

ANNUAL REPORT

2005 - 2006



National Institute of Nutrition
(Indian Council of Medical Research)
Hyderabad - 500 007

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National Institute of Nutrition
(Indian Council of Medical Research)
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आचार्य एन. के. गांगुली
महानिर्देशक

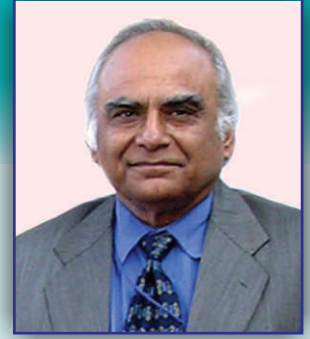
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MESSAGE

The Eleventh Five Year Plan places firm emphasis on alleviation of major nutritional problems prevalent in the country today, especially those arising due to micronutrient deficiency. Dietary inadequacy of certain micronutrients like iron, iodine and vitamin A among our population groups has been a major issue. This silent hunger having deleterious effect on the physical as well as economic health of our people, especially women and children, has become a major cause for concern to the academicians as well as policy makers. The need for well-devised research programmes to provide cost-effective, pragmatic solutions to combat multifactorial nutritional problems is being largely met through the research endeavors of NIN.

The laboratory, clinical and community studies being carried out at this premier nutrition research institute are indeed need-based and aim at providing much awaited answers to several nutritional complexities affecting our society.

I am happy to state that the institute has readily responded to assist in disaster management programme by assessing health and nutritional status of Tsunami-affected population groups. Their studies on tribal population groups also speak of the priority accorded to the health of the underprivileged sections of our society. There is a lot to deduce from the studies carried out at NIN on the molecular basis of non-communicable degenerative diseases such as link of -Crystallin protein between diabetes and cataract and role of resistin in diabetes. This year's research has unearthed some interesting facts relating to the prevalence of hypertension, diabetes, obesity, cancers and other degenerative diseases in the rural communities. Some basic studies involving micronutrients, dietary fats and food fortification hold the potential of impacting on large-scale nutritional interventions in the country. NIN's studies in the areas of pre-clinical toxicology, laboratory animal science and nutrition extension also help to devise better research models for wider use.

I earnestly hope that the institute's research programmes turns out to be more need-based in the coming years and help solve different nutrition-related problems affecting our communities.

Prof. N.K. Ganguly

CONTENTS

Page No.

RESEARCH STAFF	i
RESEARCH HIGHLIGHTS	iii
I. COMMUNITY STUDIES	
1. Health and nutrition status of Tsunami affected population living in the relief camps in Andaman & Nicobar Islands	1
2. Prevalence of Vitamin A Deficiency (VAD) among preschool children of rural India	4
3. Assessment of diet and nutritional status of individuals and prevalence of hypertension in adults & anaemia among adult men and NPWL women in rural communities	6
4. Acceptability of micronutrient fortified millet based biscuits - A study among primary school children	8
5. Nutritional status of tribal population in ITDA project of Bhadrachalam in Khammam District, Andhra Pradesh	10
II. CLINICAL STUDIES	
MATERNAL HEALTH	
1. Obstetric outcome and proinflammatory cytokine response in women with genital tract infections	13
2. Effects of maternal nutrition in early pregnancy on placental development	15
III. BASIC STUDIES	
1. External validation of the National Facility for Dried Blood Spot Technology for Vitamin A Estimation	18
2. Iron and zinc bioavailability of representative Indian and US diets : Regional distribution and availability of iron and zinc from representative Indian diets	18
3. Wheat flour atta food fortification with micronutrients iron, folic acid and Vitamin A - Public Private Partnership	27
4. Effect of sesame lignans on the oxidative stability of edible vegetable oils	28
5. Role of n-3 PUFA in fetal programming of insulin resistance in off-spring : Biochemical and molecular mechanisms	29
6. Health beneficial effects of fruits and vegetables : Total phenolic content and antioxidant activity of dry fruits	33
7. Development of antioxidant rich recipes utilizing legumes as the base	34
8. B-Crystallin assisted reactivation of glucose-6-phosphate dehydrogenase	35
9. Effect of hyperglycemia on the expression of A- and B- crystallins under diabetic conditions	38
10. Effect of non-enzymatic glycation on structure and molecular chaperone function of -crystallin	40
11. Transcriptional analysis of resistin and identification of <i>cis</i> - and <i>trans</i> acting factors regulating resistin expression	42
12. Comparative structural analyses of human, mouse and rat resistin	44
IV. PATHOLOGY	
Cytokines profile and micronutrients in <i>plasmodium vivax</i> infection	47

V. EXTENSION & TRAINING	
A. Service Activities	49
B. Research Activities	
Development of communication strategies to improve nutrition and health related knowledge of NSS volunteers	52
VI. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE	
A. FOOD SAFETY	
1. Effect of magnesium compounds on mobilization of deposited fluoride in rabbits	54
B.CANCER AND XENOBIOTICS	
1. Antimutagenicity of heat processed ginger	57
2. <i>H Pylori</i> infection and <i>in vivo</i> nitrosation	58
3. <i>In vivo</i> nitrosation potential of population at high risk for gastric cancer	59
4. Ethnopharmacological validation of biodynamic compounds in traditional medicine	60
5. Role of nutrients in environmental toxicity	62
VII. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES (NCLAS)	
A. Service Activities	65
B. Research Activities	
1. PCR based DNA fingerprinting of WNIN strain and its obese mutants	69
2. Studies on energy metabolism in WNIN obese rat mutants	70
3. Genetic typing of WNIN/Ob and WNIN/GR-Ob strains using microsatellite markers	72
4. Establishment of baseline values of body composition and blood pressure in different species of laboratory animals maintained at NCLAS, NIN - A study in rat strains	74
VIII. PRE-CLINICAL TOXICOLOGICAL STUDIES	
1. Safety/ Toxicity Studies of Ayurvedic Formulations (a,b,c,d,e) (WHO Biennium Programme)	79
 INSTRUMENTATION SERVICES	 81
 LIBRARY AND DOCUMENTATION SERVICES	 83
 Ph.D PROGRAMMES	 85
 AWARDS/ HONOURS CONFERRED ON SCIENTISTS	 88
 PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS	 89
 WORKSHOP/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN	 90
 SERVICES RENDERED TOWARDS INCOME GENERATION	 91
 SCIENTIFIC PUBLICATIONS	 92
 SCIENTIFIC ADVISORY COMMITTEE	 96

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RESEARCH HIGHLIGHTS

In its relentless efforts, the National Institute of Nutrition (NIN) continued its research in different thrust areas pertaining to human nutrition and health. As the life expectancy is increasing the prevalence of degenerative diseases is also on the rise. Keeping this in view, National Nutrition Monitoring Bureau (NNMB) carried out surveys on the prevalence of degenerative diseases and the dietary intake of the population in different states of the country. During this year under the Country Investment Plan, technology transfer for the development of micronutrient-fortified foods to the State Government enterprise was given priority. In addition, technical assistance was also provided to the AP State Civil supplies department to fortify and supply wheat flour to the community through Public Distribution System. Emphasis was given to other priority areas like tribal nutrition, beneficial effects of antioxidants in human health and also as stabilizing agents in edible vegetable oils. In addition, there is a paradigm shift in the nutrition research related to degenerative diseases. Fostering this vision, NIN has taken up new approaches in identifying molecular basis of degenerative diseases such as link of α -Crystallin between diabetes and cataract and role of resistin in diabetes. A series of studies on dietary fats, micronutrients, women's health, food safety and health benefits of ginger were also carried out. The role of NSS volunteers as change agents in nutrition education in the community was studied. Here are the highlights of the research carried out during the year:

1. COMMUNITY STUDIES

1.1 Health and nutrition status of Tsunami affected population living in the relief camps in Andaman & Nicobar Islands

A rapid survey was carried out during the months of April/May 2005, to assess the health and nutritional status of Tsunami affected population living in the relief camps. In addition, a survey was carried out in the two hostels (one each for boys and girls) in students studying in 10th and 12th standards

in the affected islands, to assess their nutritional status. A total of 2513 individuals from 28 relief camps established in nine Islands for Tsunami affected population in Andaman and Nicobar Islands were covered in the survey.

The levels of consumption of various food groups observed in the current survey were, however, better than those reported for their rural counterparts of mainland except other vegetables, milk & milk products, the intake of which was low. The median intake of various nutrients (per CU/day) by the households barring proteins was less than the RDA. The data revealed that the extent of undernutrition among preschool children in the relief camps of Andaman and Nicobar Islands was significantly lower than that reported for their rural counter parts of mainland. The girls were nutritionally at a disadvantage as compared to boys among both the Settlers and Nicobarese. The adult Nicobarese were better in their nutritional status as compared to the settlers.

1.2 Prevalence of Vitamin A deficiency (VAD) among preschool children of rural India

As a part of the survey on "Prevalence of micronutrient deficiencies" Vitamin A deficiency (VAD) was investigated among the vulnerable groups of rural population in the States of Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu and West Bengal. Out of the total of 71,591 preschool children from 633 villages 3,934 samples were analyzed for blood Vitamin A levels using Dry Blood Spot (DBS) technique to assess the sub-clinical Vitamin A deficiency.

The result suggest that about 62% (CI: 60.3, 63.3) of children in general, had blood vitamin A levels of <20 g/dL, indicative of high prevalence of sub-clinical vitamin A deficiency. The proportion ranged from a high of about 88% in Madhya Pradesh, through 79% in Kerala to about 50-60% in the remaining States. The prevalence of sub-clinical VAD (<20 g/dL) was significantly ($p < 0.05$) higher among 3-5 year children (63.1%, CI: 61.2, 65.0)

compared to 1-3 year children (59.6%, CI: 57.1, 62.1), with no significant gender differentials (Boys: 60.8%; Girls: 62.8%).

The prevalence was relatively higher (66.7%) among children who did not receive massive dose of vitamin A during the past 12 months, compared to those who received either one (61.6%) or two doses (56.3%). In the State of Kerala, where the prevalence of sub-clinical VAD was highest (79.4%), the proportion of children with dietary intake of vitamin A in amounts of <50% RDA was also high (91.8%) and that of coverage for massive dose of vitamin A was least (38.5%), compared to the remaining states surveyed.

The study revealed that the magnitude of prevalence of sub-clinical vitamin A deficiency was quite high in all the States including those states where the prevalence of clinical deficiency signs were either absent or very low. Therefore, there is a need to strengthen all the components of the National Programme for Prevention of Nutritional Blindness in the country.

1.3 Assessment of diet and nutritional status of individuals and prevalence of hypertension in adults & anaemia among adult men and NPNL women in rural communities

In view of increasing problems of diet related chronic diseases such as obesity, hypertension, etc a survey was carried out to assess the prevalence of obesity, hypertension among adult men and women (≥ 20 years). The prevalence of diabetes mellitus (DM)/ hyperglycemia was assessed in the State of Andhra Pradesh on a pilot basis. Estimation of haemoglobin was also carried out among adult men and NPNL women. A 24hr. dietary recall survey was also carried out to assess the food and nutrient intake of all the individuals, in every alternate HH [covered for socio-demographic information].

Obesity

The overall prevalence of abdominal obesity in terms of waist circumference (≥ 102 cms) was about 1% among men, while it was 7% among women (≥ 88 cms). The prevalence of abdominal obesity in terms of waist hip ratio (WHR) was 25% and ranged from as low as 10.1% in Madhya Pradesh to a high of 40.5% in the State of Kerala among men. It was 69%

in women and ranged from a low of 36.7% in the State of Maharashtra to a high 91.8% in Kerala.

Hypertension

The overall prevalence of hypertension (SBP ≥ 140 and/or DBP ≥ 90 mm of Hg) was about 25%. No gender differentials were observed in the prevalence of hypertension. The prevalence tended to increase with age, from 13.6% in 20-30 years group to 56.4% in ≥ 80 years age group. About 60-67% of the adults were aware of hypertension, while 2-3% were currently on treatment. The prevalence of hypertension was high among the adults, who were suffering from overweight/obesity (48%) compared to normals (26%).

Diabetes mellitus

Estimation of fasting blood glucose among adults was carried out only in the State of Andhra Pradesh. The prevalence of diabetes mellitus (FBS levels of ≥ 126 mg%) was about 4% among adult men, and about 3% among women. The proportion of impaired fasting glucose FBS levels of 110 to 126 mg% was about 2% each among men and women. About 48-56% were aware of diabetes mellitus, and about 2% were currently on treatment.

