Earlier, in spite of a very high incidence of type 2 diabetes, efforts to detect any association of a common variant Pro12Ala (rs1801282) of PPARγ with type 2 diabetes failed, indicating that the Indian population is unique in its genomic architecture and possibly some yet uncharacterized genetic variants may be responsible for predisposing them to type 2 diabetes. Therefore, whether other polymorphisms in candidate genes such as TNFα, adiponectin and resistin are associated with type 2 diabetes in the Indian population was investigated.

Hence, four polymorphisms in the adiponectin gene and one in TNFα?n a cohort (n=699) from Hyderabad were screened. It was found that G308A variant in TNFα was significantly associated with waist circumference (p=0.0125) but not with T2DM, hypertension and BMI. On the other hand, the T+45G variant of the adiponectin gene was not found to be associated with any of the mentioned disease conditions. Interestingly, in contrast to other populations, any of the G+276T, G-11377A and C-11391G variants of adiponectin gene in this cohort could not be detected. These data suggest a certain level of genetic uniqueness in Indian population and varied effects of different factors on different populations. It will be interesting to screen the Indian population for novel variants, which might be responsible for making it vulnerable to metabolic disorders as a result of changing environmental factors including demographic and life style changes.

5. FOOD COMPOSITION AND NUTRIENT AVAILABILITY

5.1 Evaluation of the nutritional potential of Eri Silkworm Pupae

Studies on the nutritional potential of eri silk worm pupae, a byproduct of the silk industry has shown that it offers tremendous scope for the utilization of eri protein and fat as food source. Nutritional and toxicological evaluation of eri silk worm oil has shown that it is safe for consumption and the high α-LNA in its oil could be used to nutritional and commercial advantage.

6. PATHOLOGY

6.1 Role of type of dietary fat in the etiopathogenesis of carcinogen – induced breast neoplasm in Fischer female rats

The study was undertaken to investigate the possible etiological roles played by different types of dietary fats in female Fisher 344 rats using a carcinogen and also to study the role of insulin resistance and hyperinsulinemia in mammary carcinogenesis in these animals.

A total of 80 animals were divided into 5 groups and given either Transfats / saturated / n3 rich / n6 rich and n6 + n3 fats in their diets at 10% level for 4 months followed by administration of carcinogen DiMethylBenzAntherecene (DMBA) in 50% of animals in each group. DMBA was given (40mg/kg body weight per dose) at weekly intervals for 4 weeks by oral route and all animals were continued on their respective diets for a further period of 8 months (32 weeks).

Based on the results obtained, it appears that the **body weights** of animals not given carcinogen (C-) were, as expected, significantly higher than in carcinogen administered (C+) animals. When both the above groups were analysed separately, it was seen that n3 + n6 diet fed animals showed better body weights than others and that n3+n6 diet was much better acceptable than other diets. As regards **tumor load** and **tumor typing**, adenocarcinomas, squamous cell carcinomas and benign tumors were more in n6 fed groups as compared to others. Both the above parameters were found to be lower in n3 group as compared to n3+n6 group, suggesting that n3 may probably have a protective effect. **Immunohistochemistry** study of adenocarcinomas, showed that n3+n6 diet followed by n3 diet alone had better results than other groups studied.

Biochemical estimates indicated that carcinogen administration did not have much effect on glucose and lipid metabolism. **Haematological parameters** were unremarkable in all groups studied.

Fatty acid analysis revealed that TFA which is known to induce/promote carcinogenesis did not show increased tumor incidence, thereby suggesting that at the level of consumption (10%) it may not be promoting tumor formation. As anticipated, SFA levels were more in PO fed animals as compared to others. With respect to n3 fatty acid levels, the lower tumor incidence may be because of its anti-inflammatory role which was also evident in n3+n6 fed animals as compared to n6 only fed animals. This could also be because of lower n6 : n3 ratio in SFO + FO group. N6 rich diet consumption showed highest tumor incidence and density which could be attributed to increased inflammation and this in turn could be due to increased oxidative stress/arachidonic acid. Mammary adipose tissue in C+ animals was surprisingly very minimal / not present and hence no analysis could be undertaken which could have otherwise added valuable information to data generated from this study.

Finally, it appears that n6 rich diet has deleterious effects in relation to tumorogenesis while n3 alone diet has a better outlook with respect to the same. Apart from n3 diet, n3 + n6 diet was also observed to be beneficial. SFA and TFA diets that are associated with increased incidence of CVD and other chronic diseases do not seem to show a similar trend with respect to carcinogen induced mammary neoplasms.

7. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

7.1 Genotoxicological effects of pesticides in agricultural farmers in Guntur district of Andhra Pradesh

The widespread use of pesticides and exposure is a health hazard. Although a million

cases of pesticide toxicity are documented every year around the World, there is only limited data available on its cytogentetic effects. In addition, acute exposure to pesticides leads to generation of free radicals which include oxidative stress, lipid peroxidation and alterations in antioxidant status in animals and humans**.** Hence, a study was conducted in cotton growing farmers in Guntur district of A.P. These cotton growing farmers use more complex mixture of pesticides when compared to the farmers of other districts of the State. Therefore, the study was taken up to assess the extent of toxicity by analyzing the different test parameters which are the best toxicity indicators of exposure assessment.

Aims & Objectives of this study were to assess the toxicity of the commonly used pesticides viz., organochlorines, organophosphates etc in the agricultural farmers of Guntur district by AchE inhibition and to assess the cytogenetic changes and also the DNA damage in the blood of agricultural farmers by chromosomal aberrations (CAs), lymophocyte micronucleus test, and sister chromatid exchange.

The results of the cytogenetic analysis indicated that out of the 4,547 metaphase plates scored in the exposed subjects (312), 213 (4.7%) found to have CAs. Similarly, in the un-exposed group (312) out of the 3,267 metaphase plates analysed 55 (1.7%) were found to be positive for CA. A significant increase in CAs in the agricultural farmers exposed to pesticides indicates that chronic/sub chronic occupational exposure to complex mixture of pesticides is genotoxic. In the present investigation, 102 of the subjects from exposed group were found to be positive for micronuclei (0.15%) whereas in the un exposed group 89 (0.13%) subjects were found to be positive for micronuclei.

The results of the AchE activity in RBC indicated that there was a significant ($p<0.0.5$) decrease in the RBC AchE activity of the experimental subjects when compared to controls. Lipid peroxidation in terms of thiobarbituric acid reactive substance (2.68 ± 0.056) was significantly increased when compared to control subjects ($p<0.01$), while the antioxidants such as reduced glutathione (40.52 ± 1.50), and α-tocopherol in experimental group (7.58 ± 0.22) were significantly reduced ($p<0.01$) when compared to control subjects. There was a significant reduction in the level of GSH in the experimental subjects, while the activity of catalase in the experimental subjects increased when compared to control subjects. The liver and kidney function tests such as SGPT and urea were normal in both groups.

The studies suggest that there is a need to curtail the indiscriminate use of pesticides and that farmers should be enlightened to follow good agricultural practices.

8. EXTENSION AND TRAINING

8.1 Content analysis of nutrition component in School Science textbooks

Development of innovative nutrition education curricula is a continuous and demanding process. Before developing the nutrition content that can be effectively blended into the school science curricula, the first step would be to evaluate the nutrition component in the existing school science textbooks. A study was carried out with an aim to assess the over all nutrition component in the school science curricula. NCERT and AP State Education Board text books were considered for the study. The study which systematically applied quantitative and qualitative methods of Content Analysis concluded that the space allocated for biology in relation to physical sciences was lesser in higher classes (VI and above). Nutrition component is systematically organized at primary school level (till V Class), but not so much at high school level. Nutrition, in whichever class it is covered after class III in both NCERT and AP syllabi, deals only with – food groups or nutrient deficiency disorders. The study found that many important topics such as nutrition and growth, link between childhood malnutrition and non-communicable diseases in adulthood, adolescent nutrition, nutrition for girl child, hidden hunger, lifestyle factors and obesity, nutrition during pregnancy and lactation, importance of breast feeding, unhealthy foods, fortification etc. are not covered in the curricula. Considering that many earlier studies indicated that school based nutrition education is preferred mode of learning and

effective way of education, the results of this study will be useful during future revisions of the textbooks for strengthening the nutrition component.

9. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

9.1 Effect of long-term exercise in WNIN obese rats

From the original WNIN parental stock, two obese mutant strains were developed and designated as WNIN/Ob and WNIN/GR-Ob. These strains showed hyperphagia, higher body fat, hypertriglyceridemia, hyperinsulinemia, hypercholesterolemia and hyperleptinemia. Additionally, impaired glucose tolerance was observed in WNIN/GR-Ob rats. The present study is aimed to check whether long-term exercise will have any marked effect on the body composition, insulin resistance and lipid profile of these mutant rats.

So, a total of 72 animals from these two mutants along with parental strain WNIN were taken at 35 days of age and divided into 3 groups. Two groups of animals were taken for exercise on Rota-rod treadmill and the remaining served as controls. Exercise was carried out at two speeds for two different durations. Daily food intake and weekly body weights were measured and body compositions of these animals were analyzed by using total body electrical conductivity (TOBEC). Insulin resistance, lipid profile and serum lactate levels were measured by standard procedures. The study showed that exercise improves glucose tolerance and reduced insulin resistance in all three strains of rats tested.

There were only marginal changes by doubling the intensity of exercise. Simultaneous decrease in LBM, total body sodium and water suggests that there is extra cellular fluid loss in exercised animals when compared to controls. This is also reflected in terms of increased total body potassium. Exercise also had positive effect on reducing plasma triglyceride levels. The alterations in plasma lactate levels suggest that the glucose utilization is higher with low intensity exercise when compared to high intensity exercise.

10. PRE-CLINICAL TOXICOLOGY

10.1 Pre-clinical toxicity evaluation of Tetravalant Vaccine (DPT+Hep B)

Tetravalent Vaccine (Diphtheria, Tetanus, Pertussis + Recombinant Hepatitis B antigen) is a prophylactic agent against Diphtheria, Tetanus, Pertussis and Hepatitis B by eliciting immunity in sufficient doses. Tetravalent vaccines are very important for preventing diseases like Diphtheria, Tetanus, Pertussis and Hepatitis B. Indian Immunologicals has prepared this Tetravalent vaccine as per DCGI guidelines, Schedule Y, Drugs and Cosmetic (Second Amendment) Rules, 2005, Government of India following GMP. The objectives of the study were to assess the safety profile of Tetravalent vaccine which elicits immunity against the four diseases (Diphtheria, Pertussis, Tetanus, Hepatitis B) and to Test the allergenic potential of tetravalent vaccine.

The test material tetravalent vaccine (TV), DPT and Hep-B was tested for acute toxicity test (14 days) in swiss albino mice & Sprauge Dawley rats. Sub chronic toxicity test was carried out in Swiss albino mice and Guinea pigs. In acute toxicity test, mice and rats were exposed once to highest dose (10 times of intended therapeutic dose) by sub cutaneous route and observed for lethality. In sub chronic toxicity test Therapeutic Dose (TD) of DPT, TD of Hep- B, TD of TV and five times of TD of TV were tested. The results showed no abnormalities in physical, physiological, clinical chemistry, hematological, pathological, immunotoxicological and genotoxicological parameters.

10.2 Safety / toxicity studies of Ayurvedic bhasmas (Vn & Wn)

Ayurvedic formulations are classified into various groups viz., Kadla (decoction), churnas (Powder), Bhasmas (Mineral + herbal preparations) etc. Bhasmas are calcined powder of metals, minerals, gems etc. Traditional literature has provided standard guidelines to prepare such formulations in non-toxic, therapeutically potential formulation. As per the traditional system, these formulations are recommended to treat chronic neurological diseases viz., liver disorder, arthritis, diabetes, neurological disorders. These are sold as rejuvenator substances. However, in the recent

past, presence of metals in such formulations sold at grocery shops in international market are reported and so these can be potential toxicants. In view of this observation, CCRAS has proposed a multicentric pre-clinical safety evaluation of various herbomineral formulations as per inter-national guidelines. The present investigation was carried out to assess the safety of products as per the international guidelines. The Objective was to assess the Pre-clinical Toxicity of coded Vn & Wn Ayurvedic Bhasmas as per the International Guidelines.

Two test formulations coded as Vn & Wn recommended in a clinical dose of 30mg and 60mg respectively for 3-4 weeks were provided by the sponsor. The present investigation involved acute toxicity test (14 days) in swiss albino mice, sub acute toxicity test (30 days) and long-term term toxicity test (120 days) in Swiss Albino mice & WNIN Rats. In acute toxicity test, mice were exposed once to highest dose (10 times of intended therapeutic dose) by oral gavage and observed for lethality. The test compound was administered daily with 33% honey water (v/v) for 15 days and 30 days in Sub acute toxicity, long term toxicity test respectively in various dose levels viz. therapeutic dose (TD), average dose (TDx5) and high dose (TDx10). The results of acute toxicity test showed no lethality in mice and rats after a single exposure to 50 times of therapeutic dose till 14th day.

In subacute toxicity test, 10% pre-terminal deaths were recorded in mice, exposed to TD & 5 times of TD in both Vn and Wn Bhasmas. No pre-terminal deaths were recorded in rats. No significant treatment related effect were seen on food intake, body weight gain, clinical signs, behavioral activity etc in the survived animals. No significant changes in hematological parameters and clinical chemistry parameters were observed. In long term toxicity test, pre-terminal deaths were recorded in VC (10%), TD (15%), AD (10%), HD (10%) in mice which received Vn and Wn Bhasmas for 90 days. Mortality was 5% in rats exposed to Wn. No significant treatment related effects were seen on food intake, body weight gain, clinical signs, behavioral activity etc. No significant changes in hematological and clinical chemistry parameters were found. Genotoxicity effect was observed in those which were given ten times of recorded therapeutic dose.

10.3 Pre-clinical toxicity evaluation of Skimmed Milk Fermentate (SMF)

The Skimmed Milk Fermentate (SMF) having bacteriocin type activity has been developed by indigenous technology, with an intention to promote it as a bio-preservative for Indian dairy products. The SMF has been produced by fermenting skim milk with a bacteriocinogenic (bacteriocin producing) strain of food grade lactic acid bacterium, *Pediococcus pentosaceus*, isolated from Cheddar cheese. National Dairy Development Board is keen to exploit this product for its use as a preservative in commonly consumed dairy products in India.

The Objective was to carry out preclinical toxicology of SMF to ensure its safety. The intended daily dietary intake (DDI) of SMF was calculated (1.2gm/day i.e.0.001%w/w). The investigation involved acute toxicity test (14 days) in swiss albino mice and WNIN rats and Sub chronic toxicity test in WNIN Rats. In acute toxicity test, mice and rats were exposed once to highest dose of test material (10 times of intended therapeutic dose) by oral gavage and observed for lethality. Sub chronic test has been conducted in Rats (Wistar NIN) receiving the diet containing 0.2%, 1%, 2% SMF. In addition, a group of animals received the diet with low protein (30%), and less fat (15%) which is considered equivalent to poor man's diet.

The results of this study showed no lethality in mice/rats after a single exposure to maximum quantity of SMF in acute toxicity test. In sub-chronic toxicity test, there were no pre-terminal deaths except one animal which died on 62nd day of drug exposure receiving 2% SMF and no significant treatment related effect on food intake, body weight gain, clinical signs, behavioral activity etc. was noticed. There were no abnormalities in hematological, clinical chemistry, histopathological parameters. At higher dose, some genotoxic effect was observed which was not significant.

I. COMMUNITY STUDIES

1. MAPPING, SIZE ESTIMATION AND INTEGRATED BEHAVIORAL AND BIOLOGICAL ASSESSMENT (IBBA) IN HIGH HIV PREVALENCE SETTINGS IN INDIA

In view of the growing epidemic of AIDS in India, the Bill & Mellinda Gates Foundation (BMGF) had initiated a programme "Avahan AIDS India Initiative" in order to control and prevent AIDS, in high-risk States in the country. The program is being implemented in 71 districts across 6 states and 4 Highways sites in the country. At the instance of BMGF the study titled "Integrated Behavioral and Biological Assessment" was carried out at the request of Family Health International (FHI) an US based International agency and ICMR, in close collaboration with National AIDS Control Organization (NACO) and State AIDS Control societies (SACS), to generate data base that will allow BMGF and its Governmental and non-governmental partners to follow key trends in HIV, STIs and risk behaviors and also use the data to project trends in the future.

The study was conducted in six States of Andhra Pradesh, Maharastra, Tamil Nadu, Karnataka, Nagaland & Manipur, and four National highway segments, adopting a uniform protocol. The overall implementation of the study, in all the study States and national highways was the responsibility of the National AIDS Research Institute (NARI). It was proposed to conduct the study at three points of time during the project period, the baseline survey was undertaken during 2005-07 while mid-line and the end line surveys are proposed to be conducted during 2009 and 2011 respectively.

The National Institute of Nutrition carried out the baseline survey in Andhra pradesh in the eight high prevalence districts of Visakhapatnam, East-Godavari, Guntur, Prakasam, Chittoor, Warangal, Karimnagar and Hyderabad. The results of the baseline study conducted on Female sex workers, Men having sex with Men and the clients of Female sex workers are presented in the current report.

Objectives

The specific objectives of the study were,

i) To map areas (in terms of geographic location and characteristics) where population sub-groups at risk for HIV are present,

ii) To obtain accurate estimate of size of the population sub-groups at risk of HIV, and

iii) To carry out behavioral and biological assessment in populations targeted by the interventions,

- to obtain measures of risk behaviors of population sub-groups at risk of HIV,
- to provide indicators for assessing program success and identifying bottlenecks,
- to determine HIV prevalence and incidence in all sampled groups,
- to estimate the prevalence of STIs, specially Syphilis (Tp), N.gonnorrhoea (Ng), Chlamydia trachomatis (Ct) and Herpes Simplex Virus type-2 (HSV2) in all the sampled groups, and to assess serological evidence of previous infection with HSV2 on 20% of total sample, preferably in younger age groups as a proxy indicator of fall in the prevalence over a five year period.

METHODOLOGY

Study Design

It is a cross sectional study to be conducted at three points of time during the project period, using two stage sampling procedure. The districts were selected based on criteria of socio-cultural regions, high proportion of female sex worker population and the higher prevalence of HIV.

Sampling Frame

The sampling frame comprised of physical locations, where sub-population members tend to congregate like brothels, bars and public places.

Survey population

The survey population included

- Female Sex Workers (FSW)
- Men having sex with Men (MSM)
- Clients of Female Sex Workers

Sampling procedure

Two alternative sampling approaches Viz; “conventional cluster sampling” for the selection of Brothel based FSWs and Clients of FSWs and 'Time location cluster sampling' (TLCS) for the selection of street based FSWs and MSM, were used in the study. Within each of the selected cluster, simple random sampling procedure was used for the selection of study subjects.

Sample Size

Sample sizes for each population sub-group included in the survey have been calculated on the basis of ;

- Expected baseline value of key indicator, Viz; consistent condom use as 50%, at which the sample size will be maximum.
- Ability to detect a change of 15 percentage points,
- 95% of confidence limits (level set at 0.05, corresponding to 95%),
- Statistical power (level set at 10%, corresponding to 90% power) and
- Design effect of 1.7 for TLCS.

The sample size estimated based on the above criteria was 400 respondents for each sub-group of 'at risk population' for each district and for calculation of HIV incidence rates about 1200 to 1500 subjects were considered per target group.

Indicators

Behavioral indicators

- Socio-demographic characteristics,
- Sexual history and behavior.
- Knowledge, attitude and practices about STI and its prevention

Biological indicators

Prevalence of HIV and STIs such as, Syphilis, Neisseria Gonorrhea, Chlamydia Trachomatis and Herpes simplex virus type 2 (HSV-2).

Laboratory Procedures

- HIV screening using ELISA and confirmation of all positive cases with second ELISA.
- Serologic tests for syphilis using quantitative rapid plasma regain (RPR) screening test and a qualitative Treponema Pallidum Hemagglutination Assay (TPHA) confirmation test.
- Transcription Mediated Amplification (TMA) by APTIMA combat for Ng and Ct.
- Multiplex PCR on samples collected from all reported and clinically identified genital or anal ulcers for Treponema pallidum (Tp), HSV-2 (carried out by NARI),
- HSV-2 serology,
- HIV incidence testing by BED-CEIA (HIV-1 subtypes B, E, and D, IgG-Capture Enzyme Immuno Assay, will be carried out by NARI).

Ethical issues

The ethical issues included, voluntary nature of participation of the respondents in the study, written informed consent, maintaining anonymity, confidentiality. The survey being sensitive in nature, the psychological risks involved in the behavioral questionnaires and biological procedures were conducted in strict privacy. Test results of syphilis were provided to all the respondents and necessary treatment was also provided whenever it was necessary. Syndromic treatment was given if required by the medical officer, to those who needed.

Behavioral survey

The consent forms and standardized questionnaires were developed by the FHI and NIN, translated into 'Telugu' and back translated into English, for correctness, applicability, reliability and better comprehension and were pre-tested in field conditions. The final versions of questionnaire were made in bilingual formats.

The private research agency Vis; “AC NEILSEN ORG–MARG PVT Ltd” carried out the field activities, which included mapping of sites for high risk population for sampling frame development, community preparations, collection of behavioral data, biological samples (blood, urine and swab) transportation of the same to the district laboratory and to the State laboratory and provided syndromic treatment as per the protocol. All the respondents were referred to NGO clinics for receiving the syphilis test results and treatment and to VCCTCs for HIV testing.

The division of field studies at NIN carried out training and standardization of the investigators in data collection, supervision of field and laboratory activities.

During the study period data collection was carried out on the "Female sex workers" (FSWs) in all the eight selected districts, the category of "Men having sex with men" (MSMs) was covered in four districts (Hyderabad, Guntur, East-Godavari and Visakhapatnam). In addition, the clients of the female sex workers were also covered in the above four districts and Warangal.

Biological survey

The biological component of the study comprised of establishment of State laboratory at NIN, which was responsible for carrying out analysis of biological samples and & quality control, was the responsibility of department of Microbiology, National Institute of Nutrition. Eight district laboratories were also established in the microbiology departments of six medical colleges and two private laboratories (Prakasam and Chittoor districts) in the study districts, which carried out the RPR test for syphilis and dispatched the samples to the State laboratory at NIN. The blood samples were tested for HIV at State laboratory and the aliquots were dispatched to NARI for quality control and BED-CEIA test for HIV incidence.

Data entry and analysis

The data on behavioral, biological, clinical aspects was checked and subjected to double data entry (one entry by the research agency and other by the NIN), as a check for entry errors. The errors were rectified and the data was sent to the Data management Group (DMG) at National Institute of Epidemiology, Chennai for analysis.

Results

About 400 respondents of each category of high-risk groups were covered in each of the district selected for behavioral and biological data collection. Salient observations with regard to socio-economic,mographic particulars, risk behavior, prevalence of STI and knowledge, attitude about STI & HIV and practices towards control & prevention of STI & HIV across the select districts in Andhra Pradesh are presented by category of study subjects are given below.

Female Sex Workers (Table-1)

- The mean age of the FSWs surveyed was about 30 years. The illiteracy rate ranged from a high of 86% in Hyderabad district to a low of 40% in Guntur district. A large proportion of FSWs reported that sex work was the main source of income in Visakhapatnam district (64%) compared to Karimnagar (27%). About 50-80% of FSWs across all the districts, were married and were living with either spouse or a regular sexual partner and were engaged in sex work. The mean age of first sexual contact ranged from 15 to 17 years, while the mean age of initiation of commercial sex ranged from 21 to 26 years across the districts.
- The average number of clients entertained on the last day worked by a FSW varied from 1.7 in Hyderabad district to 3.0 in Visakhapatnam and Guntur districts. Similarly the volume of clients entertained during the past week also ranged from a low of 5.4 in Hyderabad to a high of 12.1 in Prakasam district. The mean duration of sex work among the FSWs surveyed ranged from 5.1 years in the districts of Chittoor, and Prakasam to 7.6 in E.Godavari and Warangal districts.
- Barring Hyderabad (16%), about 50-78% of the FSWs across the districts reported that their clients requested for having anal sex with them. While, the proportion of FSWs who actually ever had anal sex was low (2.6%) in Hyderabad district through 13% in Visakhapatnam to about 33% in Karimnagar district.
- Most of the FSWs (>90%) had occasional / regular clients while 70-82% of FSWs also had regular non-commercial partner. A majority (42-76%) of the FSWs reported that they had sex when they visited places outside the current place of residence, during the past 12 months.
- Barring Hyderabad (34%) and East Godavari districts (46%), most of the FSWs (77 to 98%) in the other districts carried condom at the time of interview. The extent of consistent condom use

Table 1.Distribution (%) of FSWs According to Socio – Demographic, Behavioral Characteristics, Knowledge & Practices about STIs and Prevalnce of STIs – By District in Andhra Pradesh

Particulars	Visakha patnam	East Godavari	Gun-tur	Praka sam	Chit-tor	Wara ngal	Karim nagar	Hydera bad
Mean Age (yrs)	29.9	30.5	30.8	29.1	29.8	28.7	29.1	30.3
Literacy rate	45.9	37.7	59.6	36.2	40.0	21.6	22.7	14.3
Sex Work as main Sources of Income	63.5	55.5	29.9	52.6	47.9	40.4	27.3	44.3
Currently Married	75.5	58.7	65.4	74.5	70.9	64.5	50.1	80.6
Living with regular sexual partner	76.0	69.0	77.0	79.0	80.0	74.0	73.0	76.0
Mean age (Yrs) at first sexual act	16.4	15.4	16.9	16.2	16.2	15.5	15.2	14.8
Mean age (Yrs) at initiation of commercial sex	23.5	22.9	24.6	23.9	24.7	21.0	23.0	25.5
Mean no. of clients last/day (Nos)	3.0	2.9	3.0	2.6	2.5	2.2	2.1	1.7
Mean no.of clients past week (Nos)	11.5	10.9	11.4	12.1	10.1	7.0	5.9	5.4
Duration of sex work (Yrs)	6.4	7.6	6.2	5.1	5.1	7.7	6.1	4.9
Ever asked by clients for anal sex	51.2	78.4	74.3	58.1	63.4	76.1	58.2	51.5
Ever had anal sex	12.6	19.6	29.6	20.8	29.4	29.1	32.8	2.6
Occasional clients	99.0	93.2	97.6	99.8	98.8	100.0	88.5	93.1
Regular clients	96.9	93.8	99.7	96.8	99.5	99.5	99.6	81.3
Regular Non-commercial partner	73.5	69.6	77.7	79.8	80.2	76.1	82.4	77.7
Had sex during Travel in past 12 months	42.0	75.0	76.0	71.0	67.0	ND	ND	ND
Currently carrying condom	81.4	46.4	83.0	77.3	52.7	78.0	98.2	33.7
	Consistent condom use with							
Occasional clients	89.0	82.0	85.0	39.9	36.1	84.9	73.0	55.6
Regular clients	80.9	76.0	84.9	17.2	14.9	80.9	62.8	64.2
Regular Non-commercial partner	8.2	17.2	15.1	0.6	0.8	2.0	9.1	4.3
Aware of at least two STI Signs/ symptoms among Women	98.4	95.3	96.7	96.2	95.0	84.7	78.9	85.9
Aware of at least two STI Signs/ symptoms among Men	91.1	80.1	72.9	89.7	86.9	58.1	61.7	68.6
Ever heard of HIV/AIDS	99.9	99.7	99.8	98.1	97.6	96.8	95.6	93.2
Aware that HIV/AIDS can be prevented	98.4	94.4	93.4	99.9	97.0	90.7	87.1	83.0
Aware of correct methods of prevention	26.9	18.8	17.9	13.0	10.2	18.6	14.5	21.7
Transmission by mosquito/insect bite	36.0	45.0	43.0	61.0	65.0	55.0	52.0	28.0
Transmission by sharing clothes/ utensils	23.0	22.0	24.0	19.0	22.0	41.0	24.0	14.0
Ever contacted by Peer/Outreach Worker	41.6	31.9	0.8	69.0	54.5	70.4	57.7	71.0
Received condom	47.0	30.7	6.0	68.1	54.0	58.1	59.7	52.2
Received information on STI/HIV/AIDS	42.9	29.6	4.1	65.8	53.5	64.2	57.4	63.6
HIV	14.2	26.3	21.3	11.1	8.0	10.8	21.1	14.3
Syphilis	7.1	15.0	8.6	5.2	10.3	10.2	6.4	17.4
Chlamydia Trachomatis	3.6	3.2	1.7	3.4	3.1	2.9	3.0	6.5
Gonorrhea	1.4	1.2	1.3	0.2	2.5	1.9	1.6	6.4
HSV-2*	58.6	88.7	85.4	65.8	80.8	55.7	74.2	79.8

* - Based on a random sample of 10% of sera specimens selected for HSV-2 testing, ND- Not Done

with different types of clients ranged from a high 36-89% with occasional clients, through 15-85% with regular clients to a low <1-17% with regular non-commercial partners, in various districts.

- Majority of FSWs surveyed reported that they were aware of signs and symptoms of STIs among women as well as men and also were aware of HIV/AIDS. A small proportion of FSWs (10-27%) were aware of correct methods of prevention of HIV/AIDS.
- A considerable proportion of subjects had misconceptions regarding transmission of HIV, they believed that HIV can be transmitted through mosquito/insect bite (28-65%) and by sharing clothes/utensils (14-41%).
- A maximum (71%) of the FSWs were reportedly approached by peer / outreach worker in Hyderabad district, compared to a low (1%) in Guntur district. The proportion of FSWs who received condom from peer / outreach workers ranged from 6-68% in Guntur and Prakasam district. About 43-65% of FSWs received information on STI/ HIV/ AIDS 'through peer/ outreach worker across the districts, barring Guntur (4%) and East Godavari district (30%).
- The prevalence of HIV among FSWs surveyed was maximum in East Godavari district (26.3%) compared to only 8% in Chittoor district. The prevalence of syphilis ranged from a high 17.4% in Hyderabad district to 5.2% in Prakasam district. Similarly the prevalence of Chlamydia Trachomitis (6.5%) and Gonorrhea was (6.4%) highest in Hyderabad district. The prevalence of HSV-2 ranged from about 56% in warangal district, to 89% in East Godavari district.

Men having Sex with Men (Table-2)

- A majority (51 to 84%) of MSM surveyed were bi-sexual in nature, followed by Kothis (5 to 32%). The mean age of MSM ranged from 25-30 years and a majority of them were literate.
- The proportion of currently married MSM ranged from 27% in Hyderabad to 60% in Guntur district. Among currently married, about 60% of MSM were living with either spouse or other regular partner in Guntur district as compared to 27% in Hyderabad district. The proportion of unmarried MSM who were living with others ranged from 36% in Guntur to 58% in Visakhapatnam district.
- A small proportion of MSMs reported that sex work was the main source of income. Majority of MSMs (99%) from Visakhapatnam district had regular male/ Hijara partner compared to 39% each in Guntur and Hyderabad districts. A large proportion of MSMs (88% to 95%) had other than non-commercial male / Hijara partners. About 66% of MSM from Guntur district had regular female partner compared to 34% in Hyderabad district.
- A maximum of 90% of MSM from Visakha-patnam district reported that they had anal sex during the past twelve months, when they traveled out side the current place of residence compared to 53% of MSMs in Hyderabad.
- A small proportion of MSM (10%) of Guntur district reported that they were carrying condom at the time of survey compared to 44% in Visakhapatnam district. About 20 to 43% of MSM of Guntur district used condom consistently with all types of partners as compared to <1 to 5% of MSMs from Visakhapatnam district. A small proportion, <1 to 4% of MSM were using condom with regular female partner.
- A smaller proportion of MSM had misconcep-tions about transmission of HIV, they believed that HIV can be transmitted through mosquito/ insect bite (14-26%) and by sharing clothes/ utensils (14-28%).
- Majority of MSM (65 to 100%) reported that they were aware of atleast two signs and symptoms of STIs. However, only a small proportion of MSM (3.0 to 22%) were aware of correct methods of prevention of HIV/AIDS.
- Almost all the MSM surveyed, reported that they were contacted by the local NGOs and a majority of them received condoms from them.
- The prevalence of HIV is high among MSM of Hyderabad district (24.7%) compared to 9.3% in Visakhapatnam district. Similarly, the prevalence of syphilis was also high in Hyderabad district (15.7%) compared to Guntur 3.5%.
- The prevalence of HSV -2 is high (78%) among the MSM of E.Godavari district, as compared to a low 29% in Guntur district.

Table 2. Distribution (%) of MSMs According to Socio – Demographic, Behavioral Characteristics, Knowledge & Practices about STIs and Prevalnce of STIs – By District in Andhra Pradesh

Particulars	Visakha patnam	East Godavari	Guntur	Hyderabad
Category of MSM				
Kothi	32.0	18.9	5.3	23.0
Panthi	0.5	2.9	5.8	9.6
DD	10.8	8.6	4.0	14.8
Bisexual	55.9	68.8	83.6	50.8
Hijra	0.7	0.8	1.3	1.9
Mean Age (yrs)	25.0	29.5	27.3	27.7
Literacy rate	83.6	78.5	65.1	78.6
Currently Married	38.5	46.4	60.0	26.8
Married & Living with either spouse or others	38.1	45.8	60.1	27.4
Unmarried – Living with others	57.8	43.3	36.2	48.9
Sex Work as main Source of Income	1.5	6.7	3.6	8.1
Regular male/ hijra partner	98.7	77.5	39.1	39.3
Paying male partners	89.6	35.1	26.0	36.3
Paid male/ hijra partners	31.4	48.0	46.2	46.2
Paid female partners	27.5	39.7	63.6	31.8
Other non-commercial male/ hirja partners	95.4	88.6	87.5	88.8
Regular female partner	50.9	52.6	65.7	34.1
Currently Carrying Condom	44.2	34.3	9.6	34.3
Had anal sex during Travel in past 12 months	90.0	60.0	70.0	53.0
Consistent condom use with				
Regular male/ hijra partner	2.2	8.7	22.0	12.0
Paid male/ hijra partners	1.3	13.0	40.0	17.6
Paid female partners	4.5	15.3	43.3	23.4
Other non-commercial male/ hijra partners	0.7	6.6	32.0	14.7
Regular female partner	0.2	4.0	2.6	0.8
Aware of at least two STI signs/ symptoms among men	83.6	80.9	66.4	65.3
Ever heard of HIV/AIDS	98.7	99.4	99.7	100.0
Aware that HIV/AIDS can be prevented	95.3	94.5	86.0	87.2
Aware of correct methods of prevention	3.0	7.6	16.8	22.3
Transmission by mosquito/insect bite	13.7	22.1	22.4	26.2
Transmission by sharing clothes/ utensils	23.1	16.3	13.7	27.8
Ever contacted by NGO staff	100.0	100.0	100.0	100.0
Received condom from Avahan staff	98.1	90.1	81.8	95.8
HIV	9.3	22.2	13.1	24.7
Syphilis	5.6	13.0	3.5	15.7
Chlamydia Trachomatis	1.2	1.0	1.4	2.0
Gonorrhea	0.5	0.0	0.4	0.9
HSV-2*	37.0	77.7	29.1	69.0

* - Based on a random sample of 10% of sera specimens selected for HSV-2 testing.

Table 3. Distribution (%) Clients of FSWs According to Socio – Demographic, Behavioral Characteristics, Knowledge & Practices about STIs and Prevalnce of STIs – By District in Andhra Pradesh

Particulars	Visakha patnam	East Godavari	Guntur	Warangal	Hyderabad
Mean Age (yrs)	28.0	30.0	31.0	29.7	30.5
Literacy rate	86.5	63.8	63.7	90.3	81.0
Currently Married	59.7	70.2	70.2	71.3	62.7
Unmarried – Living with main sexual partner	56.1	29.5	17.9	62.6	14.7
Mean age at first sexual act (Yrs)	18.2	18.8	18.2	17.7	19.0
Mean age at initiation of commercial sex (Yrs)	19.5	20.3	19.3	19.4	19.9
Mean No. of FSWs visited during past 1 month	2.0	2.1	3.0	4.5	2.9
Mean No. of FSWs visited during past 6 month	5.0	4.8	7.4	10.6	7.6
Mean No. of commercial sex acts had in past 1 month	3.0	4.0	3.8	27.5	3.7
Mean No. of commercial sex acts had in past 6 month	8.1	9.1	9.7	50.1	9.5
Occasional FSWs	100.0	98.4	100.0	99.1	100.0
Regular FSWs	71.7	80.3	58.8	90.8	58.4
Type of partners					
Non-paid main/ regular female partners	82.6	77.7	26.3	86.4	66.2
Other non-paid casual female partners	21.3	49.3	45.4	51.1	33.6
Male/ hijra partners	16.0	1.4	4.2	5.8	0.9
Currently carrying condom	13.3	10.6	4.6	14.3	11.7
Consistent condom use with					
Occasional FSW	22.6	37.6	28.2	18.7	18.9
Regular FSW	23.1	26.2	24.0	16.4	16.0
Non-paid main/ steady female partner	4.1	1.2	0.0	1.2	0.0
Male/ hijra partner	18.6	27.8	18.8	1.0	56.2
Awareness of HIV/AIDS	99.1	100.0	100.0	98.5	100.0
Presently feel at risk to be infected	54.9	22.5	55.6	57.0	59.6
HIV	8.0	8.3	6.6	6.7	2.4
Syphilis	3.4	4.8	10.1	5.5	3.1
Chlamydia Trachomatis	0.4	0.9	0.8	0.4	2.1
Gonorrhea	1.3	0.0	0.0	1.6	0.0
HSV-2*	78.0	44.8	69.2	18.9	27.4

* - Based on a random sample of 10% of sera specimens selected for HSV-2 testing.

Clients of Female Sex Workers (Table-3)

- The mean age of clients of FSWs across the districts surveyed ranged from 28 years to about 31 years. A majority of them (64%in E.Godavari & Guntur to 90% in Warangal) were literate.
- About 60 to 70% of clients of FSWs were married, while the unmarried clients of FSWs ranged from a high of 63% in Warangal district and a low of 15% in Hyderabad district.
- The mean age at first sexual act was about 18 to 19 years and the initiation of commercial sex was about 20 years across the districts. On an average each client had sex with 2 FSWs per month in Visakhapatnam & East Godavari districts and 4.5 FSWs in Warangal district.
- The proportion of clients having regular FSWs was (91%) in Warangal district and low (58%) in Hyderabad district. Almost all of the clients had occasional FSWs as their sexual partners. Clients of FSWs belonging to Warangal district had higher (86%) proportion of non-paid main regular female partner as compared to Hyderabad district (66%). The proportion of clients of FSWs who also had male/hijra partners is high in Visakhapatnam (16%), while it is lowest (<1%) in Hyderabad.
- A very low proportion of clients of FSWs (5 to 14%) were carrying condom at the time of survey, across the districts. Consistent use of condom with occasional as well as regular FSWs ranged from a low of 16% in Hyderabad and Warangal districts to a high of 38% in East Godavari district. Consistent use of condom with a regular non- paid steady main partner was very low (<1 to 4%).
- Almost all of the clients of FSWs were aware of HIV / AIDS. Barring East Godavari district (23%) about a half of the respondents from the remaining districts felt that they were at risk of being infected with HIV.

The prevalence of HIV among clients of FSWs is relatively low (2.4 in Hyderabad, 8.3% in East Godavari) across districts as compared to the other risk groups studied. The prevalence of syphilis ranged from a low of 3.1% in Hyderabad district to a high of 10.1% in Guntur district. The prevalence of HSV -2 is high (78%) among the clients of FSWs who belonged to Visakhapatnam district compared to a low 19% in Warangal district.

2. PREVALENCE AND DETERMINANTS OF OVERWEIGHT AND OBESITY AMONG URBAN ADOLESCENTS IN ANDHRA PRADESH

Obesity is fast emerging as an important public health problem among children, adolescents and adults, both in the developed as well as developing countries, contributing significantly to high risk of chronic degenerative disorders such as hypertension, type2 diabetes, coronary artery diseases and stroke. The present study, was therefore, carried out during 2006 - 07 to assess the prevalence and determinants of overweight and among urban adolescents of 12 to 17 years old in the urban areas of State of Andhra Pradesh. It was a cross sectional, nested case-control study, carried out by adopting multistage stratified random sampling procedure. A total of 8,142 adolescents of 12–17 years age group were covered under the study from 66 institutions of the State.

The salient findings of the study are as follows:

The overall prevalence of overweight was about 6%, which was significantly ($p< 0.05$) higher among girls (7.1%) compared to boys (4.4%). In general, the prevalence was significantly higher ($p< 0.05$) among the adolescents of high socio-economic status (9.6%) compared to low socioeconomic status (2.1%)(Fig.1). The proportion of adolescents participating in the outdoor games and sports was significantly higher ($p< 0.05$) among normal (control) group (66.7%) as compared to Overweight/obese (cases) children (54.5%).

Fig 1. Prevalence (%) of Overweight and Obesity among Adolescents of 12-17 years by Composite Socioeconomic Index

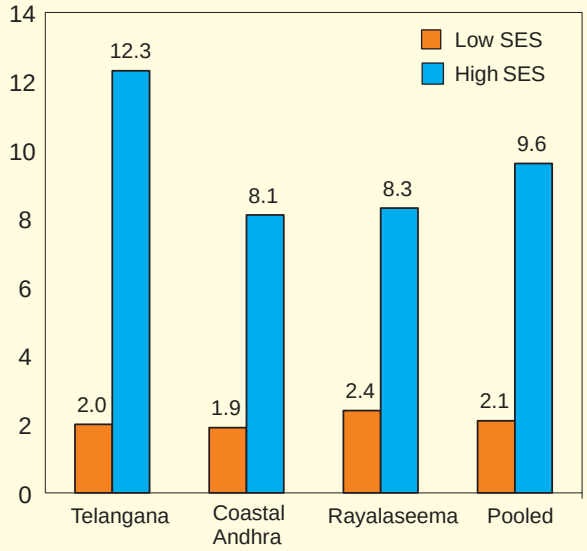
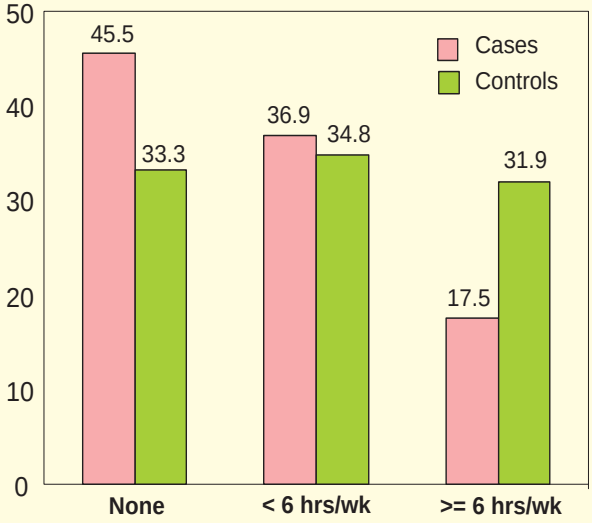


Fig 2. Distribution (%) of Adolescents according to duration of play (hrs/week) in out door games and sports



The frequency of consumption of fast foods such as burgers/noodles/fried foods was significantly ($p<0.05$) higher among the cases, compared to controls. In contrast, the consumption of milk & milk products and fish was also higher among controls compared to cases.

The average duration of 'TV watching' was significantly higher ($p< 0.05$) among cases (1.4 hrs/day) as compared to controls (1.2 hrs/day). The proportion of adolescents, who used scooter/car as mode of transport to go to school/college was significantly higher ($p< 0.05$) among the cases as compared to control group. The proportion of adolescents participating in outdoor games and sports for $\geq$6hrs/week was significantly ($p< 0.05$) higher among the control group (31.9%) compared to cases (17.5%) (Fig.2). Similarly, the proportion of children watching television for $\geq$3hrs/day was significantly ($p< 0.05$) higher among cases as compared to controls. The prevalence of hypertension (JNC Criteria VII) was significantly ($p<0.05$) higher among the cases (6.2%) as compared to control group (1.8%).

Multivariate logistic regression analysis revealed that the risk of overweight/obesity was 3 times higher among the adolescents, who were not participating in out door games and sports, 2 times higher among those who participated <6hrs/week, 2 times higher among adolescents who did not participate in household activities and/ or participated <2hrs/day, and who were watching television $\geq$3hrs/day and 1.5 times more among the adolescents who belonged to high socioeconomic status.

The study highlights the need to educate the adolescents to promote physical activity, healthy food habits & life style practices to prevent obesity and overweight and consequent diet related chronic degenerative diseases to promote physical activity, healthy food habits and life style practices to prevent obesity and overweight and consequent diet related chronic degenerative diseases.

II. CLINICAL STUDIES

1. NEONATAL ANTHROPOMETRIC DATA AND BODY COMPOSITION IN SMALL FOR GESTATIONAL AGE (SGA) BABIES COMPARED TO APPROPRIATE FOR GESTATIONAL AGE (AGA) BABIES

Low birth weight accounts for more than 1/3rd of deliveries in India and the most common cause is Intra uterine growth retardation (IUGR) primarily due to nutritional inadequacy in the pregnant mother. This has gained importance in recent decades due to the fetal origins hypothesis. This hypothesis is popularly called as Barker's Hypothesis, which states that nutritional deprivation in early life, when followed by nutritional excess later leads to abdominal adiposity and adult chronic diseases. Under nutrition in early pregnancy affects all aspects of fetal growth leading to symmetrical IUGR. Whereas in later part of pregnancy under nutrition causes reduction in weight, without affecting the length, thus leading to disproportionate or asymmetrical IUGR.

Under nutrition in intra uterine life, programs the individual to survive with low calorie intakes, and when energy intake increases in later life it may lead excessive adiposity. This might happen even if there is undernutrition in early and mid pregnancy and calorie sufficiency in later part of pregnancy, resulting in excessive fat deposition even before birth leading to altered body composition of the new born at birth.

Some recent studies using skin fold thickness techniques and unpublished studies from NIN have shown relatively more fat in new borns with birth weight less than 2,500 grams. It was demonstrated that maternal and paternal body size, glycemia and Insulin resistance are directly related to baby's birth weight and Mid upper Arm Circumference (MAC).

In another study, it was shown that adiposity and hyperinsulinemia in Indians is present at birth. Adjusted for gestational age Indian babies were lighter but their skin folds were relatively preserved, Cord leptin and insulin levels were comparable but were higher when adjusted for birth weight.

A study from St. John's Medical college, Bangalore involving a study of 429 newborns have reported that with comparable birth weights the skinfold thickness of Indian babies are similar to western populations.

The pattern of IUGR i.e., symmetrical or asymmetrical depending on the timing of nutritional insult in pregnancy, may have differences in body composition, with different consequences in later life. Literature shows that coronary heart disease (CHD) may be associated with symmetrical IUGR where as Insulin resistance is associated with asymmetrical growth retardation.

Some animal studies done at NIN have shown maternal magnesium and copper levels to be associated with fetal adiposity.

With this background it was hypothesized that neonatal body composition, Cord blood insulin and glucose levels, differ based on the pattern of IUGR, and these may be related to intra uterine fetal nutrition and timing of nutritional deprivation and growth.

Sample size of 97 per group was calculated taking mean body fat percentage as 11, in the study sample, expecting a difference of 1.5% (LBW and normal babies in an earlier study) between the groups with a precision of 0.05 and a power of 80.

Aims & Objectives

1. To determine the Body composition of FT IUGR / SGA babies and compare with AGA babies
2. To record maternal height and weight soon after delivery

3. To collect maternal and cord blood for Zinc, Copper, Calcium, Magnesium, vitamin D, glucose and Insulin estimation
4. To study the association of maternal BMI with neonatal body composition and also relate with cord blood trace elements, glucose and insulin concentrations.

Methodology

All the infants delivered at full term in a Government Maternity Hospital were recruited, Maternal age, hemoglobin, height and weight were recorded soon after delivery.

Pre-term babies and infants admitted to sick nursery for complications during delivery and babies delivered to mothers with complications were excluded from study.

Anthropometric data was recorded and Body composition of all infants was determined by using M.J.Dauncey's method within 24 hours of delivery.

Maternal and cord blood was collected for Glucose, insulin, trace elements and Vitamin D estimation.

Results

A total of 626 full term newborns were recruited, 55.5 % were male babies and 45.5% were female. Mean birth weight of these babies was 2.78 ± 0.37 kgs. 17.3% of them were low birth weight babies and about 1/3rd (208) of all babies were small for gestational age (SGA) babies (<10th centile). Among the SGA babies 85 (37%) were asymmetrical IUGR and the rest were symmetrical.

Mean antenatal visits were 3.86 (SD±2.37). Mean maternal hemoglobin was 11.1 ±1.26 gms/dl. in the first trimester and 10.6±1.52 gms/dl in the last trimester. 34.8% of them were primies (Table 4).

Mean maternal BMI was 21.3 (SD±2.99) Mean postpartum weight and heights of the mothers were 49.3 kgs and 151.2 cms respectively. Birth weights increased significantly with increasing parity, (2.72 and 2.88kgs in primipara and multipara respectively) similarly birth weights and all the anthropometric parameters increased significantly with increase in BMI of the mothers.

Among the biochemistry parameters cord blood trace elements (485) insulin and glucose (521) and Vitamin D (98) values are available for analysis (Table 5).

Table 4. Maternal and neonatal characteristics of study subjects

	Mean n = 618	95% CI
Antenatal visits number	3.86	3.7, 4.04
HB % g/dl	10.6	10.5, 10.8
IFA consumption (tablets)	51.6	48.8, 54.3
Calcium consumption (tablets)	45.9	42.6, 49.2
B complex consumption (tablets)	42.1	39.4, 44.7
Maternal weight (Kgs)	49.3	48.7, 49.98
Maternal height (Cms)	151.8	151.4, 152.3
Birth weight (Grams)	2777	2748, 2806
Length of baby (Cms)	48.05	47.88, 48.22
Head circumference (Cms)	32.7	32.5, 32.8
Mid arm circumference(Cms)	9.69	9.63, 9.76
Subscapular skinfold thickness (mm)	3.81	3.74, 3.87
Triceps skinfold (mm) thickness	4.7	4.62, 4.77
Body fat %	10.5	10.2, 10.8

Body fat percent (BF) of these infants was 10.5± 3.6. When analysed by ponderal index for all the babies the Total body fat, Fat free mass and BF% were significantly higher in babies with higher ponderal index. FFM (2.1 vs 2.6 kg) and Body fat percent (8.3vs 10.9) were significantly low in LBW babies ($p<0.001$) (Table 6).

Table 5 Maternal and neonatal biochemical parameters at birth

	Mother	New born cord blood
Calcium (n=485) mg/dl	8.8 (8.7, 8.9)	9.5 (9.4, 9.6)
Zinc (n=485) µg /dl	73.4 (71.2, 75.5)	106.6 (04.5,108.8)
Copper (n=485) µg /dl	197.5 (192.4, 202.6)	58.7 (56.0, 61.5)
Magnesium, (n=485) mg/dl	1.79 (1.77,1.82)	1.95 (1.93,1.98)
Vitamin D ng/ml	19.9(n=103) (17.4, 22.4)	11.3 (n=98) (9.9, 12.7)
Glucose mg/dl, n=521		72.9 (70.8, 75.1)
Insulin,µ units/ml (n= 513)		15.5 (14.6, 16.4)

Figures in parenthesis are 95% CI

Table 6 Comparison of neonatal Anthropometric parameters of Hyderabad with Pune and Southampton babies

Infant parameters	NIN (n= 216) Mean	SD	PUNE (n= 162) Mean	SD	Southhampton (n=114) Mean	SD
Birth Weight (Grams)	3020 (3000, 3039)	122.7	3008	116	3066	139
Length (Cms)	48.9 (48.7, 49.15)	1.7	49.1	1.5	48.4	1.2
Head circumference (Cms)	33.3 (32.8, 33.45)	1.28	33.9	0.9	34.4	0.9
Ponderal Index	25.98 (25.53, 26.44)	3.17	25.6	2.3	27	1.8
Triceps skin fold thickness (mm)	5.0 (4.93, 5.13)	0.77	4.6 (4.0, 5.2)	-	Not done	-
Sub scapular skin fold thickness (mm)	4.2 (4.0, 4.2)	0.84	4.6 (4.2, 5.2)	-	4.1 (3.7, 4.5)	-
MUAC (Cms)	10.1 (10.04, 10.23)	0.72	10.3	0.6	10.8	0.5

Figures in parenthesis are 95% CI

When analyzed by tertiles of birth weights the lowest tertile group has significantly higher cord blood insulin levels (16.9, to 14.9 u /ml, p=<0.05). But cord blood glucose levels were not significantly different 73 vs 72.8 mg/dl. Trace element levels were not different in the 3 tertile groups. Mean vitamin D levels of maternal and cord blood (20 and 12 ng/ml, respectively) have shown significant positive association. Only 10 new borns out of 98 had cord blood levels of more than 20ng/ml.

When analyzed based on vitamin D levels (>20 vs <20) of cord blood the group having more than 20ng/ml vitamin D levels have shown a significantly higher birth weight (2895 gms vs 2636 gms, p=<0.05) and head circumference, triceps skin fold thickness and body fat percentage, but no significant association was found with length, mid arm circumference or sub scapular skin fold thickness.

2. WEIGHT GAIN, IGF-1 STATUS AND BODY COMPOSITIONAL CHANGES OF UNDERNOURISHED CHILDREN DURING NUTRITIONAL REHABILITATION

Moderate to severe under nutrition manifesting as stunting and wasting in preschool children is quite high in India, the main cause being chronic protein energy deficiency due to inadequate food intakes (both qualitatively and quantitatively). The clinical manifestations may range from marasmus to kwashiorkor with altered body composition in these children. It is known that extra cellular fluid (ECF) compartment is expanded, lean mass is reduced, and total body water (TBW) expressed as percentage of body weight is increased in these conditions. In the initial phases of rehabilitation there is reduction in weight due to reduction in ECF and TBW, followed by weight gain due to new tissue deposition.

Earlier studies done at NIN demonstrated significant weight gain when malnourished children were given diets high in calories and protein. Study reported from elsewhere demonstrated an increase in height and weight with increase in fat free mass (FFM), when malnourished children were given a high protein diet (15% of energy from protein Vs 7.5% in controls). This suggests that high protein diets accelerate growth and help in restoring optimum body composition in children during recovery from malnutrition.

In another study, it was shown that high fat content in the new tissue deposited during the initial phases of rehabilitation of undernourished children, fed on high calorie diets. The cost of tissue deposition was around 7.2 Kcal/gram of tissue gained. Also, another study demonstrated a reduction of 25% in protein synthesis, with low protein diets. It was also demonstrated that energy cost of tissue deposition depends on the type of tissue deposited which is related to the quality of diet.

Studies have shown that with high protein intake urea excretion increases and urea recycling in GI tract is reduced. Some studies have suggested that increased urea excretion and increased solute load might affect the kidneys.

Due to chronic energy deficiency in severely malnourished children the body metabolism is adapted to low intakes in early life and when there is energy balance in later life they tend to develop obesity. Some studies have demonstrated that children with undernutrition / stunting in early infancy and childhood are more at risk of developing obesity. This could lead to chronic diseases with associated complications once they are exposed to better living conditions and access to high energy yielding foods.

Hypothesis

Weight gain and body compositional changes in severely malnourished children depend on the quality and quantity of diets consumed by the children.

Aims & Objectives

1. To determine the nutritional status and body composition of all children admitted for nutritional rehabilitation, at baseline, at discharge after one month and every month during follow up for 6 months.
2. To record their dietary intake on admission, and during their stay in the hospital at 15 days intervals.
3. To study the biochemical parameters of nutritional status, renal function & growth factors on admission & after one month at discharge.
4. To study the relationship between dietary composition and body compositional changes during rehabilitation and recovery phase.

Methodology

Children with moderate to severe under nutrition and without any complications admitted to the nutrition ward at Niloufer Hospital for rehabilitation were enrolled for the study.

Their initial nutritional status and body composition by Skin fold thickness method, was determined soon after admission to the nutrition ward. The dietary intake before admission was determined by 24 hour questionnaire and they were given the regular rehabilitation diet, consisting of bread, rice, dhal, eggs and milk fortified with cooking oil to make it calorie dense. The average calorie consumption in the hospital ranged from 100 to 170 Kcal / kg / day, of current body weight. The diet supplied around 9-10% of energy from protein. The quantity consumed (ad lib) was recorded daily, and 24 hour record maintained during the period of hospitalisation. Routine Vitamin and mineral supplements were given to all these children as per the WHO guidelines for treating malnourished children and record maintained. Their weight was recorded daily and anthropometric measurements for body composition were done every 15 days during admission and during recovery at home for six months.

Study was initiated in 2005 after standardization of dietary intake anthropometric measurements and other parameters. Inter rater reliability tested every 3 months. Measurements were taken by 2 persons and the average has been used for analysis. Till December 2007, a total 80 children were admitted in the nutrition ward for the study. Their mean duration of stay was 30.7 days. Follow up after discharge was possible in 24 children at monthly intervals upto 6 months. Dietary intakes and body composition measurements were carried out in these children.

Results

Mean age at admission was 25 months (SD 15.2, range 6-60 months) and mean duration of admission was 30.7 days (SD 3.76). Twelve (15%) children were under 1 year of age, 32 (40%) from 1-2 years and 36 (45%) above 3 years of age.

During the period of hospital admission, the mean weight increased from 6.3 kgs. to 7.4 kgs. In absolute terms of the total weight gain of 1.1 kgs in 30 days, total body fat increased from 0.56 to 1.01 kgs, whereas fat free mass increased from 5.8 kgs to 6.4kgs, In terms of weight gain children above 2 years of age gained around 1.4kg (SD±0.8) compared to 0.83 kg (SD±0.46) in less than 2 years of age. When expressed as percent weight gain from initial weight it was not significantly different in these two groups. (20.6% vs 17.2%). Weight gain per kg body weight per day was around 6.1 gm in both the groups. Mid-arm circumference (MAC) also increased significantly from 10.2 Cms (SD ± 1.71) to 11.7 (SD ±1.54) Cms.

Mean body fat at baseline was 8.0 %, and increased to 13.1% by one month. However, it was still short of the average of 18-20% for normal children of that age group.

S. albumin rose from 4.2 ± 1.24 to 4.7 ± 0.97 gms /dl and blood urea from 18.6 ± 7.58 to 21.3± 6.34 mg/dl. by 30 days and the maximum blood urea levels were 33.2 mg/dl. Urinary creatinine levels could be done in only 9 subjects and the mean value was 39.3 ± 21.9 mg / dl., with a range of 5 to 93.

The baseline serum zinc levels were 78.3µg /dl (SD 24.66), and increased to 96.3µg /dl (SD 22.05) by 30 days post admission. Similarly mean IGF-1 levels rose from 10.6 ng/ml at admission to 38.4 ng/ml by one month.

Mean dietary intakes at home before admission to the Nutrition Ward in the hospital were 772 calories (CI 701, 843) and 21.6 gm (CI 19.4, 23.8) of protein per day, with proteins account for 11% of the total energy. This increased to 1212 calories (CI 1146, 1279) and 39 gms of protein (CI 37.1, 41.5) per day, with energy from protein contributing 12.9%. Expressed as protein intake, per kg body weight before admission was 3.7 gm/kg/day which increased to 6.1 gm/kg/day. At follow up after 6 months after discharge the mean calories intake at home was 1169 cal per day and protein intake was 6.2 gm/kg/day. Inspite of increase in protein intake expressed as per kg body weight per day from 3 gm to > 6 gm/day there was not much of an increase in the blood urea levels as mentioned earlier.

3. PEAK BONE MASS (PBM) IN OVERWEIGHT AND OBESE MEN AND WOMEN FROM THE HIGH SOCIO-ECONOMIC GROUP

Body weight increases bone loading and mineral accretion. A positive association between body weight and bone mass in all age groups has been shown from this Institute and other studies.

Among the three components of body weight detected by DXA i.e. lean body mass, fat mass, and mineral mass together accounts for >95% of body weight. Several studies have reported that both fat & lean mass contribute to increased bone mineral density (BMD).

Apart from in mechanical influence weight, particularly contributed by fat mass, also influences BMD positively due to the aromatization of gonadal steroids in the fat tissue resulting in reduced osteoclast activity.

However, many studies have shown that despite increased mechanical load by lean mass, adipose tissue is not beneficial to bones. Only the lean body mass was found to correlate positively with bone mass with an inverse relation with fat mass.

Obesity may also be associated with Vitamin D insufficiency and secondary hyper parathyroidism due to reduced availability of Vit. D, because of its deposition in body fat compartments.

Studies describing the relationship of the individual components of body composition, (Fat &

Lean tissue) with bone mass were equivocal. Over weight and obesity is a rapidly escalating problem in India especially in the high income group. The prevalence of over weight was 4 times higher among the adolescents of high socio economic group when compared to the low income group.

Asians including Indians have a higher proportion of body fat for a given BMI than other ethnic groups. Hence, it was decided to study the effect of over weight & obesity on the development of peak bone mass in men & women of this region. The study was undertaken in the age group of 20-35 years, because it is known from earlier studies conducted at NIN that PBM is achieved in this age group and age related losses occur only after 40 years in the HIG.

Aims and Objectives

1. To study the BMD, whole body mineral content (WBBMC) and body composition by Dual Energy X-ray absorptiometer (DEXA) in over weight and obese (with BMI >25) men and women in the age group of 20-35 from high socio-economic group who have no constraints to growth.
2. To compare these parameters with those of subjects having normal BMI (18.5 to 25) from the same age and socio-economic group.
3. To determine the correlation of Peak Bone Mass with
   a) body composition measurements
   b) dietary calcium intake
   c) biochemical parameters of bone metabolism
   d) physical activity

Hypothesis

Obesity interferes with the attainment of peak bone mass.

Methodology

Sample Size: assuming 95% CI, 80% power, SD of spine BMD - 0.106 g/cm^2, the expected difference - 0.04 g/cm^2, the required sample was estimated as 75 men and 77 women with normal BMI and an equal number with BMI higher than 25. A total of 303 healthy men and women with no constraints to growth in the age group of 20-35 years from the high socio-economic group with a BMI of >25 were recruited for this study. High BMI 74 males and 77 females, Normal BMI-75 males and 77 females.

Presence of medical conditions and intake of drugs affecting bone metabolism was regarded as an exclusion criterion.

Background information and anthropometric measurements like weight, height and waist-hip ratio were recorded.

Anthropometry

A general physical examination was conducted for each participant. Height (m) was measured to the nearest 0.1 cm on a portable stadiometer (GPM Swiss make) and weight was measured with Seca 882 Electronic balance (Seca GMBH & Co Hamburg) to the nearest 0.1 Kg with minimum clothing and without footwear. Body mass index (BMI) was calculated as weight/ height^2. Blood pressures of subjects were measured according to standard procedure.

Bone and body composition measurements

Bone densitometry at three sites (hip, spine, forearm), whole body and body composition studies were done for total lean body mass and fat mass using DXA (Hologic 4500W), in all the subjects.

Biochemical parameters of bone metabolism

Overnight fasting blood samples were collected from all the subjects for analysis of bone related parameters like serum calcium, phosphorous, 25 (OH) Vitamin D, Alkaline Phosphatase (Total and bone specific), PTH and acid phosphatase. Urinary fluoride was also estimated to rule out effect of excess fluoride intake.

Dietary Calcium intake was assessed by pre-tested food frequency questionnaire (FFQ) method. Similarly, subject's physical activity, estimation of the body exposed to the sun light, total time of exposure and frequency were measured by pre-tested questionnaire.

Physical activity was assessed by a questionnaire which was designed to capture subjects previous 24 hrs. physical activity as well as to pick up any other heavy work regularly done by the subject over past one year. All the physical activity data was converted into bone loading activity and graded into 0-4 scale.

Results and Discussion

Anthropometry

There were no significant differences in mean (±SD) age, and heights, between normal BMI (NBMI) males, and high BMI (HBMI) males, (mean age 27.0±4.42yrs and heights 172.8±6.39cm) and also between female NBMI and HBMI (mean±SD mean age and height being 28.6±4.67 yrs, 158.1±5.88cm respectively). As expected mean weight and BMI were significantly higher (p<.001) in HBMI males (wt.85.8±10.24kg and BMI 28.8±2.71kg/ht² Vs 69.2±8.06kg and BMI of 23.0±1.86 in normal BMI men). Female HBMI group were also significantly (p>.001) heavier with weights of 72.5±9.33kg and BMI of 29.1±3.21 kg/ht² as compared to normal females with weights of 56.6±6.40 kg and BMI of 22.4±1.9 kg/ht².

Mean sunlight exposure time and percent of body surface area exposed to the sun was not different between NBMI and HBMI groups in both men and women. Significantly higher percent of subjects belonging to HBMI, both male and female were physically active as compared to normal BMI group. This may be partially explained by the fact that HBMI subjects were trying to loose body weights by regular exercises.

Biochemistry

There were no differences in bone related bio-chemical parameters like serum calcium, phosphorous, and alkaline phosphatase between the groups, but serum Vit.D was significantly (p<0.001) lower in female HBMI group compared to normal BMI group (9.8±13.43 vs 20.3± 17.05ng/ml). Similarly immunoreactive parathyroid hormone (PTH) was significantly higher (p<0.05) in female HBMI group compared to female NBMI group (61.7±39.75 Vs 43.1±31.34). Vitamin D and PTH were not different between male HBMI and NBMI. Similar observations have been reported in other studies.

Bone parameters

It was found that BMD's of the neck, hip, spine and forearm were significantly higher in the HBMI groups in men and women when compared to NBMI.

Neck of the femur and Total Hip Parameters: In the case of neck of the femur BMD's of male (0.91±0.121 g/cm^2 HBMI vs 0.85±0.126 g/cm^2 NBMI), the total increase in BMDs in the HBMI was due to significant increase in BMC with no changes in the neck area. A similar pattern was observed in the case of BMD of neck of femur in women (HBMI), (BMD of HBMI women was 0.88±0.107 g/cm^2 vs 0.81±0.105 g/cm^2 (P<0.01) in the NBMI) and total hip BMD of men and women (male HBMI BMD was 1.02±0.123 g/cm^2 vs 0.97±0.119 g/cm^{2}in normal BMI P<0.001, Women 0.89±0.101 g/cm^2 NBMI vs 0.96±0.94 g/cm^2 P<0.05).

Spine: The BMDs of total spine were also significantly higher in the HBMI, men and women P<0.05 (Male HBMI BMD 1.0±0.107 g/cm^2 vs 0.96±0.098 g/cm^2 NBMI. Women NBMI 0.96±0.096 g/cm^2 vs 1.0±0.100 g/cm^2 in HBMI).

Whole Body : The whole body BMC was significantly (P<0.05) increased in the HBMI subjects (male and female). Men from HBMI had an excess of 150gm of mineral where as it was 125gm in case of women HBMI women compared to NBMI. The whole body BMC area was also significantly higher among HBMI men and women when compared to NBMI men and women. (P<0.01). Therefore, when analyses were performed after controlling for whole body bone area, BMDs at lumbar spine and whole body were not significantly different among the two BMI groups in both men and women.

Body composition

The body composition parameters like total body fat, total lean mass and percent body fat were significantly high (p<.001) in HBMI group of both sexes. The HBMI male and female had around 9 kg excess fat compared to NBMI. The percent fat as expected was significantly higher in the HBMI subjects (6% higher in men and women of HBMI P<0.05). The difference in the mean lean mass in the HBMI group was 7.6 and 5.7kg respectively for male and female compared to NBMI. The percent fat of HBMI was (30.0±4.89and 39.1±3.68for male and female) as compared to that of NBMI (23.9±5.13 and 33.4±5.06 respectively).

Conclusion

The results suggest that increase in BMI was associated with increase in BMD at femoral neck and hip regions in both men and women. However, higher BMI was not associated with better BMDs at other skeletal sites where skeletal size was taken into account.

4. BONE PARAMETERS OF MEN AND WOMEN WITH OSTEOPOROTIC HIP FRACTURES

Osteoporosis is one of the most common causes of disability and major contributor to medical cost in many regions of the world. After menopause in women and with advancing age in men, bones weaken and neuromuscular functions become sluggish. These changes together produce a rapid rise in the risk of fractures. Osteoporosis has clinical and public health importance because of these fractures.

National status

Osteoporosis is widely prevalent in India and osteoporotic fractures are common cause of morbidity and mortality in adult men and women. Several studies demonstrated that hip fractures occur at relatively earlier age in Indian men and women compared to their counterparts in the west. Studies from NIN which analysed the anthropo-metric, biochemical and radiological profile of women with confirmed osteoporotic fractures show that the nutritional status of woman with osteoporotic fractures was poorer as assessed by mid arm circumference, skin fold at triceps, hemoglobin, serum albumin, and serum total protein. These studies also confirm the female preponde-rance and early age of occurrence of fracture of neck of femur. Bone densitometer measurements are essential for guiding therapies directed specifically at reversing the bone loss. But other factors pre-disposing to falls such as loss of muscle tone, loss of reflexes or sluggish reflexes, poor vision, coordination may be irreversible. It is therefore, important to study the BMD fracture relationship in men and women.

Hypothesis

It is hypothesized that the BMD of patients with confirmed fresh osteoporotic fractures are lower than the BMDs of normal age and sex matched controls.

Objectives

1. It is proposed to study the bone parameters (BMD, BMC and area) of men and women with confirmed osteoporotic fractures at the hip and forearm.
2. To compare the BMDs thus generated with the BMD at the hip and forearm in age and sex matched controls, and also of young adults with hip fractures due to traumatic injuries.
3. To study the associated factors contributing to fracture.

Material and Methods

Subjects who had fractures of hip within the last three months with history of trivial injury will be recruited for the study. Nutritional status, history of fracture and immobilization will be recorded. Measurements of the BMD of the femoral neck, trochanter, wards triangle, inter trochanter and total hip of the non-fractured bone was obtained by DXA. Based on the variations in BMD of the patients the sample size will be calculated, midway.

Sample size

Assessing 95% CI, 80% power, SD of hip BMD 0.15 and expected differences 0.07 required sample sizes is 73 for each gender.

Results

The total sample size of 292 subjects have been recruited in the study, of these 146 had a recent history of osteoporotic fracture (73 male and 73 female), and 146 were age and sex matched controls (70 male and 76 female). In addition 7 subjects with traumatic fractures, were also recruited. Analysis has been completed in 90 cases (Males 43 and Females 47) and 88 controls (47 Male and 41 Female). The background characteristics of the cases and the controls were not different from each other. The hip BMD in the

cases was 0.71±0.139 in men and 0.55±0.126 in women versus 0.84±0.116 in control men and 0.64±0.121 in control women.

There were significant difference between the BMD values of the hip between the fracture cases and the controls in both men and women (P<0.05). The T scores at the hip were –2.27±0.972 in men with fractures vs –1.26±0.768 in control subjects (P<0.05), and –3.31±1.064 in women with fractures vs –2.50±0.989 in control women. The bone densities at the spine were in the osteoporotic range in all the women (cases and controls) and they were not different.

However, men with osteoporotic fractures also had spinal densities in the osteoporotic range but they were significantly different from male controls (P<0.05) (Men spinal T score cases –2.52±1.685 versus controls –1.86±1.371 (P<0.05), women cases –3.60±1.546 versus-3.54±1.151control (NS).

There were no differences in the serum calcium levels between the cases and controls in men and women. However, serum vitamin D levels (ng/ml) were significantly lower in the fracture cases when compared to controls in both men and women (Men cases 16.62±9.499 in cases vs 39.5±16.03 in controls, women cases 11.64±10.82 versus 36.03±12.16 controls (P<0.05). The serum albumin levels were also significantly lower in the fracture cases when compared to controls in both the sexes.

III. BASIC STUDIES

1. PHYTOFERRITIN CONTENT AS AN INDICATOR OF IRON DENSITY FROM PLANT FOODS: DEVELOPMENT OF A COMMON IMMUNOASSAY METHOD FOR MEASURING PLANT FERRITIN CONCENTRATIONS

Biofortification is an emerging strategy, aims at improving nutrient density and bioavailability in staple crops through plant breeding techniques. Phytoferritin is an iron storage protein present in all plants and appears to be well conserved among plant species. The project envisages developing a common immunoassay to estimate plant ferritin (Phyto-ferritin) concentration and to use it as a screening tool to select germplasm of staple crops such as wheat, rice, maize, common bean and legumes for crop biofortification. On the basis of antigenic index amongst plant ferritins (Fig.3), it is assumed that antisera produced against pea ferritin can be used to develop a common immunoassay method for quantitation of phytoferritins in majority of the staple food crops.

Aims & Objectives

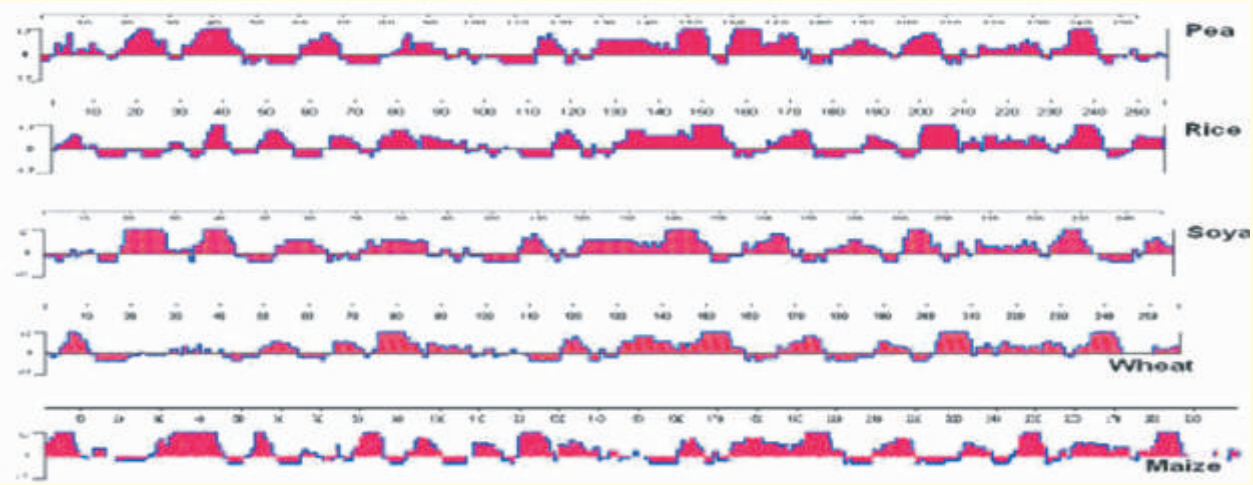
To develop and validate a common immunoassay method for phytoferritin in staple crops.

Methods

Sandwich ELISA: Pea seed ferritin was purified and characterized. Polyclonal antisera against this protein was produced in New Zealand white rabbits used for preparing a IgG-HRPO conjugate. The titers for coating antiserum and conjugate dilution were determined by checkerboard titration.

A sandwich ELISA was developed according to the method described for human ferritin ELISA. Briefly, the method consisted of coating of polystyrene microtiter plates with 1:40,000 dilutions of the antisera in a carbonate buffer pH 9.5 on the day of the assay followed by blocking with 0.5% BSA. The plate was then incubated with pea ferritin standards (0-500 ng/ml) or sample extract (prepared as described below and diluted to 1:10) followed by the further incubation with IgG-HRPO conjugate (1:40,000) for 2h. The enzyme activity was assessed using H_2O_2-OPD substrate and the colour intensity measured at 492nm (Biotek).

Figure 3. Antigenicity index (Jameson-Wolf plot) comparison of select plant ferritin sequence using protean module of Laser gene software, Ver. 6.0. (courtesy, M/s DNAstar). Peaks represent hydrophilicity & valley hydrophobicity



Extraction of phytoferritin from seeds : About 10-50g of powdered rice, wheat and maize, pea, soya bean, lentil, green gram, black gram and red gram were homogenized using polytron for 3 min at high speed in extraction buffer (10mM PBST, 1mM EDTA) and filtered. The filtrate was then centrifuged at 25,000 rpm for 20 min and the supernatant further centrifuged at 10,000 rpm for 10 min. The supernatant subjected to $MgCl_2$ salt fractionation followed by ultracentrifugation at 100,000g for 2h. The pellet thus obtained dissolved in 2M urea and analyzed for phytoferritin content. The recovery of phytoferritin from the sample was monitored with purified pea ferritin (50 ng/mL) added during the extraction.

Protein blotting : Partially purified proteins from all the samples were also subjected to protein blotting and probed with pea ferritin antisera.

Iron content: Iron content in these samples was estimated by bathophe-nanthroline method.

Statistical analysis: The concentration of pea ferritin vs optical density was plotted on log-log graph to obtain the phytoferritin content in the samples. Correlation between the iron and phytoferritin contents was determined using the Sigma Plot software.

Results

Characteristic features of sandwich ELISA: The dose response was linear in the range of 0-500 ng of pea ferritin /mL with r^2 of 0.94. The sensitivity was found to be10 ng/mL. Reproducibility of the ELISA method was acceptable and showed coefficient of variation for both inter and intra assay variability of less then 12% (Fig 4A).