About one third of adult men were currently smoking, out of them three fourths were smoking since more than 10 years. The prevalence of diabetes mellitus was high among adult men and women who were overweight/obese (9%) compared to normals (2-4%).

1.4 Nutritional status of tribal population in ITDA project of Bhadrachalam in Khammam District, Andhra Pradesh

A special survey was carried out to assess the health and nutritional status of the tribal population in the project area. The district has a total tribal population of about 4.5 lakhs, mainly constituted by Koyas, Lambadas/Sugalis, and Kondareddis. The study revealed high rate of adult illiteracy, poor economic status, dependency on agriculture and allied occupations for livelihood, low intake of protective foods and gross inadequacy in the intake of micronutrients. The overall prevalence of undernutrition, though low compared to their tribal counterparts of the State, it was however, higher than

their rural counterparts. The prevalence of morbidities such as fever and diarrhoea was also relatively higher compared to that reported in rural areas, indicating the problem of poor environmental sanitation and personal hygiene. The coverage of beneficiaries for immunization and supplementation of massive dose vitamin A and IFA tablets was relatively good. Poverty and poor health seeking behaviour probably contributed to aggravate the situation.

1.5 Acceptability of micronutrient fortified millet based biscuits-A study among primary school children

Undernutrition continues to be a major public health problem in India, affecting a large section of the communities, the most vulnerable being young children and women of reproductive age groups. In addition to the existing mid day meal (MDM) programme, the Commissioner of Civil Supplies, Government of Andhra Pradesh proposed to supplement micronutrient fortified millet based biscuits as snacks to the primary school children. The biscuits were fortified with ragi/maize/jowar in levels, so as to provide 50% of the recommended allowances per day/child, by consumption of three biscuits with a total weight of 24 g. Sensory evaluation of the fortified biscuits was evaluated. All the three varieties of biscuits viz. jowar, maize and ragi based ones were found to be equally good.

With regard to the acceptability of the micronutrient-fortified biscuits among primary school children, about 90% of the children gave a score of 'good' to 'very good' for all the characteristics studied viz. appearance, colour, texture, flavour and taste with respect to all the three types of micronutrient fortified biscuits.

2. CLINICAL STUDIES

2.1 Obstetric outcome and proinflammatory cytokine response in women with genital tract infections

There has been extensive research on maternal infection and pregnancy outcomes in developed countries, relatively, little is known in the underprivileged poor communities of India, where the problem of infections is much greater. Association of IUGR and PTD with histological chorioamnionitis and local cytokine (IL8, TNFa) response was determined.

The results revealed that height and weight of mothers were associated with birth outcome, histologic chorioamnionitis was significantly associated with linear growth of babies. In addition to nutritional factors, other factors such as inflammatory response due to genital tract infections might play an equally important role in adverse birth outcome.

2.2. Maternal nutrition in early pregnancy affects placental development

A study was carried out to assess and compare the placental morphology by measuring the villous structure and vascular endothelial growth factor (VEGF) and placental growth factor (PLGF) expression from conception at 7-10 weeks of gestation, of low socio-economic status (LSES) and high socio-economic status (HSES) groups, in relation to their nutritional status. The study indicated the significant disparity in placental structure between the undernourished and well nourished at a comparable gestational period and is suggestive of a predominant hypoxemic placental development in these LSES women under the stress of undernutrition.

3. BASIC STUDIES

A. Micronutrients

3.1 External validation of the National Facility for Dried Blood Spot Technology for Vitamin A Estimation

The National facility for Dried Blood Spot (DBS) Technology for vitamin A estimation established at the Institute is operational since March 2004.

There was a good agreement between the DBS and plasma retinol analyzed at the facility at various time points and that analyzed at the Croft Technologies after a period of one year. Thus the performance of NIN DBS facility has been externally validated.

3.2 Iron and zinc bioavailability of representative Indian and US diets : Regional distribution and availability of iron and zinc from representative Indian diets

One of the main causes of iron deficiency anaemia is low dietary bioavailability of iron. It is, generally, accepted that iron and zinc deficiencies

frequently occur together because the dietary factors that impair iron absorption also affect zinc absorption. There are no RDAs for zinc in India. Therefore, it is important to obtain regional data on dietary intake and food composition and to measure iron and zinc absorption from several days of dietary consumption as meals.

There are regional, rural and urban differences in iron and zinc density and their in vitro availability, which are mainly due to the composition of major staple and phytate content in the diet. Modification of diet to improve iron and zinc availability can be achieved by replacing major staple either by improving iron content and/or minimizing inhibitor phytate. Ironically good sources of minerals are also good in phytates and the intake of absorption promoters such as fish, meat and ascorbic acid is very low.

B. Food fortification

3.3 Fortification of whole wheat flour (atta) with micronutrients iron, folic acid and vitamin A - Public Private Partnership

As part of the Public Private Partnership and under Country Investment Plan, the technology of fortification of whole wheat flour (atta) with micronutrients developed by NIN has been translated to fortify and supply wheat flour through fair price shop in the state of Andhra Pradesh on a pilot scale by the AP State Civil Supplies Department, Government of Andhra Pradesh. The fortified atta branded 'VIJAYA ENRICHED ATTA' provides iron - 60 mg, folic acid - 1.5 mg and vitamin A 3300 IU per kg of atta and is priced Rs.12/kg.

C. Dietary fats

3.4 Effect of sesame lignans on the oxidative stability of edible vegetable oils

Sesame (*Sesamum Indicum* Linn) has long been used as a traditional health food in India for its nutritional and medicinal value. Sesame contains substantial amounts of unique components, namely sesamin and sesamol. The higher stability of sesame oil has been attributed to its inherent lignans. The effect of sesamin and sesamol in enhancing the stability of edible vegetable oils was evaluated. The increase in antioxidant potential and

Radical Scavenging Activity (RSA) of Soyabean oil (SBO) or Sunflower oil (SFO) due to addition of lignans may possibly be due to synergism among sesame lignans and non-glyceride components of SBO (soya lignans, isoflavanoids) or SFO (phytosterols).

3.5 Role of n-3 PUFA in foetal programming of insulin resistance in offspring: Biochemical and molecular mechanisms

Long chain polyunsaturated fatty acids (LC-PUFA) of both n-6 and n-3 series are integral components of cell membrane and are important determinants of fetal growth and development. Studies on the effects of n-3 PUFA on fetal programming of biochemical and molecular parameters associated with insulin resistance suggests that maternal intake of Trans fatty acids (TFA) (from hydrogenated vegetable oils) may increase the susceptibility to biochemical/metabolic alterations known to be associated with increase in risk of chronic diseases.

ELOVL4 is a novel member of family of human fatty acid elongases involved in long chain fatty acids and whose function is essential for photoreceptor maintenance. These observations suggest that ELOVL4 expression may be related to n-3 PUFA nutritional status. Further, the decrease in retinal ELOVL4 expression associated with abnormality in retinal morphology in TFA fed groups suggests that TFA may affect retinal function and metabolism of long chain PUFA.

D. Antioxidants

3.6 Health beneficial effects of fruits and vegetables: Total phenolic content and antioxidant activity of dry fruits

Phenolic compounds present in fruits and vegetables are reported to have multiple biological effects including antioxidant activity (AOA). The phenolic content and antioxidant activity of some commonly consumed plant foods and some preliminary data on the antioxidant activity of a few fresh fruits as natural sources of antioxidants has been generated. Dry fruits are rich in antioxidant activity and phenolic compounds appear to be significant contributors to their antioxidant activity. Consumption of dry fruits may therefore augment the antioxidant status and protect against chronic diseases.

3.7 Development of antioxidant rich recipes utilizing legumes as the base

Phenolic compounds are the potent ubiquitous antioxidant substances present in plant foods. An attempt was made to generate the data base on the antioxidant activity (AOA) and phenolic content (PC) of plant foods commonly consumed by the Indian population and assess the effects of different types of domestic processing on these parameters. It also involves formulating AOA rich recipes based on the data generated and assessing the effect of the consumption of these recipes on AO status in human volunteers. The AOA of salad prepared with green gram sprouts with lemon, salt and pepper was the highest among the different salad recipes tested and the one prepared from Bengal gram sprouts was the next best.

E. Degenerative diseases

3.8 Diabetic cataract and chaperone function of α -crystallin

Prolonged diabetes, without proper management, can lead to various short-term and long-term secondary complications, including diabetic cataract. Accumulation of modified proteins due to unfolding and aggregation is the major molecular event in cataractogenesis. Chaperone-like function of one of the lens proteins, α -crystallin, is believed to be vital for not only to prevent protein aggregation in cataract formation but also to function as a stress mediator in many other stress conditions. Studies indicate that post translational modifications such as nonenzymatic glycation under diabetic conditions has a negative impact on the chaperone function of α -crystallin in terms of protecting enzymes against inactivation. Though, expression of α -crystallin is increased due to hyperglycemia induced stress in many tissues including lens, there is enhanced degradation and modification. These studies provide a link between chaperone function of α -crystallin and diabetic cataract. Further studies are under way to manipulate the expression and modification *in-vivo* chaperone function of α -crystallin by dietary agents.

3.9. Transcriptional analysis of resistin and identification of *cis*- and *trans* acting factors regulating resistin expression

The adipocytokine resistin, a member of a family of cysteine-rich proteins known as resistin-like molecules (RELM) is also shown to be involved in inflammatory processes. Previous studies have however highlighted that resistin impairs glucose tolerance and insulin action in mice. In addition, resistin also inhibits adipogenesis in murine 3T3-L1 cells. In order to further evaluate the role of these transcription factors in the expression of human resistin, an electrophoretic mobility shift assay was performed wherein the binding of AP-1, C/EBP and c-Rel to their respective cognate oligonucleotides was characterized.

Resistin promoter sequences containing the binding sites for C/EBP, AP-1 and c-Rel shows binding with nuclear extracts prepared from corresponding cells. These experiments clearly demonstrate that AP-1, C/EBP and c-Rel present in the nucleus bind to the resistin promoter and could thereby modulate the expression of human resistin.

4. EXTENSION & TRAINING DIVISION

In addition to the extension and training activities, the division has carried out research activities pertaining to nutrition education.

4.1 Development of communication strategies to improve nutrition and health related knowledge of NSS volunteers

A study (Phase-I) using NSS volunteers as change agents to educate the community on various aspects of nutrition was conducted. The NSS volunteers were selected from the colleges of urban and rural areas around Hyderabad.

The initial knowledge levels were significantly different among the NSS volunteers of rural and urban areas (ANOVA, $p < 0.001$). Intervention through nutrition education by using suitable communication materials improved the nutrition knowledge of NSS volunteers of degree colleges. Since the NSS volunteers are involved in community education programmes, such programmes help them to gain the nutrition knowledge which may in turn help them to educate the community on health and nutrition aspects.

5. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

A. Food safety

5.1 Effect of magnesium compounds on mobilization of deposited fluoride in rabbits

A study was conducted to assess the possible benefits of magnesium compound administration in fluorosis and its capacity to mobilize already deposited fluoride from the bones as well as to prevent new fluoride deposition and, toxicological potential of Mg salts on various organs. Simultaneous feeding of magnesium compound (milk of magnesia) reduces fluoride absorption suggesting a beneficial effect of magnesium hydroxide ingestion on fluoride retention and toxicity. Histopathology and haematological study showed that there was no adverse effect of magnesium compound in experimental animals.

B. Cancer and xenobiotics

5.2. Antimutagenicity of heat processed ginger

Spices are important constituents in the preparation of various foods in Indian culinary practices. A study was undertaken on the antimutagenicity of fresh and dry forms of ginger under commonly practiced culinary conditions. The antimutagenic effect of ginger was not altered in the extracts of ginger subjected to normal cooking conditions.

5.3. Ethnopharmacological validation of bio-dynamic compounds in traditional medicine

The results of earlier studies indicate that extracts (coded 4308,4212,3107,3223 & 5322) of plants, which are traditionally used as anti-inflammatory drugs have potential antioxidant activity as evaluated by battery of in vitro tests and ex vivo test (AR. 2002-04). The present investigation was therefore undertaken to validate its anti-inflammatory potential using standard experimental animal models.

The study results suggest that aqueous and alcoholic aqueous extracts of traditional preparations Rasna panchaka has potential anti-inflammatory activity as evident from decreased

exudate volume, reduced oxidative stress and modulate levels of TNF- α and IL-6. The biological plausibility as evident from the study suggest that water and water plus methanol extracts of Rasna panchaka can be considered as potential candidates in the treatment of rheumatoid arthritis.