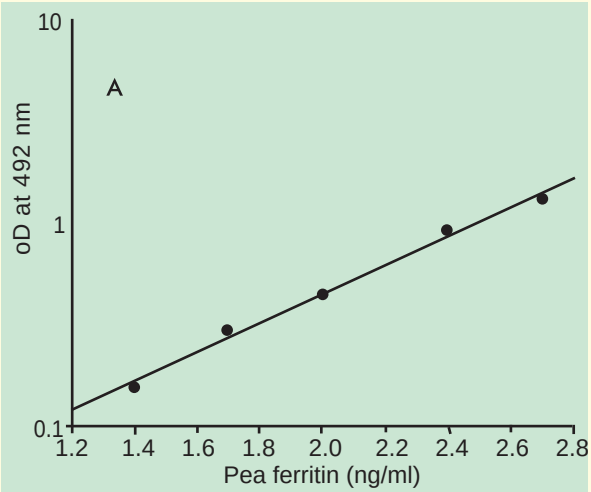
Phytoferritin content: The extracts of samples showed linearity with phytoferritin and protein content. The recovery of extraction based on the pea ferritin ranged from 68 - 100%. The phytoferritin content showed high variability among legumes and cereals. The protein extract of lentil had the highest phytoferritin content followed by black gram, green gram, soybean and red gram and the lowest - to non-detectable levels in rice, wheat and maize.

Correlation between phytoferritin and iron content of legumes: The phytoferritin and iron contents of legumes showed positive correlation with r^2 of 0.80. (Fig 4B).

On protein blotting of the extracts with pea ferritin antiserum showed good immuno cross reactivity with all the extracts. The extracts of cereals not only cross-reacted with a band corresponding to pea ferritin but also with other proteins (Fig 5A).

However, the extracts of legumes cross-reacted with only one major band corresponding to pea ferritin (Fig 5B). These results indicate that the sandwich ELISA can be used for estimating phytoferritin content of legumes.

Figure 4. Log-Log dose response of pea ferritin concentration and optical density. X-axis concentration (ng/ml) and Y-axis OD at 492 nm. The regression coefficient for dose response r^2 = 0.940 (A). Correlation of phytoferritin concentration in legume and iron control r^2 = 0.80 (B)



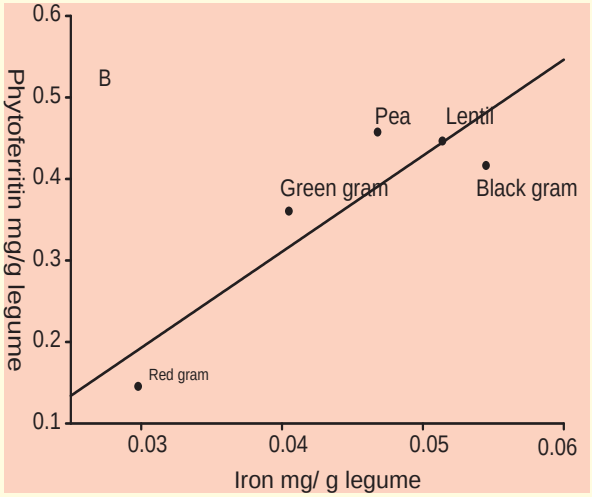
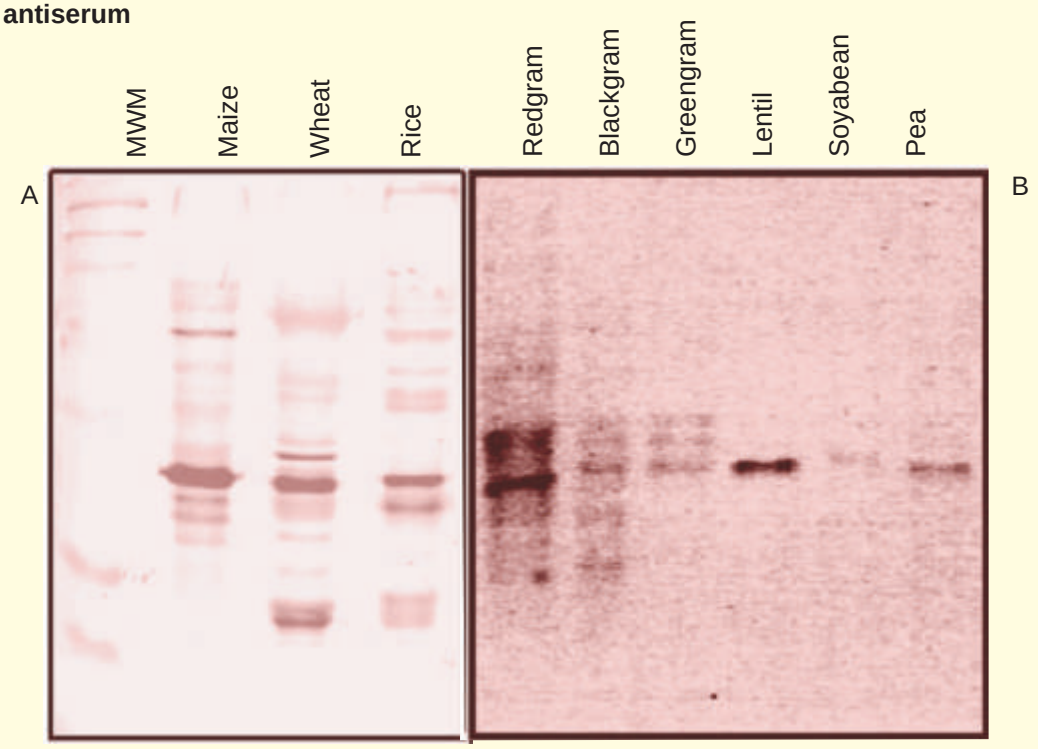


Figure 5. Immuno-cross reactivity of protein extracts of cereals (A) and legumes (B) with pea ferritin antiserum



Conclusions

A sensitive sandwich ELISA method to quantitate phytoferritin levels in legumes was developed. The assay can be used as a screening method for iron density and will have application in crop biofortification program.

2. STANDARDIZATION & VALIDATION ZINC BIOAVAILABILITY SCREENING METHOD USING Caco-2 CELL MODEL

Zinc deficiency is a public health problem in populations subsisting on vegetarian diets and is due to poor density and low bioavailability of dietary zinc. Therefore, increasing the density and bioavailability of zinc in staple crops such as wheat through crop biofortification is considered as an emerging strategy to prevent and control zinc deficiency. Deprtatment of Biotechnology, GOI, has recently initiated the 'India Crop-biofortification network program" under which wheat has been identified as the target crop . The network project objectives are to identifying potential breeder lines for high zinc through screening the germplasm and biofortification through conventional plant breeding technology and to evaluate the bioavailability of zinc from such crops.

Aims and objectives

To establish and validate Caco-2 cell line as a tool for assessing zinc bioavailability in humans.

Methods

^{65}Zn Uptake in the presence of ligands: Caco-2 cells were obtained from National Center for Cell Sciences, Pune. Cells were grown in 6-well culture plates and used 12-14 days post confluence. To establish the time and dose response, Caco-2 cells were incubated with ^{65}Zn (1µCi) for 0 - 240 min or with 0-200 µmole/L Zn for 2 h. The uptake process was assessed in Zn repleted (25 µmole/L Zn) or depleted (10 µmole/L TPEN) cells for 3 h and also with 1:1, 1:5 and 1:10 molar ratio of the dietary ligands; inositol hexa phosphate (IP6), tannic acid, L-ascorbic acid,

L-arginine, L- methionine, L-histidine HCl, L(+) tartaric acid and (+) catechin. The radioactivity taken up was counted in gamma ray spectrometer and expressed as % of control without ligands, control being 100% and also fold change over control expressed in percentage.

^{65}Zn Uptake in the presence of food matrices: Uptake of zinc from food matrix containing two levels of phytate was compared against that of iron under identical conditions by the coupled *in vitro* digestion and uptake by Caco-2 cells. Indian bread (chapatti) prepared from high (>90%, HEWF) and low (<70%, LEWF) extraction wheat flour along with extract of black tea or red grape juice as sources of polyphenols was used.

Food matrix traced with either ^{65}Zn or ^{59}Fe was subjected to the gastric and intestinal digestion to obtain "digesta". The digesta was then fed to the cells and the radioactivity measured as described above and expressed as percentage of respective controls with out food matrix.

Statistical analysis: All the experiments were performed in triplicates and replicated at least once to generate 6 observations. Kinetic parameters were calculated using Sigma Plot (Ver 7.0). One way ANOVA followed by least significant differences (LSD) post-hoc 't' test was performed using the SPSS (ver 11.0) statistical package to assess the effect of treatments (ligands and food matrix) on uptake and P value <0.05 was considered significant.

Results

Characteristics of zinc uptake by Caco-2 cells: ^{65}Zn uptake increased with time and reached plateau at 2 h. Zinc uptake process was saturable at low concentrations (0-50 µmole/L) and non-saturable above 50 µmole Zn/L with Km of 65 µmolar and Vmax 93 nmole/mg protein/2h. Intracellular depletion of zinc increased zinc uptake whereas zinc loading did not influence uptake (P<0.05, Fig. 6).

IP-6 dose dependently inhibited zinc uptake while histidine inhibited zinc uptake at 1:10 molar ratio. Tannic acid, tartaric acid, and methionine increased the uptake of zinc in a dose dependent manner while catechin and ascorbic acid did not influence zinc uptake (Fig 7).

Figure 6. Effect of zinc depletion (10 µ mole/L TPEN) or repletion (25 µ mole Zn /L) on ^{65}Zn uptake in Caco-2 cells. The bars represent mean uptake ±SD. The bars without common superscript differ significantly (P<0.05)

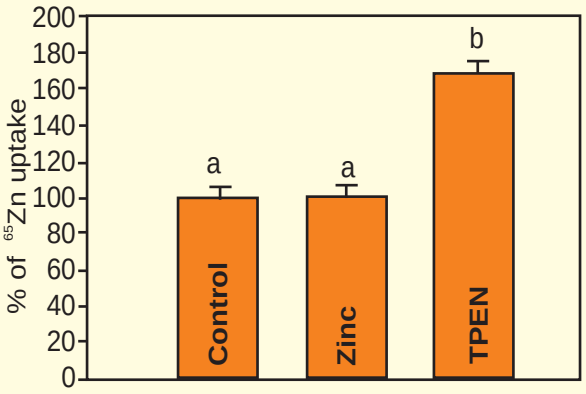
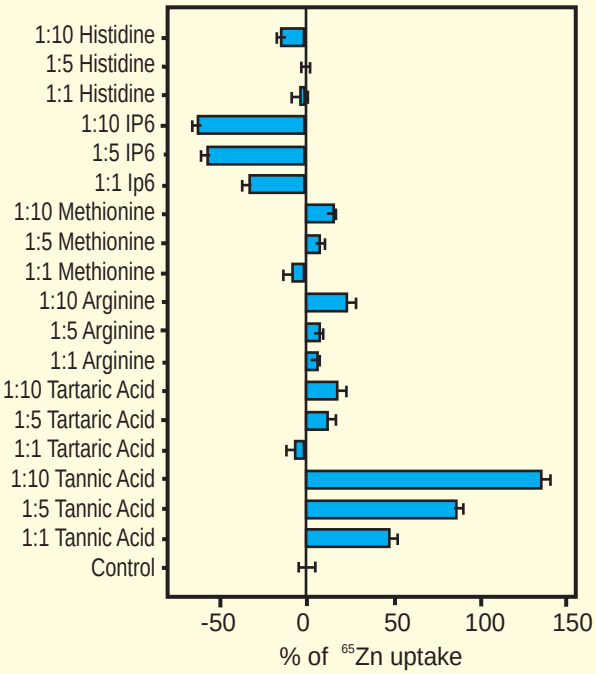
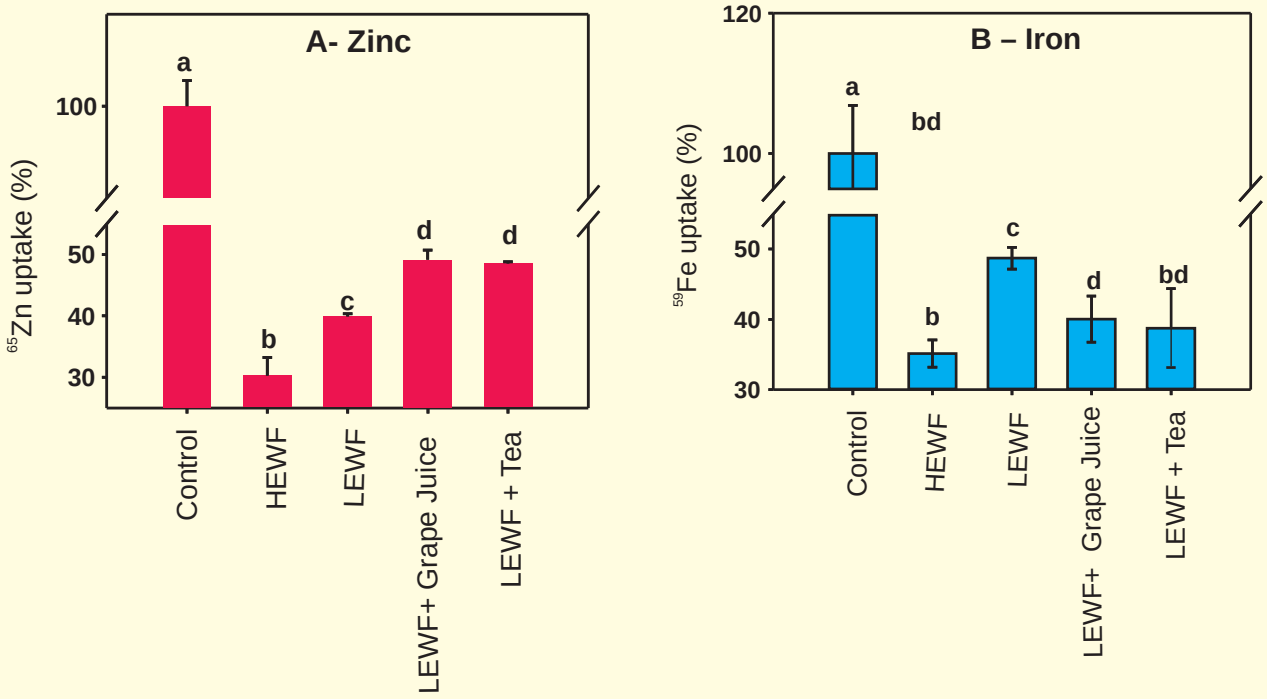


Figure 7. Effect of dietary ligands on ^{65}Zn uptake. The uptake is calculated from the dpm and expressed as percentage (dpm of ligand - dpm of control / dpm of control). Bars represent mean±SD of 6 observations and bars that do not share common superscript differ significantly (P<0.05)



These results are in agreement with the studies in humans and animals reported in the literature.

Figure 8. Influence of food matrix on ^{65}Zn (A) and ^{59}Fe (B) uptake from high (HEWF) and low (LEWF) extraction wheat flour in Caco-2 cells. Tea extract and grape juice was used as sources of polyphenols. The percentage uptake of the minerals was calculated as % of control (without food matrix as 100%). Bars represent mean+SD and bars that do not share common superscript differ significantly (P<0.05)



Validation of Caco-2 cells for zinc bio-availability: The uptake of zinc (31.7±1.53%) and iron (38.6±4.43%) was high (p<0.05) from LEWF compared to HEWF Indian bread (Fig 8). Tea extract and grape juice significantly increased (P<0.05) zinc uptake (Fig. 8A) from LEWF while it decreased (P<0.05) that of iron (Fig. 8B). These results are in agreement with the known effects of phytate and polyphenol- rich foods, inhibiting the absorption of iron but not that of Zn in humans and animals. In order to assess the suitability of the Caco-2 cell based Zn bioavailability method, the absorption ratios of Zn from low and high phytate based foods obtained in the present study was compared with the studies reported in the literature for humans. The absorption ratio of Zn from low and high phytate foods obtained in the present study (1.46) was similar with the data obtained from human studies.

Conclusions

Caco-2 cells respond to intracellular zinc status and the uptake of zinc is modulated with the type of dietary ligands. The most promising promoter of zinc uptake is tannic acid. By validating the cell line with that of iron, with respect to the direction of response to various food matrices suggest that the Caco-2 cells can be used as screening tool for assessing bioavailability of zinc from foods.

3. STUDIES ON THE RESPONSE AND INTERACTIONS OF IRON AND ZINC IN Caco-2 CELLS: DOSE AND TIME DEPENDANT MODULATION OF IRON AND ZINC BIORESPONSE IN Caco-2 CELLS

It is now widely recognized that iron and zinc interact at the site of absorption (intestine) and influence the uptake of one another. Earlier studies conducted at NIN have established that increased oxidative stress induced damage during iron repletion is alleviated by natural antioxidants such as ascorbic acid, α-tocopherol and by zinc. Also, a negative interaction between iron and zinc, as evidenced by the decreased uptake of mineral in the presence of the other and in induction of ferritin and metallothionein, under conditions of a zinc depletion-repletion study in rats has been recently reported. The aim was therefore to understand how iron and zinc interact during uptake. The Caco-2 cell line is an ex vivo model for bioavailability determination. DMT-1 is the apical iron transporter and IRP-2 is known to be rapidly synthesized de novo in response to iron status of the cell. The ZnT family (SLC30A) of efflux transporters decreases cellular zinc concentration; ZnT-1 and ZnT-4 have been shown to be physiologically relevant and are highly expressed in the small intestine of rodents and human duodenal biopsy samples.

Aims and objectives

To elucidate the dose and time dependant modulation of iron and zinc bioresponse in Caco-2 cells.

Methods

Caco-2 cell culture: Caco-2 cells experiments were seeded in six-well plates and used the monolayer for fourteen days post-seeding. All the experiments were performed in triplicates and repeated at least once to generate six observations.

Mineral uptake studies: For time – dependent uptake studies, DMEM supplemented with either 50 µmolar iron and or zinc with ^{59}Fe or ^{65}Zn for 0-120 min were used. For dose – dependent uptake studies, cells were incubated with 0 – 200 µM iron or zinc for 2 h. After the incubation, medium was aspirated and the monolayers were washed, cells scraped, ^{59}Fe (Packard Tri-Carb 2100 TR) and ^{65}Zinc (GRS – 201L, PLA electro-appliances, Mumbai, India) counted. Actual uptake (nmol/mg protein/2h for iron or pmol/mg protein/2h for zinc) was calculated.

Interaction studies: Cells were supplemented with 1:0, 1:0.25, 1:0.5, 1:1, 1:2 and 1:4 molar ratio of iron (50 µM Fe with ^{59}Fe): zinc for 2h. Cells were processed as above. For studying the effect of iron on zinc uptake, essentially the same procedure was carried out with ^{65}Zinc. Actual uptake was calculated as above and converted to percentage of 1:0 molar ratios, which was taken as control or 100%.

Kinetic studies: To study the interaction between iron and zinc, cells were incubated with 5, 20, 50 or 100 µM iron along with 1:0, 1:0.25, 1:0.5, 1:1, 1:2 and 1:4 molar ratio of Zn as above. For studying the kinetics of zinc inhibition of iron uptake, cells were incubated with 50 µM Fe with 1:0, 1:0.25, 1:0.5, 1:1, 1:2 and 1:4 molar ratio of zinc for time points of 5, 10, 15, 30, 60 and 120 min.

Kinetic data modeling: Uptake data were subjected to data fit using Sigma Plot software (ver 7.1) to derive K_m and V_{max} values. Dose-dependent uptake data were plotted and the resultant uptake curve was best fit using non-linear regression equations to model the uptake process using mean of all replicates. Accuracy of curve fit was ensured by r^2 values greater than 0.90.

The uptake curve was resolved into its individual linear components by subtracting the extrapolated value from the observed uptake. The same process was repeated till all components were resolved to linearity. The slope is an indicator of the relative contribution of that component to total uptake and y0 value is the level of uptake from which that component contributes to total uptake.

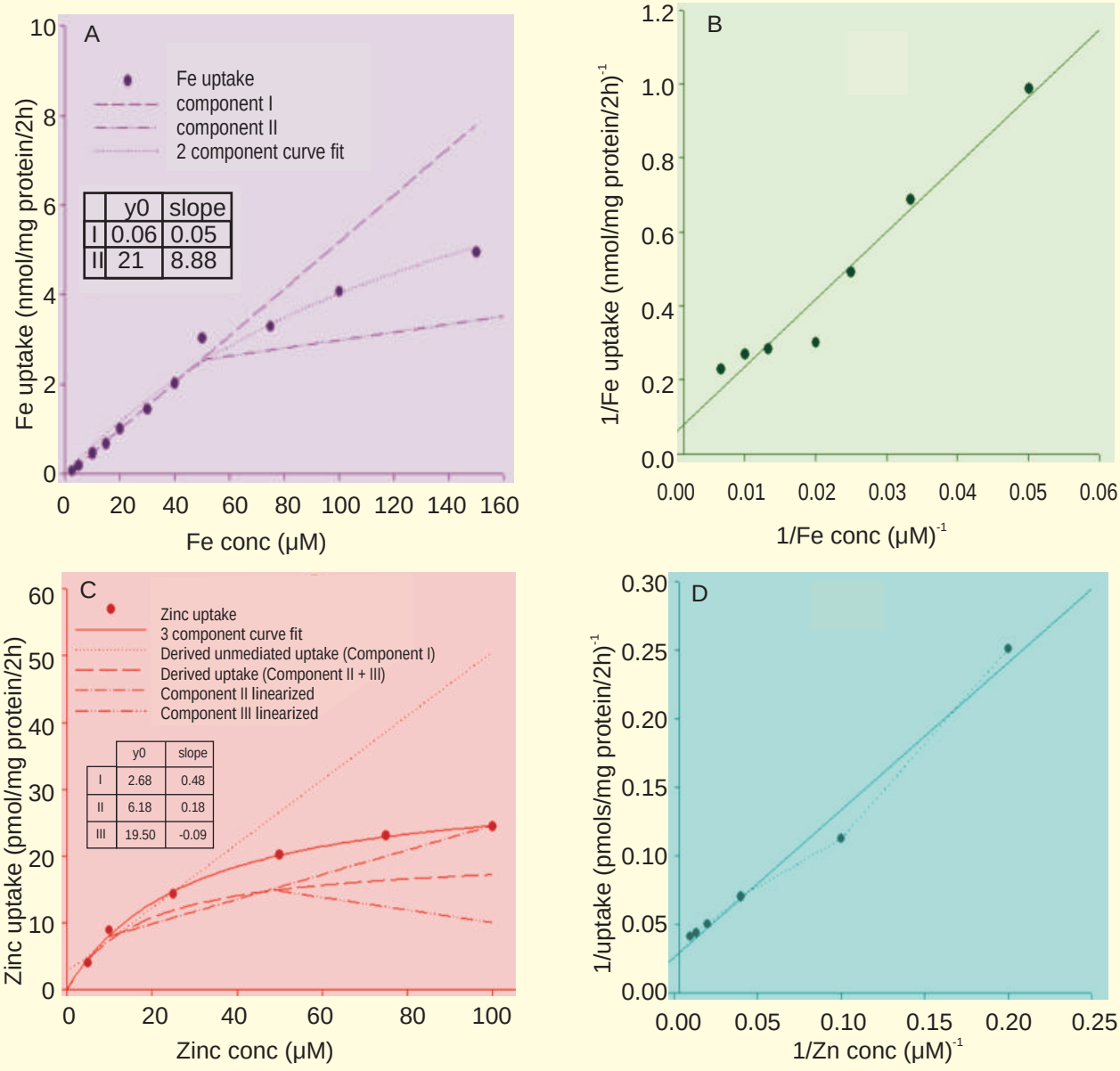
The double reciprocal plot of mean of uptake with 1/Fe conc. or 1/time was subjected to linear regression to obtain K_m and V_{max} from the slope (K_m x V_{max}) and y_0 ($1/V_{max}$). In case of 1/Fe conc, the data points of 5µM were not considered as no inhibition was observed.

Effect of iron / zinc repletion: Cells were either left untreated or pretreated with 50 µmolar zinc for 2,3 or 4 h and iron uptake studied in the presence and absence of zinc as above. Similarly, cells were pretreated with iron and zinc uptake quantitated.

Cellular zinc depletion: Cells were depleted of cellular zinc by pre-treatment with 10µM TPEN (N, N, N', N'-Teterakis [2-pyridylmethyl] ethylene diamine, an intracellular zinc chelator) for 2 h prior to quantitation of iron or zinc uptake as above.

Protein expression: Cells were either left untreated or treated with 50 µM iron or zinc for

Figure 9. Iron and zinc uptake in Caco-2 cells: Iron (A) and zinc (C) uptake data were curve fit and individual components linearized. Inset table gives slope and intercept values of individual components. A double reciprocal plot of dose – dependent iron (B) and zinc uptake (D) was plotted to obtain K_m and V_{max} values.



2, 4 or 6h. Cells were lysed in RIPA, protein estimated, proteins separated on 10% SDS-PAGE, electro-transferred on to nitrocellulose membrane, probed with DMT-1, IRP-2, ZnT-1, ZnT-4 primary antibodies followed by enzyme conjugated anti-species antibodies and the blots visualized using TMB substrate system & quantitated using a GS-710 densitometric scanner (Bio–Rad, Hercules, CA, USA).

Statistics: Means between treatments or time – points were compared by one-way ANOVA and post-hoc't' test, P<0.05 was considered significant (SPSS software, ver 11.0).

Results

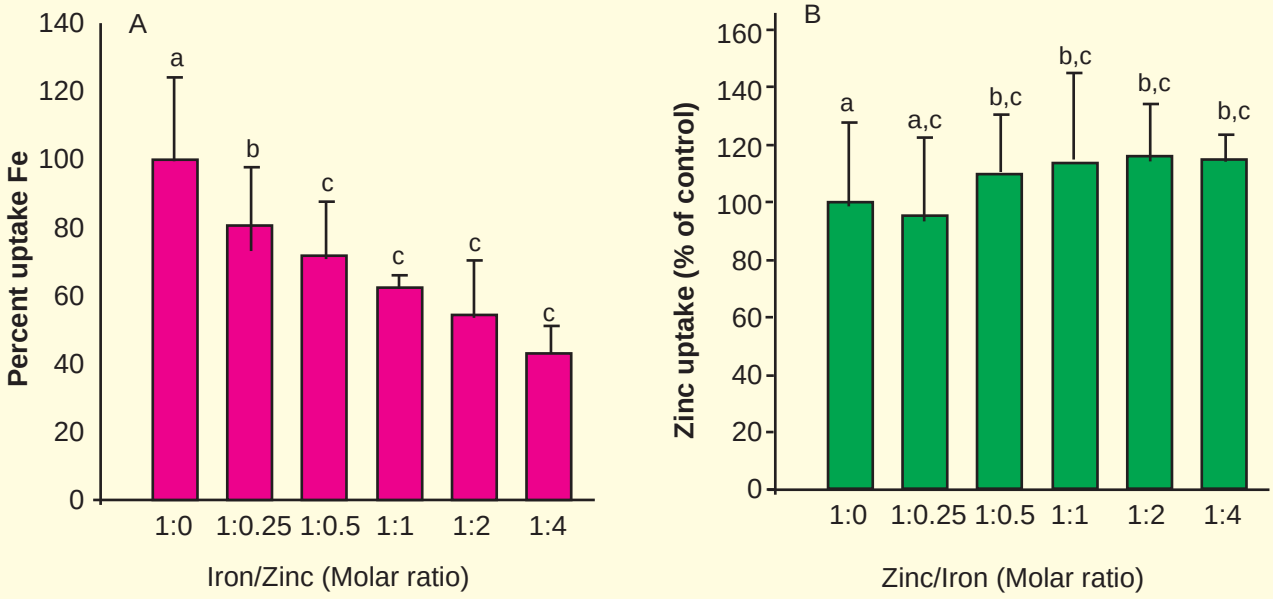
Iron uptake in Caco-2 cells: Dose– dependent uptake data best fitted a two – compartment hyperbolic equation. Resolution of uptake into individual components gave y_0 (nmol/mg protein) values of 0.06 (I) and 2.1 (II) (Fig 9A). A double reciprocal plot of the dose–time uptake data gave a K_m of 2.89 µM and a V_{max} of 156 pmol/min/mg protein for iron uptake (Fig.9B). The obtained y_0 and slope values for the individual components point towards these uptake processes being mediated through transporters.

Zinc uptake in Caco-2 cells: The dose-dependent uptake data fitted a three component model with y_0 (pmol / mg protein) values of 2.88 (I) 6.18(II) and 19.50 (III) (Fig 9C). Double – reciprocal plot of dose – dependent zinc uptake gave a K_m of 42.1 µM and a V_{max} value of 3.09 pmol / mg protein/min (Fig 9D). The obtained y_0 and slope values for the individual components suggest that uptake processes are both mediated (II, III) through transporters with an unmediated component (I).

Iron / zinc interactions during uptake: Zinc dose – dependently and significantly decreased iron uptake at all ratios tested during simultaneous incubation of iron:zinc (Fig 10A). Maximum inhibition of uptake at 42% of control (0.92±0.2 vs. 0.384±0.06 nmol/mg protein; P<0.001) was seen at 1:4 molar ratio. Under similar conditions zinc uptake increased (Fig 10B). This effect of iron is not dose-dependent. Zinc inhibited iron uptake but iron did not inhibit zinc uptake, suggestive of non-competitive inhibition.

Kinetics of zinc inhibition of iron uptake: Zinc inhibited iron uptake only at 50µM and 100 µM concentration and at greater than the ratio of 1:1 (Fig 11A). Iron uptake and at greater than molar ratio of 1:1 was significantly different from the 15 min time point onwards (Fig 11C). Double reciprocal plots of the kinetic data showed

Figure 10. Uptake of ^{59}Fe (A) or ^{65}Zn (B) in the concomitant presence of the other. Bars (mean ±SD) that do not share a common letter are significantly different at P<0.05 by one-way ANOVA and post-hoc't' test



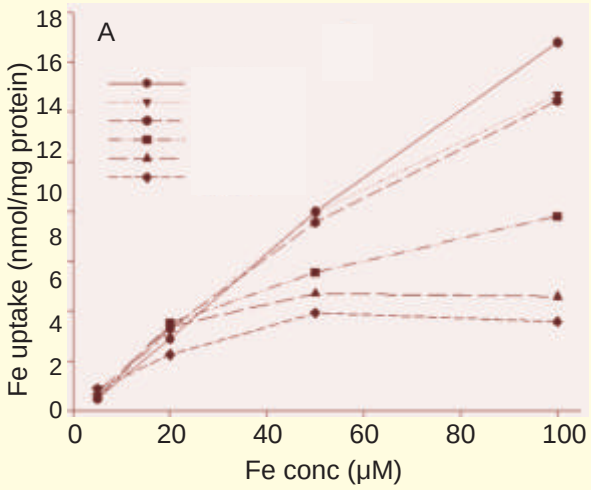
decrease in both K_m and V_{max} values implying mixed inhibition kinetics (Fig 11 B&D). There was a 40% decrease in V_{max} and K_m at 1:1 and above compared to 1:0 Fe:Zn.

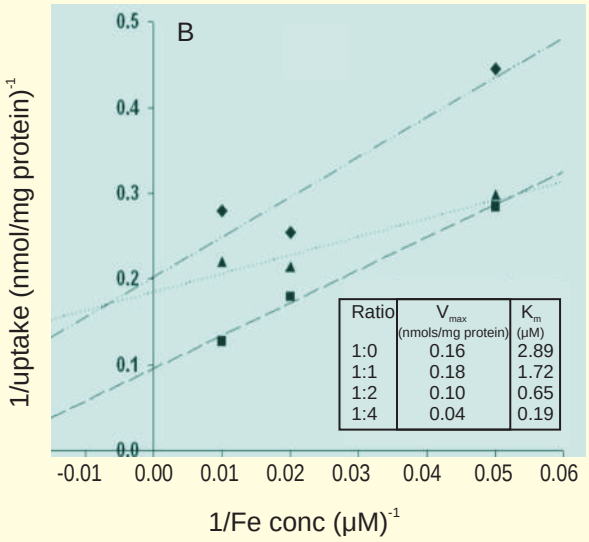
Effect of altered cellular mineral status: Pre-treatment of cells with 50 µM zinc increased iron uptake per se (Fig 12A). It also abrogated the negative interactions between iron and zinc during uptake observed previously (compare Figs 12A and 10A). Iron pre-treatment did not significantly affect zinc uptake or its interactions with iron (Fig12B).

TPEN treatment decreased iron uptake irrespective of the presence or absence of concomitant zinc (Fig 12C). Treatment of cells with TPEN led to increase of zinc uptake (120% of control). These data firmly establish the importance of cellular zinc status on iron uptake and interactions during uptake. On the contrary, iron loading or intracellular chelation using Desferoxamine did not significantly affect zinc uptake or its interactions with iron during uptake.

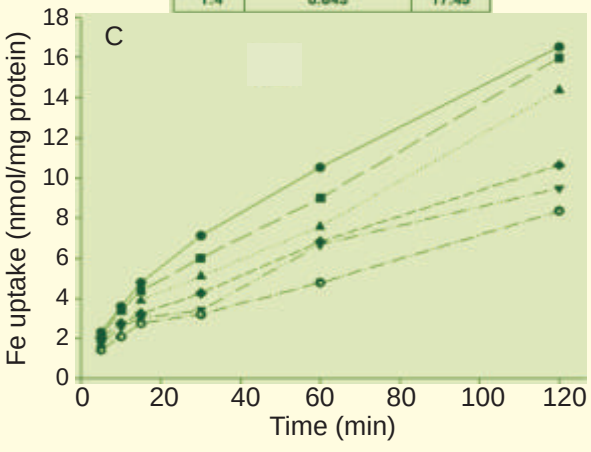
Effect of cellular iron / zinc concentration on iron/ zinc responsive proteins: In order to

Figure 11. Iron uptake as a function of iron concentration (A) and its double reciprocal plot (B) time (C) and its double reciprocal plot (D) in the presence of various molar ratios of zinc. Inset table gives Vmax and Km values





Ratio	Vmax (nmols/mg protein)	Kt (min)
1:0	1.523	27.14
1:0.25	1.333	25.65
1:0.5	1.128	29.15
1:1	0.775	14.23
1:2	0.722	15.66
1:4	0.643	17.45



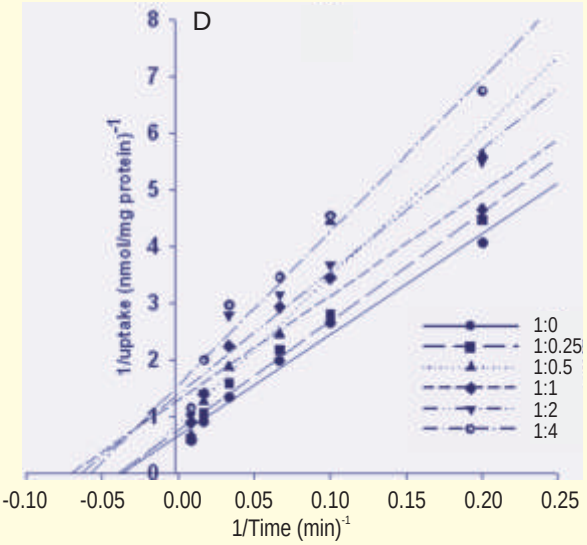
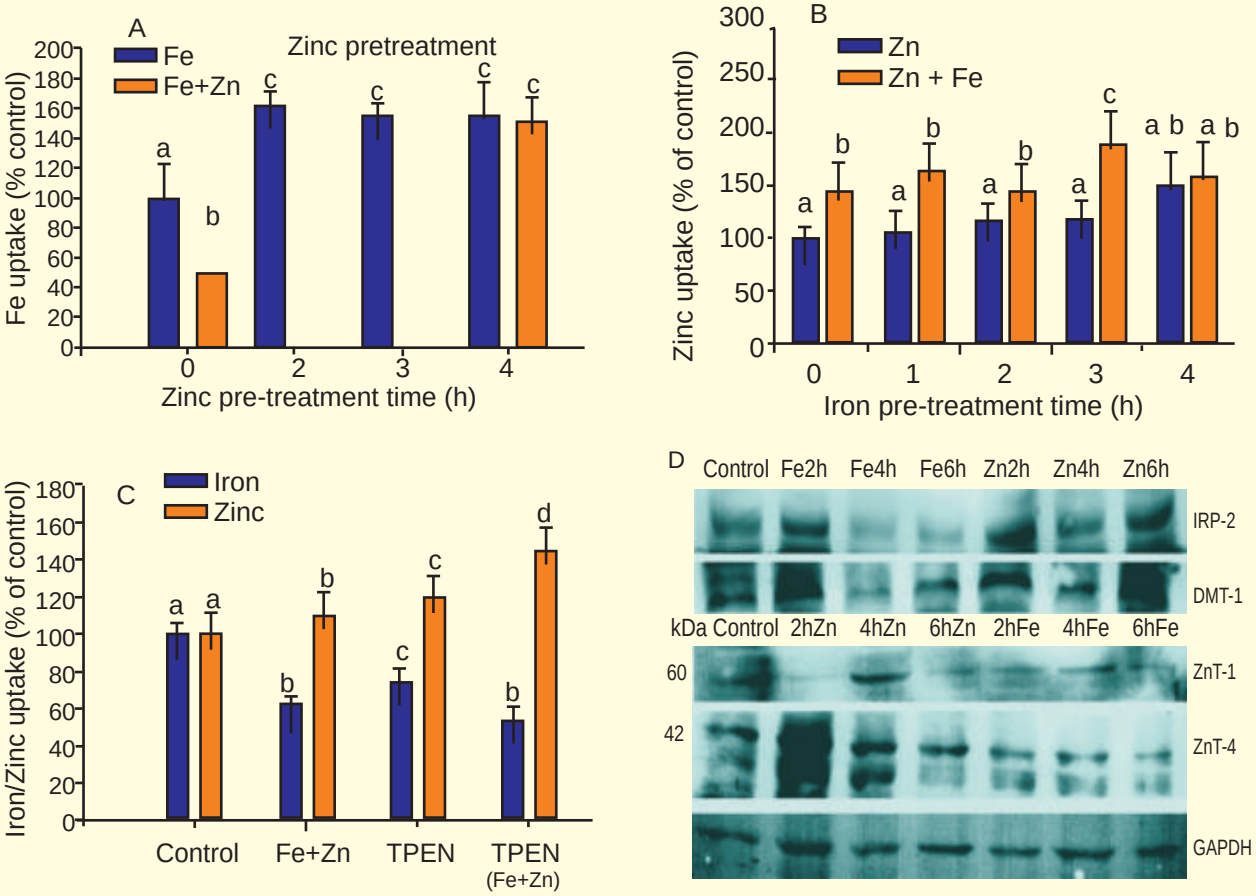


Figure 12. Effect of altered mineral status upon pre-treatment on Iron (A) and / or zinc (B) or together (C) and protein expression (panel D). Bars (mean ± SD) that do not share a common letter are significantly different at P<0.05 by one-way ANOVA and post-hoc't' test



understand the role of known transport and responsive proteins of iron and zinc, cellular mineral status was altered and expression of these analyzed. Iron pre-treatment decreased IRP-2 expression levels but did not alter DMT-1 expression, while zinc increased expression of both. Upon zinc pre-treatment ZnT-1 expression increased till 4h, ZnT-4 expression increased at all time points. Upon iron pretreatment, no significant change was found in expression of ZnT-1 or ZnT-4. Upon TPEN pre-treatment, there was no significant change in DMT-1 expression (Fig. 12D). These changes observed in ZnT-1,-4 may reflect increase in cellular zinc concentrations.

Conclusions

The changes in K_m, V_{max} and y_0 and slope values of individual components of iron and zinc uptake suggest mixed inhibition meaning a single transporter alone may not be involved in the inhibition process, DMT-1, the apical iron transporter. If DMT-1 was transporting both iron and zinc, zinc inhibition of iron uptake should persist with increased DMT-1 expression; but abrogation of negative interactions is observed. Zinc and iron therefore may not be simultaneously transported by DMT-1.

4. METABOLIC PROGRAMMING OF INSULIN RESISTANCE: ROLE OF MATERNAL AND PERI / POSTNATAL CHROMIUM STATUS IN THE OFFSPRING: MUSCLE DEVELOPMENT & FUNCTION

Epidemiological studies have revealed strong relationship between maternal undernutrition, poor fetal growth and subsequent development of metabolic syndrome, glucose intolerance and type 2 diabetes. Micronutrient deficiencies can have profound and persistent effects on foetal tissues and organs. It was shown earlier that maternal micronutrient restriction alters the body composition (muscle, bone and adipose) of the offspring and may predispose them to adult onset diseases *(Annual Report 2006)*. Cr participates in glucose and lipid metabolism, enhances insulin binding to its receptors and amplifies all its known effects including activation of insulin receptor kinase and inhibition of insulin receptor phosphatase.

Although there is no epidemiological evidence for Cr deficiency *per se*, abundant literature demonstrates that Cr supplementation improves growth, longevity and increase lean body mass (LBM) in obese subjects. However, it is not clear whether or not the effect of Cr on LBM is due to its effect on muscle development and / or function. Although Cr picolinate is a widely used supplement for optimal insulin function, there are no reports on the effects of maternal Cr restriction on growth, development and function of muscle or on the capacity of the offspring to secrete insulin to a challenge of glucose. Hence, the present study was undertaken to test the ***hypothesis that maternal Cr restriction alters muscle development and function in the offspring.***

The objectives of the study are:

Assess the effect of maternal and peri / postnatal dietary restriction of chromium on body composition and development of muscle in the offspring in its later life.

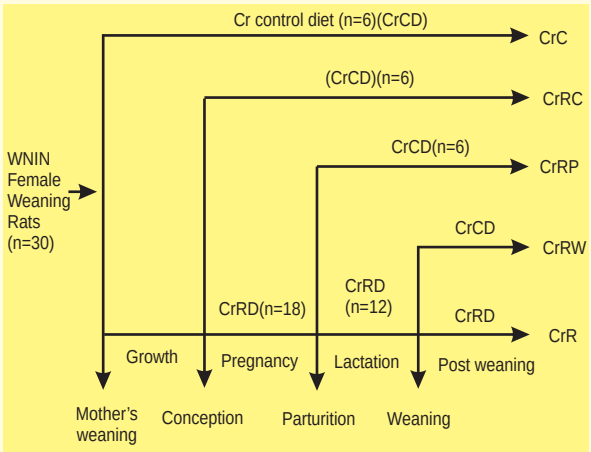
If the body composition is altered in the offspring, study the probable biochemical mechanisms involved.

Assess the effects if any of chromium rehabilitation to restricted mothers and offspring from different time points of initiation and duration on muscle growth / development in the offspring.

Experimental design

Female weanling Wistar NIN (WNIN) rats (n=30) were divided into two groups of 6 and 24. The group of 24 rats was fed for twelve weeks a casein based 18% protein Cr restricted diet containing 0.51mg Cr/kg diet. The other group of 6 rats received the control diet with 1.56 mg Cr per kg diet. The animals Cr status monitored after 12 weeks of feeding the control or restricted diets, their oral glucose tolerance determined and then mated with control males. From conception, six pregnant dams from the restricted group were switched to control diet (CrRC) while the remaining mothers continued on restricted diet throughout pregnancy. At parturition, six mothers from restricted group were supplemented with control diet (CrRP) and the remaining mothers continued on restricted diet till weaning. From day 3 of their life, 8 offspring were maintained with each mother during the course of lactation. At weaning, half the number of restricted offspring were weaned onto control diet (CrRW), while the remaining offspring continued on restricted diet (CrR). The feeding protocol used in this experiment is represented schematically in fig. 13.

Figure 13. Schematic representation of the feeding protocol of different groups of Cr mothers and their offspring



Food intake, body weight gain, plasma and urinary chromium concentrations, parameters related to glucose tolerance, insulin resistance and plasma lipid profile were monitored in WNIN female rats before mating as also their reproductive performance.

In both male and female offspring body composition changes were monitored using TOBEC at 3 monthly intervals. Due to high morbidity / mortality in them beyond this time point, the male offspring were followed up to / sacrificed at 18 months of their life whereas the female offspring were sacrificed at 15 months of age.

Results

In WNIN female rats

At the level of restriction employed in this study, chronic dietary Cr restriction did not affect any of the parameters in the WNIN female rats including their reproductive performance.

In the offspring

As expected, CrR offspring had significantly lower plasma Cr levels ($p < 0.05$) compared to CrC till 18 months of age, while CrRC, CrRP and CrRW offspring caught up with CrC as early as postnatal day 90 and continued so thereafter both in male and female offspring (data not given).

Body weight

Body weight of male CrR offspring was significantly higher than those of CrC at 12 and 18 months of age. Although all three rehabilitation regimes corrected the change at 12 months, only CrRP but not CrRC or CrRW appeared to correct the change at 18 months [Figure 14A]. Body weights of female offspring were comparable among groups till 9 months of age. At one year of age female CrR offspring had significantly [$p < 0.05$] higher body weight than CrC and this continued till 15 months of age [Figure 14B]. Here again, CrRP but not CrRC or CrRW appeared to restore the bodyweights only at 12 months of age but not 15 months [Figure 14B].

Since altered body composition is associated with altered body weight and increased adiposity and decreased muscle mass are known forerunners of insulin resistance. Hence, whether or not the increased body weight of the offspring was associated with altered body composition as determined by TOBEC measurement was verified.

Lean Body Mass [LBM] % and Fat Free Mass [FFM] %

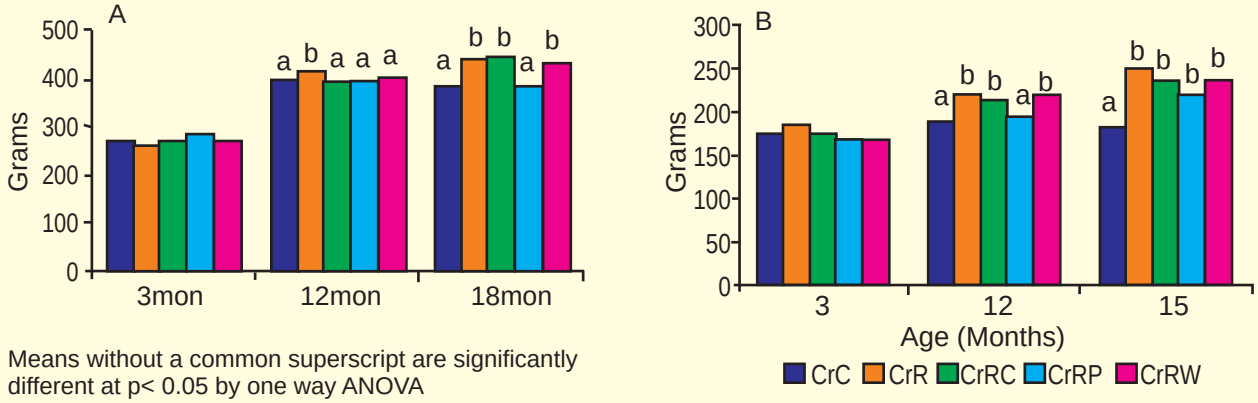
Males:

Percent of lean body mass [LBM%] and fat free mass [FFM%] were significantly lower [$p < 0.05$] in CrR than CrC offspring at 18 months of age but not earlier [Figure 15A & 15B]. It was interesting that CrRP but not CrRW appeared to correct the changes in LBM % and FFM %. However, it was surprising that CrRC did not correct the change.

Females:

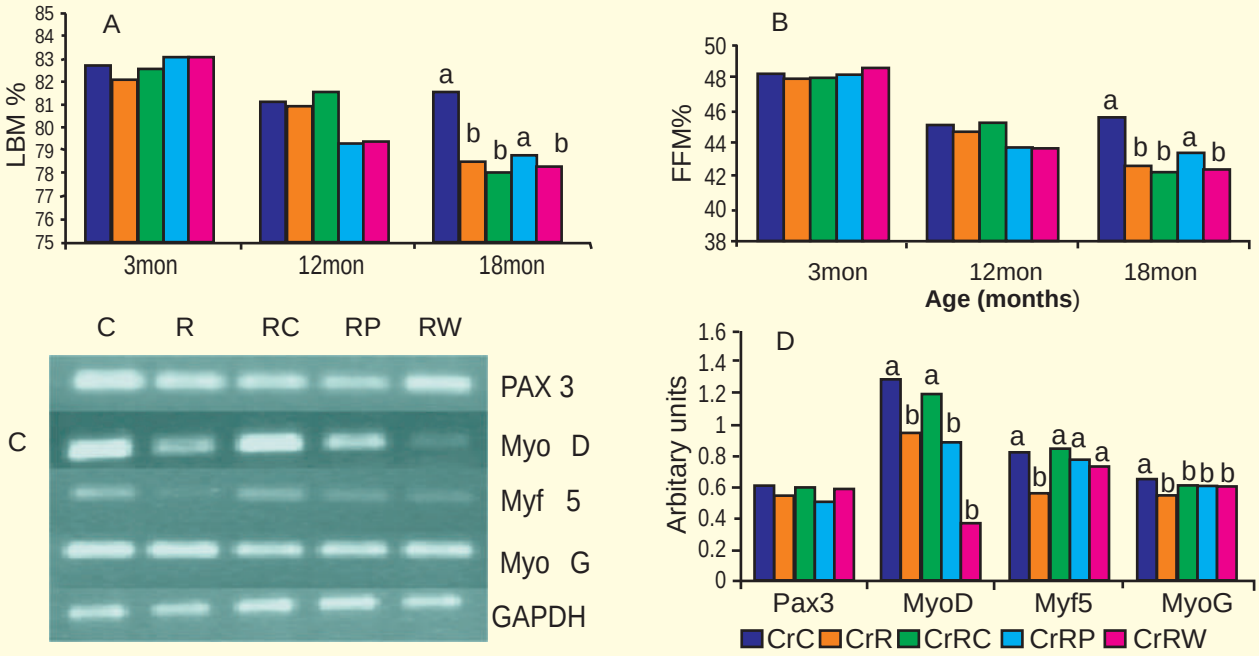
LBM % and FFM % were significantly lower [$p < 0.05$] in CrR than CrC at three months of age and it continued so till 15 months of age. While CrRC corrected these changes at all the time points studied, CrRP and CrRW could not correct the change, specially at 15 months of age [Figure 16A & 16B].

Figure 14. Body weights of male [A] and female [B] offspring of different groups



Means without a common superscript are significantly different at $p < 0.05$ by one way ANOVA

Figure 15. LBM % [A, n=6], FFM % [B, n=6] and myogenic gene expression in the muscle [C, n=3] of male offspring



Values are mean ± SE
Means without a common superscript are significantly different at p< 0.05 by one way ANOVA

Myogenic gene expression in the muscle

Considering that LBM and FFM represent muscle and bone essentially, the expression of genes that regulate myogenesis to determine whether or not impaired muscle development was associated with decreased LBM % and FFM % was next assessed.

Males:

Chronic maternal Cr restriction significantly lowered in the offspring, the expression of MyoD, Myf 5 and MyoG , the genes involved in muscle development but had no effect on the expression of Pax3. All rehabilitation regimes appeared to correct the expression of Myf 5 but not that of MyoG. While CrRC and CrRP partially corrected MyoD expression, CrRW appeared to worsen it [Figure16C].

Females:

Expression of myogenic genes i.e., Pax3, MyoD, Myf5 and MyoG was significantly decreased in CrR compared to CrC and different rehabilitation regimes had varied effects on the expression of different genes. Changes in Pax3 expression were not corrected by rehabilitation in general. CrRC but not CrRP and CrRW mitigated the changes in MyoD expression. On the other hand CrRC and CrRP but not CrRW corrected the changes in the expression of Myf5 & MyoG genes [Figure 16C].

3-O-methyl glucose uptake by muscle [diaphragm]:

Uptake of ^{3}H labeled 3–O–methyl D glucose by the muscle (diaphragm) was determined to assess the alteration if any in the function of the muscle of these offspring.

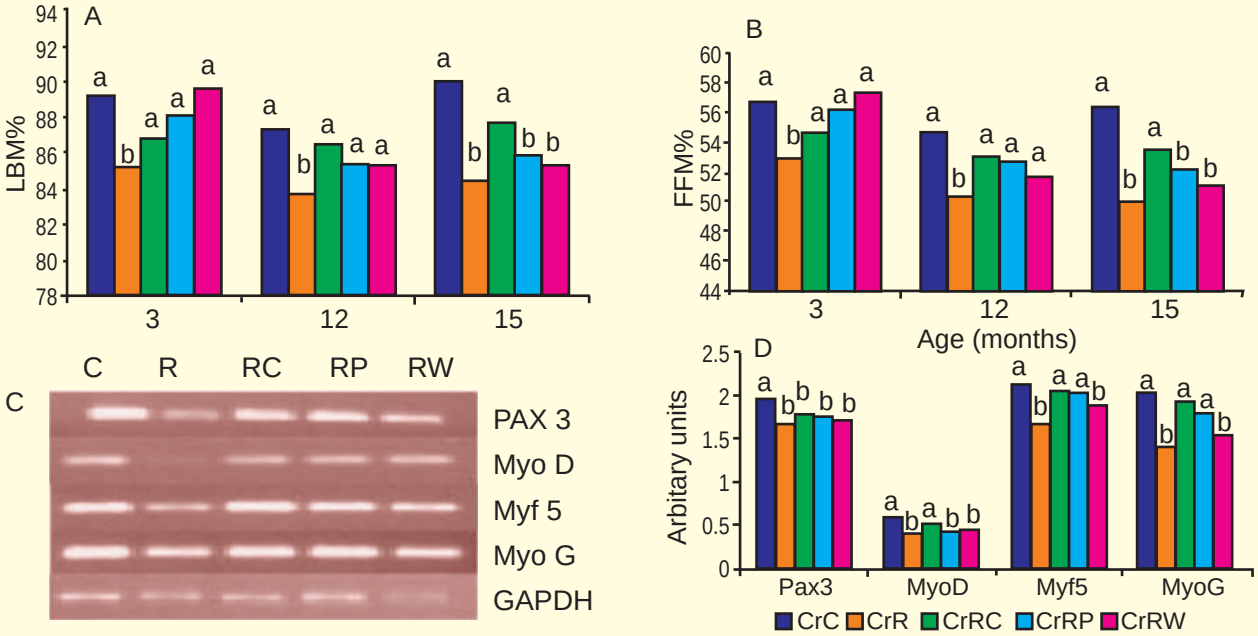
Males:

Basal uptake of 3-O-methyl glucose was significantly higher in CrR than CrC offspring. Interestingly CrRC and CrRP but not CrRW could mitigate this change. In line with higher basal uptake of 3-O-methyl glucose, insulin stimulated 3-O-methyl glucose uptake was also higher in CrR than CrC offspring. However, the fold increase in 3-O-methyl glucose uptake with insulin was comparable among groups [Table 7].

Females:

As in the male offspring, basal as well as insulin stimulated uptake of 3-O-methyl glucose was

Figure 16. LBM % [A, n=6], FFM % [B, n=6] and myogenic gene expression in the muscle [C, n=3] of female offspring



Values are mean ± SE
Means without a common superscript are significantly different at p< 0.05 by one way ANOVA

significantly higher in CrR than CrC offspring. However, unlike in male offspring, none of the rehabilitation regimes appeared to correct the change in basal or insulin stimulated 3-O-methyl glucose uptake.

Interestingly again, the fold increase of 3-O-methyl glucose uptake with insulin was in general comparable among all the five groups [except CrRP] [Table 8].

Conclusion

Chronic maternal Cr restriction had moderate but significant effects on body weight and composition (LBM&FFM), muscle development & its function in the offspring. Although the effects of maternal Cr deficiency appeared earlier in female offspring than males, in both they continued till late in life. Rehabilitation had varied effects on different effects of maternal Cr restriction in the offspring of the two sexes.

Table 7. 3-O-methyl glucose uptake by diaphragm of male offspring

	CrC	CrR	CrRC	CrRP	CrRW
Basal	0.136?0.019[a]	0.296?0.007[b]	0.147?0.016[a]	0.170?0.042[a]	0.280?0.041[b]
Insulin stimulated	0.138?0.018[a]	0.341?0.022[b]	0.151?0.017[a]	0.186?0.038[a]	0.306?0.037[b]
Insulin stimulated/ basal	1.02?0.031	1.14?0.064	1.03?0.015	1.13?0.058	1.10?0.040

Table 8. 3-O-methyl glucose uptake by diaphragm of female offspring

	CrC	CrR	CrRC	CrRP	CrRW
Basal	0.067?0.005[a]	0.254?0.004[b]	0.196?0.003[b]	0.206?0.002[b]	0.202?0.007[b]
Insulin stimulated	0.083?0.004[a]	0.280?0.006[b]	0.223?0.005[b]	0.212?0.005[b]	0.235?0.005[b]
Insulin stimulated / basal	1.25?0.092[a]	1.10?0.025[a]	1.13?0.045[a]	1.02?0.021[b]	1.17?0.069[a]

Units : nmol/hr/gm tissue, Values are mean±SE [n=4]
Means without a common superscript are significantly different at p< 0.05 by one way ANOVA

5. HYPOGLYCEMIC / INSULIN LIKE ACTIVITY IN CAMEL MILK: QUANTIFICATION OF THE EFFECT IN ANIMAL MODELS OF DIABETES / INSULIN RESISTANCE

Aim of the study was to evaluate/ quantify the hypoglycemic / anti-diabetic effect of camel milk using drug/ diet induced rat models of type 1 and type 2 diabetes/ insulin resistance respectively and assess the heat stability / susceptibility of the effect.

Medicinal properties of camel milk are known for centuries and it is believed to be more potent than cow or goat milk. Traditionally, it has been used in the treatment of diabetes, asthma, ulcers, milk allergies etc. The anti-diabetic activity of camel milk has been reported recently in type 1 diabetic subjects and is attributed to the significant amounts of immuno-reactive insulin present in it. The hypoglycemic effect of camel milk in streptozotocin induced hypergly-cemia in rats was reported earlier. However, the scientific evidence available so far, for the anti-diabetic activity of camel milk is scanty and equivocal. Therefore the following studies were conducted in diet and drug induced models of hyperglycemia / insulin resistance in rat models.

This study evaluated the hypoglycemic effect of camel milk using drug (Streptozotocin) and diet (high sucrose) induced rat models for type 1 diabetes / insulin resistance (type 2 diabetes mellitus) respectively.

The heat stability / susceptibility of the effect if any was assessed by subjecting the camel milk to pasteurization and boiling. Appropriate controls were maintained on cow milk subjected to similar treatments.

Methodology and Techniques

Experimental

A. Freeze Drying of camel and cow milk (raw, pasteurized and boiled)

Fifty liters each of camel milk and cow milk were used for this study. While camel milk was supplied frozen by Dr.R.P.Agrawal, S.P. Medical College, Bikaner, Rajasthan, cows milk was purchased from local sources. Nutrient composition of the two milk samples were determined by standard methods and is given in table 1. The two milk samples were divided in to three parts of 17, 17 and 16 liters. While one of the aliquots of each milk (raw) was freeze dried, the other two aliquots were pasteurized (heating at 62^{0} C for 30 minutes) or boiled for 10 minutes, cooled to room temperature and subjected to Freeze drying.

As NIN does not have industrial freeze dryers to freeze dry such large volumes of samples, they were freeze dried in the Industrial freeze driers in the Defense Food research Laboratory (DFRL), Mysore and the Central Food Technological Research Institute (CFTRI), Mysore. The freeze dried powders were stored in vacuum sealed pouches at 4^{0} C till use.

B. WNIN rat models for Diabetes / insulin resistance

Type 1 or type 2 diabetes / insulin resistance were induced in male, weanling WNIN rats by injecting a single intra-peritoneal dose of Streptozotocin (STZ) (40 mg/kg body weight) to rats receiving AIN 93 control diet or by feeding AIN 93 diet containing 65% sucrose respectively. The composition of the two AIN 93 diets used in these studies is given in tables 2 and 3.

Camel / cow milk (raw or pasteurized or boiled) were administered to rats in the form of freeze-dried powders mixed with diet, at a dose of one gram / rat / day, starting from the day they were injected STZ or received high sucrose diet. The quantity of camel / cow milk fed to the rats in this study corresponds approximately to the administration of 500 ml of raw camel milk to type 1 diabetic subjects, which has been reported earlier to decrease their daily requirement of insulin and improve their fasting plasma glucose levels.

I) Streptozotocin induced model for type 1 Diabetes

Male weanling WNIN rats (eighty) were divided in to eight groups of ten each and fed the

control AIN 93 diet as mentioned below for a period of sixty days. Type 1 diabetes was induced in seven groups of these rats (groups 2 to 8) by a single intra-peritoneal injection of Streptozotocin (STZ @ 40mg/Kg body weight) on day one of the experiment and the animals received their respective diets as mentioned below for sixty days from the day STZ was injected.

Group I : Control (AIN 93) diet

Group II : Control diet + STZ

Group III : ,, + camel milk (raw)

Group IV : ,, + camel milk (pasteurized)

Group V : ,, + camel milk (boiled)

Group VI : ,, + cow milk (raw)

Group VII : ,, + cow milk (pasteurized)

Group VIII : ,, + cow milk (boiled)

ii) Diet induced model for type 2 Diabetes/ insulin resistance

Eighty male weanling WNIN rats were divided into eight groups of ten each. While group 1 received the control AIN 93 diet , insulin resistance / type 2 diabetes was induced in seven groups of these rats (groups 2 – 8) by feeding 65 % sucrose containing AIN 93 diet for sixty days from day one of the experiment as mentioned below.

Group I : Control (AIN 93) diet

Group II : High (65 %) sucrose diet

Group III : ,, + camel milk (raw)

Group IV : ,, + camel milk (pasteurized)

Group V : ,, + camel milk (boiled)

Group VI : ,, + cow milk (raw)

Group VII : ,, + cow milk (pasteurized)

Group VIII : ,, + cow milk (boiled)

Ten rats were maintained in each treatment group (to account for any mortality of the animals during the experiment) for two months. Following parameters were determined on day 30 and 60 of feeding, in six animals per treatment group.

C. Hypoglycemic / insulin like effect of camel / cow milk

Insulin Resistance / HOMA IR: The animals were fasted overnight and blood samples drawn by puncturing the orbital sinus. Plasma glucose (glucose oxidase/peroxidase kit, Biosystems, Spain) and insulin (RIA kit for rat insulin from Linco Research, USA) were determined in these samples and the animals insulin resistance status assessed by computing the HOMA IR index and the formula for its computation is given below.

$$\text{HOMA IR} = \frac{\text{Fasting plasma glucose mmol/l} \times \text{Fasting plasma Insulin U/l}}{22.5}$$

Oral Glucose Tolerance test (OGTT): The animals were then administered a glucose solution (40 g / dl) through an oral gavage @ 250 mg / Kg body weight and blood samples were drawn at 30, 60 and 120 minutes after the glucose load. Plasma glucose and insulin levels were determined in all the blood samples as mentioned above and the area under the curve (AUC) for glucose and insulin during the OGTT computed using the Trapezoid formula. While insulin AUC was used to assess the animals' capacity for insulin secretion to an acute challenge of glucose load, the ratio of glucose AUC / insulin AUC during the OGTT was used as another measure of assessing insulin resistance in the animals.

Heat sensitivity / stability of the hypoglycemic effect: The heat sensitivity/ stability of the hypoglycemic effect was assessed by determining the above parameters in STZ treated / high sucrose diet fed rats which received camel/ cow milk (pasteurized / boiled) along with their diet.

Plasma lipid profile: The effects, if any of STZ treatment/ high sucrose feeding on the rats' plasma lipid profile and its modulation by feeding camel/ cow milk was assessed by determining the levels of triglycerides (Glycerol phosphate oxidase/ peroxidase kit by BioSystems S.A., Barcelona, Spain), non esterified fatty acids (kit supplied by RANDOX Laboratories, Antrim, UK), total cholesterol and HDL – cholesterol (kits supplied by BioSystems

S.A., Barcelona, Spain), in the fasting blood plasma.

Salient findings:

- ❖The two milk samples were comparable in general excepting that cow milk had a slightly higher amount of protein and carbohydrate than the camel milk.
- ❖It was observed in general that at 30 days of feeding, neither camel milk nor cow milk (raw, pasteurized or boiled) had any significant effect on the parameters studied.

A. Streptozotocin induced / type 1 diabetes model

As expected Streptozotocin (STZ) injection (group 2 compared to controls i.e., group 1) resulted in

- ❖ Significant decrease in the body weight of the rats at the end of 30 days of feeding and it persisted till 60 days .
- ❖ Significant increase in fasting plasma glucose and glucose AUC during the oral glucose tolerance test (OGTT)
- ❖ Significant decrease in fasting plasma insulin and insulin AUC during OGTT.
- ❖ HOMA IR and the ratio of glucose AUC / insulin AUC during OGTT were higher
- ❖ However, it had no significant effect on the plasma lipid profile of the rats

At the dose used in this study in the WNIN rats, feeding camel milk (raw, pasteurized or boiled : groups 3 – 5) for two months neither alleviated nor aggravated the changes caused by the STZ in the glucose metabolism parameters mentioned above compared to group 2 (STZ treated controls).

- ❖ Feeding cow milk (raw, pasteurized or boiled : groups 6 – 8) also had no significant effect in general on any of the parameters of glucose metabolism compared to group 2, (STZ treated controls) but for the stray instance of increased fasting plasma insulin levels in the STZ treated rats fed pasteurized cow milk (group 7).
- ❖ Feeding neither camel milk nor cow milk (raw, pasteurized or boiled : groups 3 – 8) for two months in general had any significant effect on the plasma lipid profile or free fatty acid levels. However, feeding pasteurized cow milk to STZ treated rats (group 7) caused a significant increase in total cholesterol and free fatty acids in plasma compared to STZ treated controls.

B. High sucrose feeding / Insulin resistance / type 2 diabetes model

Feeding 65% sucrose in the diet for two months (group 2 compared to controls i.e., group 1) had no significant effect on the body weights of WNIN male rats.

Compared to Controls (Group 1) Feeding high Sucrose diet resulted in

i. Increased fasting plasma glucose levels significantly

ii. However, the increase observed in fasting plasma insulin and the computed HOMA IR values were not significant.

iii. Increased plasma total cholesterol, HDL cholesterol, triglycerides and free fatty acids, but the differences were not statistically significant.

Compared to high sucrose diet alone fed rats (group 2)

I. Feeding raw camel milk to rats receiving high sucrose diet (group 3) significantly decreased fasting plasma glucose levels.

ii. Feeding boiled camel milk (group 5) did not affect this hypoglycemic effect whereas pasteurizing (group 4) affected it partially, appears to suggest the relative heat stability of the effect.

iii. None of the three camel milk regimes (groups 3 – 5) affected the fasting plasma insulin levels.

iv. However, rats fed cow milk (raw, pasteurized and boiled : groups 6 – 8) also showed similar hypoglycemic effects at two months of feeding.

v. In general glucose AUC during OGTT was decreased and insulin AUC increased (except group 5 : boiled camel milk) in high sucrose fed rats receiving camel milk (raw, pasteurized or boiled : groups 3 – 5) but the differences were not statistically significant.

vi. Almost similar changes were observed in the glucose tolerance parameters of high sucrose diet fed rats given cow milk (raw, pasteurized or boiled : groups 6 – 8).

vii. Feeding camel milk or cow milk (raw, pasteurized or boiled : groups 3 – 8) to high sucrose diet fed rats for two months decreased total and HDL cholesterol levels. While the changes in total cholesterol levels were significant in all the groups, that in HDL cholesterol was significant in only the rats fed pasteurized camel milk (group 4) or raw cow milk (group 6). On the other hand changes caused in the levels of plasma triglycerides and free fatty acids by feeding camel or cow milk were in general not significant statistically.

Conclusions

1. At the dosage level tested in this study, camel milk or cow milk (raw, pasteurized or boiled) has no hypoglycemic effect in the streptozotocin induced model of hyperglycemia / type 1 diabetes in WNIN rats .
2. However, they have a comparable hypoglycemic effect in high sucrose diet induced model of hyperglycemia / type 2 diabetes in WNIN rats and the effect is heat stable in general in both the milks. Further, the hypoglycemic effect of both these milk samples, appears not to be due to their ability to modulate basal levels of plasma insulin or the secretion to a challenge of oral glucose load.

From the results it appears that at the dosage level employed in these studies, the hypoglycemic effect of camel milk does not seem to be greatly different from that of cow milk in both the models of hyperglycemia (ie, drug or diet induced).

6. GENERATION OF DATABASE ON HEALTH BENEFICIAL EFFECTS OF PLANT FOODS COMMONLY CONSUMED IN INDIA: ROOTS, TUBERS AND OTHER VEGETABLES

Epidemiological evidence demonstrates that diets rich in fruits and vegetables promote health and reduce the risk of degenerative diseases. Plant foods are rich sources of phenolic compounds with potent antioxidant activity, which are responsible for health benefits. Recently, much work is focused on antioxidant properties of natural sources. This study attempts to generate a data base on the phenolic content and antioxidant activity of plant foods commonly consumed in India. So far data was generated on the antioxidant activities of green leafy vegetables, fresh and dry fruits, cereals, millets, pulses and legumes (Annual report 2005-2007). During this year, studies were taken up to determine the antioxidant activity of roots, tubers and other vegetables.

Materials & Methods

Commonly consumed (based on the NNMB survey) varieties of roots, tubers and vegetables were collected from four different local markets of the twin cities. Standard extraction and estimation protocols described earlier were adopted (Annual reports 2005-2007). While the total phenolic content (TPC) was determined by the Folin's method, the anti-oxidant activity (AOA) was determined by two different methods. 1. FRAP (Ferric Reducing antioxidant power) 2. DPPH Radical Scavenging. Mean phenolic content and AOA by DPPH, FRAP of the different foods analysed along with their botanical names is given in table 9 and table10 and the salient findings are :

Results

a) Roots and Tubers (Table 9)

1. Phenolic content of roots and tubers ranged from 22.21 to 169.41mg/100g on fresh weight basis. Beet root had the highest (169.41) total phenolic content, followed by tapioca (137.55 mg/100g) while carrot had the least (22.21 mg/100g).
2. DPPH radical scavenging activity ranged from 0.11-1.25 mg trolox equivalent/g with the highest activity being seen in Beet root (1.25) and the least in carrot (0.11 mg Trolox equivalents / g).

Tables 9. Antioxidant content of roots and tubers

Name of the food	Botanical name	Phenolic content mg/100g	DPPH scavenging activity (trolox equivalent mg/g)	FRAP (µmol/g)
Beet root (red)	*Beta vulgaris*	169.41 ± 40.19	1.25 ± 1.34	226.91 ± 32.96
Carrot	*Daucus carota*	22.21 ± 5.51	0.11 ± 0.03	9.22 ± 2.33
Colacasia	*Colacasia antiquorum*	81.59 ± 21.03	0.71 ± 0.17	120.59 ± 15.10
Onions (big)	*Allium cepa*	64.16 ± 3.69	0.23 ± 0.02	51.69 ± 12.39
Spring onions	*Allium cepa*	73.55 ± 8.68	0.12 ± 0.03	25.86 ± 3.35
Potato	*Solanum tuberosum*	38.42 ± 0.62	0.16 ± 0.08	25.35 ± 3.69
Radish (white)	*Raphanus sativus*	66.73 ± 18.46	0.29 ± 0.05	46.56 ± 10.04
Sweet Potato	*Ipomoes batatas*	53.70 ± 3.44	0.25 ± 0.04	15.20 ± 11.33
Tapioca	*Manihot esculenta*	137.55 ± 6.04	0.51 ± 0.07	109.00 ± 2.62
Yam (ordinary)	*Typhonium trilobatum*	54.92 ± 8.15	0.74 ± 0.10	104.01 ± 11.15
	Range	22.21-169.4	0.11-1.25	9.22-226.91

Values are Mean ± SD

Correlation	r	r^2%
TPC Vs DPPH	0.76	57.52
TPC Vs FRAP	0.86	73.26
DPPH Vs FRAP	0.97	94.87

3. FRAP activity showed a broad range from 9.22 - 226.91µmoles/g. Here again the highest and least activities were seen in Beet root and carrot respectively.

Tables 10. Antioxidant content of Vegetables

Name of the vegetable	Botanical name	Phenolic content mg/100g	DPPH scavenging activity (trolox equivalent mg/g)	FRAP (µmol/g)
Beans	*Phaseolus coccineus*	129.41 ± 14.93	0.83 ± 0.11	37.33 ± 6.97
Bitter gourd	*Momordica charantia*	139.67 ± 12.20	0.18 ± 0.02	24.99 ± 3.07
Bottle gourd	*Lagenaria vulgaris*	50.64 ± 6.72	0.36 ± 0.03	37.41 ± 6.22
Broad beans	*Vicia faba*	188.09 ± 9.40	3.33 ± 1.40	82.16 ± 9.36
Brinjal	*Solanum melongena*	123.77 ± 10.62	1.50 ± 0.13	75.53 ± 8.74
Cababge (Green)	*Brossica oleracea var. capitata*	85.58 ± 9.18	0.78 ± 0.21	44.82 ± 8.07
Cababge (Red)	*Brossica oleracea var. capitata*	339.00 ± 19.51	4.05 ± 0.68	378.08 ± 51.25
Capsicum	*Capsicum annuum var. grossa*	82.30 ± 3.31	0.96 ± 0.17	24.66 ± 4.22
Cauliflower	*Brassica oleracea, var. botrytis*	94.84 ± 4.34	0.66 ± 0.00	48.44 ± 11.10

(Contd.)

Tables 10. Antioxidant content of Vegetables (Contd.)

Name of the vegetable	Botanical name	Phenolic content mg/100g	DPPH scavenging activity (trolox equivalent mg/g)	FRAP (µmol/g)
Cluster beans	*Cyamopsis tetragonoloba*	97.92 ± 14.11	1.02 ± 0.15	41.38 ± 5.75
Cucumber	*Cucumis sativus*	31.46 ± 7.17	0.63 ± 0.13	75.15 ± 17.46
Drumstick	*Moringa oleifera*	88.76 ± 9.34	0.52 ± 0.16	17.30 ± 3.85
Kovai	*Coccinia cordifolia*	50.39 ± 9.77	0.78 ± 0.06	24.37 ± 5.65
Ladies finger	*Abelmoschus esculentus*	167.70 ± 39.63	4.66 ± 0.65	107.96 ± 4.69
Mango raw (green)	*Mangifera indica*	130.10 ± 12.30	2.76 ± 0.45	166.92 ± 14.27
Plantain, green	*Musa sapientum*	30.63 ± 1.57	0.34 ± 0.25	25.84 ± 11.88
Pumpkin	*Cucurbita maxima*	46.43 ± 12.95	0.38 ± 0.07	8.77 ± 1.72
Ridge gourd	*Luffa acutangula*	27.04 ± 6.12	0.12 ± 0.03	8.88 ± 1.37
Snake gourd	*Trichosanthes anguina*	29.60 ± 1.60	0.38 ± 0.06	13.54 ± 4.46
	RANGE	27-339	0.12 - 4.66	8.7 ± 378

Values are Mean ± SD

Correlation	r	r^2%
TPC Vs DPPH	0.78	61.89
TPC Vs FRAP	0.85	72.32
DPPH Vs FRAP	0.75	56.89

4. A significant correlation was observed between TPC and AOA, with the r values being 0.76 and 0.86 respectively with FRAP and DPPH scavenging.

b. Vegetables (Table 10)

1. Phenolic content of vegetables showed a wide range 27-339 mg/100g. Out of 19 commonly consumed vegetables studied, Cabbage (red) had the highest total phenolic content (339.00) followed by Broad beans (188.09). The lowest total phenolic content (TPC) was seen in ridge gourd (27.04 mg/100g).
2. DPPH scavenging activity ranged from 0.12 - 4.66 mg trolox equivalent / g. Ladies finger had shown highest DPPH activity 4.66 followed by red cabbage 4.05 and the lowest was in ridge gourd 0.12 mg/g.
3. FRAP activity in vegetables showed a wide range 8.7 - 378 µmoles/g. The highest FRAP activity was seen in red cabbage (378) followed by raw mangoes (166.9) and the lowest was in pumpkin (8.77 moles/g).
4. Significant correlation was observed between TPC and AOA with r values being 0.78 and 0.85 respectively with DPPH and FRAP.
5. In general, correlation between TPC and AOA by different methods was comparable among vegetables, roots and tubers, and these findings are in agreement with literature .

7. ERYTHROCYTE ALDOSE REDUCTASE ACTIVITY AND SORBITOL LEVELS IN DIABETIC RETINOPATHY

Prolonged exposure to chronic hyperglycemia, without proper management, can lead to various short-term & long-term secondary complications, both of macro and microvascular nature, which represent the main cause of morbidity and mortality in diabetic patients. Diabetic retinopathy (DR), a vascular disorder affecting the microvasculature of the retina, is a leading cause of adult blindness and is the most common complication of diabetes. Based on observational study, the prevalence of DR in India was reported to be about 10% amongst diabetic subjects and a population-based study reported about 17% DR in urban India. In a clinical study the prevalence was 34% among type-2 diabetic patients. Although, the pathogenesis is not known, many biochemical pathways associated with hyperglycemia, among these, polyol pathway received maximum attention. Aldose reductase (ALR2; EC:1.1.1.21), the first and rate-limiting enzyme in the pathway, reduces glucose to sorbitol. Studies on animal models of diabetes and galactose feeding suggest increased polyol pathway activity in the pathogenesis of DR. Several studies based on specific inhibitors of ALR2, support the involvement of polyol pathway in the pathology. Retinal capillary pericytes contain ALR2 and the accumulation of sorbitol in pericytes has been linked to their degeneration and selective death. Pericyte loss, the major event of early diabetic retinopathy, also occurs in diabetic and galactose fed dogs that develop retinopathy. Furthermore, evidence for the involvement of ALR2 as risk factor for DR and other diabetic complications comes from polymorphism studies. Although a majority of the animal studies with ALR2 inhibitors (ARI) have yielded encouraging results, on the whole, clinical trials of ARI have failed to show efficacy against various diabetic complications.