5.4 Role of nutrients in environmental toxicity

The use of heavy metals like Lead (Pb), Mercury (Hg), Cadmium (Cd), Arsenic (As) etc. has resulted in the rise of their levels in environment resulting in exposure that is toxic to human health. Since one decade, reports mostly from developed countries suggest that the heavy metals (Pb, Cd, Hg, As etc.) used in industries, induce slow progressive and most of the times, irreversible damage to the nervous, haemopoietic and renal systems in population. In addition, few reports indicate their interaction with nutrients (Fe, Zn, Cu, Mg, Ca etc.) and alteration in biochemical functions specially at sub-cellular /cellular levels. The important physiological functions of essential metal ions like Iron (Fe), Zinc (Zn), Copper (Cu), Magnesium (Mg) etc. have been well established. Among the various heavy metal toxicities reported, lead toxicity is reported from all parts of the world. The study suggested that among those screened 70% had lead level above 10 μ g/dl. The haemoglobin was inversely correlated with blood lead levels of 15 μ g/dl. The serum iron levels were found to be high with blood lead levels. The correlation between zinc, Iron and lead levels indicates the interaction of nutrients and pollutants.

6. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

6.1 PCR based DNA fingerprinting of WNIN strain and its obese mutants

Two mutant obese rat strains, WNIN/Ob and WNIN/GR-Ob were developed from the existing WNIN rat colony, which is being maintained at NCLAS in an inbred status for the past 84 years. Both the mutants are obese, but WNIN/GR-Ob has impaired glucose tolerance additionally. A study was undertaken to establish genetic identity for these two obese mutant rats. The cloned product from WNIN/GR-Ob were expressed both in mutant and parental strain and thus not unique to the mutant.

6.2. Establishment of baseline values of body composition and blood pressure in different species of laboratory animals maintained at NCLAS, NIN - A study in rat strains

National Centre for Laboratory Animal Sciences (NCLAS) is maintaining different species of laboratory animals for biomedical research both for in house use as well as supply to other institutions. As the center is catering to the needs of several institutions including for pre-clinical toxicology testing, it has become necessary to establish normal physiological and biochemical values in the most commonly used strains of laboratory animals. Since rat strains are the most frequently used animals, initial studies were taken up in different strains maintained at the centre viz., Wistar/NIN (WNIN), Sprague Dawely (SD), Fischer - 344N (F-344N), Wistar Kyoto (WKY), CFY and Holtzman.

The study showed that there were significant differences between strains of rats in terms of body composition, physical activity, serum clinical chemistry and blood pressure. By virtue of higher body weight for age in SD rats, their total body fat was also significantly higher than other strain of rats. However, it is WNIN male rats, which had higher percentage of body fat, higher resting time, higher plasma tryglycerides, higher heart rate when compared to other strain of rats. This was followed by Wistar Kyoto strain. The Fischer-344N rats showed the least growth rate, higher night time activity. These studies show that there were differences between strains and between genders in the same strain.

7. PRE-CLINICAL TOXICOLOGY

7.1. Safety/toxicity studies of ayurvedic formulations (a,b,c,d,e) (WHO Biennium Programme)

The traditional use of Ayurvedic formulations is one of the widely accepted therapy especially in the treatment of chronic diseases viz. arthritis, asthma, infertility, rejuvenation etc. The data on safety of the Ayurvedic formulations has become important for wider global acceptance of these products.

The coded Ayurvedic formulations developed by CCRAS, MoH & FW are reported to have potential therapeutic activity in chronic diseases and hence were taken up for pre-clinical toxicity screening as per WHO guidelines. Safety of five Ayurvedic formulations "a,b,c,d&e" by acute/sub-acute toxicity tests in mice/rats were evaluated as per the protocols suggested by sponsor.

7.1.1. Acute

No mortality, morbidity, weight loss and abnormal behaviour was recorded after a single exposure of a test compound with ten times of the recommended therapeutic dose after 14 days in Swiss albino mice which were exposed to the test formulations.

7.1.2. Sub-Acute

Pre-terminal deaths occurred in animals receiving (therapeutic dose) 1XTD (10%), 5XTD (30%) and 10XTD (60-70%) of formulation **a** between 14th day to 28th day, while the test compound **b, c, d** and **e** did not show any behavioral changes.

There were no pre-terminal deaths in animals receiving **b** test formulations at various dose levels. The physical and physiological activities, food intake and gain in body weights were not significantly different between groups exposed to **b** test compound and animals receiving vehicle. There were pre-terminal deaths with formulation **c** of males only (10%) in all the groups of animals receiving test formulations at various dose levels. There were no histopathological changes due to formulation **c, d** & **e** in all the major organs studied.

The Institute continued its endeavours to meet its mandate through various research projects that were initiated in IX Five Year Plan. Efforts are continued to identify newer emerging areas in different fields of nutrition research from time to time to develop need-based strategies to combat nutrient deficiency disorders in the country so as to meet the goals of the Country's National Nutrition Policy.

I. COMMUNITY STUDIES

1. Health and nutrition status of Tsunami affected population living in the relief camps in Andaman & Nicobar Islands

INTRODUCTION

Natural calamities like droughts, famines, cyclones, floods, earth quakes etc., in addition to causing devastating effects like loss of life and properties, also affects country's economy adversely, by decreasing agricultural and industrial output, increasing rural unemployment, migration of the poor to urban areas etc. thereby reducing purchasing power and increasing household food and nutrition insecurity. In addition, acute shortage of drinking water and inaccessibility to food contribute significantly to increased undernutrition, morbidities and mortalities in the communities.

On the morning of 26th December 2004, the country experienced a less known phenomena called 'Tsunami', meaning "harbor waves" in Japanese, which were generated due to earthquakes in the sea floor near Indonesia. These giant waves travelled across the sea and hit Andaman & Nicobar Islands and several villages in the coastal areas of Tamil Nadu, Pondicherry and Andhra Pradesh States. The most severely affected one was Union Territory of Andaman & Nicobar Islands, viz., Carnicobar, Katchal, Little Andaman, Kamorta, Teressa, Chowra and Great Nicobar.

The Administration of Andaman & Nicobar, Government of India and several national and international NGOs swung into action to provide immediate relief measures such as shelter, food, water, clothing and medical care to the affected populations by establishing relief camps at various sites. Subsequently, the Andaman & Nicobar administration, with the assistance of Government of India and several NGOs initiated various welfare programmes such as food for work, provision of rations including vegetables, construction of temporary shelters, establishment of dispensaries etc. In addition, 10th and 12th class boys and girls appearing for Board examinations in various

Islands for the current academic year were brought to Port Blair and were accommodated in hostels. They were provided with free food, new set of books etc., as well as special coaching classes, so as to enable them to appear for final examinations.

At the request of Andaman & Nicobar Administration, the National Institute of Nutrition, Hyderabad, carried out a rapid survey during the months of April/May 2005, to assess the health and nutritional status of Tsunami affected population living in the relief camps.

GENERAL OBJECTIVE

To assess the health and nutritional status of Tsunami affected population living in selected relief camps and temporary shelters in Andaman and Nicobar districts.

SPECIFIC OBJECTIVES

1. To assess the nutritional status in terms of anthropometry and prevalence of clinical signs of nutritional deficiency of Tsunami affected population living in relief camps.
2. To assess the average food and nutrient consumption at the household level and relief camps by weighment method of diet survey, and
3. To assess the prevalence of morbidities in the community during the previous fortnight.

STUDY DESIGN

It was a cross sectional study, carried out among inmates of relief camps established by Andaman and Nicobar administration.

Sample size and sampling procedure

Keeping in view the rapid nature of survey and constraints in the inter island transportation, it was decided to cover a sub-set of 28 relief camps out of 149, spread over in different parts of the Andaman & Nicobar Islands. In addition, a survey was carried out in the two hostels (one each for boys and girls)

wherein students studying in 10th and 12th standards in the affected islands, to assess their nutritional status in terms of anthropometry and clinical examination, and history of morbidity during previous fortnight.

INVESTIGATIONS

The following investigations were carried out during the survey:

- Measurement of heights and weights of all the individuals using standard equipment and techniques
- Clinical examination of all those covered for anthropometry for the presence of nutritional deficiency signs
- History of morbidity during the previous fortnight
- Average intake of foods and nutrients at the household level by carrying one day weighment diet survey in a sub-sample of HHs and at the relief camps by carrying out institutional diet survey in the community kitchens.

The salient findings were as follows:

A total of 2513 individuals from 28 relief camps established in nine Islands for Tsunami affected population in Andaman and Nicobar Islands were covered in the survey. Of these, 1485 were Nicobarese while the remaining 1028 were settlers from mainland, belonging to Andhra Pradesh, Tamil Nadu or Orissa.

Profile of the community

Among Nicobarese, a third of adults (34.3%) were cultivators, while about 11% were engaged either in agricultural labour or other labour. The proportion of individuals engaged in service and business were 8.1 and 1.1% respectively. A majority of the adult females (82.4%) were housewives. About half of the adult male settlers (52.8%) were engaged either in agricultural labour or other labour. Only negligible proportion (3.3%) was engaged in cultivation, while about 15% were in service or business.

FOOD INTAKE

In general, the average consumption of various foods (g/CU/day) at the household level (n=43), barring cereals & millets, pulses & legumes and roots & tubers was less than the recommended levels. The intake of protective foods like green leafy vegetables, other vegetables and milk & milk products was grossly inadequate. The levels of intakes were essentially similar between Nicobarese and settlers, except for other vegetables, roots & tubers and fats & oils, the consumption of which was relatively higher among the Nicobarese. The levels of consumption of various food groups observed in the current survey were however, better than those reported for their rural counterparts of mainland by NNMB except other vegetables, milk and milk products, the intake of which was low.

NUTRIENT INTAKE

The median intake of various nutrients (per CU/day) by the households barring proteins was less than the RDA. The intake of micronutrients such as vitamin A, iron, free folic acid and riboflavin was grossly inadequate in both the groups. The levels of intake of various nutrients were comparable between the Nicobarese and the settlers, barring vitamin C, which was grossly inadequate among Nicobares. This could be attributed to low intake of protective foods such as vegetables and fresh fruits.

The proportion of households with consumption levels of nutrients in amounts of < 50% RDA was maximum for vitamin A (92%), free folic acid (76%) and iron (63%) compared to other nutrients. Only about 4% of the households, at the time of survey, were found to be consuming protein and energy in amounts of less than 50% of RDA.

NUTRITIONAL STATUS

Prevalence of Clinical signs of nutritional deficiency

None of the infants examined exhibited clinical signs of nutritional deficiency. About 0.5% of preschool children (1-5 years) were emaciated. The prevalence of vitamin A deficiency signs such as conjunctival xerosis was higher (2.7%) as

compared to that reported for their rural counterparts of mainland (1.3%). The prevalence of clinical signs of B complex deficiencies such as angular stomatitis and glossitis was about 0.5% each. About 2.3% had dental caries. Among school age children, about 8.6% had conjunctival xerosis, the prevalence of which was higher than that reported for mainland rural counter parts (5%). The prevalence of Bitot spots was about 0.4%.

The prevalence of conjunctival xerosis and dental caries among adolescent children was about 3-4%, while that of Bitot spots was 0.6%. The prevalence of total goitre rate (TGR) was 7.8%. About 6% of adults had pallor, 3.2% had goitre and 2.9% had dental caries.

Anthropometry

Preschool children

Weight for age

The overall prevalence of underweight (weight for age < Median- 2SD) was about 48%, while that of severe underweight (< Median- 3SD) was about 11%. The prevalence of underweight observed was significantly lower ($p < 0.01$) than that reported for their rural counter parts in mainland (48% Vs 60%). The prevalence of underweight (< Median- 2SD) was significantly higher ($p < 0.01$) among girls (53%) compared to boys (42%) and among children of Settlers (58%) compared to Nicobarese (41%).

Height for age

The overall prevalence of stunting (height for age < Median - 2SD), an indicator of long duration malnutrition, was about 37%, while the extent of severe stunting (<Median - 3SD) was about 13%. The prevalence of stunting was significantly lower ($p < 0.01$) compared to that reported for their rural counter parts of mainland (37% vs 49%).

Weight for height

In general, the prevalence of overall wasting (Weight for height < Median-2SD), an indicator of short duration undernutrition, was about 16%, while that of severe grade was about 3%. The prevalence was significantly lower ($p < 0.01$) than that reported by NNMB for rural children (16% vs 23%).

Thus, the data revealed that the extent of undernutrition among preschool children in the relief camps of Andaman and Nicobar Islands was significantly lower than their rural counter parts of mainland, the Nicobarese children were better off compared to those of settlers. The girls were nutritionally at a disadvantage compared to boys among both the Settlers and Nicobarese.

Adolescents

The overall prevalence of undernutrition (those below 5th percentile of BMI) among adolescents was about 22%. A higher proportion of boys were undernourished (27%) compared to girls (16%). A significantly higher ($p < 0.01$) proportion of adolescents among Settlers (42%) were undernourished as compared to Nicobarese (5%). Similarly, the prevalence of overweight/ obesity was about 3%, which was higher among Nicobarese (4.8%) compared to Settlers (1.4%).

About 17% males and 19% females had varying degrees of chronic energy deficiency (BMI < 18.5), the levels of which were significantly ($p < 0.01$) lower than that reported by NNMB for their rural counterparts of main land (37% and 39% respectively). The prevalence of overweight and obesity (≥ 25 BMI) was 13% and 21% among adult males and females respectively, which was significantly higher ($p < 0.01$) as compared to their rural counter parts of mainland (6% and 8% respectively). The prevalence of CED was however; lower among adult males and females of Nicobarese (10 & 11%) as compared to the Settlers (28 & 31%). Conversely, the prevalence of overweight and obesity was higher among adult males and females of Nicobarese compared to settlers (17 & 25% vs 7% & 15%).

Thus, the data reveals that among those living in Tsunami relief camps, the adults of Andaman & Nicobar Islands covered in the present survey were nutritionally better off compared to their rural counterparts of mainland, and the adult Nicobarese were better off than to the settlers.

Nutritional status of hostellers

Among the adolescents, the common nutritional deficiency signs observed were goitre (8.1%), conjunctival xerosis (3.6%), pallor (1.8%), and dental fluorosis (1.3%).

The most common morbidities reported were fever (4%), acute respiratory infection (2%) and diarrhoea (0.5%) among adolescents, 2.4%, 1.5% and 0.5% respectively for adults. About 88% were normal ($\geq 5^{\text{th}}$ - $< 85^{\text{th}}$ centile of age/sex specific BMI). The overall extent of overweight/obesity ($\geq 85^{\text{th}}$ centile of BMI) was about 8%. The overall prevalence of undernutrition ($< 5^{\text{th}}$ centile of BMI) was about 5%, with the proportion being higher among boys (8.3%) compared to girls (2.3%).

In general, about 14% of adult men and 10% of women had chronic energy deficiency (< 18.5 BMI), while 83% of men and 80% women were normal ($18.5 - < 25$ BMI). The prevalence of overweight/obesity was relatively higher among women (10.1%) compared to men (3.4%).

Thus, the data revealed that the inmates of both boys and girls hostels were nutritionally better off compared to their mainland rural counter parts.

RECOMMENDATIONS

- ❖ There is an urgent need to improve the micronutrient status of the community. The programme of supplementation of micro-nutrient fortified biscuits to preschool children under ICDS, initiated by UNICEF in some areas may be strengthened and extended to all areas.
- ❖ IEC activities have to be strengthened to ensure better nutrition of girl child, especially in < 5 year age group, through ICDS services.
- ❖ High prevalence of overweight and obesity among the adults of Nicobarese signifies the need for health and nutrition education to impart better dietary practices and lifestyle patterns among them to prevent chronic degenerative diseases at a later part of life.

2. Prevalence of Vitamin A Deficiency (VAD) among preschool children of rural India

INTRODUCTION

The NNMB undertook the survey on “Prevalence of Micronutrient deficiencies” viz.,

Vitamin A Deficiency (VAD), Iron Deficiency Anaemia (IDA) and Iodine Deficiency Disorders (IDD) among the vulnerable groups of rural population in the State of Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu and West Bengal during 2002-2003. The objectives, study design, sampling procedures, survey methodology and the results of the survey were published earlier (NIN Annual Report 2004-05 & NNMB Technical Report No. 22). However, the data on sub-clinical vitamin A deficiency among preschool children could not be included in the above publication, due to the delay in establishing the Dried Blood Spot (DBS) facility at the Institute for vitamin A analysis, which became operational in March 2004 (Annual report: 2004-2005). The results on both clinical and sub-clinical vitamin A deficiency along with its association with socio-economic variables are presented in this report.

METHODOLOGY

Sample collection, transport, storage and analysis

A free falling drop of blood from finger-prick from the selected preschool child was collected on a pre-coded special chromatography paper. It was shade dried, wrapped in black paper, kept in an envelope and sent to NIN by courier from the field, every third day. The samples were protected from light and preserved in a deep freezer at -20°C till analysis at the DBS facility. The survey extended over a period of one and a half years and the duration between collection of sample and analysis ranged from about 6 months to 2 years, with an average period of about 21 months.

Salient findings of the survey were as follows:

COVERAGE

A total of 71,591 preschool children from 633 villages in the eight States were covered for VAD survey. The survey could not be conducted in the States of Gujarat and Uttar Pradesh due to logistic reasons. A total of 3,934 samples from 8 States were analyzed for blood vitamin A levels.

Sample Characteristics

A majority of the children covered belonged to Hindu (85.3%) households (HHs), followed by Muslims (10.6%) and Christians (3.6%). The proportion of Muslims was relatively higher in the States of Kerala (31.6%) and West Bengal (22.5%). About 42% of the HHs belonged to other backward communities (OBCs), while about 30% belonged to Scheduled caste/Scheduled tribe. The proportion of tribal households was relatively higher in the States of Madhya Pradesh (28.4%) and Orissa (19.5%), while the proportion of Scheduled caste HHs was relatively higher in the States of West Bengal (27.5%), Andhra Pradesh (25.1%) and Tamil Nadu (24.6%). The major occupation of the head of HHs was Labour (45%, agricultural labour: 15.9% and other labour: 29.5%), followed by agriculture (27%), business (10.2%) or service (9.7%).

About 52% of the adult females in the HHs surveyed were illiterate, the proportion of which was maximum in the State of Madhya Pradesh (76%), followed by Karnataka (71%), Andhra Pradesh and Orissa (60% each), West Bengal (57%) and 6% in Kerala. In general, only about a fourth of the HHs (24.2%) had sanitary latrine, the proportion of which was maximum in Kerala (94%) and least in Orissa and Madhya Pradesh (8-9%).

Prevalence of Clinical VAD

The overall prevalence of Bitot spots among 1-5 year children, an objective sign of vitamin A deficiency, was 0.8% (CI: 0.73, 0.87). The prevalence was more than 1% in the States of Madhya Pradesh (1.4%, CI: 1.15, 1.65), Maharashtra (1.3% CI: 1.06, 1.54) and Andhra Pradesh (1.2%, CI: 0.98, 1.42). While none of the preschool children examined in Kerala had Bitot spots, its prevalence in the State of Orissa was observed to be about 0.3%.

The overall prevalence of night blindness and conjunctival xerosis was 0.3% (CI: 0.26, 0.34) and 1.8% (CI: 1.70, 1.90) respectively.

The prevalence of Bitot spots was significantly ($p < 0.05$) higher among children from households belonging to Hindus (0.8%), Scheduled Castes

(1.4%), Scheduled Tribes (1.2%), agricultural labour (1.3%), non-agricultural labour (0.9%), HHs with an illiterate mother (1.1%) and those HHs not having sanitary latrine (0.9%).

Sub-clinical VAD

The overall median blood vitamin A level was 17 g/dL, which ranged from a low 9 g/dL in the State of Madhya Pradesh to a high of about 20 g/dL in the States of Tamil Nadu & Karnataka. No significant age (1-3 years: 17.2 g/dL and 3-5 year: 16.6 g/dL) and gender (boys: 17.2 g/dL and girls: 16.5 g/dL) differentials were observed in the median blood vitamin A levels.

About 62% (CI: 60.3, 63.3) of children in general, had blood vitamin A levels of < 20 g/dL, indicating high prevalence of sub-clinical vitamin A deficiency. Their proportion was highest in Madhya Pradesh (88%), followed by 79% in Kerala and about 50-60% in the remaining States. The prevalence of sub-clinical VAD was significantly ($p < 0.05$) higher among 3-5 year children (63.1%, CI: 61.2, 65.0) compared to 1-3 year children (59.6%, CI: 57.1, 62.1), with no significant gender differentials (Boys: 60.8%; Girls: 62.8%).

The prevalence was significantly ($p < 0.05$) higher among children belonging to Christians (68.8%) and Muslims (69.3%), Scheduled Tribes (74.1%) and backward communities (62.9%), households with illiterate adult woman (62.8%) and those having no sanitary latrine (64.6%). The prevalence, though not statistically significant, was relatively higher (66.7%) among those children who did not receive massive dose of vitamin A during the past 12 months, compared to those who received either one (61.6%) or two doses (56.3%).

In the State of Kerala, where the prevalence of sub-clinical VAD was highest (79.4%), the proportion of children with dietary intake of vitamin A in amounts of $< 50\%$ RDA was also highest (91.8%) and that of coverage for massive dose of vitamin A was lowest (38.5%) compared to other States.

Multiple logistic regression analysis revealed that the risk of having sub-clinical vitamin A deficiency was 2.0 (CI: 1.6, 2.6) times higher for Scheduled Tribe children compared to other

community, 1.5 (CI: 1.2, 1.8) times higher in Muslims compared to Hindus and 1.3 (CI: 1.0, 1.5) times higher for HHs having a family size of ≥ 8 compared to small family size (1-4) and 1.5 (CI: 1.0, 2.3) times higher among those children who did not receive massive dose of vitamin A compared to those who received the same during past one year.

The study revealed that the magnitude of prevalence of sub-clinical vitamin A deficiency was high (62%) in all the States irrespective of extent of prevalence of clinical deficiency signs. Similar high prevalence of sub-clinical VAD (64%) was reported in another large scale study carried out in Orissa, under the aegis of WHO during 2003.

In the present study, the prevalence of sub-clinical VAD was observed to be very high in the States of Kerala despite absence of clinical signs of VAD, which could be due to low dietary intakes of Vitamin A as well as low coverage for massive dose Vitamin A supplementation. Several other studies conducted during nineties have also revealed a very high prevalence of sub-clinical VAD despite absence of clinical signs in countries such as Lesotho (78%), Senegal (72%), Cote D'ivore (68%) and Congo (98%) (WHO MIDIS Working paper No.2, 1995, WHO/Nut/95.3).

Thus the study revealed that the prevalence of sub-clinical VAD is significantly higher among the preschool children in all the States surveyed, and therefore there is an urgent need to strengthen the national programme of supplementation of massive dose vitamin A to young children and to extend the same up to 5 years of age. The IEC activities need to be intensified to bring in dietary diversification by encouraging the community to grow kitchen gardens and to include locally available vitamin A rich foods in their daily diets, more frequently. The scope of fortifying foods with vitamin A, wherever possible, should also be explored.

3. Assessment of diet and nutritional status of individuals and prevalence of hypertension in adults & anaemia among adult men and NPNL women in rural communities

The National Nutrition Monitoring Bureau (NNMB), which was established in the year 1972 by

the ICMR in the States of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa, West Bengal and Uttar Pradesh has been carrying out diet and nutrition surveys on a regular basis. During the year 2000-01, the NNMB undertook the diet and nutrition surveys in the rural areas of all the States, except Uttar Pradesh. In view of increasing problems of diet related chronic diseases such as obesity, hypertension, etc being reported in the developing countries, the components such as assessment of prevalence of obesity, hypertension among adult men and women (≥ 20 years) were also included in the survey. In addition, estimation of haemoglobin was also carried out among adult men and Non-pregnant and Non-lactating (NPNL) women, the groups which were not covered in the micronutrient deficiency survey carried out earlier (2002-03). The prevalence of diabetes mellitus (DM)/ hyperglycaemia was assessed in the State of Andhra Pradesh on a pilot basis.

METHODOLOGY

Sampling design

The villages surveyed by the National Sample Survey Organization (NSSO) in its 54th Round of Consumer Expenditure Survey, during the year 1998 formed the sample frame and a sub sample of villages were covered in the present survey. A total of sixteen strata (1.8 million population/ per strata) were selected from each State and from each of these strata, five villages were selected randomly. Thus, a total of eighty villages were covered in each State. In each of the selected villages, twenty households (HHs) were covered for survey by adopting cluster-sampling method. For this purpose, the village was divided into five geographical areas (cluster of HHs), one of which consisted of households belonging to SC/ST community. From each geographical area, four consecutive HHs were surveyed, by selecting random start.