In principle, all diabetic patients might be expected to develop diabetic microvascular complications if activation of polyol pathway were the triggering factor. Hence, correlating total ALR2 activity with DR prevalence may provide an important link between an easily measurable marker in peripheral blood and risk of progression toward eye disease. In this study, we examined the activity of ALR2 in erythrocytes obtained from diabetic patients with and without retinopathy. Further, we also measured the levels of sorbitol as a surrogate marker for ALR2 activity levels in erythrocytes.

Methods

Design: A hospital based case-control study was conducted. The study protocols are approved by the Institutional Ethics Committees of Sarojini Devi Eye Hospitals and Institute of Ophthalmology, Hyderabad. Subjects were recruited from the patients who visited the Sarojini Devi Eye Hospitals and Institute of Ophthalmology, Hyderabad. A total of 356 type 2 diabetic subjects (198 with diabetic retinopathy, 164 diabetic subjects without any complications) and 66 normal subjects were investigated. The written consent of the subjects has been obtained after explaining the study details. Full history of the subject with respect to age, sex, clinical symptoms, duration & type of diabetes, medication and socioeconomic back ground with the help of a well-designed questionnaire was collected. The fundus of each subject was evaluated by both direct and indirect ophthalmoscopy. The presence of retinal micro aneurysm, dot and slot hemorrhages, intraretinal micro vascular abnormalities and cotton wool spots was defined as non-proliferative DR (NPDR), which is again categorized as mild, moderate, severe and diabetic maculopathy. Formation of new vessels with and without bleeding and production of hemorrhage into vitreous is defined as proliferative DR (PDR) and classified either grades as neovascularisation elsewhere (NVE) and those on the optic disc are called neovascularisation of the disc (NVD).

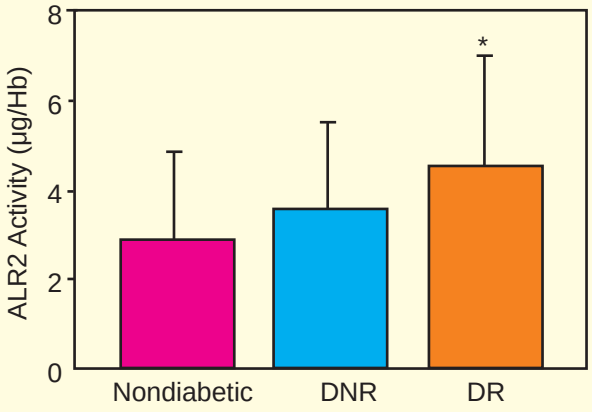
Methods: Blood was drawn and RBC and plasma were separated by centrifugation and stored at –85°C until further analysis. ALR2 activity and sorbitol levels (by spectrophotometric method) along with random glucose and glycosylated hemoglobin (HbA1C, by kit) levels in erythrocytes were determined. Glucose was estimated in plasma by GOD-POD method using a kit. The data

were expressed as mean standard deviation (S. D.). Mean values were compared by one-way ANOVA with post hoc tests of least significant difference (LSD) method. Correlations were calculated to study relationship of ALR2 and sorbitol with other variables. P values were also calculated for AR in these groups.

Results

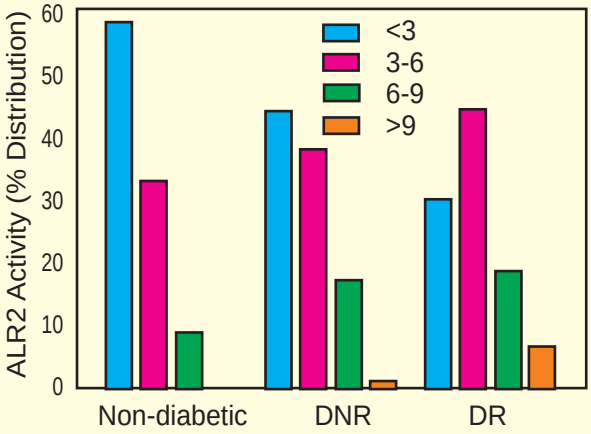
1. Erythrocyte ALR2 activity in diabetic group was not significantly different from control (P>0.05). Interestingly, ALR2 activity in DR group was significantly higher not only from control group but also from diabetic group (P<0.05) (Figure17).

Figure 17. Erythrocyte aldose reductase activity. Data represent mean±SD of ALR2 activity in nondiabetic control (n=66) and diabetic patients without (DNR; n=164) and with retinopathy (DR; n=182).*Designates statistical significance (p<0.05) in comparison to the other groups



2. However, there was considerable overlap of ALR2 activity between the groups. Therefore, the data was examined after distribution of individuals into one of four sub groups defined according to level of ALR2 activity (Figure 18). A majority of subjects in the control group have <3.0 units (58 %), about 33% have 3-6 units and only 9% have activity in the category of 6-9 units. Approximately 43% of the subjects in diabetic group have <3.0 units, 35% have 3-6 units and about 18% have ALR2 activity in the category 6-9 units. Whereas a majority of the DR subjects (46%) have 3-6 units of ALR2 activity, a substantial proportion (20%) has 6-9 units activity and those with >9.0 units of ALR2 activity are found predominantly in this group (6%). These results suggest that prevalence of DR is associated with higher ALR2 activity.

Figure 18. Percentage distribution of aldose reductase activity levels. ALR2 activity is distributed into <3, 3-6, 6-9 and >9 units/g Hb subgroups in nondiabetic control and diabetic patients without (DNR) and with retinopathy (DR). Data represent mean of each subgroup in nondiabetic control and DNR and DR groups



3. Levels of sorbitol, product of ALR2 reaction, were determined in a sub set of subjects in all three groups. Though, the levels of sorbitol were significantly high in diabetic group compared to control group (P<0.05), sorbitol levels were further high in DR group as compared to diabetic as well as control group (P<0.05) (Figure 19).

Figure 19. Erythrocyte sorbitol levels. Data are mean±SD in nondiabetic control (n=24) and diabetic patients without (DNR; n=44) and with retinopathy (DR; n=52). *Designates statistical significance (p<0.05) in comparison to the other groups

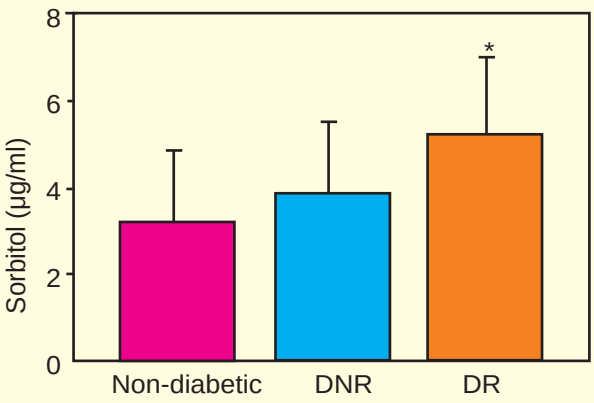
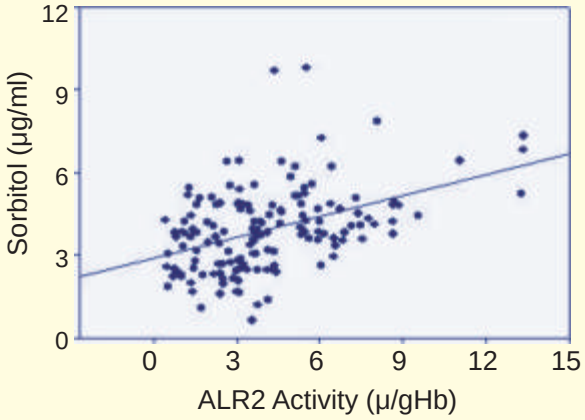


Figure 20. **Correlation between erythrocyte sorbitol levels and ALR2 activity.** Correlation (r=0.188) between erythrocyte sorbitol levels and ALR2 activity in control and diabetic patients without (DNR) and with retinopathy (DR) was found to be significant at p<0.05. Correlation was done for those samples in which sorbitol was determined.



4. Neither ALR2 nor sorbitol levels were correlated with age, glucose, duration of diabetes and HbA1C levels in all the three groups i.e. control, diabetic and DR group. However, ALR2 activity is correlated with sorbitol levels (r = 0.188; p<0.05) (Figure 20).

Conclusions

The results demonstrated that the activity of ALR2 is significantly higher in DR patients as compared to diabetic patients and suggests that ALR2 activity might serve as in independent risk identification factor for DR, irrespective of duration of diabetes and age of the patient. Based on these results it may be hypothesized that activation of polyol pathway with ALR2 above certain threshold level could predispose the diabetic patients to retinopathy. Thus, ALR2 activity and sorbitol levels either separately or together may serve as a diagnostic/ prognostic marker(s) for diabetic complications.

8. EFFECT OF TURMERIC AND CURCUMIN ON OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES IN STREPTOZOTOCIN-INDUCED DIABETIC RAT TISSUES

There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals by means of glucose autoxidation, non-enzymatic glycation, advanced glycosylation end products (AGE), and enhanced glucose flux through the polyol pathway. Elevated generation of free radicals resulting in the decrease of antioxidant defense components may lead to oxidative stress in various tissues. Under physiological conditions, a variety of antioxidants protect the body against the adverse effects of free radicals. Diabetes-induced oxidative stress has been found to affect different parts of the body directly or indirectly leading to various complications. Dietary intervention, particularly the use of traditional foods and medicines derived from natural sources, is the mainstay in the management of diabetes. In this context, there has been a growing interest in recent times in identifying as many dietary sources (including spices) as possible for their ability to control diabetes and its complications. Curcumin [1, 7-bis (4 - hydroxy - 3 - methoxyphenyl) - 1,6. heptadiene-3, 5-dione] is a yellow phenolic compound present in turmeric (*Curcuma longa*), a widely used spice in Indian cuisine. Curcumin has a number of biological applications along with a significant antioxidant activity.

Objective: The objective of the present study is to evaluate protective effect of long-term feeding of curcumin and its dietary source (turmeric) on STZ-induced oxidative stress in rats.

Methodology

Design: Three-month-old WNIN rats were made diabetic by injecting STZ (35-mg/kg body weight) dissolved in 0.1 M sodium citrate buffer, pH 4.5. The control group (Group I; n=8) rats received the vehicle alone. After 72 hr, fasting blood glucose levels were monitored and animals with blood glucose levels 145 mg/dL were considered as diabetic and were distributed into four groups (Groups II–V). Animals in these groups received either only the AIN-93 diet (Group II; *n =8*) or received the AIN - 93 diet containing 0.002%

(Group III; *n*= 8) or 0.01% (Group IV; *n*= 8) curcumin or 0.5% turmeric (Group V; *n*= 8) for a period of 8 weeks.

Methods: At the end of 8 weeks of treatment, the fasted rats were euthanized by CO_2 inhalation. The specimens like liver, kidney, pancreas, and heart were dissected out and stored at -70°C until further analysis. Levels of malondialdehyde as thiobarbutyric acid reactive substances (TBARS), protein carbonyls were measured in RBC and tissue samples by standard methods. The antioxidant enzymes in RBC and tissues were assayed by the spectrophotometric methods.

Statistical analysis: Data were analyzed with SPSS windows version, 14.0 software. Results were expressed as mean ± SD for six animals in each group. Group means were compared with one way analysis of variance (ANOVA) followed by least significance difference (LSD) method for all parameters. Non-parametric test was also performed when the homogeneity of variance was violated. P values of less than 0.05 were considered to indicate statistical significance.

Results

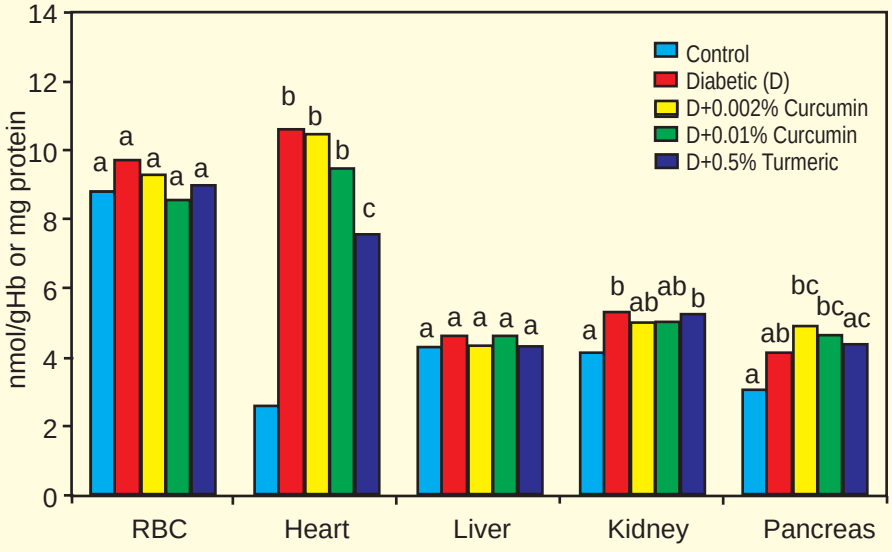
TBARS and protein carbonyls: There was a significant increase of TBARS in RBC, liver, kidney, heart and pancreas of diabetic rats compared to control rats. Interestingly, curcumin and turmeric feeding significantly inhibited formation of TBARS in these tissues (Table 11). Although, protein carbonyls content was increased in all organs of diabetic rats compared to control rats, the increase

Table 11. Effect of turmeric and curcumin on TBARS levels in streptozotocin-induced diabetic rats

Tissues	Groups				
	Control	Diabetic (D)	D+0.002% Curcumin	D+0.01% Curcumin	D + 0.5% Turmeric
RBC	6.08?1.07 [a]	10.09?2.40 [b]	8.23?1.89 [bc]	6.42?1.12 [ac]	7.29?1.95 [ac]
Heart	234?11 [a]	290?10 [b]	271?15 [c]	256?15 [c]	271?17 [c]
Liver	430?71 [a]	576?67 [b]	435?27 [a]	418?30 [a]	475?64 [a]
Kidney	878?31 [a]	1165?65 [b]	1106?11 [b]	774?88 [c]	786?52 [c]
Pancreas	59.22?13.2 [a]	89.85?19.3 [b]	56.79?13.4 [a]	56.10?22.5 [a]	36.63?5 [a]

Values are mean ± SD (n=6).
Variations in superscripts indicate significance (P<0.05) of mean differences between groups for a given tissue., TBARS were expressed as nmols/g Hb in RBC and nmol/g tissue, in tissues.

Figure 21. Effect of turmeric and curcumin on protein carbonyls levels in streptozotocin-induced diabetic rat tissues



Values are mean ± SD (n=6). Variations in superscripts indicate significance (P<0.05) of mean differences between groups for a given tissue. Protein carbonyls were expressed as nmols/g Hb in RBC and nmol/mg protein, in tissues.

was found to be statistically significant only in heart and kidney (Figure 21). Formation of protein carbonyls in heart was inhibited partially, but not completely upon feeding of curcumin and turmeric to the diabetic animals (Figure 21). These results indicate that while curcumin and turmeric are efficient in preventing increased lipid peroxidation and marginally effective against protein oxidation under hyperglycemic conditions.

Antioxidant enzymes: The activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), gluthione peroxidase (GPx) and gluthione-S-transferase (GST) in RBC, liver, heart, pancreas and kidney of control and experimental rats have been summarized in Tables 12-14. SOD activity was significantly lower in RBC but higher in heart and pancreas of diabetic animals compared to controls (Table 12). Interestingly, feeding of curcumin and turmeric to the diabetic animals prevented these changes in RBC and heart, particularly at higher level of curcumin and turmeric (Table 12). Though, curcumin and turmeric were able to bring down the SOD activity in pancreas, the effect was partial (Table 12) CAT activity was decreased in RBC and kidney, but increased in heart and pancreas of diabetic animals compared to controls (Table 13). While feeding of curcumin and turmeric improved CAT activity in kidney, the activities in heart and pancreas were not reversed (Table 13). GPx was activity increased significantly in liver, kidney and pancreas of diabetic animals compared to control (Table14). Curcumin and

Table 12. Effect of turmeric and curcumin on superoxide dismutase activity in streptozotocin-induced diabetic rats

Tissues	Control	Diabetic (D)	D+0.002% Curcumin	D+0.01% Curcumin	D+0.5% Turmeric
RBC	3338?272 ^{a}	2403?386^{b}	2902?795 ^{ab}	3298?997^{b}	3565?1503^{b}
Heart	4.68?0.85 ^{a}	6.74?0.51^{b}	6.17?0.63^{bc}	6.38?0.42^{bc}	5.67?1.06^{c}
Liver	22.01?0.99 ^{a}	22.88?1.66 ^{a}	21.78?2.75 ^{ab}	20.55?2.41 ^{b}	22.10?0.76 ^{ab}
Kidney	23.72?3.41 ^{a}	25.53?2.44 ^{ab}	26.00?1.17 ^{ab}	25.10?1.51 ^{ab}	27.00?2.18^{b}
Pancreas	2.62?1.01 ^{a}	6.35?0.53^{b}	4.59?1.26^{ac}	4.53?1.51^{d}	4.51?1.54^{d}

Values are mean ± SD (n=6).
Variations in superscripts indicate significance (P<0.05) of mean differences between groups for a given tissue. SOD activity was expressed as units/min/g Hb in RBC and units/min/mg protein in tissues

Table 13. Effect of turmeric and curcumin on catalase activity in streptozotocin-induced diabetic rats

Tissues	Control	Diabetic (D)	D+ 0.002% Curcumin	D+ 0.01% Curcumin	D + 0.5% Turmeric
RBC	39430?4796 ^{a}	25592?1006^{b}	37161?495^{a}	31319?696^{a}	41427?1237^{a}
Heart	3.72?0.34 ^{a}	6.02?1.22^{bc}	5.74?2.26^{c}	7.37?0.64^{c}	6.59?0.48^{bc}
Liver	67.66?7.6 ^{a}	65.22?3.09 ^{a}	69.42?5.36 ^{a}	69.80?8.94 ^{a}	60.04?15 ^{a}
Kidney	66.02?3.81 ^{a}	52.43?8.52^{b}	48.90?4.36^{b}	57.09?2.84^{cd}	61.03?0.81^{ad}
Pancreas	3.89?2.62 ^{a}	6.72?1.55^{b}	5.38?1.07 ^{ab}	4.36?2.47 ^{a}	5.48?0.78 ^{ab}

Values are mean ± SD (n=6).
Variations in superscripts indicate significance (P<0.05) of mean differences between groups for a given tissue. CAT activity was expressed as µmoles of hydrogen peroxide decomposed per min/g Hb in RBC and µmoles/min/mg protein in tissues.

Table 14. Effect of turmeric and curcumin on glutathione peroxidase activity in streptozotocin-induced diabetic rats

Tissues	Groups				
	Control	Diabetic (D)	D+0.002% Curcumin	D+0.01% Curcumin	D+0.5% Turmeric
RBC	523?44 [a]	506?68 [a]	508?68 [a]	444?52 [a]	516?64 [a]
Heart	28.95?4.04 [a]	32.21?3.34 [ab]	32.39?2.09 [ab]	32.17?4.58 [ab]	34.63?3.25 [b]
Liver	0.65?0.05 [a]	0.88?0.04 [b]	0.85?0.04 [b]	0.80?0.15 [b]	0.79?0.12 [b]
Kidney	0.67?0.13 [a]	1.11?0.18 [b]	1.04?0.12 [bc]	1.05?0.21 [bc]	0.90?0.07 [c]
Pancreas	2.36?0.26 [a]	5.41?0.42 [b]	4.21?0.36 [c]	3.75?0.54 [c]	3.79?0.66 [c]

Values are mean ± SD (n=6).
Variations in superscripts indicate significance (P<0.05) of mean differences between groups for a given tissue. GPx activity was expressed as µmoles of NADPH oxidized/h/g Hb in RBC and µmoles/h/mg protein in tissues.

turmeric were able to prevent the increase in GPx activity in pancreas (Table 14). However, feeding of turmeric but not curcumin prevented these changes only in kidney (Table 14). GST activity was found to be unaltered in the diabetic rat tissues, except kidney, compared control rat tissues. However, neither curcumin nor turmeric was effective in reversing these changes in kidney.

The burden of oxidative stress may play a central role in the pathogenesis and progression of diabetic complications. Hence, it is likely that antioxidant compounds would prevent and/or delay the progression of diabetic complications. These results suggest that administration of curcumin and turmeric improves but does not completely prevent oxidative stress in streptozotocin-induced diabetes, despite unaltered hyper-glycemic status and thus may have an important bearing on the management of secondary complications of diabetes. Hence, agents, which can prevent diabetic complications irrespective of glycemic control, would have advantages in the management of secondary complications.

9. ANTICATARACTOGENIC EFFECT OF GINGER AGAINST STREPTOZOTOCIN - INDUCED DIABETIC CATARACT IN RATS

Prolonged exposure to chronic hyperglycemia, without proper management, can lead to various short-term and long-term secondary complications, both of macro and micro vascular nature, which represent the main cause of morbidity and mortality in diabetic patients. Large-scale surveys have shown that without proper management diabetes can lead to various complications like nephropathy, retinopathy, neuropathy and cataract. The complications arise from various pathways; one of them is polyol pathway, where the elevated levels of glucose in tissues are converted to sorbitol by aldose reductase (ALR2). Another mechanism that is implicated in the development of diabetic complications is formation of advance glycation end products (AGE) through non-enzymatic glycation of proteins. Excess sugars under diabetic conditions react non-enzymatically with amino group of proteins to form Schiff's-base intermediates, which rearrange to form AGE. These AGE cross-link the proteins to high molecular weight aggregates which affect structure and function of many proteins. Therefore, aldose reductase inhibitors and antiglycating agents are of considerable value in ameliorating the complications of diabetes like cataract. A number of studies with experimental animals

suggest that the compounds that inhibit ALR2 and nonenzymatic glycation could be effective in the prevention of diabetic complications. Numerous ALR2 inhibitors (ARI) such as sorbinil, tolrestat and antiglycating agents such as amino guanidine have been found to improve some diabetic complications, but failed in the clinical trials. In this context, ARI and antiglycating activity of various dietary sources were investigated (*Ann Rep, 2004-05, 2005-06 & 2006-07).* Ginger is one of the agents that have shown antiglycating activity in the *in vitro* studies. In the present study, the antiglycating effect of ginger and its mechanism of action under the *in vivo* conditions using streptozotocin-induced diabetic cataract in rats as a model was investigated.

Methodology

WNIN rats were selected and diabetes was induced by streptozotocin, STZ (32 mg/kg body weight; IP) and divided into 4 groups (Groups II-IV). The control (Group I) rats received only vehicle. While Group II animals received AIN-93 diet, rats in Group III and Group IV received 0.5 % and 3 % ginger in AIN-93 diet respectively, for a period of 8 weeks. Cataract progression due to hyperglycemia was monitored by slit lamp biomicroscope and classified into 4 stages. At the end of 8 weeks, the animals were sacrificed and the biochemical pathways involved in the pathogenesis of cataract such as oxidative stress, nonenzymatic glycation and polyol pathway in the lens were investigated to understand the possible mechanism of action of ginger. Blood glucose and insulin were also determined.

Results

1. Despite the increased food intake, the body weight of Group II animals was decreased, when compared to the Group I. However, the decrease in body weight due to hyperglycemia was not ameliorated by treatment with ginger.
2. Ginger partially prevented the streptozotocin-induced hyperglycemia, as assessed by blood glucose and insulin levels, indicating that ginger may have hypoglycemic effect which needs to be confirmed by further studies (Figure 22).
3. Interestingly, ginger not only delayed progression and maturation of STZ-induced diabetic cataract, but delayed onset of the cataract (Figure 23).
4. Most importantly, ginger reduced the percentage of glycated protein in the soluble lens protein as assessed by boronate affinity chromatography (Figure 24).
5. There was a reduction in the formation of HMW aggregates on lens protein as assessed by SDS-PAGE up on ginger treatment (Figure 25), most probably due to inhibition of protein glycation.
6. Also treatment with ginger appears to have minimized osmotic stress as assessed by polyol pathway enzymes (Table 15).

Figure 22: Effect of ginger on (A) blood glucose and (B) insulin levels in streptozotocin-induced diabetic rats

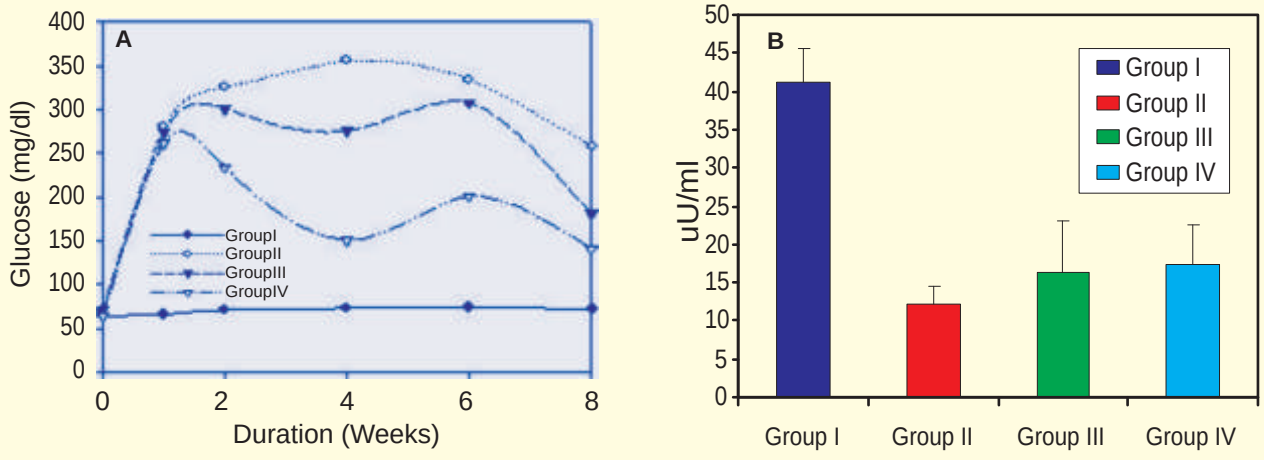


Table 15. Effect of Ginger on activities of polyol pathway enzymes, aldose reductase (AR) and sorbitol dehydrogenase (SDH), in rat lens

Enzyme	Group I	Group II	Group III	Group IV
AR	34.12 ± 1.8	53.67 ± 2.3*	41.46 ± 3.6 #	37.1 ± 3.18 #
SDH	2.40 ± 0.3	3.78 ± 0.6 *	2.77 ± 0.2 #	2.44 ± 0.2 #

The data are the mean SD (n=4). AR activity was expressed as µmoles NADPH oxidized/h/100mg protein. SDH was expressed as µmoles NADH oxidized/h/100/mg protein. The asterisk denotes that data are significantly different from Group I and the sharp denotes that data are significantly different from Group II.

Figure 23. Effect of ginger on average stage of streptozotocin-induced cataract as a function of time

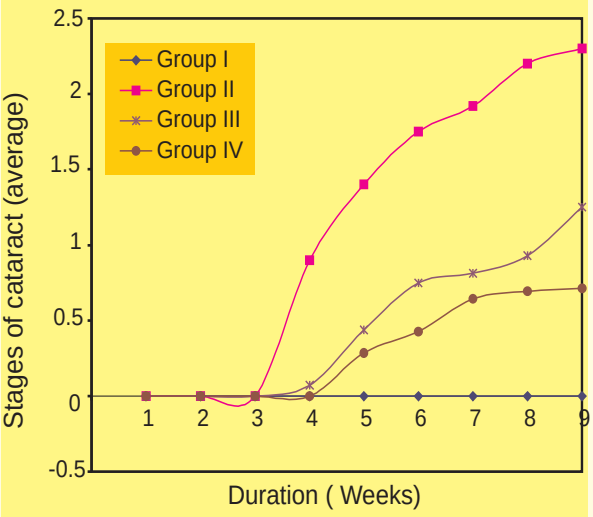


Figure 24. Effect of ginger on the percentage of glycated protein in the soluble protein fraction of lens

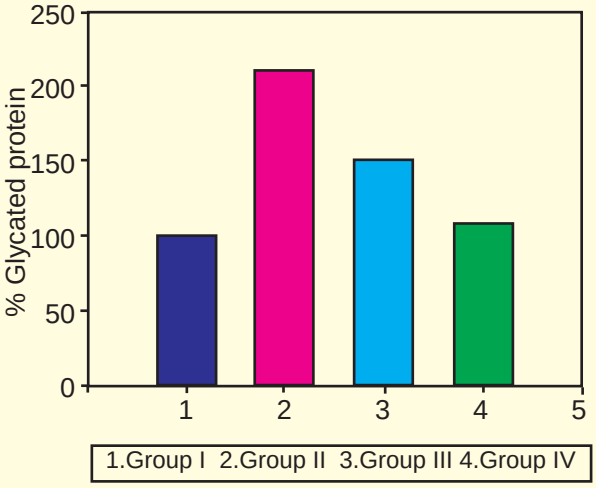
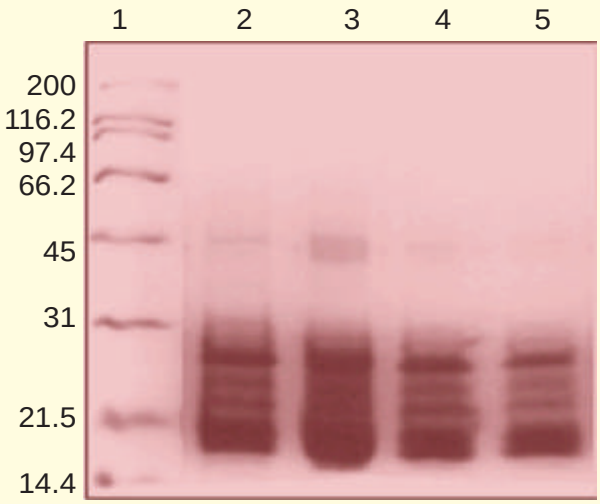


Figure 25: Effect of ginger on protein cross-linking in soluble fraction of lens. Soluble protein was loaded onto a polyacrylamide gel. Lane 1: Molecular weight markers, Lane 2: Group I, Lane 3: Group II, Lane 4: Group III, Lane 5: Group IV. Arrow indicates cross liked proteins in untreated diabetic rat lens, which were reduced in the ginger treated groups



7. Ginger feeding has countered the hyperglycemia - induced oxidative stress, since there was a reversal of changes with respect to lipid peroxidation, protein carbonyl content and activities of antioxidant enzymes in a significant manner.

Conclusions

The results indicate that ginger is effective against development of diabetic cataract in rats. Moreover, these results thus provide a basis for the antiglycating effect of ginger that may have pharmacological implications in the treatment of diabetic complications.

10. INHIBITION OF ALDOSE REDUCTASE BY RUTIN: IMPLICATION FOR THE PREVENTION OF DIABETIC COMPLICATIONS

Aldose reductase (ALR2; EC: 1.1.1.21), the first and rate-limiting enzyme in the polyol pathway, reduces glucose to sorbitol using NADPH as a cofactor. Sorbitol is then metabolized to fructose by sorbitol dehydrogenase. Studies on animal models of diabetes and galactosemia suggest increased polyol pathway activity in the pathogenesis of late onset of diabetic complications such as neuropathy, retinopathy, nephropathy and cataract. Furthermore, evidence for the involvement of ALR2 as a risk factor for diabetic complications comes from genetic polymorphism studies. Several studies indicate that the Z-2 allele and a putative protective allele, Z+2, of ALR2 are significantly associated with diabetic retinopathy. Thus, ALR2 inhibition represents a novel therapeutic modality for the treatment of diabetic vascular complications. To date a wide variety of ALR2 inhibitors (ARI) have been developed like sorbinil and tolerestat. Although a majority of the animal studies with ARI have yielded encouraging results, on the whole, clinical trials of ARI have failed to show efficacy against various diabetic complications. The likely cause of inefficacy and side effects of ARI might be due to a lack of selectivity towards related enzymes involved in the detoxification of reactive aldehydes, such as aldehyde reductase. ALR1 (EC 1.1.1.2), member of the aldo-keto reductase super family that coexists with ALR2 in most tissues. Many ARI are shown to equally interact with aldehyde reductase. Thus, intensive research continues to identify and test both synthetic as well as natural products for their therapeutic value to prevent the onset and/or progression of diabetic complications. Thus, there is still an urgent need for new ARI with improved specificity and safety. In the course of investigations on the evaluation of ARI activity of natural sources, significant inhibition with some dietary sources, which are part of routine diet such as spinach, fenugreek, fennel, lemon, orange, grapes, apple and black tea was found. Based on the data base search for chemical composition of these dietary agents it was found that rutin is a common flavonoid in these sources. Further, rutin is one of the flavonols, which is abundantly present in many fruits and vegetables. The objective of the study was to assess inhibition of ALR2 by rutin, its mechanism of inhibition and significance of inhibition.

Methodology

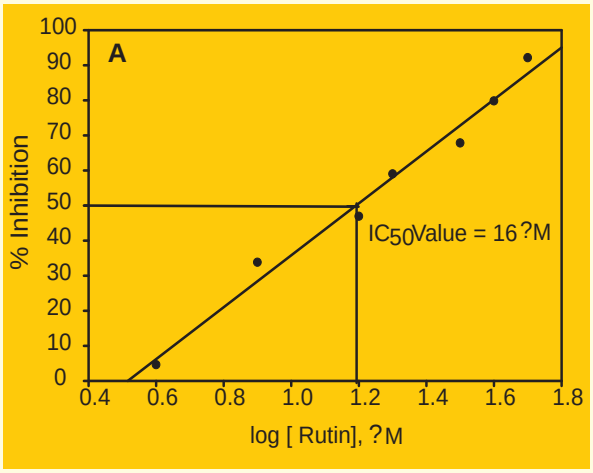
For inhibition studies concentrated stocks aqeous extract (5%) of rutin was prepared in DMSO. Inhibition of ALR2 by rutin was assessed against ALR2 isolated from WNIN rat lens as well as against recombinant human ALR2 according to the earlier reported methods. Specificity of rutin against ALR2 was evaluated by testing its activity against partially purified aldehyde reductase from goat liver. Kinetics of rutin inhibition were carried out to understand the nature of inhibition and to determine Ki (inhibition constant). The effect of rutin on intracellular sorbitol formation in RBC under high glucose conditions was evaluated to understand the significance of ALR2 inhibition.

Results

1. Rutin inhibited both rat lens and human recombinant ALR2 with an IC_{50} value of 16 µM (Figure 26).
2. Rutin was found to be more potent than the known flavonoid inhibitor of ALR2, quercetin (Table 16**).**
3. Interestingly, rutin had shown specificity towards ALR2 as compared to ALR1 and its selectivity ratio was six times higher than quercetin (Table 17).
4. Decrease in Vmax and Km of recombinant human ALR2 in the presence of rutin indicates uncompetitive inhibition of ALR2 by rutin (Figure 27A & Table 18).
5. High inhibitory constant (KI =5 x 10^{-6} M) value for recombinant human ALR2 indicates strong affinity of the inhibitor towards the enzyme. (Figure 27B).
6. Accumulation of sorbitol in RBC under high (50 mM) glucose conditions was prevented in the presence of rutin.

Figure 26. Inhibition of aldose reductase by rutin. Representative dose response curves for the inhibition of rat lens AR **(A)** and human recombinant AR **(B)** by rutin. AR activity in the absence of rutin was considered as 100% to calculate the percentage of inhibition.

Inhibition of rat lens aldose reductase by Rutin



Inhibition of recombinant human aldose reductase by rutin

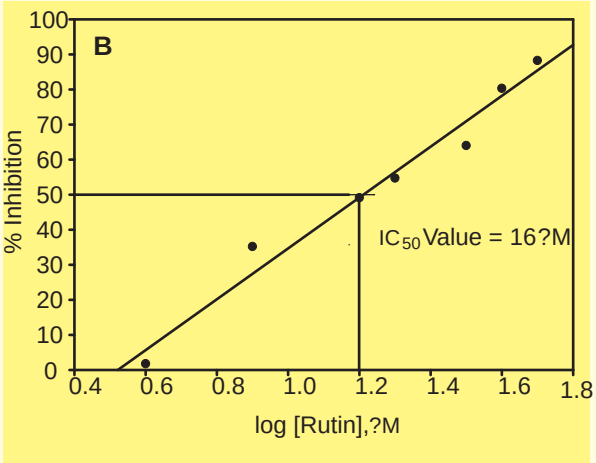
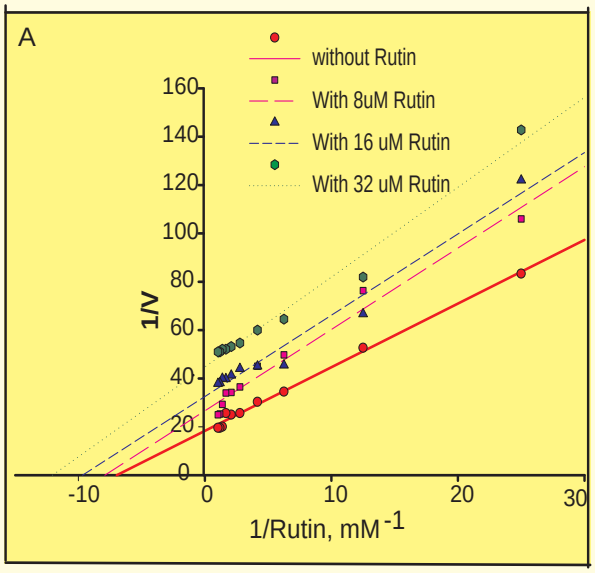


Figure 27. Kinetics of recombinant human ALR2 inhibition. (**A**) Representative double reciprocal plot of recombinant human ALR2 in the absence (circles) and presence of 0.008 mM (squares), 0.016 mM (triangles) and 0.032 mM rutin (oval). (**B**) Determination of Ki by plotting slopes obtained from double-reciprocal plot versus inhibitor concentration.



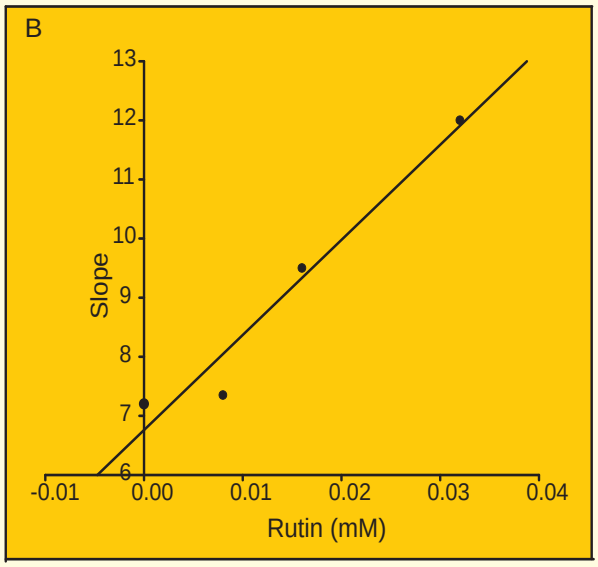


Table 16. IC_{50} vlues of rutin and quercetin for inhibition of rat lens and recombinant human ALR2. Values are average of three independent experiments

Inhibitor	Rat ALR2	Human recombinant ALR2
Rutin	16 µM	16 µM
Quercetin	29.2 µM	39.9 µM

Table 17. Specificity of rutin and quercetin with ALR1 and ALR2. Values are average of three independent experiments.

Inhibitor	ALR2	ALR1	Selectivity ratio (ALR1/ALR2)
Rutin	16 µM	200 µM	12.5
Quercetin	29.2 µM	60.4 µM	2.0

Table 18. Kinetic parameters of recombinant human ALR2 in the presence and absence of rutin. Asterisks (*) indicate a statistically significant difference from the values in the absence of rutin. Values are Mean ± standard deviation (n=3).

Human recombinant ALR2	No inhibitor	Rutin		
		8 µM	16 µM	32 µM
Km	0.14 ± 0.011	0.135* ±0.0025	0.105* ±0.0087	0.083* ±0.0056
Vmax	0.054	0.039*	0.028*	0.022*

7. At 50 and 100 µM, rutin was more effective in preventing the accumulation of intracellular sorbitol in RBC compared to quercetin (Table 19).

Table 19. Effect of rutin and quercetin on intracellular red cell sorbitol level. Sorbitol levels were measured in RBC incubated in the presence of normal (5.5 mM) and high (50 mM) glucose for 3 h. Values are in µg/ml RBC and are mean ± standard deviation of three independent experiments. The asterisks (*) indicate a statistically significant difference from the control group and the sharps (#) indicate a statistically significant difference from the glucose 50 mM group (ANOVA, $p<0.05$)

Group	No inhibitor	Rutin	Quercetin
Control	4.5 ± 0.36	---	---
Glucose 50 mM	11.4* ± 0.53	---	---
Glucose 50 mM + Inhibitor (50 µM)	---	6.5 ± 0.63#	8.7 ± 0.57#
Glucose 50 mM + Inhibitor (100µM)	---	4.4 ± 0.20#	6.6 ± 0.34#

Conclusion: Rutin (quercetin-3-rutinoside), is a flavonol glycoside composed of flavonol (quercetin) and the disaccharide (rutinose), found in many fruits, vegetables and spices. Rutin inhibited ALR2 with an IC_{50} value 16 µM. Its specificity towards ALR2 (over ALR1) and ability to prevent the accumulation of sorbitol in RBC suggests the significance of rutin as a specific and potent ALR2 inhibitor, which could be explored further for preventing or delaying of diabetic complications.

11. EFFECT OF CURCUMIN ON HYPERGLYCEMIA-INDUCED VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN STREPTOZOTOCIN-INDUCED DIABETIC RAT RETINA

One of the most common secondary complications of type 1 and type 2 diabetes is diabetic retinopathy, a leading cause of blindness in middle age and older people. Hyperglycemia and poor metabolic control are important factors in the development of diabetic retinopathy. Diabetic retinopathy is characterized by retinal edema, increased neovascularization and neuronal degeneration in the retina. Neovascularization is essential for normal eye development, however in diseases like age related macular degeneration and diabetic retinopathy this process becomes uncontrolled. One of the main angiogenic factors involved in intraocular neovascularization is vascular endothelial growth factor (VEGF). VEGF is an endothelial mitogen and vascular permeability factor. In retina VEGF is secreted by retinal pigment epithelial cells (RPE) under both physiological and pathological condition. Although, the exact mechanism involved in the pathogenesis of diabetic complications including diabetic retinopathy is not known, many biochemical pathways associated with hyperglycemia; glucose autoxidation, polyol pathway and PKC activation are implicated. Studies indicate that each of these pathogenic mechanisms reflects a single process, oxidative stress. Elevated VEGF expression in diabetic retina has been linked to enhanced oxidative stress. Under such hypoxic conditions, the expression of VEGF is regulated by hypoxia inducible factor 1 (HIF-1), which binds to hypoxia response element on VEGF promoter. Involvement of advanced glycation endproducts (AGE) in the

development of diabetic complications has been well documented. Moreover, AGE induce VEGF expression in cell cultures and animal models, thus being considered to be involved in the pathogenesis of diabetic retinopathy. Various small molecules have been investigated for their ability to inhibit angiogenesis. One such molecule is curcumin, the active principle present in the widely used yellow spice turmeric. Curcumin has been shown to have significant antioxidant activity both *in vitro* and *in vivo*, in addition to its anti-inflammatory, anticarcinogenic and antiviral activities. Furthermore, curcumin is reported to inhibit hypoxia-induced angiogenesis via down-regulation of HIF-1. In the present study, the effect of curcumin and its dietary source turmeric on VEGF expression in streptozotocin (STZ)-induced diabetic rat retina was investigated.

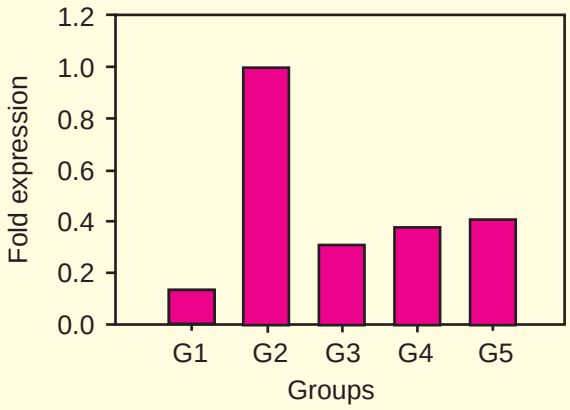
Methods

Three-month-old male WNIN rats with an average body weight of 238 g were used in the study. All the animals were fed with AIN-93 diet ad libitum. The control (group I; n=8) rats received 0.1 M citrate buffer, pH 4.5 as a vehicle, whereas the experimental rats received a single intraperitoneal injection of STZ (35mg/kg body weight) in citrate buffer. After 72 h, fasting blood glucose levels were monitored and diabetic animals were distributed into four groups (groups II-V). Animals in these groups received either only the AIN-93 diet (group II; n=13) or received the AIN-93 diet containing 0.002% (group III; N=9) or 0.01% (group IV; n=9) curcumin or 0.5% turmeric (group V; n=8) for a period of 8 weeks. Turmeric contains 1-2% curcumin and hence 0.5% turmeric corresponds to approximately 0.01% curcumin. At the end of 8 weeks animals were sacrificed and retinas were dissected. Total RNA was isolated from retinal tissues and real time PCR analysis was performed for quantifying Vegf expression. The Vegf expression was normalized to house keeping gene (GAPDH) according to comparative Ct-value method for relative quantification. Equal amounts of protein from total retinal lysates were separated on 10% SDS-PAGE and blotted on to immobilon PVDF membrane to quantitate VEGF by western blot. CML levels were also quantified by western blot similarly. The immunoreactive bands were quantified using Quantity One software (Bio-Rad).

Results

1. There was an increase in Vegf expression (~5 folds) in diabetic retina as compared to control retina. Notably, feeding of curcumin and turmeric to diabetic rats inhibited expression of Vegf and expression levels in curcumin and turmeric fed diabetic rats were almost similar to that of control rats (Figure 28).

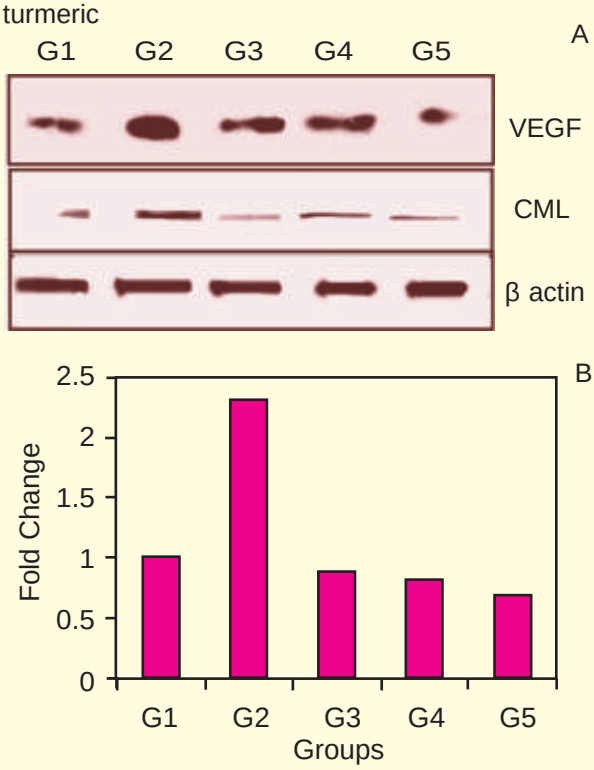
Figure 28. Expression of Vegf in control, untreated and treated diabetic rat retina by real time-PCR. Results are average of three independent experiments. G1 – Control group; G2 - Diabetic group; G3 - Diabetic + 0.002% curcumin; G4 - Diabetic +0.01% curcumin; G5 - Diabetic + 0.5% turmeric

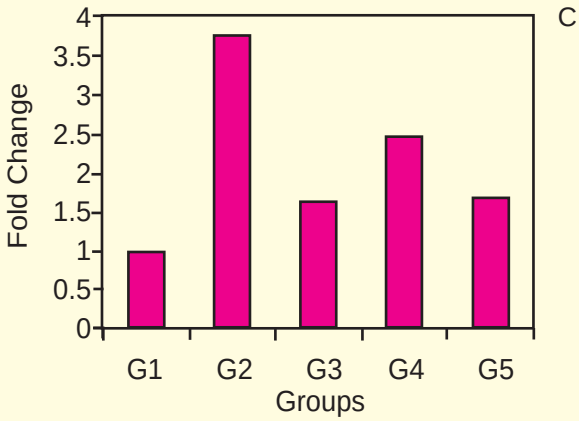


2. Increase in VEGF expression in diabetic rat retina was also shown at protein level by immunoblotting using VEGF specific antibody (Figure 29). This increase in VEGF at protein levels was also prevented by supplementation of curcumin and turmeric to diabetic rats (Figure 29).

3. Advanced glycation end products (AGE) are known to induce Vegf expression through transcription factor AP-1 and curcumin has been shown to inhibit AP-1 activation. Increased oxidative stress has been shown to enhance AGE formation. Therefore, the possibility of curcumin modulating the increased VEGF mRNA expression in diabetic retina by inhibiting AGE formation due to its antioxidant activity was assessed.

Figure 29. A - Immunodetection of VEGF and CML-antigen in control, untreated and treated diabetic rat retina. **B & C** - Densitometric quantification of VEGF and CML. Quantification of VEGF and CML in untreated and treated diabetic groups was plotted as fold over control group. Data in panel B and C are average of three independent experiments. G1 – Control group; G2 - Diabetic group; G3 - Diabetic + 0.002% curcumin; G4 - Diabetic +0.01% curcumin; G5 - Diabetic + 0.5% turmeric





4. There was an increase in CML (a predominant AGE) levels in STZ induced diabetic rat retina as compared to control rat retina (Figure 29). Interestingly, feeding of curcumin and turmeric to diabetic rats results in considerable inhibition of CML formation (Figure 29).

Conclusions

In this study it was demonstrated that curcumin and its dietary source turmeric inhibit VEGF expression in retina under hyperglycemic conditions. AGE are known to induce VEGF expression through transcription factor AP-1 and some studies indicate that curcumin has been shown to inhibit AP-1 activation. It appears from the study that in addition to its direct effect on AP-1, curcumin may also influence the formation/ inhibition of AGE (CML) probably by reducing free radical production.

12. GENETIC POLYMORPHISM IN PPARγ & ITS ASSOCIATION WITH INSULIN RESISTANCE CARDIOVASCULAR DISEASES AND HYPERTENSION

The metabolic syndrome is a condition that promotes atherosclerosis and increases the risk of cardiovascular events through the aggregation of independent metabolic disorders. The hallmark features of the metabolic syndrome include atherogenic dyslipidemia, a prothrombotic state, insulin resistance, hypertension, and abdominal obesity. Other disorders associated with the metabolic syndrome include elevated microalbuminuria, increased fibrinogen, decreased plasminogen activator, elevated plasminogen activator inhibitor-1 (PAI-1), increased blood viscosity, and increased uric acid. Each abnormality contributes independently to the development of atherosclerosis, but when clustered together, these metabolic disorders are synergistically atherogenic. Metabolic syndrome hastens the development of micro and macrovascular disorders. Many studies have shown that elevated systolic and diastolic blood pressure

independently increases the risk for coronary heart disease (CHD). Increased blood pressure independently increases the risk of athero-sclerosis, presumably by promoting the entry of LDL into the subendothelial space, and may exacerbate other metabolic abnormalities. Type 2 diabetes is a multifactorial, polygenic disorder characterized by chronic hyperglycemia arising from insulin resistance where target tissues fail to respond to a normal level of insulin in terms of clearing off glucose from circulation. Obesity is the most potent risk factor for type 2 diabetes. Epidemiological observations underscore a strong correlation of obesity with type 2 diabetes. The pattern of development of the metabolic syndrome in Indians is found to be different from other populations. The anthropometric variables, body fat patterns and plasma lipid are different and need to be validated in Asian Indian population. A typical obesity phenotype observed in Asian Indians consists of higher percentage of body fat at a lower value of body mass index (BMI), high waist–hip ratio (W–HR) at a relatively low waist circumference and less lean body mass as compared to Caucasians and other Asian ethnic groups. Indian population possibly possesses some novel genetic component(s), which give it the uniqueness in terms of propensity to develop metabolism related disorders. Causative factors responsible for metabolic syndrome are complex and thought to involve metabolic, hormonal, genetic and lifestyle interactions. The metabolic, physiologic and genetic mechanisms underlying the clustering of the components of the metabolic syndrome have been widely studied. Prospective twin studies, familial segregation and heredity studies clearly support a genetic basis for the metabolic syndrome and its components. As mentioned in earlier reports, the role of Pro12Ala and C1431T SNPs of PPAR, Trp64Arg polymorphism of ADRB3, C-420G polymorphism of resistin gene and I/D polymorphism in ACE gene in relation to T2DM, hypertension and obesity was studied. While other polymorphisms didn't show any association, C-420G SNP in resistin gene was found to be strongly associated with T2DM (p=0.013).

Genotypings of all the samples for four polymorphisms in adiponectin gene and one SNP in TNFα were completed. As shown in Table 20, G-308A SNP in TNFα was found to be significantly associated with waist circumference (p=0.0125) but not with T2DM, hypertension and BMI. T+45G SNP (Table 21) in adiponectin gene was not associated with any of the mentioned disease conditions. Interestingly, in contrast to other populations, and did not find three of the four variants, namely, G+276T, G-11377A and C-11391G (Table 22) in this population. These data suggest a certain level of genetic uniqueness in Indian population and varied effects of different factors on different populations. It will be interesting

Table 20. Distribution of genotype and allele frequencies of G-308A polymorphism in *TNFα* gene according to clinical characteristics of the subjects

	G/G	G/A	MAF
Diabetic (350)	323 (92.3%)	27 (7.7%)	0.039
Non-Diabetic (348) (p>0.05)	316 (90.8%)	32 (9.2%)	0.046
Hypertensives (350)	322 (92%)	28 (8%)	0.040
Normotensives (348) (p>0.05)	317 (91%)	31 (9%)	0.045
BMI ≥23 kg/m^2 (452)	419 (92.6%)	33 (7.3%)	0.037
BMI <23 kg/m^2 (246) (p>0.05)	220 (89.4%)	26 (10.6%)	0.052
High WC (458)	428 (93.4%)	30 (6.6%)	0.033
Low WC (240) (p=0.0125*)	211 (87.9%)	29 (12.1%)	0.060

Table 21. Distribution of genotype and allele frequencies of T45G polymorphism in *Adiponectin* gene according to clinical characteristics of the subjects

	T/T	T/G+G/G	MAF
Diabetic (348)	271 (77.8%)	77 (22.2%)	0.120
Non-Diabetic (348) (p>0.05)	260 (74.7%)	88 (25.3%)	0.135
Hypertensives (350)	270 (77.1%)	80 (22.9%)	0.122
Normotensives (346) (p>0.05)	261 (75.5%)	85 (24.5%)	0.132
BMI ≥23 kg/m^2 (450)	338 (75%)	112 (25%)	0.134
BMI <23 kg/m^2 (246) (p>0.05)	193 (78.5%)	53 (21.5%)	0.115
High WC (456)	350 (76.8%)	106 (23.2%)	0.126
Low WC (240) (p>0.05)	181 (75%)	59 (25%)	0.131

Table 22. Distribution of minor allele frequencies of SNPs in adiponectin gene in different populations

	T+45G	G+276T	G-11377A	C-11391G
French	14%	28%	25%	10%
Danish	9.9%	29%	26%	7.8%
Japanese	31%	26%	-	-
Koreans	31.3%	28.3%	-	-
Hyderabad (NIN)	10.9%	0	0	0

to screen many more genetic factors and to look for novel factors, which might be affecting this population in making it vulnerable to metabolic disorders as a result of changing environmental factors including demographic and life style changes.

IV. FOOD COMPOSITION AND NUTRIENT AVAILABILITY

NUTRITIONAL AND TOXICOLOGICAL EVALUATION OF ERI SILK-WORM PUPAE

Eri Silkworm pupae (Phylosamia ricini) a by-product of the silk industry is regarded as a delicacy in Northeast India. The Central Silk Board (CSB) has introduced Eri cultivation in Andhra Pradesh, Jharkand and Bihar, where vast tracks of land are under Castor and Tapioca cultivation. Large amounts of spent silkworm are available as by product, which can be used for animal feed or processed for human consumption. Therefore, it was deemed important to do a systematic study of the nutritional and toxicological profile of Eri silkworm pupae.

Aims and Objectives

Nutritional potential and toxicological evaluation of Eri Silkworm pupae.

Specific objectives of the study are

1) To determine the nutrient composition of Eri pupae and prepupae grown on host plants, Tapioca and Castor.
2) To study the protein quality of Eri Silkworm pupae by amino acid analysis and *in vivo* protein quality evaluation.
3) Short term toxicological evaluation of eri silkworm pupae.

Nutrient composition: Proximate composition has shown that eri silkworm prepupae and pupae are both good sources of protein and fat and with exceptionally high zinc content. It is also a good source of calcium and magnesium. Pre pupae and pupae grown on either castor or tapioca showed no significant difference in the nutrient composition (Table 23).

Fatty acid composition showed that the unsaturated fatty acids comprised of approximately 67-69% of total fat with alpha linolenic acid being the major fatty acid which could be used for nutritional advantage.

Short term nutritional and toxicological evaluation of eri silkworm pupae oil is in progress.

Protein quality evaluation: Amino acid composition of the eri silkworm pupae has shown that eri pupae and prepupae are good sources of essential amino acids particularly rich in

Table 23. Proximate composition and mineral content of Eri silkworm pupae

Nutrient	Pupae (Wet weight)		Pupae (Dry weight)	
	Castor	Tapioca	Castor	Tapioca
Moisture (g/100g)	8.8	9.0	8.5	8.8
Protein (g/100g)	54.2	54.0	54.6	54.8
Fat (g/100g)	26.2	26.2	26.2	25.0
Total ash (g/100g)	4.0	4.1	3.8	4.2
Fiber (g/100g)	3.54	3.56	3.45	3.62
Carbohydrate (g/100g)	3.26	3.14	3.45	3.58
Iron (mg/100g)	2.54	2.52	2.40	2.34
Zinc (mg/100g)	7.10	7.10	7.24	7.02
Calcium(mg/100g)	75.4	76.8	74.2	71.2
Magnesium (mg/100g)	180.0	196.4	178.2	188.6
Phosphorus (mg/100g)	585	572	584	570

Table 24. *In vivo* Protein Quality evaluation (Values are mean ± SD (n = 6)

Parameters	Casein	Eri silkworm	
		Prepupae	Pupae
Food Intake g/2weeks	121 ± 10.03	117 ± 10.08	117 ± 9.14
Gain in body weight g/2weeks	40 ± 7.21	26 ± 4.16	24 ± 4.8
Feed efficiency ratio %	33 ± 4.11	22 ± 2.05	21 ± 3.63
Protein digestibility %	92 ± 1.63	87 ± 1.99	87 ± 3.92
Dry matter digestibility %	96 ± 0.44	96 ± 0.52	96 ± 1.43
Net Protein Utilization %	61 ± 4.64	41 ± 1.62	38 ± 4.44

Table 25. Nutritional evaluation of eri silkworm pupae [values are mean±SD (n=12)]

Group	Control		Experimental	
Food intake (g)	1652 ± 68.7	1280 ± 68.6	1559 ± 76.2	1282 ± 89.1
Gain in body weight (g)	353 ± 24.2	195 ± 17.5	299 ± 27.2	179 ± 22.4
Dry matter digestibility (%)	95.6 ± 0.9	95.9 ± 1.0	95.0 ± 1.0	95.3 ± 1.0
Serum Triglycerides (mg/dl)	86.2 ± 15.3	69.6 ± 10.3	63.2 ± 4.4	53.7 ± 8.4
Serum cholesterol (mg/dl)	169 ± 2.8	128 ± 15.1	107 ± 17.4	99 ± 3.6
HDL cholesterol (mg/dl)	10.3 ± 2.1	11.0 ± 2.1	22.7 ± 3.1	20.2 ± 3.1
Alkaline phosphotase (u/L)	91.1 ± 18.0	81.3 ± 11.7	91.4 ± 15.0	87.7 ± 6.1

phenylalanine, valine and isoleucine. The average chemical score was 60 with lysine as the limiting amino acid (Table 24).

Defatted eri prepupae and pupae were taken up for protein quality evaluation (Table – 25). Food intake was comparable between casein and eri silkworm prepupae/pupae. Though the protein quality of eri silk worm prepupae/pupae was significantly lower than that of casein as judged by feed efficiency ratio, protein digestibility and NPU it was comparable to grain legumes.

Nutritional and toxicological evaluation of eri silkworm defatted oil (Table 25): The fatty acid composition showed that the pupal fat was a rich source of alpha linolenic acid (44%) that could be used to nutritional advantage. Therefore, an 18 week nutritional and toxicological evaluation was carried out with the oil defatted from eri silkworm. The study was carried out using weanling NIN wistar rats divided into two groups of 24 animals each (12 males and 12 females) and fed a diet containing 10% eri pupal oil or groundnut oil.

The results showed a significantly ($P<0.05$) lower food intake and body weight gain among male animals fed pupal oil compared to the groundnut oil fed group. Dry matter digestibility was similar in both the experimental as well as control group. The cholesterol and triglyceride levels in serum was significantly reduced in animals fed pupal oil but not in liver and heart. Decrease in plasma cholesterol often accompany hepatic cholesterol accumulation, however in this study decrease in plasma cholesterol resulted without significant accumulation in the tissues. This may be due to the high alpha linolenic acid which may inhibit cholesterol synthesis or stimulate cholesterol metabolism into bile acids and neutral sterols which are excreted.

There was no apparent significant differences in the organ weights of the animals fed pupal oil or groundnut oil. Histopathological examination of the hearts, lungs, liver, spleen, kidney, brain, testis and ovaries revealed no abnormality. Some changes seen in some organs are features commonly seen in colony bred animals and common to both the groups and do not have any great significance. Therefore it may be concluded that pupal oil did not cause any toxic changes in the tissues examined. The study confirms that pupal oil had comparable nutritional qualities to groundnut oil and can be used safely for nutritional benefits.

V. PATHOLOGY

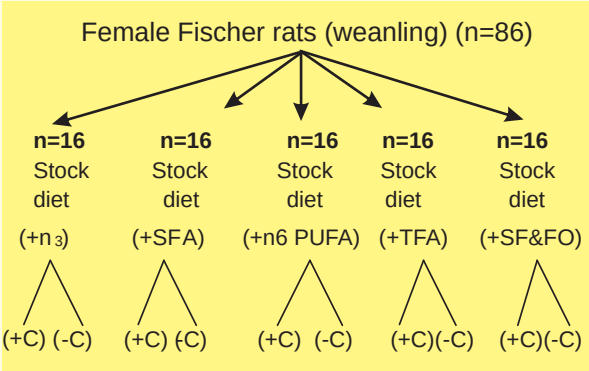
ROLE OF TYPE OF DIETARY FAT IN THE ETIOPATHOGENESIS OF CARCINOGEN – INDUCED BREAST NEOPLASM IN FISCHER FEMALE RATS

The relationship between obesity and breast cancer is still not clearly known. Though life style changes and dietary fat intake are known to increase the incidence of female breast cancer, a cause and effect relationship does not seem to exist. One of the reasons is due to the multifactorial nature of the disease itself. However the aspect of fat quality i.e. saturated vs unsaturated (N_6 vs N_3) and transfat etc. has not been studied. Hence, the present project is undertaken to investigate the possible etiological roles played by the different types of dietary fats in female Fisher 344 rats using a carcinogen.

Objectives

- To study the effect of qualitative differences in dietary fat i.e. saturated vs polyunsaturated vs transfats in experimental mammary carcinogenesis.
- To study the role of insulin resistance and hyperinsulinemia in mammary carcinogenesis in these animals.

Study design



© – Carcinogen; **SFA** – Saturated Fatty Acid., **TFA** – Trans Fatty Acids, **FO** – Fish oil).

Source of fats : Total fat content in diet – 10%
SFA – Palmolein oil (PO), **TFA**– Vanaspathi (V),
n_6 PUFA – Sun flower oil (SFO),
n_3 – Soya bean oil (SBO), **SFO + Fish oil** (SFO+FO)

All the animals were put on their respective diets for 4 months followed by administration of carcinogen DiMethylBenzAntherecene (DMBA)–40mg/kg bodyweight/dose at weekly intervals for 4 weeks by oral route and continued on the same diet for a further period of 8 months (32 weeks) (Table 26).

All the animals were put on their respective diets for 4 months followed by administration of carcinogen DiMethylBenzAntherecene (DMBA) – 40mg/kg body weight per dose at weekly intervals for 4 weeks by oral route and continued on the same diet for a further period of 8 months (32 weeks).

Parameters studied

1. Plasma lipids – TG, total cholesterol and HDL
2. Insulin, glucose & insulin – glucose ratio estimations
3. Plasma phospholipids and mammary adipose tissue fatty acid composition
4. Tumor characteristics
   - * *Type, number, size*
   - * *Morphological sub category*
   - * *Tumor grade*
5. IHC
   - * *Estrogen and progesterone receptor status*
   - * *Peritumor breast adipose tissue aromatase presence*

Results

A) Body weights data

Between carcinogen administered (C+) and carcinogen not administered (C-) of all groups –Significantly higher ($p < 0.05$) in (C-) group than C+) group.

Between the carcinogen administration (C+) groups - SFO + FO group significantly higher ($p < 0.05$) than all other groups before carcinogen administration and before euthanization.

Table 26. Oil analysis used in the diet preparation (in percentage)

Fatty acid	Vanaspati (TFA) (I)	Palmolein (SFA) (II)	Sunflower (n6) (III)	Soyabean (n3) (IV)	Sunflower FO (n6+n3) (V)
!4:0	0.9	0.79	---	0.07	---
16:0	38.46	35.65	6.55	10.84	5.3
16:1	----	0.16	0.13	--	---
18:0	5.16	4.50	4.67	3.94	3.66
18:1t	20.14	---	---	---	---
18:1c	29.4	45.64	44.09	21.73	45.5
18:2	4.31	12.43	43.6	55.2	38.95
20:0	0.48	0.55	0.45	2.78	0.39
18:3	----	0.25	0.44	5.3	0.38
Other trans	1.05	---	------	---	--
Others	---	---	---	0.4	---
20:1	---	---	---	--	0.54
20:4	--	--	--	--	0.84
20:5	--	--	--	--	1.74
24:1	--	--	--	--	0.24
22:5 n-3	--	--	--	--	0.18
22:6 n-3	--	--	--	--	1.16
T.SFA	45.0	41.49	11.67	18.06	9.6
MUFA	29.4 (21.2)	45.64	44.09	21.3	46.04
LA n-6	4.31	12.43	43.6	55.2	39.8
ALNA n-3	---	0.25	0.44	5.3	3.46 *
P/S	0.09	0.30	3.77	3.34	4.48
n-6 / n-3	---	41.4	99	10.4	11.5

** Includes LC n-3 PUFA*

All the diets were identical (casein -based semi synthetic) expect for the type of fat used.

Between the carcinogen not administered (C-) groups - SFO + FO was significantly higher than other groups ($p<0.05$).

B) Tumor load in carcinogen treated group

- Tumor load was highest in n6 rich diet fed animals (100%) and least in n3 rich diet (50%). Incidence in SFA and n3 + n6 diet animals was 75% followed by TFA (62.8%).
- Tumor density per animal was highest in n6 fed animals and least in TFA fed animals. SFA and n3 fed animals also had high tumor density. n3 + n6 group was closer to the TFA group.

C) Typing of tumors

Adenocarcinomas were the most predominant tumors followed by squamous cell carcinomas. Nine percent of tumors were benign in nature while others abscesses & granulomas across all carcinogen treated groups.

*D) Histological grading of tumors **

Based on tubular differentiation, nuclear morphology and mitoses, it was seen that only n6 fed group had tumors spread across all 3 histological grades (1 - 3) while all the other dietary groups had tumors in grades 1 and 2 only. Most of the tumors across all groups were in grade 1 followed by grade 2. The only grade 3 tumor was seen in n6 fed animals.

E) Immunohistochemistry parameters in tumors

It was observed that 62.5% of tumors in n3 + n6 fed animals followed by 50% in n3 fed animals and 43.75% in n6 group were positive for both ER and PR. However, PR positivity with no ER presence

was seen in SFA and TFA group. Aromatase positivity was seen across all the dietary groups with the highest in TFA group and least in n3 fed group.

F) Haematological parameters:

There were no significant differences between the groups with regard to all parameters studied.

G) Biochemical parameters:

1. *Glucose, insulin, insulin:glucose ratio and HOMA index*

In the carcinogen fed groups, there were no differences with respect to glucose levels while insulin, insulin:glucose ratio and HOMA index were found to be significantly increased ($p<0.05$) in group IV (n3-SBO) as compared to other groups. In the non-carcinogen administered groups, there were no differences among the above parameters studied (Table 27).

2. *Plasma total cholesterol, triglycerides and HDL levels*

It was observed that there were no significant differences in any of the parameters studied in relation to carcinogen treated groups. However, in the non-carcinogen treated groups, total cholesterol and HDL were significantly higher in groups II & III (SFA & n6) as compared to other groups while triglycerides were significantly higher in group I as compared to other groups (Table28).

3. *Fatty acid analysis*

a) Total SFA content of plasma phospholipids in C+ rats was not different between the groups while in the C- group it was higher in PO fed animals as compared to the others.

b) Total MUFA in C+ group was higher in TFA fed animals as compared to SFO + FO group. In C- group they were not different in any of the diets.

c) Total LCn3 PUFA – C+ group significantly lower in in PO fed rats as compared to SFO + FO animals. C- group highest in SFO + FO group. Total LCn6 PUFA – C+ group significantly increased as compared to C- group. TFA group and SFO + FO were less than other groups.

4. *Mammary adipose tissue analysis* : Could not be undertaken as adipose tissue content was inadequate for analysis to be done.

INFERENCE

Based on the results obtained, it appears that the **body weights** of animals not given carcinogen (C-) were, as expected, significantly higher than in carcinogen administered (C+) animals. When both the above groups were analysed separately, it was seen that n3 + n6 diet fed animals showed better body weights than others fed on different diets,

Table 27. Effect of different types of oils on insulin, insulin/glucose and HOMA index in rats

Parameters	Vanaspati (I)	Palmolein (II)	Sunflower (III)	Soyabean (IV)	Sunflower+FO (V)
	Non-carcinogen groups (n=5)				
Glucose (mg %)	68 ? 2.0^{a}	71 ? 3.3^{a}	69 ? 4.2^{a}	70 ? 6.0^{a}	65 ? 4.5^{a}
Insulin (µu /ml)	28 ? 3	34 ? 5	33 ? 3	32 ? 4	29 ? 1.9
Insulin/ Glucose ratio	0.40 ? 0.03^{ab}	0.47 ? 0.06^{b}	0.48 ? 0.04^{b}	0.44 ? 0.03^{ab}	0.47 ? 0.03^{ab}
HOMA Index	56.7 ? 3.7^{ab}	52.7 ? 6.1^{a}	48.12? 3.9^{a}	53.7 ? 5.3^{a}	49.87 ? 2.9^{a}
	Carcinogen administered groups (n=3)				
Glucose (mg %)	137 ? 21.9	117 ? 6.8	112 ? 12.8	125 ? 2.5	117 ? 2.8
Insulin (µu /ml)	63 ? 17.7^{a}	62 ? 8.5^{a}	70 ? 7.1^{ab}	107 ? 24^{b}	94 ? 9^{ab}
Insulin/ Glucose ratio	0.44 ? 0.08^{a}	0.47 ? 0.04^{ac}	0.58 ? 0.07^{abc}	0.85 ? 0.19^{b}	0.78 ? 0.12^{bc}
HOMA Index	59.9 ? 13.1^{b}	40.6 ? 8.8^{ab}	43.4 ? 6.5^{ab}	31 ? 7.4^{a}	20.4 ? 6.9^{a}

Values are means ± SE number in parenthesis
Values in row having different superscripts letters are significantly different by one-way ANOVA and LSD test, ($P < 0.05$). * $P<0.05$ independent t-test as compared to normal

Table 28. Effect of different types of oils and lipid profile of rats

Parameters	Vanaspati (I)	Palmolein (II)	Sunflower (III)	Soyabean (IV)	Sunflower+FO (V)
	Non-carcinogen groups (n=5)				
Cholesterol (mg%)	91 ? 4.2^{a}	115 ? 6.3^{b}	115 ? 7.8^{b}	82 ? 5.6^{a}	91 ? 4.2^{a}
Triglycerides (mg%)	75 ? 21.8^{b}	65 ? 4.8^{bc}	54 ? 5.5^{ab}	37 ? 3.1^{a}	43 ? 7.3^{ac}
HDL (mg%)	69 ? 6.5^{a}	94 ? 4.1^{b}	87 ? 9.6^{ab}	67 ? 3.3^{a}	71 ? 6.1^{a}
	Carcinogen administered groups (n=4)				
Cholesterol (mg%)	79? 7.4	86 ? 16.4	86 ? 9.4	97 ? 16.5	90 ? 6.3
Triglycerides (mg %)	78 ? 20.5	96 ? 17.7	92 ? 26	69 ? 11.1	64 ? 5.8
HDL (mg%)	57 ? 9.4	62 ? 10.2	53 ? 8.9	82 ? 23	74 ? 6.7

Values are means ± SE
Values in row having different superscripts letters are significantly different by one-way ANOVA and LSD test, ($P < 0.05$)

thus indicating that carcinogen administration probably did effect the body weights and that n3+n6 diet was much better acceptable than other diets.

With regards to **tumor load**, among various animal groups studied,n6 rich diet appears to be promoting greater tumor development as compared to other diets while it was lowest in n3 fed animals. The tumor load per animal was more in group fed n6 diet and least in TFA fed group.

As for **tumor typing**, irrespective of the diet fed, adenocarcinomas were predominant as compared to other types. Adenocarcinomas, squamous cell carcinomas and benign tumors were more in n6 fed groups as compared to others. Both the above parameters were found to be lower in n3 group as compared to n3+n6 group, suggesting that n3 may probably have a protective effect.

Immunohistochemistry study of adenocarcinomas, which indicates prognosis in various groups, showed that n3+n6 diet followed by n3 diet alone had better results than other groups studied.

Biochemical estimates in the C+ group showed that the glycemic status appeared to be maintained in all groups except for increased insulin levels in n3 fed group. Also, plasma lipid levels were not altered in any of the groups. Based on this study findings, carcinogen administration did not have much effect on glucose and lipid metabolism.

Fatty acid analysis revealed that TFA which is known to induce/promote carcinogenesis did not show increased tumor incidence thereby suggesting that at the level of consumption (10%) it may not be promoting tumor formation. As anticipated, SFA levels were more in PO fed animals as compared to others. With respect to n3 fatty acid levels, the lower tumor incidence may be because of its anti-inflammatory role which was also evident in SFO + FO fed animals as compared to SFO only fed animals. This could also be because of lower n6 : n3 ratio in SFO + FO group. N6 rich diet consumption showed highest tumor incidence and density which could be attributed to increased inflammation and this in turn could be due to increased oxidative stress/arachidonic acid. Mammary adipose tissue in C+ animals was surprisingly very minimal / not present and hence no analysis could be undertaken which could have otherwise added valuable information to data generated from this study.

Haematological parameters were unremarkable in all groups studied.

Finally, it appears that n6 rich diet has deleterious effects in relation to tumorogenesis while n3 alone diet has a better outlook with respect to the same. Apart from n3 diet, n3 + n6 diet was also observed to be beneficial. SFA and TFA diets that are associated with increased incidence of CVD and other chronic diseases do not seem to show a similar trend with respect to carcinogen induced mammary neoplasms.

VI. EXTENSION & TRAINING

A. SERVICE ACTIVITIES

1. Publications

The quarterly periodicals, namely, Nutrition (English), Poshan (Hindi), Poshana (Telugu) and a semi-technical bulletin Nutrition News, covering popular articles of public interest and scientific information on nutrition are being published.

The other titles which were reprinted, on popular demand include "Dietary Guidelines for Indians – A Manual (English & Telugu)", Diet and Diabetes (English), Diet and Heart Disease (English).

2. Training programmes

2.1. Regular Training Programmes: This year a total of twenty eight candidates have attended the regular training programmes of the Institute viz. (i) Post-Graduate Certificate Course in Nutrition (15 participants) (ii) Annual Training Course in Endocrinological Techniques (10 participants) and (iii) Techniques for Assessment of Nutritional Anaemias (3 participants).

2.2 Adhoc Training Programmes:

An adhoc training programme was conducted for two WHO fellows from Myanmar in the field of "Public health and nutrition" (4th June – 27th July, 2007).

About 31 PG students underwent training in various disciplines like Biotechnology, Microbiology, Biochemistry, Foods and Nutrition, Computers etc as part of their dissertation work from different institutes of the country.

3. Extension activities

3.1 Exhibitions

1. Organized an exhibition stall at Gudur as part of **Bharat Nirman Celebrations**. People from different walks of life visited the stall. Posters and the information given in the handouts of NIN were explained to the visitors. About 500 people visited the stall. (August. 7-11, 2007)

2. A portable exhibition set was put up as part of Open space activity organized by FAO Solutions Exchange. Participated in the event and discussed about various IEC strategies to disseminate knowledge related to dietary guidelines. (19th August 2007)

3. An exhibition stall was put up in **Bhartiya Vigyan Sammelan and Expo**, organized by Madhya Pradesh Council of Science & Technology in Bhopal. Different posters related to Nutrition and Health were explained to the visitors. Over 1000 people from different walks of life visited the stall during the event. (November 21-26, 2007)

4. An exhibition stall was arranged as part of **"19th Krishi Shilpa O Banijya Mela",** organized by Agragami Handicapped Samity, at Medinapur, Kolkatta. Over 3000 rural folk from different walks of life participated in the Mela (December 8 – 15, 2007)

5. As Part of Nutrition Awareness Programme, organized by an NGO, World Vision, at Shadnagar, Hyderabad, posters were displayed in the event. The posters were explained to the visitors. A hand out was prepared on tips to lead healthy life and distributed to the visitors. About 70 participants visited the stall.