INVESTIGATIONS

In each village, socio-demographic particulars were collected from all the 20 HHs. Anthropometric measurements, such as, height, weight, mid upper arm circumference (MUAC) and fat fold thickness at

triceps (FFT) were taken on all the individuals from these HHs. They were also examined for the presence of clinical signs of nutritional deficiency. Information on morbidity such as fever, acute respiratory infections, measles, and diarrhoea during the preceding 15 days of visit was also collected from all the individuals. Twenty four hour dietary recall diet survey was carried out to assess the food and nutrient intake of all the individuals, in every alternate HH (covered for socio demographic information).

About 51,705 individuals of different age /sex groups from 14,256 HHs of 713 villages were covered for anthropometry, clinical examination, and prevalence of morbidity. Data on food and nutrient intake was collected on 30,244 individuals from 7078 HHs. Blood samples were collected on 3391 men and 3384 NPWL women for the estimation of haemoglobin levels. Measurement of blood pressure was carried out on 11,923 men and 13,702 women and fasting blood samples were also collected from 1803 men and 1883 women only in the State of Andhra Pradesh for estimation of blood glucose levels.

Socio-economic profile

About 37% of the HHs belonged to backward communities, 22% belonged to SC and 11% ST, while the rest (30%) were of other castes. About two thirds of HHs (62.4%) lived in semi pucca houses, while about 21% were in kutcha houses.

The average family size was 4.9. About 49% of the HHs did not possess any agricultural land. About 32% of adult men ranged from a low 7.7% in Kerala to a high 46% each in the State of Andhra Pradesh and West Bengal and 48% of adult women were illiterate ranged from a low 11.5% in Kerala to a high 65% in the State of Madhya Pradesh. About a third of the households each were fetching drinking water either from public taps (39%) or tube wells (35%). In general, about 27% of the HHs had sanitary latrine, about 76% of the HHs possessed separate kitchen and about two thirds of the houses (72.1%) had electricity.

Food intake

Cereals formed the bulk of the rural dietaries while millets constituted 13%. The average intake (g/CU/day) of all the foodstuffs, except roots & tubers formed males and other vegetables was

below the RDI in all age/sex/physiological groups. The mean consumption of cereals & millets (418 g) among males was below the recommended level of 460 g in majority of the States, except in the States of Orissa (472 g) and West Bengal (509 g). Similarly, in case of females, the States of Orissa and West Bengal were meeting the RDI.

The average intake of green leafy vegetables is grossly deficit when compared to RDIs of both males and females in all the States barring among males in the States of Orissa and West Bengal. Consumption of other protective / income elastic foods such as milk & milk products (80 ml), sugar & jaggery (14 g) and fats & oils (13 g) was also grossly deficit when compared to the recommended levels among both sexes.

Nutrient Intake

The median intake (CU/day) of all the nutrients was lower than the recommended levels in both sexes in all the States, except for niacin among the females. The median intake of all the nutrients was lower than the recommended levels among all the age/sex/physiological and activity groups especially the consumption of micronutrients such as iron, vitamin A, riboflavin and folic acid was grossly deficit compared to RDAs.

Clinical examination

None of the preschool children exhibited signs of kwashiorkor and marasmus. The prevalence of Bitot spots, the objective sign of vitamin A deficiency, was 0.6% ranged from nil in the State of Kerala to 1.3% in Maharashtra, while 0.8% had angular stomatitis, indicative of B-complex deficiency. Among the school age children, the common deficiency signs observed were conjunctival xerosis (1.9%), Bitot spots (1.6%), angular stomatitis (1.9%), phrynoderma (0.9%) and goitre (1.4%). About 13% had dental caries.

Morbidity

The most common forms of morbidities among different age groups were fever, diarrhoea, dysentery, and acute respiratory infections, the prevalence of which ranged from 0.1-5 %.

Anthropometry

The mean heights and weights of individuals of different age / sex groups were considerably lower

than the reference values (NCHS). Overall prevalence of underweight (weight for age <Median-2SD) was 55% ranged from a low 35.0% in Kerala to a high 64% in the State of Madhya Pradesh. The prevalence of stunting (height for age < Median- 2SD) was 52%, while wasting (weight for height <Median- 2SD) was 15%. No significant gender differences were observed.

The prevalence of undernutrition based on BMI (<5th centile of age and sex specific BMI) was 57% among 10-13 years and 30% among 14-17 years, while the prevalence of overweight/obesity was about 1%.

About 33% adult men (a low 27.7% in Kerala to a high 42.4% in Karnataka) and 36% (a low 21.1% in Kerala to a high 47.6% in Orissa) of the women had different grades of chronic energy deficiency (CED) as measured by BMI (<18.5). About 59% of men and 53% of the women had normal body mass index (18.5-25.0). The prevalence of overweight/obesity (BMI >25) was high among women (11%) compared to men (8%).

Abdominal obesity

The prevalence of abdominal obesity in terms of waist circumference (>102 cms) was about 1% among men, while it was 7% among women (≥ 88 cms). The prevalence of abdominal obesity in terms of waist hip ratio (WHR) was 25% ranged from a low 10.1% in Madhya Pradesh to a high 40.5% in the State of Kerala among men and 69% ranged from a low 36.7% in the State of Maharashtra to a high 91.8% in Kerala among women.

Undernutrition vs. socioeconomic status

The overall prevalence of undernutrition (weight for age <Median - 2SD) among preschool children was significantly higher among the children belonging to Scheduled Tribes and households living in kutcha houses. The prevalence of undernutrition among preschool children was relatively high, in those with average household income of <Rs.300/- p.m.

Anaemia

The overall prevalence of anaemia was about 55% in adult men and 75% among women (NPNL). The prevalence was very high in the States of West Bengal (84% in males vs 93% in females), Kerala (68% in males vs 89% in females) and

Madhya Pradesh (68% in males vs. 87.4% in females).

DIET RELATED CHRONIC DISEASES

Hypertension

The overall prevalence of hypertension (SBP ≥ 140 or/and DBP ≥ 90 mm of Hg) was about 25% among men and 24% in women. The prevalence tended to increase with age, from 13.6% in 20-30 years group to 56.4% in ≥ 80 year age group. About 60-67% of the adults was aware of hypertension, while 2-3% were currently on treatment. The prevalence of hypertension was high among the adults, who were suffering from overweight/obesity (48%) compared to normals (26%).

Diabetes mellitus

Estimation of fasting blood glucose among adults was carried out only in the State of Andhra Pradesh. The prevalence of diabetes mellitus (FBS levels of ≥ 126 mg%) was about 4% among adult men, and about 3% among women. The proportion of impaired fasting glucose FBS levels of 110 to 126 mg% was about 2% each among men and women. About 48-56% were aware of diabetes mellitus and about 2% were currently on treatment for diabetes mellitus.

The prevalence of diabetes mellitus was high among those who were suffering from overweight and obese (9%) among adult men as well as women compared to normal adults (2.4%).

4. Acceptability of micronutrient fortified millet based biscuits - A study among primary school children

Undernutrition continues to be major public health problem in India, affecting a large section of the communities, the most vulnerable being young children and women of reproductive age groups. Recently, the problem of micronutrient deficiency disorders such as iron deficiency anaemia (IDA), vitamin A deficiency (VAD) and iodine deficiency disorders (IDD) are attracting the attention of both policy makers as well as public health administrators. The studies carried out by the National Nutrition Monitoring Bureau (NNMB) in

several States including Andhra Pradesh have shown that the diets of population in general and those of young children in particular are grossly deficient in micronutrients such as iron, calcium, vitamin A, riboflavin, folic acid and vitamin C.

Since micronutrient deficiencies are identified as silent epidemics affecting the growth, development and well being of the populations, diverse intervention programmes are being explored for supplementation of micronutrients. The Commissioner of Civil Supplies, Government of Andhra Pradesh had proposed to supplement micronutrient fortified millet based biscuits as snacks to the primary school children, in addition to the existing mid-day meal (MDM) programme.

The A.P foods, a Government of Andhra Pradesh enterprise, Nacharam, Hyderabad, under the technical guidance of NIN produced micronutrient fortified millet based biscuits using jowar, maize or ragi. Based on the initial sensory evaluation carried out at the NIN, steps were taken to improve the organoleptic characteristics of these biscuits in terms of texture, taste and flavour.

The biscuits were fortified with ragi/maize/jowar in levels, so as to get 50% of the recommended allowances per day/child, by consumption of three biscuits with a total weight of 24 g. The A.P Foods prepared the fortified biscuits and sensory evaluation of the same was carried out successfully at NIN. All the three varieties of biscuits viz. jowar, maize and ragi based ones were found to be equally good.

However, before initiating the large-scale production of these biscuits and implementation of the programme, it was proposed to study the acceptability of the micronutrient-fortified biscuits among primary school children. Therefore the current study was carried out with the objective to assess the acceptability of the micronutrient fortified millet biscuits among preschool children.

METHODOLOGY

It was a cross sectional study carried out in a total of 14 primary schools @ 7 each from Ibrahimpatnam and Shameerpet Mandals of Ranga

Reddy district. In each of the selected schools, about 70 children (35 boys and 35 girls) of 9 to 11 year age groups were included in the study. The acceptability was assessed among a total of 330 children for jowar biscuits, 350 children for maize biscuits and 314 children for ragi biscuits. To avoid cross over effect in reporting of acceptability levels, each group was included for evaluation of only one type of biscuit.

Informed written consent was obtained from the Directorate of School Education, Hyderabad and Heads of the Institutions.

The salient findings of the study are as follows:

In each of the selected schools, required number of children in the age group of 9-11 years was randomly selected. After explaining the purpose of the study to the group, each child was provided a copy of the pre-tested questionnaire in local language and contents of the same were explained to them. They were asked to record their opinion in the questionnaire in terms of 1-5 score after consuming the biscuits.

The analysis of data revealed that, about 90% of the children gave a score of 'good' to 'very good' for all the characteristics studied viz. appearance, colour, texture, flavour and taste with respect to all the three types of micronutrient fortified biscuits. The mean score (in a scale ranging from a low '1' to high '5') obtained for colour was maximum for maize (4.5), followed by ragi (4.3) and jowar (4.2). With respect to the 'appearance', the mean score was 4.2 for jowar biscuits, 4.1 for ragi, and 4 for maize biscuits. The score for 'texture' ranged from 4.1 for maize, 4.2 for jowar and 4.3 to ragi biscuits. A maximum score of 4.4 was observed with respect to 'flavour' for ragi, followed by 4.3 for maize and 4.2 for jowar. Ragi biscuits scored highest of 4.5 followed by maize (4.4) and jowar (4.2) with respect to the 'taste'. The mean overall score was maximum for maize (4.6), followed by ragi (4.5) and jowar (4.3) biscuits.

Some of the suggestions given by the students for improving the overall quality were;

- (i) to increase the sweetness,
- (ii) softness of the biscuits and,
- (iii) addition of cream to the biscuits.

5. Nutritional status of tribal population in ITDA project of Bhadrachalam in Khammam District, Andhra Pradesh

The tribes live in isolation from the general population and are socially and economically disadvantaged. The health and nutritional status of tribal population depend on the ecosystem they live in. Geographical isolation, unique cultural and social practices, lack of formal education, poor infrastructural facilities, lack of better health seeking behaviour, poverty etc. expose them to higher risk of undernutrition, morbidities and mortality. National as well as State governments have been implementing several programmes under Tribal Sub Plan approach (TSP) for the overall development of tribes.

Andhra Pradesh is homeland of nearly 33 tribal groups accounting for 6.6% of the total population of the State and they are inhabiting the north and northeastern parts of the State as well as border States of Orissa, Maharashtra and Chattisgarh.

Earlier surveys carried out by National Nutrition Monitoring Bureau (NNMB) among the tribal population in the State revealed higher prevalence of underweight among preschool children compared to their rural counterparts. At the request of the Project Officer (PO), Integrated Tribal Development Agency (ITDA), Bhadrachalam, the National Institute of Nutrition (NIN) carried out a special survey to assess the health and nutritional status of the tribal population in the project area. The district has a total tribal population of about 4.5 lakhs, mainly constituted by Koyas, Lambadas/Sugalis and Kondareddis.

GENERAL OBJECTIVE

To assess the health and nutritional status of tribals living in Bhadrachalam ITDA area of Khammam district.

SPECIFIC OBJECTIVES

To assess :

- ❖ Food and nutrient intake at household level among the tribal population.