6. Participated in the Science & Technology Exhibition, "Pride of India Science Expo", organized as part of the 95th Session of the Indian Science Congress, at Andhra University, Visakhapatnam (Jan. 3-7, 2008).

3.2 Popular Lectures/ Awareness Camps

1. Delivered extension lectures (3) on "Nutrition and Health" in summer camps by displaying the posters related to Nutrition and Health for school children and adolescent girls living in and around Charminar area as part of the 17th Annual Summer Camps organized by Confederation of Voluntary Associations (COVA) in association with Greater Hyderabad Municipal Corporation. (2nd and 10th May 2007)
2. Delivered an extension lecture on "Fats for Health" to the members of Association of Food Scientists and Technologists, Hyderabad at CFTRI Regional Station. About 30 scientists attended the programme. (10th July 2007)
3. Delivered extension lectures (4) on "Nutrition and Health" to the 4 batches of cabin crew of Indian Airlines, Hyderabad. In each batch about 50 members cabin crew participated in the programme. (8th & 28th July, 4th Aug. & 18th Oct. 2007)

4. Delivered a talk about the research activities of NIN to senior medical officers representing different states of the country. About 50 senior medical officers participated in the programme (22nd Oct 2007).
5. Delivered a lecture on "Fitness, Nutrition and Health", to a batch of senior executives at New Delhi as part of the training programme organized by Air-India. About 40 senior executives participated in the programme (29th November 2007)
6. A popular talk on "Nutrition and Health" was given to the constables of Railway Protection Force. About 25 Head Constables and 4 Inspectors participated in the programme. The programme was followed by an interactive session with them (3rd March 2008)
7. An inspirational talk was given to the school children on "Nutrition and Health" and also scope for the children to take-up science as the subject for future career buildup, organized by Matrushri E & L school, Hyderebad. About 100 students from the classes from 6th to 9th participated in the programme. (14th Feb-08)

3.3 Nutrition Awareness Camps

In association with Food and Nutrition Board, Hyderabad, two Nutrition Awareness Programmes were held for Anganwadi workers/teachers, ICDS functionaries, at Shadnagar, RR Dist. On the first day 60 SHG women participated in the programme while on the second day 120 anganwadi and ICDS functionaries participated in the programme. Low cost nutritious recipes like ragi ladu and upma made of broken wheat (*dahlia*) were demonstrated (10th and 13th September 2007).

3.4 Radio talks

1. A popular talk was broadcast on All India Radio about the **"Importance of micronutrients in diet"** in Telugu on 14th June 2007.
2. Two Radio talks on **"Blending of oils and low cost nutrition foods"** and **"About NIN and its achievements; balanced diet"** were

broadcast in "Vanitha Lokam" on 3rd and 7th September 2007 respectively on All India Radio.

DOORDARSHAN (TV PROGRAMMES)

A byte on **"Importance of vegetables in a balance diet"** with special reference to Brinjal and Okra and their nutritive values was broadcast in 30 minutes programme by TV 9 on 25th and 26th March 2008.

4. SPECIAL EVENTS

4.1 National Technology Day Celebrations (11th May 2007)

To commemorate 'National Technology Day" a nutrition awareness camp was organized by an NGO, COVA for school children and discussed about the technologies developed by NIN such as food fortification, and other achievements. About 50 school children participated in the programme.

4.2 National Nutrition Week Celebrations (Sep 3rd – 7th, 2007)

- Radio talks were delivered on the occasion of National Nutrition Week (Sept.1).
- A Brainstorming session on "Nutrition Promotion among School Children" was organised at the institute. The session was attended by Principals of local schools, representatives from Recognised School Teachers Association, NGOs (Sept. 1).
- A one day State-level Workshop was held on "Nutrition Promotion for a Stronger Nation" in association with Food and Nutrition Board (Govt. of India) and Department of Women Development and Child Welfare (Govt. of A.P) (Sept. 4)
- A Nutrition awareness programme was organized for school children at Railway Girls High School, Lallaguda, Hyderabad (Sept.4)

- A popular article on "Functions of foods and balanced diet" was published in Telugu magazine *Ujwala*.

4.3 World Food Day celebrations (16th October 2007)

A one-day Workshop was organized on "Right to Food", as part of World Food Day celebrations, in association with Association of Food Scientists and Technologists (Hyderabad Chapter) and Oil Technologists Association of India (South zone), Hyderabad.

4.4 National Science Day (NSD-28th Feb-08)

Participated in NSD celebration and organized nutrition awareness programme for School children of Department of Atomic Energy. Apart from nutrition awareness programme few points related to global warming related to the National Science Day's theme ie., "Understanding the Planet" was also covered in the talk. About 100 students of 8th and 9th classes participated in the programme.

4.5 International Womens' Day (8th March 08)

Nutrition awareness camp was organized in association with ICRISAT in a Residential Government School for girl children. Concepts related to school gardening, nutrition and health during adolescence were discussed during the programme. About 60 girl students participated in the event.

5. ACTIVITIES OF SECRETARIAT FOR WHOSEA NUTRITION RESEARCH-CUM-ACTION NETWORK

The Extension and Training Division has been carrying out the activities of the Secretariat for WHO Southeast Asia Nutrition Research-Cum-Action Network since 2004. As part of the day-to-day activities, correspondence related to the Secretariat of the WHO South East Asia Nutrition Research-cum-Action Network was carried out. The January 2008 issue of the SEA NETWORK – the bi-annual newsletter was brought out and disseminated. **The Joint WHO-FAO Inter-country Workshop on Food and Nutrition Policy and Plans of Action, 17-21st December 2007 was organized at the Institute**.

Over 30 participants from 4 Southeast Asian Countries, representatives from FAO and WHO took part in the Workshop. Scientists from the Extension and Training Division acted as facilitators and conducted sessions during the workshop. In order to revitalize the activities of the Network, a meeting with the representatives of WHO Southeast Asia Regional Office (WHOSEARO), FAO and WHO Collaborating Centers in Nutrition was organized.

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SEA Network

IX Meeting of SEA Nutrition Network – A Curtain Raiser

The IX meeting of the Joint WHO- FAO Southeast Asia Nutrition Research-cum-Action Network will be held at the National Institute of Nutrition (NIN), Hyderabad, India from 24th to 26th September 2008.

The three-day meeting is an endeavour to promote effective exchange of information among the member countries and partnering organizations/ institutions. Participants drawn from diverse fields of food and nutrition like researchers, academia, and policy implementation of the 11 member states will take part in the meeting. Representatives from UN organizations like WHO, FAO, UNICEF, ICCIDD and World Food Programme will also participate in the meeting.

NIN to host the IX meeting of SEA Nutrition Network

Alongside discussing the ways and means to revitalize the Network, the meeting would also focus on the rising food prices in the region and its impact on household food insecurity. The participants would also share some successful programmes and experiences in the management of mild, moderate and severe malnutrition using community-based strategies. Some sessions are also planned to discuss recent interventions to combat and control micronutrient malnutrition especially Iodine Deficiency Disorders (IDD) and Iron Deficiency Anaemia. Through the group activities that are planned for the workshop the participants would look into future research needs and evidence-based interventions for management of malnutrition; exchange views on making the micronutrient deficiency control and prevention programmes successful by bridging the gap between research and action and explore the possibility of behavioral change communication in control and prevention of anemia.

FAO meet discusses soaring food prices in Asian countries

The one day emergency brainstorming session on the Food and Agriculture Organisation's (FAO) Initiative on Soaring Food Prices (ISFP) in select Asian countries was held at the FAO Regional Office, Bangkok, Thailand on 25th April 2008. Representatives of the Governments of Bangladesh, Cambodia, Indonesia, Philippines, Timor-Leste and Thailand along with officers from the FAO Regional Office for Asia and the Pacific (FAO RAP) attended the meeting. The aim of the meeting was to identify actionable country-specific plans to counter the ill effects of soaring food prices.

In a brief welcome session, the aims of FAO ISFP and its role in assisting the member governments to tackle the problem of soaring food prices was explained to the participants.

In a presentation on 'Agricultural price movements and their underlying reasons, the outlook for next yea crops, regional policy responses and areas of concer Asia', the representative from FAO RAP observe the international prices of fats/oils, cereals, su meat products were all increasing while tha products seemed to be declining in some From the 1st quarter of 2007 to the 1st qu wheat prices increased by 131%, maize 222%, chicken by 16% and palm oil by factors responsible for rising prices increasing crude oil prices, energ cereals outpacing supply, droughts speculators and demand from the biofuels

B. RESEARCH ACTIVITES

Content analysis of nutrition component in school Science Textbooks

The importance of early learning of nutrition-related knowledge, attitudes and behaviours for future health is widely recognised. Nutrition education is an accessible and effective tool in developing healthy nutrition-related practices and schools provide most effective and efficient ways to reach a large segment of the population, including young people, their families and the community in general. In an earlier study conducted in the Institute, it was observed that the children prefer to learn in the classroom set up through the teacher. Nutrition education can best take place in the classroom set up, if the nutrition component can be effectively blended into the science curriculum at the school level instead of providing it as an external knowledge intervention. Hence, development of innovative nutrition education curricula is a continuous and demanding process. Before developing the nutrition content that can be effectively blended into the school science curricula, the first step would be to evaluate the nutrition component in the existing school science textbooks. The best method for doing so would be to carry out the quantitative and qualitative assessment of the nutrition component in the school science textbooks using a research method called Content Analysis, which can be defined as a technique for making replicable and valid inferences from the text/data to their context. In this context a study was conducted with the following objectives:

Objectives

1. To analyse the biological science content in relation to the over all general science component of the textbooks of all classes from primary to high school (I - X classes)
2. To do quantitative content analysis for finding out the proportion of space allocated for the nutrition component in relation to the other topics in the biology textbooks
3. To conduct the qualitative analysis of the nutrition component for finding out the topics dealt with, importance assigned to them, continuity of the topics from one class to the other.

Materials and Methods

Sample: All science text books from Classes I-X of two streams - NCERT and AP State Board were selected for the study.

Methodology and Analyses: For the purpose of the study all the content related to Physics, chemistry and environment were categorized under Physical and Environmental Sciences and content related to life sciences were categorized as Biology. Under biology, the terms 'nutrition', 'food safety', 'health' and 'others' were operationally defined before carrying out the study. Both quantitative and qualitative content analysis methods were used. Physical measurement of space (in terms of pages) allocated, number of illustrations allocated, exercise questions were measured as part of the quantitative analysis. Qualitative analysis of topics covered, quality of narration, illustrations, other general elements like font, print, etc was also carried out.

Results

Quantitative content analysis showed that the biology component occupied relatively less space in relation to physical and environmental science in the NCERT textbooks in the higher classes, while at least one chapter of them was dedicated to nutrition from I to VII classes, there is no special chapter on nutrition in the high school science textbooks (from VIII to X classes) (Table-29). Similarly, the number of chapters allocated for biology are relatively less when compared to physical and environmental science in classes VI to X even in the AP State Board Textbooks. Special chapters on nutrition appear only in classes IV and V. Although nutrition topics are dealt with in the IX and X classes they appear as a sub-units in other chapters related to health (Table-30). As regards food safety, there are hardly any dedicated chapters for the subject in the science textbooks of both NCERT and AP Board.

As regards the space allocation for each of the topics of interest, with in the biology component in the school textbooks, nutrition has been provided over 10% of the space in all classes up to VII in the NCERT curriculum. However, it is not the same in the AP State Board syllabus. Food safety got about 1% of all space allocated for biology in primary classes in NCERT (Fig-30). In AP state Board Science syllabus, it was observed that although there were no dedicated chapters for nutrition, 10-23% of the space was allocated for nutrition component in the biology content in the primary

Table-29 Distribution of number of chapters in NCERT Textbooks

Class	Total	Physical and Environmental Science	Biology	Nutrition	Health & Hygiene	Food Safety
I	18	7	11	1	3	0
II	19	9	10	1	1	0
III	18	5	13	1	4	0
IV	14	7	7	1	1	0
V	17	7	10	1	2	0
VI	16	11	5	2	0	0
VII	15	9	6	1	1	0
VIII	14	11	3	0	1	0
IX	15	11	4	0	1	0
X	17	12	5	0	0	0

Table-35 Distribution of number of chapters in AP State Board Textbooks

Class	Total	Physical & Environmental Science	Biology	Nutrition	Health & Hygiene	Food Safety
I	7	3	4	0	1	0
II	9	4	5	1 unit	1	0
III	10	6	4	0	0	0
IV	11	7	4	1	1	2
V	11	7	4	1	0	0
VI	11	8	3	0	1	0
VII	11	9	2	0	0	0
VIII	14	9	5	0	3 units	1 unit
IX	19	13	6	1 unit	1 unit	1 unit
X	15	11	4	2 units	4 units	0

classes. About 10% of the biology content was allocated for nutrition in Class –X. However there was almost no allocation of specific space for food safety in AP Textbooks at all, except in V and VII classes where 15% and 5% of space in biology component was allocated respectively (Figure-31).

Qualitative content analysis revealed that in both NCERT and AP State Board syllabi, the first nutrition related content in the first three classes was devoted to recognizing various foods, creating awareness about the foods that are derived from plant and animal sources. There after in higher

Figure-30: Subject-wise allocation of space (%) in Biology component of NCERT textbooks

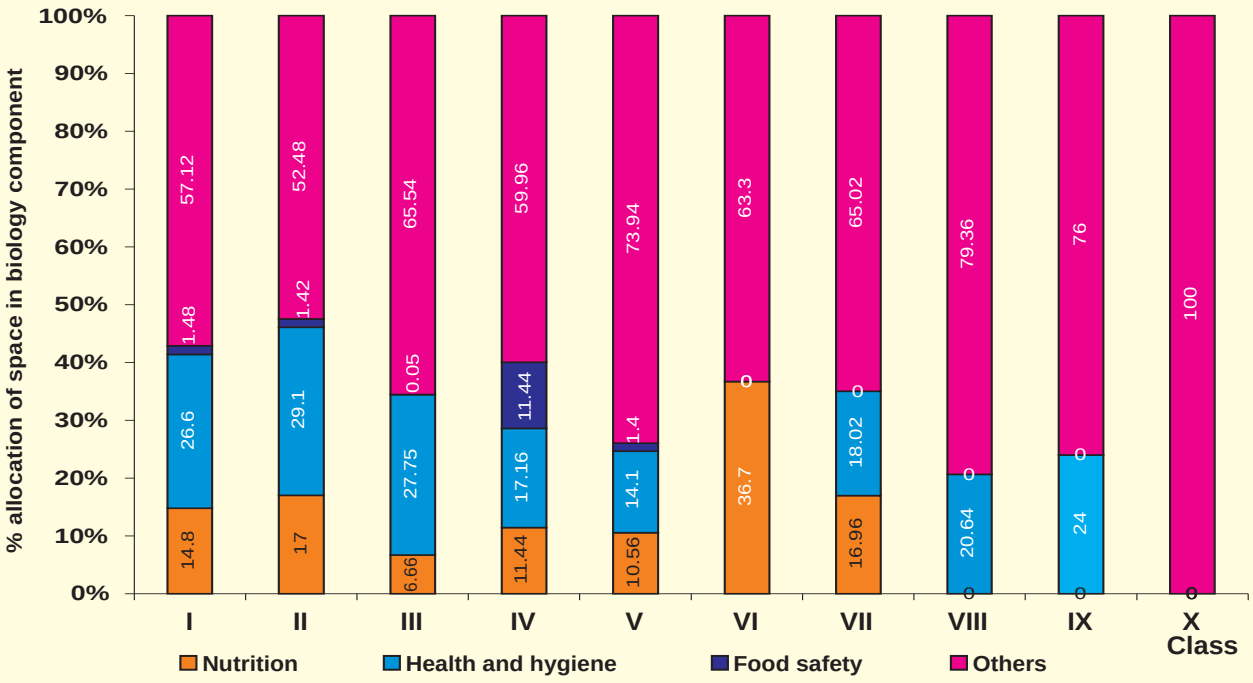
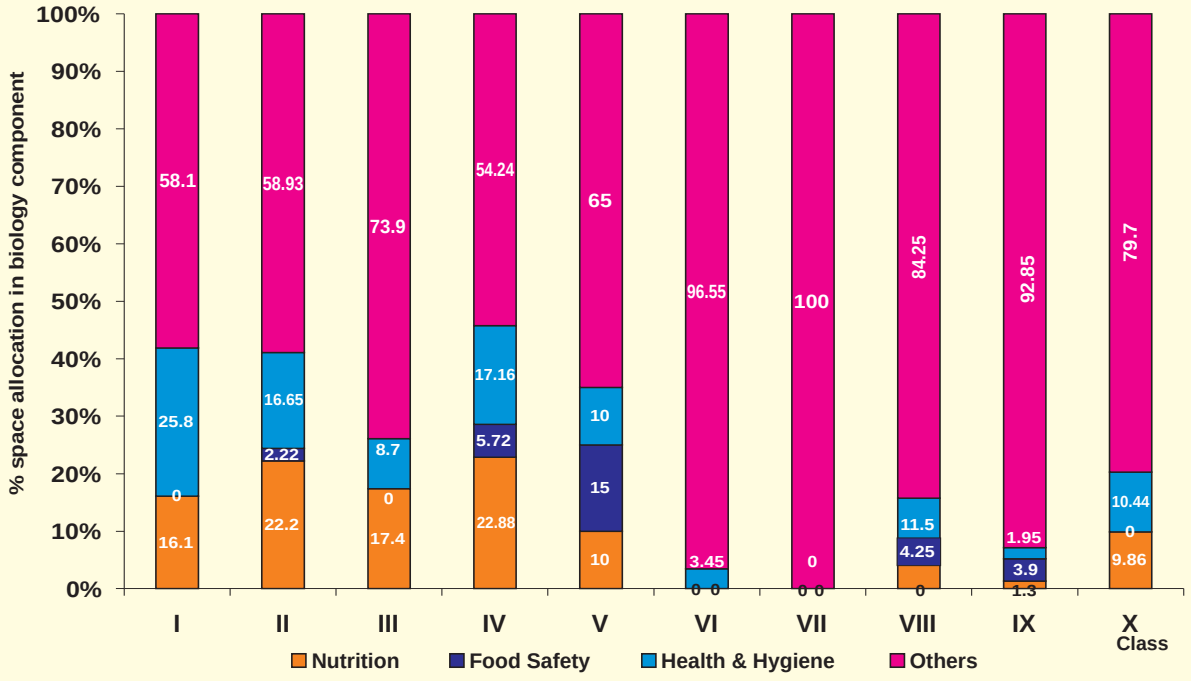


Figure-31: Subject-wise allocation of space (%) in Biology component of AP State Board textbooks



classes wherever nutrition topics are covered, topics like food groups, functions of foods and nutrition deficiency disorders are dealt with in greater or lesser detail. The data also indicated that the colour visuals and tables have been interspersed in the NCERT textbooks both for visual relief and easy understanding. However, in AP State textbooks, text dominates and visuals are simple black and white line drawings which sometimes are not easily comprehendible. In both the syllabi in all classes except in Classes I-X, the nutrition lessons appear in the back pages of the books.

Conclusions

The present study revealed that the space allocation for biology in relation to physical sciences is lesser in higher classes. Nutrition component is dealt at primary school level but very little at high school level. Nutrition in whichever class it is covered after class III in both NCERT and AP syllabi, it only deals with – Food groups or nutrient deficiency disorders. Similarly, food safety deals only with cleanliness of surroundings, not consuming fly swarming or insect infested foods.

The study clearly brings out the lacunae in the nutrition component covered in the school curricula. It could be recommended that many important topics such as nutrition and growth, link between childhood malnutrition and non-communicable diseases in adulthood, adolescent nutrition, nutrition for girl child, hidden hunger, lifestyle factors and obesity, nutrition during pregnancy and lactation, importance of breast feeding, unhealthy foods, fortification etc. be covered in the curricula. Considering that many of our earlier studies indicated that school based nutrition education is preferred mode of learning and effective way of education, the results of this study will be useful during future revisions of the textbooks for strengthening the nutrition and food safety components.

VII. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

GENOTOXICOLOGICAL EFFECTS OF PESTICIDES IN AGRICULTURAL FARMERS IN GUNTUR DISTRICT

The wide spread use of pesticides and exposure is a health hazard. Farmers are exposed to pesticides during spraying operations. Although million cases of pesticide toxicity are documented every year around the World, there is only limited data available on its cytogenetic effects. In addition acute exposure to pesticides leads to generation of free radicals which include oxidative stress, lipid peroxidation and alterations in antioxidant status in animals and humans**.** Hence, a study was conducted in cotton growing farmers in Guntur district. These cotton growing farmers use complex mixture of pesticides predominantly when compared to the other districts of Andhra Pradesh. Therefore, the study was taken up to assess the extent of toxicity in their blood samples by analyzing the different test parameters which are the best toxicity indicators of exposure assessment.

Aims & Objectives

1. To assess the toxicity of the commonly used pesticides viz., organochlorines, organophosphates etc in the agricultural farmers of Guntur district by Acetylcholine Esterate inhibition.
2. To assess the cytogenetic changes and also the DNA damage in the blood of agricultural farmers of Guntur district by chromosomal aberrations (CA), lymophocyte micronucleus (MN) test, and sister chromatid exchange (SCE).

A survey was completed in the 24 randomly selected villages of Guntur district and interview schedules were also administered to 624 (312 experimental and 312 control) subjects by adopting a stratified proportionate random sampling procedure. A statistically viable sub sample of about 400 human volunteers (about 200 each from both control and experimental groups) was selected for the collection of blood samples. All the subjects were analysed for AchE inhibition, oxidative stress parameters, liver and kidney function tests. Out of the 400 blood samples only 152 (76 each) subjects were used for cytogenetic studies due to logistics. The samples were also tested for haematological analysis such as haemoglobin, packed cell volume etc.

The results of the cytogenetic analysis indicated that out of the 4,547 metaphase plates scored in the exposed subjects, 213 (4.7%) found to have CAs. Similarly in the un-exposed group out of the 3,267 metaphase plates analysed 55 (1.7%) were found to be positive for CA (Table 31). This showed a significant difference (P<0.001) between the exposed and controls. A significant increase in CAs in the agricultural farmers exposed to pesticides indicates that chronic/sub chronic occupational exposure to complex mixture of pesticides is genotoxic. In the present investigation, 102 of the subjects from exposed group were found to be positive for micronuclei (0.15%)

Table 31. Chromosomal aberrations & Satellite Associations in the exposed and un-exposed individuals

Group	No. of subjects	No. of CA	%CA	Metaphases with SA	%SA
Experiment	76 (No. of cells scored 4547)	*213	4.7	1736 (No. of cells scored 4547)	38.1
Control	76 (No. of cells scored 3267)	55	1.7	703 (No. of cells scored 3278)	22.2

*(P<0.001)

where as in the un exposed group 89 (0.13%) subjects were found to be positive for micronuclei. In contrast to the evaluation of CA, the scoring of MN in lymphocytes is simple, sensitive and fast. Therefore, MN assay is the important biomarker that allows the evaluation of both clastogenic and aneuploidogenic effects in a wide range of cells, since they are detected in interphase (Table 32).

Table 32. Micronuclei in the agricultural and non-agricultural subjects

Group	No. of cells screened	No. of micronuclei	% of micronuclei
Tests (76)	68,153	*102	0.15%
Control (76)	69,757	89	0.13%

*(P<0.001)

The frequency of SCE in exposed group was not statistically significant when compared to control subjects (Table 33).

Table 33. SCE analysis in the study population

Group	Total no. of plates	Total no. of Exchanges	SCE/cell Mean?SD
Experiment (76)	1207	(87.3%)	2.8?1.03
Control (76)	1754	1402(80%)	3.0?2.03

It can be concluded from the present study that agricultural farmers using a complex mixture of pesticides had erythrocyte cholinesterase inhibition and increased levels of peroxidation when compared to control group.

The results of the AchE activity in RBC indicated that there was a significant ($p<0.0.5$) decrease in the RBC AchE activity of the experimental subjects when compared to controls. Lipid peroxidation in terms of thiobarbituric acid reactive substance (2.68 ± 0.056 nm) was significantly increased when compared to control subjects ($p<0.01$), while the antioxidants such as reduced glutathione (40.52 ± 1.50 µg/mg protein), and α-tocopherol in experimental group (7.58 ± 0.22 mg/ml) were significantly reduced ($p<0.01$) when compared to control subjects. There was a significant reduction in the level of GSH in the experimental subjects while the activity of catalase in the experimental subjects increased when compared to control subjects.

As regards the liver and kidney function tests such as SGPT and urea there were no significant difference observed between the control and experimental subjects. No abnormal haemato-logical profiles were found between the agricultural farmers occupationally exposed to complex mixture of pesticides and non-agricultural subjects.

The KAP studies indicated that majority of the agricultural farmers did not use any protective devices. This could be one of the important reasons for the current observations.

Biomonitoring studies suggest that there is a need for awareness campaigns and educational programmes for agricultural farmers highlighting the importance of safety measures which need to be adopted while handling the pesticides to minimize the risk of genotoxic hazards against their health at the farming community level.

VIII. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES (NCLAS)

A.SERVICE ACTIVITIES

1. Breeding and Supply of Animals

During the period 37,959 animals were bred and of which 34,356 animals were supplied including the parent institution. There was a decrease of 5.4% in breeding and 13.3% in the supply of animals. The income generated from animal supply was Rs.42.73 lakhs which was 3.5% less than the previous year. The details of individual animals species and strains bred and supplied are shown in table 34 and 35.

2. Supply of animal Feed

a. Stock animal feed

A Total of 27,914 kg of animal feed (25596 kg of rat/mouse feed; 2318 kg of g.pig/ rabbit feed) was supplied during the period generating an amount of Rs.21.83 lakhs. There was a decrease of 15.8% in the supply of feed and decrease of 11.6% in the amount generated during the reported period.

2. Experimental Animal Feed

Need based supply of experimental animal feed of 804 kg, was continued during the period and the details are shown in table 36. Income generated from this activity is Rs.2.25 lakhs.

3. Supply of Blood and Blood products

During this period 110ml blood, 40ml sera and 2712 ml plasma [total 2862ml] were supplied to 11 institutions on 62 different occasions. A sum of Rs. 4,19,560 was realized towards this activity. In addition 220 ml of blood was also supplied to internal needs of the institution.

4. Health Monitoring

A total of 925 samples from various colonies were taken for microbiological monitoring during this period. Samples were taken from following animal strains - Mice - BALB/c (50), C57/6J (56), FVB (48), Nude (02), Nude hetero (50), Swiss (78); rats - WNIN (161), SD (63), Fischer (48), Kyoto (02); Hamster – Syrian (20). Fecal samples were also taken from Rabbits (123) and G. Pigs (120). In addition other samples such as water (14), diet (13), bedding (11) and equipment (66) were also taken for testing.

The above samples were collected from (1) a sample of animals supplied (1%) from November 2007 onwards and (2) randomly from the higher age group of animals available from the colony. The results of the study are shown in table 37 (mice) and table 38 (rats).

The following organisms were found to be present both in mouse and rats colonies:

E.Coli, Kl.Spp, Pseudomonas .Spp, Proteus Spp. A calcovar anitrat, Bacillus.Spp, Corynebacterium Spp, Stephylococcus Spp, Streptococcus Spps, Listeria monocytogenes, Kluyvera Spp, Serratia Spp and *Micrococcus Spp.*were isolated from the above animals.

The results showed that most of the organisms were not present at the age of one month, they started making their appearance from the second month onwards. When you look at the animals from more than 6 months of age they harbored all the organisms that are shown in the table 37 & 38. It can be concluded that the ambient air in the facility at present favours the growth of several organisms and they are harboured by the time they become more than 6months of age. With the renovation of the facilities with higher air capacity handling unit, it is expected that by next year we will be able to bring down the incidence of several micro-organisms.

Necropsy of six-month-old rat strains like WNIN,WKY,and Fischer strains showed tumors, liver cysts due to Teania taeniformis and spleenomegaly.(Table 37 and 38). Ectoparasites Myobia and Mycoptes sp were found in general in almost all strains of Mice.

Table 34. Details of breeding and supply of different species and strains of laboratory animals eduring the period from 1.4.07 to 31.3.08

Sl. No.	Species	Strain or Breed	Stock as on 1.4.07	Total Number of animals								Balance as on 31.3.08
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Culled		
										Old age	Sick ness	
1	Mouse	BALB/c An. N (inbred)	491	7336	7827	301	7086	7387	-	-	-	440
		C57BL/6J (inbred)	724	4180	4904	108	3798	3906	4	-	-	994
		N:NIH(S) Nude (inbred)	337	316	653	286	139	425	78	-	-	150
		NCr.Nude	100	873	973	74	361	435	196	-	-	342
		FVB/N (in bred)	605	285	890	582	-	582	-	-	-	308
		Swiss (in bred)	423	8070	8493	669	6973	7642	66	-	-	785
2	G. Pig	N:HART (Hartley)	84	1538	1622	-	1299	1299	58	-	-	265
		N:NIH (Coloured)	112	807	919	-	744	744	43	-	-	132
3	Rabbit	New Zealand white	75	184	259	2	132	134	20	-	-	105
4	Monkey	Macaca mulatta (Rhesus)	24	-	24	-	-	-	-	-	-	24
	TOTAL		**2975**	**23589**	**26564**	**2022**	**20532**	**22554**	**465**			**3545**

Table 35. Details of breeding and supply of different species and strains of laboratory animals during the period from 1.4.07 to 31.3.08 .

Sl. No.	Species	Strain or Breed	Stock as on 1.4.07	Total Number of animals								Balance as on 31.3.08
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Culled		
										Old age	Sick ness	
1	Rat	CFY/NIN (inbred)	93	27	120	-	-	-	19	-	-	101
		Fischer 344 N (inbred)	154	64	218	41	8	49	63	10	-	96
		Holtzman (inbred)	55	18	73	-	-	-	20	-	-	53
		SD (Sprague Dawley) (Outbred)	398	2384	2782	780	1111	1891	123	22	-	746
		Wkyoto (inbred)	77	15	92	2	-	2	22		-	68
		WNIN (inbred)	1270	9863	11133	621	8739	9360	204	333	-	1238
		WNIN/GR-Ob	534	609	1143	70	-	70	34	411	-	628
		WNIN/Ob-Ob (inbred)	753	722	1475	62	-	62	35	576	-	802
2	Hamster	Golden (inbred)	321	668	989	32	336	368	45	208	-	368
3	Sheep		1	-	1	-	-	-	-	-	-	1
			3656	**1437.0**	**18026**	**1608**	**10194**	**11802**	**565**	**1560**	**-**	**4099**
Total of Tables 39 & 40				**37959**		**3630**	**30726**	**34356**				

Table 36. Experimental feed

No	To whom supplied	Type of diet	Qty (kgs)
1	Sri Krishnadevaraya University College, Ananthapur	Fructose	25
2	Leila Neutraceuticals, Vijayawada	High fat diet	50 30 50 40
3	Zydus, Ahmedabad	Fructose Sucrose	40 100
4	O.U., Hyderabad	Galactose control	14
5	PCT, Hyderabad	SMF Diet	30
6	CCMB, Hyderabad	High fat Cholesterol Sunflower oil	27 8 13
7	NBRC, Haryana	Iron deficient diet Protein diet	15 30
8	NIN, Hyderabad	NTP 2000 Low protein Normal DAG Oil Sheaoline oil diet	40.5 kg 40.5 kg 40.5 kg 27 kg 55.8 kg

Screening for pathogenic viruses

A total of 443 sera samples were tested for 8 different rodent viruses; of which 213 belonged to mice and 231 belonged to mice.

Rats were tested for Sendai, Lymphocytic Choromeningitis Virus [LCM] and Mouse Encephalomyelitis [MEV] viruses.

Results: It was found that Sendai was positive in WNIN-1/8, Holtzman - 2/8, and Kyoto-2/7; MEV was found in Holtzman - 3/16, Kyoto - 2/16 strains and LCM was not found in any of the rat strains tested (Table 39.)

Mice were tested for Pneumonia virus of mouse [PVM], Reo 3, Mouse Hepatitis Virus [MHV], LCM and Minute Virus of mouse [MVM] viruses.

Results: It was found that all the 29 samples tested for PVM were negative, where as out of 46 samples tested Reo 3 virus was found in BALB/c -3/10, Swiss -1/9, FVB/N - 4/9 and nude hetero - 7/9 mice strain samples.

MHV was found positive in 39 of the 46 samples of different strains; they were FVB/N - 8/8, Nude hetero-8/8, C57BL6J-7/8, Swiss - 8/8, BALB/c - 6/8 and Nude- 2/6. LCM virus was tested in 46 mice samples and 18 were positive as given; FVB/N -1/8, Nude hetero - 2/8, C57BL6J - 7/8, BALB/c -7/8, Nude -1/6 and Swiss mice- 0/8. All MVM 46 samples tested showed Negative (Table 39).

Apart from the in-house samples, mice sera [17] received from a local institution were also tested for PVM virus and found negative. This service realized Rs. 7650/-.

Sick animals

A total of 32 animals belonging to rats- SD-18, Fischer-13 and Rabbit-1 have been reported sick during this period. They were euthanized and samples were collected for Microbiology and Histopathology.

In addition a total of 36 experimental Swiss mice from PCT studies were also taken and subjected to microbiological monitoring protocols.

5. Human Resource Development:

Both the regular training programmes (supervisors and technicians) were conducted during the year.

In the junior level course, LATTC, 11 participants were trained and in the senior level course, LASTC, 9 participants were trained in laboratory animal sciences. In addition two persons from Ranbaxy and Panacea Biotech underwent ad hoc training for a period of 1-3 weeks.

Two staff from M/s.Sugan Life Sciences, Tirupathi and 3 from M/s. Claris Life Sciences, Ahmedabad underwent adhoc training. The center also provided teaching support to the students of DMLT course (from a local hospital) for a period of 3 days.

The center also took 3 students of B.Tech Biotechnology and M.Sc., for their dissertation work in animal physiology or health monitoring.

Table 37. Microbial colonies detected in different mice strains

Organisms Isolated	BALB/c (50)		C57BL/6J (56)			Swiss (78)		FVB-N (48)	Nude-Hetero (50)	Nude (2)
	1m (4)	>6m (46)	1m (4)	2m (4)	>6m (48)	1m (4)	>6m (74)	>6m (48)	>6m (50)	2m (2)
A.calcovar	N	26	N	N	15	N	30	14	22	01
Bacillus	N	30	N	N	10	N	22	12	10	01
E.coli	02	40	03	04	40	04	58	45	34	01
Listeria	N	42	N	N	30	N	60	40	26	01
Micrococcus	N	20	N	N	16	N	21	22	18	01
Klebsiella	01	22	02	02	20	02	30	20	30	01
Staphylococcus	03	46	04	04	40	04	58	40	25	02
Streptococcus	03	42	03	04	40	04	58	40	34	02
Klyuveria	N	N	N	N	N	N	N	N	N	N
Coryne	N	N	N	N	N	N	N	N	N	N
Pseudomonas	N	N	N	N	N	N	N	N	N	N
Proteus	N	N	N	N	N	N	N	N	N	N
Tumors	N	03	N	N	25	N	06	09	N	01
Spleen Enlarged	N	40	N	N	01	N	08	06	N	N
Ecto Parasites	03	40	04	04	42	04	68	40	45	N
Liver Cyst	N	08	N	N	11	N	34	12	12	N

Values in brackets are the total number of samples tested. N - Not detected

Table 38. Microbial colonies detected in different rat strains

Organisms isolated	WNIN (161)			S D (63)		Fisher (48)	Kyoto (02)	Hamster (20)
	1m (6)	2m (34)	>6m (121)	2m (2)	>1year (61)	>1year (48)	>1year (02)	>1year (20)
A.calcovar	N	N	60	N	32	20	01	08
Bacillus	N	N	42	N	18	15	01	08
Coryne	N	24	80	02	46	40	02	08
E.coli	06	24	82	02	46	40	02	08
Listeria	N	22	86	02	46	44	02	N
Klebsiella	N	14	42	N	25	20	01	08
Klyuveria	N	08	24	01	14	14	01	06
Micrococcus	N	08	22	N	16	12	01	10
Pseudomonas	N	06	28	01	18	20	01	18
Proteus	N	10	32	01	16	18	01	05
Staphylococcus	05	20	89	02	46	45	02	16
Streptococcus	05	20	86	02	44	42	02	16
Tumors	N	N	30	N	17	08	01	02
Spleen Enlarged	N	04	24	01	18	04	01	01
Giardia spp.	N	N	N	N	N	N	N	20
Liver Cyst	N	N	62	N	40	12	02	12

Values in brackets are the total number of samples tested. N - Not detected

Table 39. Prevalence of viral antibodies in rat and mouse strains

No	Virus	No. of samples tested	Rat strains						Results
			WNIN	SD	Fisher	Holtz-man	CFY	Kyoto	
1	Sendai	46	1/8	0/8	0/8	2/8	0/7	2/7	5 /46 positives
2	LCM	92	0/16	0/16	0/16	0/16	0/14	0/14	All 92 negatives
3	MEV	92	0/16	0/16	0/16	3/16	0/14	2/14	5 /92 Positives
Total		230	1/40	0/40	0/40	5/40	0/35	4/35	10/230 positives

No	Virus	No. of samples tested	Mice strains						Results
			BALB/c	Swiss	C57/6J	FVB/N	Nude Hetero	Nude	
1	PVM	29	0/6	0/6	0/6	0/5	0/6	---	All 29 negatives
2	Reo 3	46	3/10	1/9	0/9	4/9	7/9	---	15/46 positives
3	MHV	46	6/8	8/8	7/8	8/8	8/8	2/6	39/46 positives
4	LCM	46	7/8	0/8	7/8	1/8	2/8	1/6	18/46 positives
5	MVM	46	0/8	0/8	0/8	0/8	0/8	0/6	All 46 negative
Total		213	16/ 40	9/39	14/39	13/38	17/39	3/18	72/213 positives

6. Research Support

Pre Clinical Toxicology (PCT)

During the period 10 research projects from NIN and PCT were approved by IAEC for implementation. The PCT studies on Skim Milk Fermentate, ABFN and Tetravalent vaccine were completed during this period. Other studies are also in progress.

B.RESEARCH ACTIVITIES

EFFECT OF LONG TERM EXERCISE IN WNIN OBESE RATS

Exercise has been shown to be beneficial for reduction of body weight as well as to increase glucose tolerance in diabetic individuals. Since the center has been projecting that WNIN/Ob and WNIN GR-Ob rats as good models for studying human obesity and NIDDM, it was thought appropriate to study effect of exercise on these animals.

Aims and Objectives

The objective of the study is to evaluate low and high intensity exercise over a period of 90 days on the plasma glucose, insulin and lipid profile in the obese mutant rats with normal Wistar rats serving as controls.

Methodology

Twenty eight rats each from WNIN/Ob and WNIN/GR-Ob were taken at 35 days of age and divided into two groups of low (12 animals from each group) and high (16 animals from each group) intensity. Each group was again divided into half, 6 each in the case of low intensity and 8 each in the case of high intensity from all the three strains. While one group underwent exercise other group served as an unexercised control in each strain. The low intensity regime consisted of 15 minutes exercise twice a day at 15 rpm in a rota rod treadmill. The high intensity exercise consisted of 30 minutes duration twice a day at 30 rpm. At the end of the exercise the following parameters were estimated:

I. Total body composition (lean body mass, total body fat, percentage body fat, total body

sodium and potassium and water) were determined in TOBEC instruments using coil ID 3076 and 3117.

2. Glucose tolerance test: Fasting blood samples were collected from the supra orbital venous plexus of the eye. Each animal was administered oral glucose (250 mg/100 gm body weight) and the blood was drawn after 1 and 2 hours. Plasma glucose and plasma insulin were determined in all the three samples.

3. Plasma triglyceride and total cholesterol were determined in the fasting blood sample.

4. Immediately after the exercise blood was drawn from supra orbital venous plexus and collected in heparinised tubes on ice. The samples were centrifuged and plasma separated immediately and analyzed for lactate.

Results

Body composition

The body weight of all the three strains were reduced significantly (P<0.05) following exercise when compared to their unexercised controls. Similar results were observed with both low and high intensity exercise. There were also significant reductions in LBM, total fat mass and fat free mass in the exercise group when compared to controls (P<0.05). The reduction in body fat was 15.49% in WNIN rats, 14.64% in WNIN/Ob and 19.69% in WNIN GR-Ob rats when they underwent low intensity exercise. High intensity exercised animals showed the following reductions: 19.25% in WNIN, 22.56% in WNIN/Ob and 24.65% in WNIN/GR-Ob rats. There were also significant reduction in total body water and total body sodium in exercised animals (P<0.05). However, total body potassium levels were significantly increased.

Glucose tolerance and plasma insulin

Area under the curve (AUC) for glucose were significantly higher in the unexercised obese and GR-Ob groups when compared to controls. Following exercise there was a significant reduction in the AUC for glucose in all three exercised groups (both low and high intensity) except in WNIN rats in the high intensity group. Reduction in AUC was 15% in WNIN rats, 25% in WNIN Ob and 25.2% in WNIN/GR-Ob in the low intensity groups. 22.5% in WNIN Ob and 19.9% WNIN/GR-Ob in high intensity exercised animals. Similarly plasma AUC for insulin levels also decreased for following exercise in control and WNIN GR/Ob rats but not in WNIN Ob rats.

Table 40. Effect of low intensity exercise on selected biochemical parameters (n=6)

Parameters	*Unexercised animals*			*Exercised animals*		
	WNIN	WNIN/Ob	WNIN/GR-Ob	WNIN	WNIN/Ob	WNIN/ GR-Ob
Glucose(mg/dl) (AUC)	269.94 ±19.83	284.38 ± 10.21	293.2 ± 20.59	229.44* ± 15.98	213.42* ± 20.79	219.29* ± 13.92
Insulin (µU/mol) (AUC)	130.16 ±10.23	195 ± 18.25	263.5 ± 25.65	67.33* ± 8.65	191.5! ± 24.65	180.41* ±12.92
HOMA I.R	12.63 ±1.2	17.17 ±1.89	18.98 ±2.23	5.41* ±0.92	12.87* ±2.56	11.26* ±2.29
Triglycerides (mg/dl)	39.01 ± 5.27	94.68 ± 4.5	118.56 ±17.22	30.31* ± 2.68	83.53* ±4.02	100.3* ±28.13
Cholesterol (mg/dl)	89.2 ±6.22	79.4 ±8.69	76.17 ±2.17	85.8* ±2.8	56.11* ±11.15	55.72* ±8.06

Values are mean ± S.E * P < 0.05 significant by Duncans Multiple ANOVA.
! No two groups are significant

Table 41. Effect of high intensity exercise on selected biochemical parameters (n=8)

Parameters	Unexercised animals			Exercised animals		
	WNIN	WNIN/Ob	WNIN/ GR-Ob	*WNIN*	WNIN/Ob	WNIN/ GR-Ob
Glucose(mg/dl) (AUC)	189.82 ±14.65	256.88 ±60.79	335.65 ±28.35	181.30* ±23.61	199.02* ±63.64	268.75* ±25.46
Insulin (µU/mol)(AUC)	114 ±10.25	295.5 ±28.65	370.5 ±42.65	97.41* ± 8.99	273.16* ± 18.62	271.33* ± 21.05
HOMA I.R	10.60 ± 1.05	17.87 ±2.23	19.14 ±1.89	6.25* ± 1.01	8.25* ±1.52	14.89* ±2.21
Triglycerides (mg/dl)	32.8 ± 11.19	119.68 ±18.75	251.39 ± 13.58	31.43! ± 11.78	97.72* ±19.09	164.16* ±22.15
Cholesterol (mg/dl)	82.9 ±18.32	144.39 ±23.02	106.34 ±10.16	68.39* ±11.92	111.85* ±17.53	97.6* ± 6.91

Values are mean ± S.E * P < 0.05 significant by Duncans Multiple ANOVA.
! No two groups are significant

However, when insulin resistance was assessed in terms of HOMA IR index all the exercised animals showed reduction in HOMA IR when compared to controls (Tables 40 & 41).

Plasma lipid profile

There were significant differences in plasma triglyceride levels between normal WNIN rats and both the mutant rats. Following exercise, there were marginal reductions in the plasma triglyceride levels in both the exercised groups when compared to their controls. Plasma cholesterol levels was not altered in the low intensity exercised WNIN rats. Other strains of rats in the low intensity group and all the 3 strains in the high intensity group showed reduction in plasma cholesterol level (Tables 45 and 46).

Plasma lactate

Following exercise lactate levels increased significantly in the exercised animals when compared to controls. The intensity of increase is more with low intensity exercise. When compared to 63.1% in WNIN, 68% in WNIN Ob, and 72.9% in WNIN/GR-Ob at low intensity exercise, it was 27.6% in WNIN, 38.2% in WNIN/Ob and 58.2% in WNIN/GR-Ob rats at high intensity exercise levels.

Conclusion

The study showed

1. That exercise improves glucose tolerance and reduced insulin resistance in all three strains of rats tested.
2. There were only marginal changes by doubling the intensity of exercise.
3. Simultaneous decrease in LBM, total body sodium and water suggests that there is extra cellular fluid loss in exercised animals when compared to controls.
4. This is also reflected in terms of increased total body potassium.
5. Exercise also had positive effect on reducing plasma triglyceride levels.
6. The alterations in plasma lactate levels suggest that the glucose utilization is higher with low intensity exercise when compared to high intensity exercise.

IX.PRE-CLINICAL TOXICOLOGICAL STUDIES

1. PRE-CLINICAL TOXICITY EVALUATION OF TETRA-VALANT VACCINE (DPT + HEP B)

Tetravalent Vaccine (Diphtheria, Tetanus, Pertussis + Recombinant Hepatitis B antigen) is a combination-immunizing agent developed with an intention to promote as prophylactic agent against Diphtheria, Tetanus, Pertussis and Hepatitis B by eliciting immunity in sufficient doses.

There are very few Tetravalent vaccines which are very important for preventing diseases like Diphtheria, Tetanus, Pertussis and Hepatitis B. Indian Immunologicals has prepared this Tetravalent vaccine as per DCGI guidelines, Schedule Y, Drugs and Cosmetic (Second Amendment) Rules, 2005, Government of India following GMP.

Objectives

1. To assess the safety profile of Tetravalent vaccine which elicits immunity against the four diseases (Diphtheria, Pertussis, Tetanus, Hepatitis B).
2. To Test the allergenic potential of tetravalent vaccine.

Materials & Methods

The test material tetravalent vaccine (TV), DPT and Hep-B has been obtained from sponsor along with certificate of analysis. The intended clinical dosage schedule for infants is 6^{th}, 10^{th} & 14^{th} week. The present investigation involves acute toxicity test (14 days) in swiss albino mice & Sprauge dawley rats.

Sub chronic toxicity test has been carried out in Swiss albino mice and Guinea pigs. In acute toxicity test, mice and rats were exposed once to highest dose (10 times of intended therapeutic dose) by sub cutaneous route and observed for lethality. In sub chronic toxicity test Therapeutic Dose (TD) of DPT, TD of Hep- B, TD of TV and five times of TD of TV in a clinical dose.

Food intake and body weights of all the animals were monitored bi-weekly as well as live phase of animals, (cage side observations, physical and neurological examination) site of injection etc., along with clinical chemistry, Haematology, necropsy, Histopathological and genotoxicity profile.

Rectal temperature was recorded 1-day prior and one day after each administration of the Test compound. Data was compiled and analyzed for significant difference between treatment groups and vehicle control.

The results of this study are as follows:

- No pre-terminal deaths in acute toxicity test.
- No significant treatment related effect on food intake, body weight, clinical signs and behavioral activity was recorded in Mice and Guinea pigs.
- No significant changes in haematological parameters.
- No significant changes in clinical chemistry parameters.
- No specific test compound-induced pathological changes.
- No immunotoxicological effects.
- No evidence of any genotoxicological effects.

Conclusion

No abnormalities in physical, physiological, clinical chemistry, hematological, pathological, immuno-toxicological and genotoxicological parameters were observed on sub-cutaneous administration of test compounds supplied by sponsors at various doses and durations under the experimental conditions investigated as per DBT Guidelines.

2. SAFETY / TOXICITY STUDIES OF AYURVEDIC BHASMAS (VN & WN)

Ayurvedic formulations are classified in to various groups viz., Kadla (decoction), churnas (Powder), Bhasmas (Mineral + herbal preparations) etc. Bhasmas are calcined powder of metals, minerals, gems etc. Traditional literature has provided standard guidelines to prepare such formulations in non-toxic, therapeutically potential formulation. As per the traditional system, these formulations are recommended to treat chronic Neurological disorders diseases viz., Liver disorder, Arthritis, Diabetes, Neurological disorders and sold as rejuvenator substances. However In the recent past presence of metals in such formulations sold at grocery shops in international market are reported and can be potential toxicant.

In view of this CCRAS has proposed a multi–centric pre-clinical safety evaluation of various herbomineral formulations as per international guidelines. The present investigation is proposed to assess the safety of products as per the international guidelines.

Objective

To assess the Pre clinical Toxicity of coded Vn & Wn Ayurvedic Bhasmas as per the International Guidelines.

Materials & Methods

Two test formulations coded as Vn & Wn recommended in a clinical dose of 30mg and 60mg respectively for 3-4 weeks have been provided by the sponsor. The present investigation involves acute toxicity test (14 days) in swiss albino mice, Sub acute toxicity test (30 days) and long term term toxicity test (120 days) in swiss albino mice & WNIN Rats.

In acute toxicity test, mice were exposed once to highest dose (10 times of intended therapeutic dose) by oral gavage and observed for lethality. The test compound has been administered daily with 33% honey water (v/v) for 15 days and 30 days in Sub acute toxicity, long term toxicity test respectively in various dose levels viz. Therapeutic Dose (TD), Average Dose (TDx5) and High Dose (Tdx10).

Food intake and body weights of all the animals were monitored bi-weekly as well as live phase of animals, cage side observations, physical and neurological examination along with clinical chemistry, Haematology, necropsy and Histo-pathological profile. Data was compiled and analyzed for significant difference between treatment groups and vehicle control.

The results of this study are as follows

Acute Toxicity Test: No lethality was recorded in mice and rats after a single exposure to 50 times of therapeutic dose till 14th day.

Subacute Toxicity Test: 10% pre-terminal deaths were recorded in mice, exposed to TD & 5 times of TD in both Vn and Wn Bhasmas. No pre-terminal deaths were recorded in rats. No significant treatment related effect on food intake, body weight gain, clinical signs, behavioral activity etc in the survived animals, no significant changes in hematological parameters, no significant changes in clinical chemistry parameters, were observed.

Long Term Toxicity Test: Pre-terminal deaths were recorded in VC (10%), TD (15%), AD (10%), HD (10%) in mice which received Vn and Wn Bhasmas for 90 days. Mortality was 5% in rats exposed to Wn. No significant treatment related effect on food intake, body weight gain, clinical signs, behavioral activity etc. was noted. No significant changes in hematological and clinical chemistry parameters were found.

Conclusion

Maximum mortality has been recorded in mice as compared to mortality in Rats. In the animals which survived no abnormal toxicity was recorded in relation to food intake, body weight gain, clinical signs, behavioral activity etc. The Hematological and clinical chemistry profile was normal. The observations related to Histopathology and genotoxicology are in progress.

3. PRE-CLINICAL TOXICITY EVALUATION OF SKIMMED MILK FERMENTATE (SMF)

The Skimmed Milk Fermentate (SMF) having bacteriocin type activity has been developed by indigenous technology, with an intention to promote it as a bio-preservative for Indian dairy products. The SMF has been produced by fermenting skim milk with a bacteriocinogenic (bacteriocin producing) strain of food grade lactic acid bacterium, *Pediococcus pentosaceus*, isolated from Cheddar cheese. National Dairy Development Board is keen to exploit this product for its use as a preservative in commonly consumed dairy products in India. In view of this a preclinical toxicology study is proposed to ensure its safety.

Objective

To assess the safety profile of SMF a bio-preservative for dairy products.

Materials & Methods

Methodology

The test material Skimmed Milk Fermentate (SMF) has been provided by the sponsor. The intended daily dietary intake (DDI) of SMF was calculated (1.2gm/day i.e.0.001%w/w). The present investi-gation involves acute toxicity test (14 days) in swiss albino mice and WNIN rats and Sub chronic toxicity test in WNIN Rats. In acute toxicity test, mice and rats were exposed once to highest dose of test material (10 times of intended therapeutic dose) by oral gavage and observed for lethality. Sub chronic test has been conducted in Rats (Wistar NIN), received the diet containing 0.2%, 1%, 2% SMF. In addition a group of animals received the diet with low protein (30%), and less fat (15%) which is considered equivalent to poor man's diet.

Food intake and body weights of all the animals were monitored bi-weekly as well as live phase of animals, cage side observations, physical and neurological examination along with clinical chemistry, Haematology, necropsy and Histopa-thological profile. Data was compiled and analyzed for significant difference between treatment groups and vehicle control.

Results

Acute Toxicity Test: No lethality was recorded in mice/rats after a single exposure to Maximum quantity of SMF.

Subchronic Toxicity Test

There were no pre-terminal deaths except one animal which died on 62nd day of drug esposure receiving 2% SMF.

No significant treatment related effect on food intake, body weight gain, clinical signs, behavioral activity etc. were observed.

The following are in progress:

Hematological parameters.
Clinical chemistry parameters.
Histopathological Observations.
Genotoxicity Observations.

INSTRUMENTATION SERVICES

Instrumentation department is responsible for the upkeep of all Instruments in the Laboratory, new and old, and also in the preparation of technical documents for procurement of equipment. The main activities of the Department include component level trouble-shooting, installation of new equipment, imparting training to staff of the Institute and routine maintenance of all equipment.

The following new equipment were installed in the year 2007

S.No	Name of the equipment	Make & Model no.
1.	Auto sampler	Agilent
2	VAC Pressure Pump	Millipore
3	Deskjet Printer	HP
4	On-Line UPS	Power One
5	Gamma Isotope Counter	Perkin Elmer
6	UPS	ITON
7	I-125 Gamma Counter	PARA
8	Xerox Machine	Sharp
9	Software for E-800 Microscope	Media cybernitics
10	Microwave Oven -2nos.	LG
11	Digital Portable Radiation Survey Meter	Nucleomix
12	LCMS	Thermo
13	UPS On-Line	Power One
14	Fax Machine	Panasonic
15	Confocal Microscope	Leica
16	Microwave Digestion System	CEM
17	Body Composition Analyser	Tanita
18	Microwave Digestion system (HSCC supply)	CEM
19	1 KVA UPS System -4nos.	Compact
20	Gradient PCR	Eppendorf
21	Table Top Ref. Centrifuge	Eppendorf
22	Double beam UV VIS Spectrophotometer -2nos.	Hitachi
23	Microplate Washer	ThermoElectron
24	Deskjet Printer	HP
25	3KVA UPS	Power one Micro system
26	0.5KVA UPS System	Dixit Infotech
27	Reverse Osmosis Plant	Vivek
28	Refrigerator – 2 nos	LG
29	Refrigerator	Godrej
30	Refrigerator – 5 nos	Samsung
31	Deep Freezer	Vestfrost
32	Hepa Filter Module	Laminar Flow
34	Humidity Temperature Meter	Thomas Sciencitific
35	Air Curtain	Excel Engineers
36	Nitrogen Cylinder	Everest
37	Fume Hoods	Laminar Flow
38	Air-Curtain	Excel
39	-80° Deep Freezer – 4 nos	Cryo Scientific
40	-20° Deep Freezer	Vestfrost

S.No	Name of the equipment	Make & Model no.
41	Modular Cold Room	Eakcon
42	Bio-safety Cabinet	Laminar Flow
43	Split AC – 10 nos	Voltas
44	Split AC – 7 nos	Bluestar
45	Window AC	Blue Star
46	Deep Freezer	Vestfrost
47	Cryocan	Indian Oil
48	Helium Cylinder	Everest
49	Gas Cylinder	Everest
50	pH Meter – 7 nos	Orion
51	Ion selective fluoride analyzer	Orion
52	CO2 Incubator	Thermo Forma
53	Water Purification system	Millipore
54	Fat Determination system	Shimadzu
55	Microarray SAystem	Genomic Solutions
56	Iso Electric focusing Unit	Bio Rad
57	Electronic weighing scale	Eagle
58	Top loading balances – 5 nos	Sartorious
59	Vacuum pump	Promivac
60	Freeze Drier	Christ
61	Skin fold Calipers	Cranlea
62	LCD Projector	Epson
63	Shaking Water bath	Labtech
64	Roto shaker	Scientific Industries
65	Portable Hygrometer -2 nos	Extech
66	CO2 Incubator Shaker	Labline Thermo
67	Universal Bone strength Testing Machine	Lloyd Instruments
68	Conference Audio System	Beyer Dynamics
69	On-Line UPS-3 KVA -2nos.	Power One MicroSystems
70	UPS 500VA -4nos.	APC Make
71	ICP-MS	Perkin Elmer
72	5 KVA UPS – 5 Nos.	KELTRON
73	Capillary Electrophoresis	Agilent Technologies
74	Electronic Weighing Balance	Essae-Teraoka
75	Vert. dual Midi gel & Electron Transfer dual system	Bangalore Genei
76	PD Quest S/W for Gel Electrophoresis System	Bio-Rad
77	Electronic Balance Semi micro balance	Sartorious

The following staff of the department attended meetings:

B.V.Prasanna Kumar

- Participated in the Technical Committee and Negotitation Committee meetings for procurement of equipment for the year 2006-07, at ICMR Hqrs, New Delhi (23rd – 29th March 2007).
- Training programme on Calibration for Mass and Temperature Measurement at CFTRI, Mysore (3rd – 5th Oct. 2007).

LIBRARY & 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 dessimination 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>.

The Library has continued to provide an excellent Photostat support to the Scientists, technical as well as to the administrative staff. Resource Sharing and User Education Programmes etc are continuously being undertaken by the Library. One month training given to one of the Junior Assistant staff of Tuberculosis Association of Andhra Pradesh, Hyderabad in the management of Library and Automation. 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).

British Library Institutional membership is renewed for 2007 and Corporate Membership for "Universities Federation for Animal Welfare, UK" for the year 2007 has also been taken out during the year under report.

MODERNISATION OF LIBRARY AND INFORMATION NETWORK

The following work has been taken up and the equipment is procured for strengthening the services of dissemination of Information to the scientists.

a) ICMR has renewed the subscription to **Proquest Medical Library Full Text Database** of the journals. During the period total of **2250** Proquest ML Full Text Database Searches were made.

b) Subscription of JCCC@ICMR and J-Gate has been renewed by Indian Council of Medical Research through M/s. Informatics India Pvt. Ltd., Bangalore, JCCC@ICMR covers more than **475** journals received collectively at 24 Institutions/ Centres Consortia of ICMR Libraries. And **J-Gate** is an electronic gateway to global e-journals literature. It is presently has massive database of journal literature indexed from more than 10,000 e-journals with links to full text at publisher sites and provides free access to full-text of 1700+ journals with e-author e-mail address and also one can find the availability of the journal in a local library.

c) The following equipment is procured for the library

I) Mobile Compactor System, Steel Racks – 1

ii) Data Capture Unit – 1 No.

iii) HP COMPAQ PC – 4 Nos.

iv) 3M Electronic Library Security Systems – 2 Gates

v) HP DVD Writer – 1 No.

vi) UPS – 7 Nos.

NEW JOURNALS ADDED

Foreign Journals

1. Cereal Research Communication
2. Communication Research
3. CRC Critical Reviews in Toxicology
4. Food & Agricultural Immunology
5. Food Chemistry
6. Health Education and Behaviour
7. Journal of Applied Toxicology
8. Journal of Food Composition & Analysis
9. Journal of Pediatrics Gastroenterology and Nutrition
10. Journal of the Science of Food and Agriculture

11. Journalism and Mass Communication

12. Mycopathologia

13. Toxicological Letters

14. Toxicological Sciences

Indian Journals

1. Indian Development Review
2. Indian Journal of Developmental Research & Social Action
3. Indian Journal of Environmental Protection
4. Indian Journal of Millennium Developmental Studies
5. Indian Journal of Occupational and Environmental Medicine
6. Indian Journal of Occupational Health
7. Indian Journal of Social Development
8. Indian Journal of Toxicology
9. Journal of Agricultural and Food Economics
10. Journal of Ecotoxicology Environmental Monitoring
11. PEARL
12. Pestology
13. Research Journal of Biotechnology.

The following library services were expanded as detailed below:

1. NEW ADDITIONS

Books	-	215
Reports	-	529
Journals (New Subs.)	-	27
Thesis / Dissertations	-	18
CDROMS (MEDLINE)	-	119

2. OTHER ACTIVITIES

Journals Bound	-1,339
Visitors using the Library	- 2,787
Circulation of Books/Journals etc	- 1,269
MEDLINE Abstracts provided	- 2,000
No. of E-mails sent outside	- 1,454
No. of E-mails received	- 3,175
Photocopying (No.of pages)	4,52,813
Number of Annual Reports mailed	- 460
No. of Books/Journals received	- 25
No. of Duplicate Journals sent out	- 200
No. of INTERNET Searches provided	- 150
No. of Reprints sent	- 150
Proquest Full Text Database searches provided	- 2,250

3. TOTAL LIBRARY COLLECTIONS

Books	- 16,911
Journals (Bound Volumes)	- 29,342
Journals subscribed for 2007	- 312
Journals received (Gratis/Exchange)	- 310
Microforms (Microfiche)	- 1,080
Slides	- 280
Reports	- 12,050
Theses & Dissertations	- 367
MEDLINE CDROMS Discs	- 383
Current Contents on Diskettes with Abstract	- 664
Proquest (Full Text E-Journals) on CD ROMS	- 490

Ph.D PROGRAMMES

Ph.D AWARDEES

Research Scholar/ staff	Year	University	Title of thesis
1. Rita Saxena	2007	Osmania	Role of food processing on antioxidant activity and development of recipes with high antioxidant activity
2. Anil Kumar P	2008	Osmania	Molecular chaperone function of alpha crystallin under hyperglycemic condition: modulation by dietary factors

Research Scholars Registered for Ph.D

Research Scholar /staff	Title of thesis	Guide
1. Aruna B. (2002)	Biophysical characetrisation of resistin	Dr. Nasreen Z. Ehtesham
2. Haseeb A (2002)	Understanding the mechanism of action of PPAR ? as a link molecule between obesity, Type 2 diabetes and CHDs	Dr. Nasreen Z. Ehtesham
3. Kiran Kumar B. (2002)	Genetic typing of WNIN/Ob and WNIN/ GR-Ob strains using microsatellite markers	Dr.Giridharan N.V
4. Megha Saraswat (2003)	Screening of aldose reductase inhibitors and antiglycating agents from dietary sources and assessing their anticataractogenic potential	Dr.Bhanuprakash Reddy G.
5. Mrudula. T (2003)	Characterisation and significance of a novel fatty acid elongase of the eye lens	Dr.Bhanuprakash Reddy G.
6. Prashant A. (2003)	Role of scavenger receptor class B1 (SR-B1) in reticulocyte differentiation, absorption of fat and fat soluble vitamins and female infertility using WNIN/Ob rat model	Dr.Vajreswari A.
7. Md.Naseeruddin (2004)	Understanding the role of resistin in inflammatory process leading to Type 2 diabetes	Dr.Sudeep Ghosh
8. Padmavathi I.J.N. (2004)	Role of maternal chromium status in the development of insulin resistance in the offspring	Dr.Raghunath M.
9. Satyanarayana B. (2004)	Biological significance of phytoferritins	Dr.Madhavan Nair K.
10. Sreenivasulu K. (2004)	Caco -2 cell as a model to study bioavailability, mechanism of absorption and cytoprotective effects of zinc	Dr.Madhavan Nair K.
11. Vasuprada I. (2005)	Bio-response of a model Caco -2 cell system of iron and zinc	Dr.Madhavan Nair K.

Research Scholar /staff	Title of thesis	Guide
12. Shashikiran G (2005)	*In vitro* regeneration of the insulin secreting cells from the adult pancreatic ductal epithelial cells (progenirors/stem cells)- The role of specific nutrients	Dr.Vijayalakshmi V.
13. Sheril Alex (2005)	PUFA-rich oil diet supplementation on body weight regulation of obese rat model of WNIN/GR-Ob strain: A nutrient-gene interaction study	Dr.Vajreswari A.
14. Rajkumar (2005)	Charecterization and differentiation of pancreatic progenitor/stem cells (Nestin positive cells) to insulin secreting cells-the role of specific micronutrients	Dr.Vijayalakshmi V.
15.Manisha Ganeshan (2005)	Foetal origins of adiposity and insulin resistance: Role of peri/postnatal manganese status	Dr.Raghunath M.
16. Vara Prasad SSS (2005)	Role of 11?-HSD1 in pathogenesis of obesity and insulin resistance in WNIN/GR-Ob and WNIN/Ob restrains	Dr.Vajreswari A.
17. Sainath P.B (2005)	Insulin, insulin receptor and its signaling mechanism(s) in the brain and insulin sensitive target organs in the WNIN/ob & WNIN/GR-ob rats	Dr.Raghunath M.
18. Pratibha B. (2005)	Immune status of WNIN mutant rats with reference to leptin and obesity	Dr.Giridharan N.V.
19. Sreevani M. (2005)	Understanding & dissecting the role of resistin in etiology of insulin resistance using obese rat model	Dr. Nasreen Z. Ehtesham
20. Yadagiri Reddy P. (2006)	Biochemical studies on obesity induced cataractogenesis using WNIN obese rat model	Dr.Bhanuprakash Reddy G.
21. Naga Bala Shankara Srinivas P. (2006)	Studies on the significance of ?-crystallin heteropolymer in the eye lens	Dr.Bhanuprakash Reddy G.
22. Anand Kumar K. (2006)	Maternal vitamin B12 restriction induced changes in body adiposity, hyperglycemia and insulin resistance in WNIN rat offspring: Molecular basis of the changes	Dr.Raghunath M.
23. Priyanka Shanker (2006)	Study on high fluoride and low calcium on bone metabolism in rats: biochemical mechanisms	Dr.Arjun L. Khandare
24. Y. Srinivasa Reddy (2006)	Effect of environmental lead exposure on infection and immunity in undernutrition	Dr.Kalpagam Polasa
25. Kalluri Dileep (2008)	Establishment of propgable cell lines from pancreas tissue of embryo and adult WNIN Obese rats (WNIN/Ob & WNIN/GR-Ob)	Dr.Vijayalakshmi V.

AWARDS/ HONOURS CONFERRED ON SCIENTISTS

Name of the Scientist	Award/Honour
Dr.B.Sesikeran	Elected as a Fellow of Andhra Pradesh Akademi of Sciences.
Dr.G.Bhanuprakash Reddy	"DBT Overseas Associateship – 2007" for specialized training in Niche areas for a period of 3 months
	ICMR Excellent Research Output Grant", by ICMR, New Delhi.
	"Research to Prevent Blindness- International Research Scholars Award" for the year 2007 by Research to Prevent Blindness, USA
Dr.V.Sudershan Rao and Dr.S.Vasanthi	Selected as the members of the Joint FAO/WHO Expert Committee on Food Additives (2007-2011) – Food additives and contaminants.
Dr.B.Dinesh Kumar	CDRI oration award for the year 2007 by the Indian Pharmacological Society.
Dr.Devindra	Best Paper Award, 2007 (Siroh Award) of Indian Society for Plant Physiology for the Paper entitled "Reduction of raffinose oligosaccharides in red gram flour by microbial α - Galactosidase".
Ms.Vasuprada Iyengar	Best Poster award in Experimental Nutrition by Nutrition Society of India for her paper entitled "Iron and zinc interactions during uptake are determined by cellular zinc status Caco - 2 cells".
Ms. Sisha Prince Varghese	"Sagarlal Goenka Award" by Indian Dietetic Association in the national conference held at Kottayam, Kerala for her paper entitled "Evaluation of the impact of nutrition education module on life style diseases among institutionalized elderly women".
Mr.K.Sreenivasulu	Best Poster Award for the paper entitled "Zinc inhibits oxidant induced iron uptake and oxidative stress in Caco-2 cells: Role of mineral interactions and iron regulatory protein–1", at the SFRR Satellite India 2008 Conference, organized by All India Institute of Medical Sciences, New Delhi.
Sheril Alex	Young Scientists' Award in Experimental Nutrition for the Paper entitled "Diverse effects of PUFA on glucose intolerance, dyslipidemia and obesity in glucose intolerant obese rat model of WNIN/GR-Ob strain", at 39th Nutrition Society of India, 2007.

PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS/ WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING

Date	Scientist	Conference/Meeting/Workshop/Seminar
2007		
May 6 -10	Dr.G.Bhanuprakash Reddy & Ms.T.Mrudula	Annual Meeting of Association for Research in Vision and Opthalmology, at Fort Lauderdale, Florida, USA. Dr.Bhanuprakash Reddy and Ms.Mrudula presented papers on "Photoreceptor degeneration in WNIN -Ob rat" and "Effect of curcumin on retinal vascular endothelial growth factor in diabetic rat" respectively.
July 10 -13	Dr.N.Balakrishna	10th European Nutrition Conference, at Paris. Made a poster presentation on paper entitled "Association between anthropometric measurements and hypertension: A study among rural elderly in India".
June 15, 2007 - May 15, 2008	Dr.Bharathi Kulkarni	Course on Master of Public Health, at John Hopkins School of Public Health, Baltimore, USA.
July 9 – Dec. 14	Dr.R.Hari Kumar	Training Programme on "Bioethics and Ethics Committee Administration", at Seattle, USA.
Aug. 15 – Nov. 15	Dr.G.Bhanuprakash Reddy	DBT Short Term Associateship 2006 -07, at University of Michigan, USA.
Sept. 9 -13	Dr.Kalpagam Polasa, Dr.A.Lakshmaiah, DR.K.Madhavan Nair	X Asian Congress of Nutrition, at Taipei, Taiwan
	Dr.B.Sesikeran	Presented a Paper on "Constantly evolving safety assessment protocols for GM foods" in the symposium entitled "GM Foods".
	Dr.GNV.Brahmam	Prevalence of micronutrient deficiency disorders in rural areas of select States in India
	Dr.N.Harishankar	Effect of long term exercise on body composition in WNIN obese rats
	Dr.P.Ravinder	Effect of dietary factors on Caco-2 cell zinc uptake:Implications in assessing zinc bioavailability
	Dr.P.Sujatha	Characterization of procyanidins, apigenin, daidzein and genestein in pearl millet by HPLC-DAD-MS
	Dr.P.Ramulu	Hypoglycemic effects of soluble fiber (galacto-mannan) isolated from fenugreek seeds in mutant obese rats

Date	Scientist	Conference/Meeting/Workshop/Seminar
Oct. 22- Nov.1	Dr.GNV.Brahmam	"Training of Master Trainers" on WHO Growth Standards, to be organized by World Health Organisation, at Bali, Indonesia.
Dec. 12 - 14	Dr.B.Dinesh Kumar	Regional Meeting on the "Role of Education in Rational Use of Medicines", at Bangkok, Thailand.
2008		
March 3 - 7	Mr.T.Longvah	International Symposium on "Underutilized Plants for Food, Nutrition, Income and Sustainable Development", organized by AVRDC- The World Vegetable Center and International Centre for Underutilised Crops (ICUC), at Tanzania. Presented Papers on "*Perilla Frutescens* an underutilized traditional oilseed of Northeast India for food, nutrition, income and sustainable development" and "Mainstreaming the use of nutrient-rich underutilized plant food resources in diets can positively impact on family food and nutrition security – Data from Northeast India and West Africa".

WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN

I. Workshops/Conferences/Seminars

1. Brainstorming Session on Fortified Foods, sponsored by Department of Biotechnology, New Delhi (April 17-18).
2. Meeting of the Scientific Advisory Committee of NIN/FDTRC/NCLAS (Aug. 1-3).
3. Main Committee and Sub-Committee meeting of "Archives of Dr.Yellapragda Subbarow's publications at NIN" (Aug. 10).
4. 2nd Annual Forum of Food and Nutrition Security Community, organized in association with Solution Exchange and National Institute of Nutrition (Aug.17-19).
5. 2nd Sub-Committee meeting of the RCGM to finalise the protocol on "Toxicity and allergencity studies on new transgenic crops" by Dept. of Biotechnology, New Delhi. (Aug.22).
6. Meeting on "Prevalence of infertility in India", by Indian Council of Medical Research, New Delhi (Aug. 22).
7. Brainstorming session on "Nutrition Promotion among School Children". The session was attended by Principals of local schools, representatives from Recognised School Teachers Association, NGOs (Sept. 1).
8. A one day State-level Workshop on "Nutrition Promotion for a Stronger Nation" in associ-ation with Food and Nutrition Board (Govt. of India) and Department of Women Develop-ment & Child Welfare (Govt. of A.P) (Sept. 4).
9. A two-days Workshop on "Right to Information Act" for administrative staff of the institute in collaboration with the Institute of Secretariat Training and Management, DOPT, Govt. of India, New Delhi (Oct.4-5).
10. A Policy discussion on the Rs.2/ kg PDS Rice Scheme (Oct.12).
11. A one-day Workshop on "Right to Food", as part of World Food Day celebrations, in association with Association of Food Scientists and Technologists (Hyderabad Chapter) and Oil Technologists Association of India (South zone), Hyderabad (Oct. 16).
12. International Workshop on "Leadership Skills in Nutritional Sciences" (Nov. 13-14).
13. XXXIX Annual National Conference of Nutrition Society of India (Nov. 15-17).
14. Meeting of the NNMB Steering Committee (Nov. 21).
15. Brain storming session on "Setting up a Regional Fluoride Research Institute" at National Institute of Nutrition (Dec.17).
16. Joint WHO/FAO Inter-country Workshop on "Food and Nutrition Policy and Plans of Action" (Dec. 17-21).
17. Birth Anniversary of Dr. Yellapragada SubbaRow. On this occasion, Dr.S.K.Bhattacharya, Addl. Director-General, ICMR, New Delhi, laid foundation stone for the Dr.Yellapragada SubbaRow Electron Microscope Block and released a book on collected works of Dr.SubbaRow (Jan. 11, 2008).
18. FAO-NIN Sub-regional Training Workshop on "Improving the quality and safety of fresh fruits & vegetables: A practical approach"(Feb.4- 8).

II. Training Programmes

1. An adhoc training programme for two WHO fellows from Myanmar in the field of "Public health and nutrition" (4th June – 27th July, 2007).
2. Laboratory Animal Technician's Training Course. 11 participants were trained in the Course. (18th June - 31st July, 2007).
3. 35th Annual Training Course on Endocrino-logical Techniques and their Applications. Ten candidates participated in the course along with two WHO sponsored candidates (Aug 16 – Sept. 28, 2007).
4. Laboratory Animal Supervisor's Training Course. 9 participants were trained in the Course. (3rd Sept. to 30th Nov, 2007).
5. XXXXV Post-Graduate Certificate Course in Nutrition. A total of fourteen candidates from different States of the country and one candidate from Bangladesh participated in the Course (Jan. 2-March 14, 2008).

SERVICES RENDERED TOWARDS INCOME GENERATION

1. PATHOLOGY SERVICES

During the year, a total income of Rs. 3,02,520/- was generated from various projects of institute's preclinical toxicology and surgical pathology and cytology samples.

2. TRAINING PROGRAMMES

By admitiing 15 unsponsored private candidates and two WHO sponsored candidates to the three regular training courses and two WHO sponsored participants for ad-hoc training programme, an amount of Rs.1,90,000/- was generated.

SCIENTIFIC PUBLICATIONS

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

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6. Kalpagam Polasa : Nutrigenomics, diet and health. In " XL Annual National Convention of Indian Dietetics Association ", Theme: Dietetics – Itsolein changing lifestyles and diseases, 8th 9th Dec'2007 at Mammen Mappilla Hall, Kottayam, Jointly Organised by Indian Dietetic Association, Kerala Chapter & Dept. of Home Science, CMS College, Kottayam, Souvenir & Scientific Abstracts, p.31.

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SCIENTIFIC ADVISORY COMMITTEE

NIN/FDTRC

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Centre for Cellular and Molecular Biology
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New Delhi – 110 067

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Reproduction Development & genetics
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New Delhi – 110 067

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Bareilly, Uttar Pradesh – 243 122

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Project Director
National Institute of Pharmaceutical
Education and Research
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Dr.V.Ravi
Professor & Head, Neurovirology
NIMHANS, Bangalore – 560 029

Dr.T.S.Rao
Adviser, Department of Biotechnology
New Delhi – 110 003

Dr.V.V. Khole
Officer-in-Charge
National Institute for Research in Reproductive
Health,
Parel, Mumbai – 400 012

ICMR Officials

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New Delhi – 110 029

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RHN Division
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New Delhi – 110 029

Dr.S.Mokkapati
Scientist F, RHN Division
Indian Council of Medical Research
New Delhi – 110 029

Dr.G.S.Toteja
Scientist F, RHN Division
Indian Council of Medical Research
New Delhi – 110 029

Shri Sanjiv Dutta
Financial Advisor
Indian Council of Medical Research
New Delhi – 110 029