- ❖ Nutritional status of the individuals in terms of anthropometry and prevalence of clinical signs of nutritional deficiency.
- ❖ Prevalence of morbidities during the preceding 15 days of the survey.
- ❖ Knowledge and practices of mothers on infant and child feeding, and
- ❖ Extent of coverage of target population under various nutrition intervention programmes, being implemented in the area.

METHODOLOGY

It was a cross-sectional study, adopting multistage random sampling procedure. Assuming an overall prevalence of 69% of undernutrition among 1-5 year children, with 95% confidence interval and 5% relative precision, a sample of 335 children was arrived at. It was proposed to carry out survey in about 1000 HHs to cover the required number of preschool children.

For the purpose of the study, five out of total 29 mandals inhabited by tribes were randomly selected. From each mandal, four villages were selected randomly. In each of the selected villages, starting from the northeast corner of the village, 40 contiguous HHs were covered for the survey.

INVESTIGATIONS

The following investigations were carried out :
Collection of household demographic and socio-economic particulars,

- ✎ Measurement of height and weight of all the individuals in the selected households, and examination for presence of clinical signs of nutritional deficiency.
- ✎ History of morbidity during the previous fortnight.
- ✎ 24-hour recall diet survey in a sub sample of every fourth household covered for nutritional anthropometry to assess the food and nutrient intake at the household level.
- ✎ Extent of coverage of 1-2 year children, for various Immunizations during the first year of life
- ✎ Coverage of target beneficiaries under National Nutritional Programmes such as supplemen-

tation of massive dose of vitamin A and distribution of iron and folic acid tablets, and

- ✎ Assessment of breast-feeding and child rearing practices among women.

RESULTS

Following were the salient observations in the study:

COVERAGE

A total of 2751 individuals from 802 HHs in 20 villages were covered for nutrition assessment. Food and nutrient intakes were assessed in 203 Hhs.

A majority (85%) of the houses were kutchra in nature. About three fourths (72%) of the families were nuclear. The average family size was 4.6. The major occupation of the head of the household was agriculture (44%), followed by agricultural labour (28%) and other labour (20%). The average size of land holding was 2.3 acres, while one fourth of HHs did not possess any agricultural land. Illiteracy among the male head of the household and their spouses was 72% and 88% respectively. The average per capita annual income of the HHs was Rs.4,590/-.

In general, the mean consumption of all the foods (g/CU/day), barring cereals and millets was lower than the RDI. The extent of deficit was higher with respect to protective / income elastic foods such as green leafy vegetables (90%), milk & milk products (80%), sugar & jaggery (83%) and fats & oils (70%).

The median intake of all the nutrients (CU/day) was lower than the RDA. The extent of deficit ranged from 45% for fats to 88% for vitamin A. The proportion of households consuming various nutrients in amounts of less than 50% of the RDA was maximum with respect to vitamin A (96%), followed by Iron (77%), riboflavin (74%), free folic acid (71%), thiamine (40%) vitamin C (38%) and calcium (36%). In about three fourths of the households, the consumption of energy and protein was less than the recommended levels.

The prevalence of conjunctival xerosis and Bitot spots, the signs of vitamin A deficiency was 0.5% each among preschool children, while

among school age children, it was very high (6.7% and 12.4% respectively). The most commonly reported morbidities among various age groups were fever, diarrhoea and acute respiratory tract infections (ARI).

The mean heights and weights in different age and sex groups were much below the NCHS Median values but were comparable to their rural counterparts of the State (NNMB, 2000). About 65% of the preschool children were underweight (weight for age <Median- 2SD), while 18% had severe underweight (weight for age <Median -3SD). The prevalence of underweight was marginally low (65%) compared to their other tribal counterparts (69%) (NNMB Tribal Survey 2000).

The overall prevalence of stunting, an indicator of chronic undernutrition (height for age <Median- 2SD) was 46%, while that of wasting, an indicator of acute undernutrition was about 21%, which was lower than their tribal counterparts of Andhra Pradesh (57% of stunting, 27% of wasting). Significantly higher proportion of adolescent boys (45%) were undernourished compared to girls (21%) (age and sex specific BMI centiles of NHANES).

The overall prevalence of chronic energy deficiency (BMI <18.5) among adults was 51%, the prevalence being higher among females (58%) compared to males (41%). The extent of undernutrition among children and adults, however, was relatively low compared to their State counterparts.

About 90% of the mothers reportedly initiated breast-feeding within 24 hours after delivery. Only about 14% of the mothers discarded colostrum, mostly on elder's advice. The proportion of the same was lower compared to that of 30% generally encountered in the rural areas. Nearly three fourths of the mothers initiated complementary feeding to their children during 4-6 months of age, which mostly included rice/roti, bread and biscuits and milk. None of the mothers gave commercial baby foods to their children.

Majority (93%) of the children were completely immunized by 12 months of age, while the rest did not receive the third dose of DPT/Polio. About 93% of the women received TT vaccination during

previous pregnancy. Though nearly 90% of the children received massive dose of vitamin A during the previous year, only 52% reportedly received the recommended two doses against 25% in their rural counterparts (NNMB, 2003). Similarly, about 90% of the currently lactating women received iron and folic acid tablets during the last pregnancy, the corresponding figure in the rural counterparts is 62%. Only 69% received minimum of 90 tablets against 30% in rural areas. The coverage of lactating women for IFA Tablets distribution was much lower (33%).

The study revealed high rate of adult illiteracy, poor economic status, dependency on agriculture and allied occupations for livelihood, low intake of protective foods and gross inadequacy in the intake

of micronutrients. The overall prevalence of under nutrition, though low to their tribal counterparts of the State, but was higher compared to their rural counterparts. The prevalence of morbidities such as fever and diarrhoea was also relatively higher compared to that reported in rural areas, indicating the problem of poor environmental sanitation and personal hygiene. The coverage of beneficiaries for immunization and supplementation of massive dose vitamin A and IFA tablets was relatively good.

Poverty and poor health-seeking behaviour probably contributed to aggravate the situation. The study highlights the need for strengthening of the existing health facilities, health and nutrition education programmes & poverty alleviation programmes being implemented by the Government.

II. CLINICAL STUDIES

2. MATERNAL HEALTH

2.1. Obstetric outcome and proinflammatory cytokine response in women with genital tract infections

Little is known on the role of maternal infections on LBW in the underprivileged poor communities of India, where the problem of infections is huge. In the present study, histological chorioamnionitis and local cytokine (IL8, TNF) response was used as markers of chorioamnion membrane infection and determined its association with IUGR and PTD.

OBJECTIVE

To determine the association of chorioamnionitis (CA) with intrauterine growth retardation (IUGR).

METHODS

Sample selection:

Recruitment of subjects, data and sample collection were carried out at Niloufer Hospital for women and child health. Women who were less than 16 years of age, had taken antibiotics during the previous 2 weeks, had a fetus with a known congenital malformation, had a cervical cerclage and women with PIH, diabetes, abruptio placenta, placenta previa and twins were excluded from the study. Placentas were collected from women who had uncomplicated vaginal delivery after spontaneous labor and processed within 60 minutes of delivery.

Two sections of 2cm² chorioamnion membranes were cut a few cm away from the ruptured site, washed briefly in cold phosphate buffered sodium chloride solution to remove excessive amount of blood, and one section was fixed in 10% formaldehyde, which was used for scoring inflammatory cells and another section was transported in phosphate buffer saline for estimating membrane cytokines.

Screening for genital tract infections:

All the women in the study were screened for intrauterine infections at labor using markers such as bacterial vaginosis, histologic evaluation of inflammatory cells in chorioamnion membranes and IL8 concentration in chorioamnion membranes.

IL8 and TNF were measured from homogenised chorioamnion membrane supernatant by sandwich ELISA as described previously. The sensitivity of the TNF was 10pg/ml and that of the IL8 assay was 6 pg/ml. The interassay and intraassay coefficients of variation were <10% for both TNF and IL8 (9.0% and 8.6% for TNF and 7.6% and 8% for IL8) All ELISAs were calibrated against recombinant human cytokine standards.

Using Lubchenco et al., standard, percentile weight for gestation age was assessed, sexes combined. The SGA/ intrauterine growth retardation (IUGR) category consisted of infants below tenth percentile of weight for gestational age. Infants delivered before 37 weeks were considered to have PTD.

New borns were also classified into symmetrical and asymmetrical growth retarded taking birth weight, crown heels length and head circumference into consideration. Those with birth weight (BW) below 10th percentile of standard and with normal crown heel length (CHL) & head circumference (HC) were classified into asymmetrical and those with BW, CH and HC below 10th percentile of standard were classified as symmetrical growth retarded.

RESULTS

Chorioamnion membranes and new born anthropometry were collected from 73 women with term small-for-gestational age date (SGA), suggesting IUGR, 59 women with PTD and 75 term appropriate for gestational age (AGA). The mean age of mothers were 23.68 ± 0.382, 21.00 ± 0.276, 22.4 ± 0.442 for those with term AGA, term IUGR and PTD respectively. The mean gestational age in

months were 39.4 ± 0.111 and 39.04 ± 0.133 for term AGA and term IUGR and 33.0 ± 0.400 for PTD women.

Maternal and fetal characteristics are presented in table as per birth weight and gestational age. Postnatal weight (PNW) of mothers with term IUGR babies and PTD was significantly lower compared to term AGA, while height of the mothers was significantly lower in those with IUGR babies.

The birth weight (BW), crown heel length (CHL) and head circumferences (HC) were significantly lower in term IUGR and PTD compared to term AGA. Similarly, the abdominal circumference (ABC) and body fat percent were significantly lower compared to term AGA.

Table 1: Maternal and Fetal Characteristics

	Term AGA (75)	Term IUGR (73)	PTD (59)
Maternal			
Ht(cm)	152.7 \pm 0.588(68)	150.8* \pm 0.596(64)	152.1 \pm 0.828(57)
PNW (kg)	48.0 \pm 0.64 (68)	45.3** \pm 0.68 (65)	45.9* \pm 0.96 (57)
BMI	20.6 \pm 0.25 (68)	19.9 \pm 0.27 (64)	19.8 \pm 0.41 (57)
Fetal			
BW	2.8 ^a \pm 0.030(75)	2.26 ^b \pm 0.026(73)	1.78 ^c \pm 0.049 (59)
Length	47.8 ^a \pm 0.257(68)	45.9 ^b \pm 0.232(69)	43.3 ^c \pm 0.453(49)
HC	32.8 ^a \pm 0.144(68)	31.7 ^b \pm 0.134(69)	30.6 ^c \pm 0.320(49)
PI	2.6 ^a \pm 0.144(68)	2.3 ^b \pm 0.034(69)	2.2 ^c \pm 0.045(49)
ABC	27.9 ^a \pm 0.23(72)	26.0 ^b \pm 0.20(68)	24.2 ^c \pm 0.32(48)
Fat %	11.95 \pm 0.42***	9.25 \pm 0.46	7.8 \pm 0.71

ANOVA

* p<0.05 compared to FTAGA

** P<0.01 compared to FTAGA

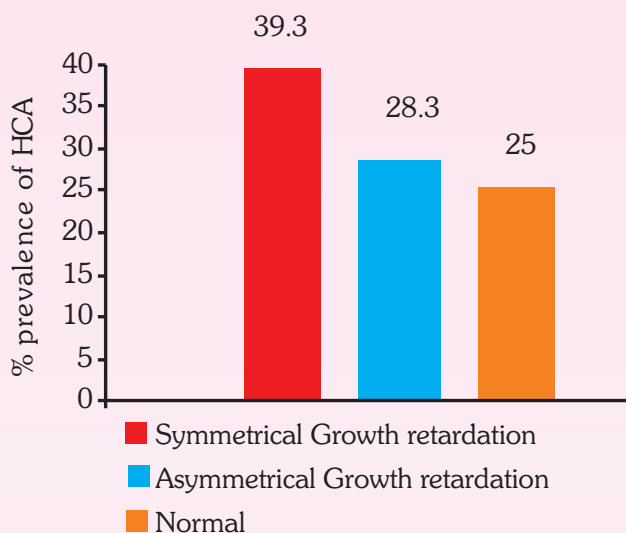
*** P<0.0001 compared to other groups

a,b,c = P<0.001 - Post Hoc Test

Of the total women in the study 28.8% had histologic chorioamnionitis. Of the term AGA, 25% had histologic chorioamnionitis, compared to 31% and 30% in IUGR & PTD respectively. When

women with IUGR babies were divided into symmetrical and asymmetrical growth retarded babies, women with symmetrically growth-retarded babies were associated with higher proportion of histologic chorioamnionitis (HCA) (Figure 1).

Figure -1 Growth Retardation and HCA



When women were divided into those with histologic chorioamnionitis and those without histologic chorioamnionitis irrespective of gestational age or birth weight, women with histologic chorioamnionitis had higher proportion of babies with less than 10th percentile birth weight, CHL and HC. Moreover, the mean birth weight, length and HC were also lower in those with histologic chorioamnionitis (Figure 2 A & B).

Twenty five out of 180 (13.8%) women had bacterial vaginosis, and the prevalence was not different between term normal, IUGR or PTD. Bacterial vaginosis was not associated with secretion of IL8 or TNF from chorioamnion membranes.

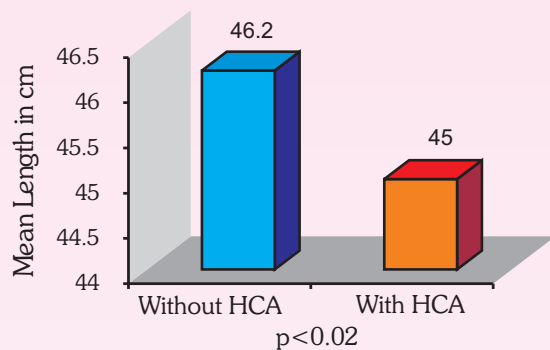
TNF was determined in 32, 56 & 58 term normal, IUGR & PTD respectively and was detectable in 60%, 51% & 59% respectively. The concentration of TNF ranged from 2.7 to 234.8, 4.0 to 891.6 and 1.72 to 375.3 pg/g protein in term normal, IUGR and PTD respectively.

IL8 was determined in 31, 56 & 58 term normal, IUGR & PTD respectively and was detectable in all except 3 term normals. It ranged

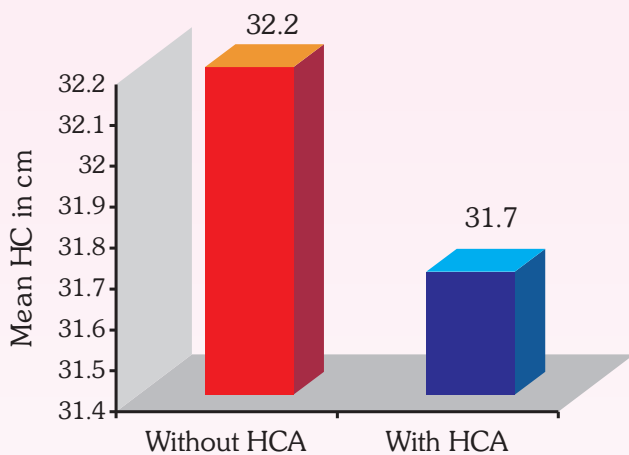
from 122.2 to 14121.0 pg/g protein in term AGA, 66.6 to 23911.0 in IUGR and 22.8 to 8550.0 in PTD.

Figure-2(A) Length and 2(B) Head circumference of new borns in women with and without histologic chorioamnionitis

(A) Length of new borns in women with and without histologic chorioamnionitis (HCA)



2(B) Head Circumference (HC) of new borns in women with and without histologic chorioamnionitis (HCA)



IL8 concentrations were increased in term normal and IUGR but more marked increase was observed in IUGR. When women were divided into those with histologic chorioamnionitis and without histologic chorioamnionitis irrespective of gestational status or birth weight, women with histologic chorioamnionitis had significantly higher concentration of IL8. When women were classified into high secretors (IL8 median) and low secretor (IL8 < median) irrespective of gestational age and BW, mean BW of babies was 120g lesser and mean CHL and HC were lower in women with high secretion of IL8 compared to those who secreted

below median. In addition, women with symmetrical growth retarded babies had significantly higher secretion of IL8 in CA membrane.

CONCLUSIONS

1. Height and weight of the mothers were associated with birth outcome.
2. Babies born to mothers with chorioamnionitis had low birth weight (LBW), significantly lower crown heel length (CHL) and head circumference (HC) compared to those without chorioamnionitis.
3. Interleukin 8 (IL8) secretions in the chorioamnion membranes was associated with chorioamnionitis. TNF, was however comparable between the two.
4. Chorioamnionitis associated with high concentration of proinflammatory chemokine such as IL8 may have an adverse effect on birth outcome. Thus, in addition to nutritional factors other factors such as inflammatory response due to genital tract infections might play an equally important role in adverse birth outcome.

2.2 Effects of maternal nutrition in early pregnancy on placental development

Recent experimental and clinical studies have identified maternal malnutrition at conception or during early period of gestation as an important factor determining the fetal growth as early as the first trimester of pregnancy. Placenta is a transient embryonic organ of communication between mother and fetus during pregnancy and is the only source of nutrient transfer to the fetus. Hence, its proper development is essential for fetal growth and development, right from embryonic stages of development.

OBJECTIVES

To assess and compare the placental morphology by measuring the Villous structure and vascular endothelial growth factor (VEGF) and

placental growth factor (PLGF) expression from conception at 7-10 weeks of gestation, of low socio-economic status (LSES) and high socio-economic status (HSES) groups, in relation to their nutritional status.

METHODOLOGY

The study was done using placental tissue collected from healthy women undergoing medical termination of unwanted pregnancy in the first trimester. Ethical committee clearance was obtained. Written informed consent was obtained from the subjects. Women testing positive for VDRL, HIV, smoking, prolonged hyperemesis, history of bacterial infections, history of congenital malformation in previous deliveries and those with previous history of abortions or chronic infections were excluded. A total of 227 samples of placental tissue were collected between 5 to 10 weeks of gestation assessed by the date of last menstrual period. Of these 127 samples were obtained from the Government Hospital (Niloufer Hospital, Hyderabad) that caters to the LSES group and 100 samples from Swapna Nursing Home, Hyderabad that caters to the HSES group.

Placental tissue were fixed in 10% buffered neutral formalin and processed paraffin blocks were cut to obtain 3 m serial sections and stained with haemotoxylin and eosin. Every fifth section was taken for the study. Slides were examined by light microscopy and those slides showing good number of villi and implantation site with anchoring villi were selected for scoring. Ten fields identified under low power and selected randomly were examined in each slide.

The scoring was done under medium power 40X. The scoring was repeated in 20% of randomly selected slides by two scientists, for inter reliability and reproducibility. Placental characteristics like mean number of villi, percentage of villi with syncytial sprouts and disposition of blood vessels like central and peripheral were scored. Based on the scores, 7-10 weeks gestational period was taken as cutoff point for further analysis. A total of 99 samples (59 from LSES and 40 from HSES group) from both groups were stained with H&E to study the morphological characters.

Slides with sections from basal plate were selected and stained for VEGF and PLGF expression using kits (Santa Cruz Biotechnology, California) for immunohistochemistry. Paraffin sections were deparaffinized using xylene and hydration was achieved using descending grades of alcohol and distilled water. Sections were transferred to 0.1M citrate buffer and autoclaved at 120°C for 5 minutes for antigen retrieval and cooled in buffer for 20- 30 minutes and washed in PBS to block endogenous peroxidase activity. Sections were layered with blocking antibody goat serum for 15 minutes and excessive serum was blotted. Sections were then layered with primary antibody (Rabbit polyclonal IgG 5 ml of 5 µg/ml concentration) and incubated for 2 hours at room temperature and washed with PBS, drained and wiped.

Sections were layered with biotinylated 2nd antibody for 30 minutes, washed with PBS, drained and wiped. Avidin biotin peroxidase reagent was applied for 30 minutes and washed with PBS. DAB solution was applied for 7 minutes and the slides were dipped in distilled water for 5 minutes. Sections were counter stained with Meyers haemotoxylin for 5 minutes and dipped in distilled water for 6 minutes. Dehydration was accomplished using ascending grades of alcohol and the slides were cleared in 1:1 xylene and alcohol and xylene for 5 minutes each and mounted in DPX mountant.

PLGF staining was done using donkey serum as serum block and affinity purified goat polyclonal antibody raised against a peptide mapping at the carboxy terminus of placental growth factor (PLGF) of human origin was used as primary antibody. The remaining steps were similar to VEGF staining. Scoring was done in four peripheral and one central fields of each slide to cover 100 villi from each sample. Thyroid tissue was taken as positive control and neurofibroma as negative control.

OUTCOME

In haemotoxylin stained slides morphological characters of the mean number of floating villi was significantly ($p < 0.01$) higher in LSES group when compared with HSES group. Villous vascular density was significantly ($p < 0.001$) higher in LSES group when compared with HSES group. Central

disposition of the blood vessels was also significantly ($p < 0.05$) higher in the LSES group. The syncytial sprouts were significantly ($p < 0.05$) higher in LSES when compared with HSES group. Decidual vascular density was significantly ($p < 0.01$) higher in HSES women compared to LSES.

The percentage of floating villi with positive staining for VEGF were significantly ($p < 0.05$) more in LSES compared to the HSES group. VEGF expression in cytotrophoblast and in blood vessels was more in HSES group compared to LSES group. The staining pattern for VEGF was significantly

($p < 0.001$) more in LSEG group when compared to the HSEG group. Floating villi, CTB expression and staining intensity pattern for PLGF were comparable between the two socioeconomic groups.

The significant disparity in placental structure observed in this study between the undernourished and well nourished at a comparable gestational period is interesting and is suggestive of a predominant hypoxemic placental development in these LSES women under the stress of under nutrition.

III. BASIC STUDIES

1. External validation of the National Facility for Dried Blood Spot Technology for Vitamin A Estimation

The National facility for Dried Blood Spot (DBS) Technology for vitamin A Estimation has been established at the Institute is operational since March 2004. During the year external validation of the facility, stability of retinol in DBS and technical services were undertaken.

MATERIALS AND METHODS

External validation and Stability

Eight pairs of serum and DBS samples collected from healthy human volunteers and stored in the laboratory were analysed at various time points to assess the stability of retinol. One such set of eight pairs of samples stored for more than a year was sent to Craft Technologies, USA for the external validation of the facility.

Technical services for analysis of vitamin A

A kit consisting of special filter paper for blood collection, silica gel bag, lancet, cotton swab, black paper and instructions for collection of blood was prepared. This was used to collect about 150 DBS samples from the states of Kerala, Andhra Pradesh and Madhya Pradesh (N=50/state) to find out the feasibility of collection and transportation of the DBS samples. The samples were transported to the laboratory within 4 days, stored at - 20°C and analysed within 22-27 days.

RESULTS

There was a good agreement between the DBS and plasma retinol analyzed at the NIN facility at various time points and that analyzed at the Craft Technologies after a period of one year (Table 2). Results on the mean and the median DBS retinol in preschool children were comparable among the three States (Table 3). The mean values are comparable to those reported earlier.

Table 2. External validation of serum and DBS retinol estimation by DBS facility

Sample ID	NIN DBS Retinol (mol/L)	Craft DBS Retinol (mol/L)	NIN Serum Retinol (mol/L)	Craft Serum Retinol (mol/L)
1	1.63	1.70	2.16	2.13
2	1.72	1.74	1.71	1.45
3	1.35	1.82	1.74	1.39
4	1.31	1.38	1.29	1.36
5	1.37	1.35	1.39	1.25
6	1.45	1.29	1.18	1.08
7	1.78	1.60	1.53	1.67
8	1.91	1.68	1.74	1.60

Table 3. Mean (SD), Median and Range of DBS retinol concentration (g/dl)

State	Retinol		Range
	N=50 Mean (SD)	Median	
Kerala 50	20.2 8.4	19.7	5 - 36.9
AP 49	20.2 8.2	20.5	5 - 40.4
MP 50	21.6 8.0	20.4	8 - 42.7

CONCLUSION

The performance of NIN DBS facility has been externally validated. A sample collection kit can be used for the collection of blood and transportation of DBS to the laboratory from the field is feasible within 5 days of collection.

2. Iron and zinc bioavailability of representative Indian and US diets : Regional distribution and availability of iron and zinc from representative Indian diets

There is a consensus that the main cause of iron deficiency anemia is low dietary bioavailability of iron. It is, generally, accepted that iron and zinc deficiencies frequently occur together because the

dietary factors that impair iron absorption also affect zinc absorption. There are no RDAs for zinc in India. With the newer methodology of using stable isotopes of iron and zinc it is now possible to generate precise data on the extent of iron and zinc absorption. This will form the basis for formulating RDA of iron and also to make recommendations on dietary diversification for making iron more bioavailable from Indian diets. Therefore, it is important to obtain regional data on dietary intake and food composition and to measure iron and zinc absorption from several days of dietary consumption as meals.

AIMS AND OBJECTIVES

- ✎ To measure the food composition of diets as consumed by the populations in the major regions of India and within them, by income groups.
- ✎ To estimate iron and zinc availability from these representative diets and their modified diets by *in vitro* method.
- ✎ To measure iron and zinc absorption from these “representative” Indian diets by members of the population with well defined general and specific iron and zinc nutritional status.
- ✎ To measure the changes in bioavailability of both iron and zinc that can be achieved by feasible dietary modifications of such diets (“improved representative” Indian diets).
- ✎ To compare the iron and zinc absorption of Indian pregnant women consuming “representative” and “improved-representative” Indian diets with those of US women consuming their habitual US diets.

Work carried out (during 2004-2006)

The first two objectives were completed and a brief report of the same is reported here. As envisaged the collaborative part could not be taken up with the US PI.

METHODOLOGY

Selection of Regional Representative Diet: Diets from four geographical zones of South - Andhra Pradesh (AP), North - Madhya Pradesh (MP), West - Gujarat (GJ) and East - West Bengal (WB) were chosen to represent the regional diets of India. From

each region, based on income, diets of two socio-economic groups, rural low-income group (RURAL) and urban middle-income group (URBAN) were considered.

Selection of the representative diet was based on the National Nutrition Monitoring Bureau (NNMB) diet survey records collected during its rural survey “Diet and nutritional status of rural population during 2000-01”. The procedure adopted for this survey was a two stage stratified random sampling method in which the village (N=80) selected based on agro economic regions formed the first stage unit while the household (N=10 household / village) formed the second stage unit. A 24-hour recall method of diet survey was administered to assess the food and nutrient intakes of individuals among these selected households.

Selection of Regional Diet for the study: The diet records of the selected 4 states were retrieved. Further to represent the entire State or a region and a State, 5 zones viz. South, West, North, East and Central were marked in the physical map of these States. Household diet records of these 5 zones were taken out and randomly selected the diet record of one household from each zone. These 5 household diet records were considered to represent 5 rural representative diets of each region. Thus there were 20 household diet records of low-income rural households. Similarly the representative diets from the same state urban middle income households were obtained from a fresh diet survey conducted near the headquarters of the state NNMB units.

Preparation of representative diets: A day's diet of the entire household was cooked and duplicate equivalent to that consumed by a male adult of each household were sampled out. They were homogenized, lyophilized, powdered and stored at -20°C till analysis.

Analysis: Analysis of proximate principles, iron, zinc, copper, calcium, β -carotene, phytates, tannins, total fiber were carried out as per the standardized AOAC procedures. Analysis of folic acid and vitamin B₁₂ was done using RIA kit method standardized for foods. Vitamin C content was assessed by HPLC method. *In vitro* iron and zinc availability was estimated by simulated intestinal

conditions. All the analytical methods have been validated using the standard reference material and internal quality controls.

Statistical analysis: Two-way ANOVA with post hoc multiple comparison 't' test and non-parametric 'Mann-Whitney U' test was carried out. To understand the contribution of dietary factors on availability multiple comparison univariate analysis was carried out.

RESULTS

Food consumption pattern: There were regional differences in the type of major staples. A distinct difference was consumption of Bajra (pearl millet), among the West (Gujarat) and rice among South (AP) regional rural households. The pattern of consumption of a combination of wheat and rice as staple with the quantity of rice among the households varying greatly with less of rice being consumed in the North was also seen. Rural and urban households had distinct food consumption pattern as cereal consumption was significantly higher in rural while vegetables, fruits and milk consumption was higher in urban households.

Proximate composition: In general, rural diets had higher protein content, whereas that of fat was significantly higher in urban households (Table 4, Figure 3).

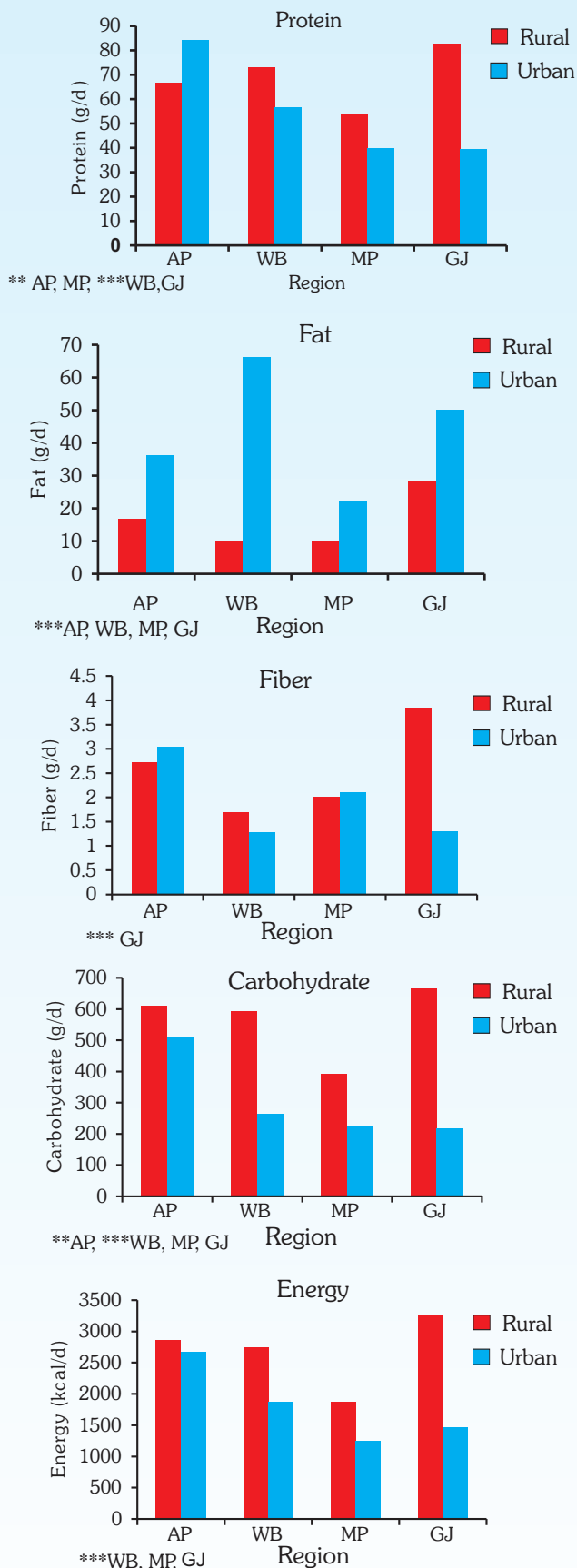
Table 4. Proximate Composition of representative Indian Diets

Parameter	Rural (g/d)	Urban (g/d)
Protein	69 ± 22.9 ^a	55 ± 24.5
Fat	16 ± 13.7 ^{***}	44 ± 28.8
Fiber	2.5 ± 1.73	2.0 ± 1.16
Ash	13.1 ± 6.43	11.8 ± 6.06
Carbohydrate	565 ± 163.3 ^{***}	304 ± 148.7
Energy (Kcal/d)	2680 ± 793.5 ^{***}	1832 ± 761.1

Values are Mean ± SD (n=20), t-test : ^{***}p<0.001 and ^ap<0.05 by Non Parametric Mann-Whitney U Test

Most of the diets were adequate in protein, with Gujarat urban diets having the least (39.6 g/d) and AP urban (84.0 g/d) having the highest protein content. Intake of fat in urban households was about 2-3 fold higher in all the regions except in East (WB), where it was 10 fold. On the other hand the intake of carbohydrate was higher in rural households. About 50% of the households' diets

Fig 3. Proximate composition of regional diets



were inadequate in energy content. The intake of energy was highest in Gujarat rural diets (3246 kcal/d) and lowest in MP urban diets (1243 kcal/d). Energy intake was significantly higher in rural than urban WB, MP and GJ households.

Iron and Zinc and other Minerals: Mean intake of iron and zinc were similar in both rural (iron 26.1 and zinc 11.5 mg/d) and urban (22.4 and 10.0 mg/d). AP rural household diets with rice as the staple had lowest mean iron content (17.3 mg/d) while Gujarat with bajra and wheat as staples had the highest (48.9 mg/d). Zinc content was highest in Gujarat rural diets (17.94 mg/d) and lowest in WB rural diets (6.2 mg/d). However, their densities were significantly lower in rural (iron 9.4 and 4.3 mg/ 1000 kcal) compared to urban (13.5 and 5.8) households diets (Table-5, Figure 4)

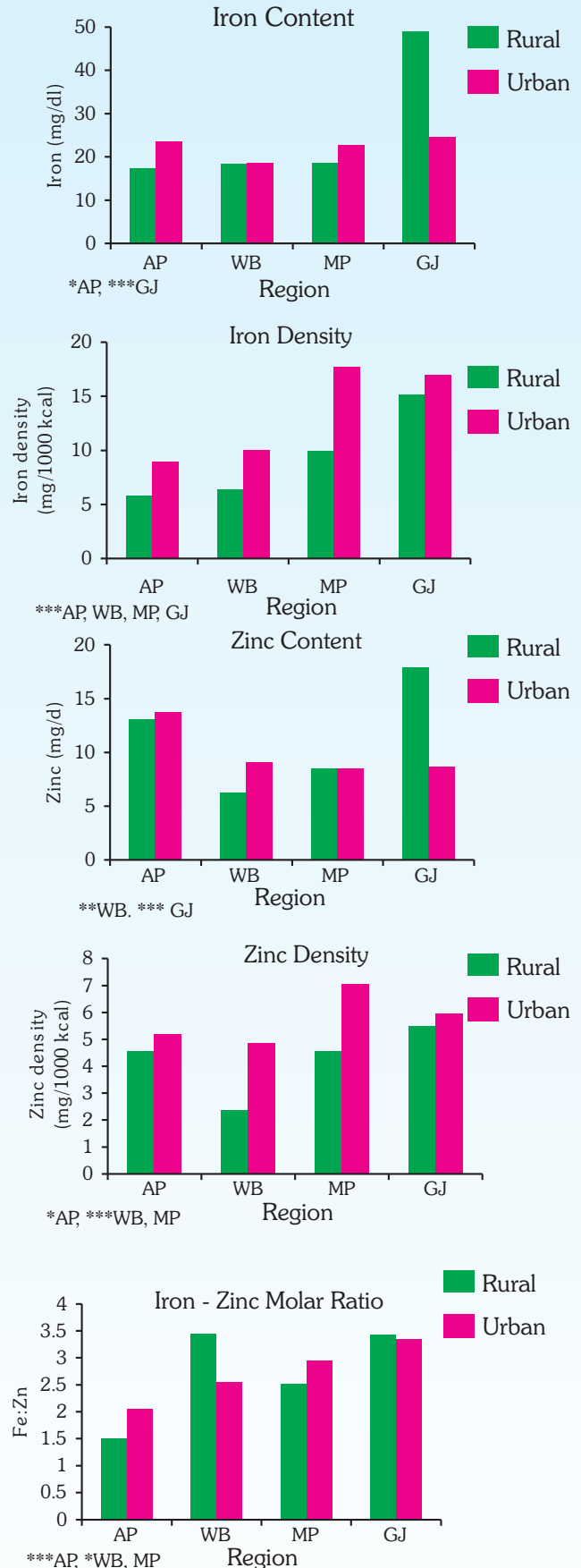
Table 5. Mineral content in representative Indian Diets

Parameter	Rural Households	Urban Households
Iron (mg/d)	26.1 ± 16.43	22.4 ± 7.88
Iron Density (mg/1000 Kcal)	9.4 ± 4.54*	13.5 ± 5.30
Zinc (mg/d)	11.5 ± 6.20	10.0 ± 3.82
Zinc Density (mg/1000 Kcal)	4.3 ± 1.57**	5.8 ± 1.39
Calcium (mg/d)	342.4 ± 144.63***	834.4 ± 471.64
Calcium Density(mg/1000 Kcal)	133.7 ± 62.41***	488.7 ± 229.62
Copper (mg/d)	4.0 ± 1.89*	2.9 ± 0.80
Copper Density (mg/1000 Kcal)	1.5 ± 0.75	1.8 ± 0.63
Fe:Zn Molar Ratio	2.7 ± 1.17	2.7 ± 0.66
Fe:Ca Molar Ratio	0.065 ± 0.049***	0.023 ± 0.011
Fe:Cu Molar Ratio	8.0 ± 4.50	8.8 ± 2.14

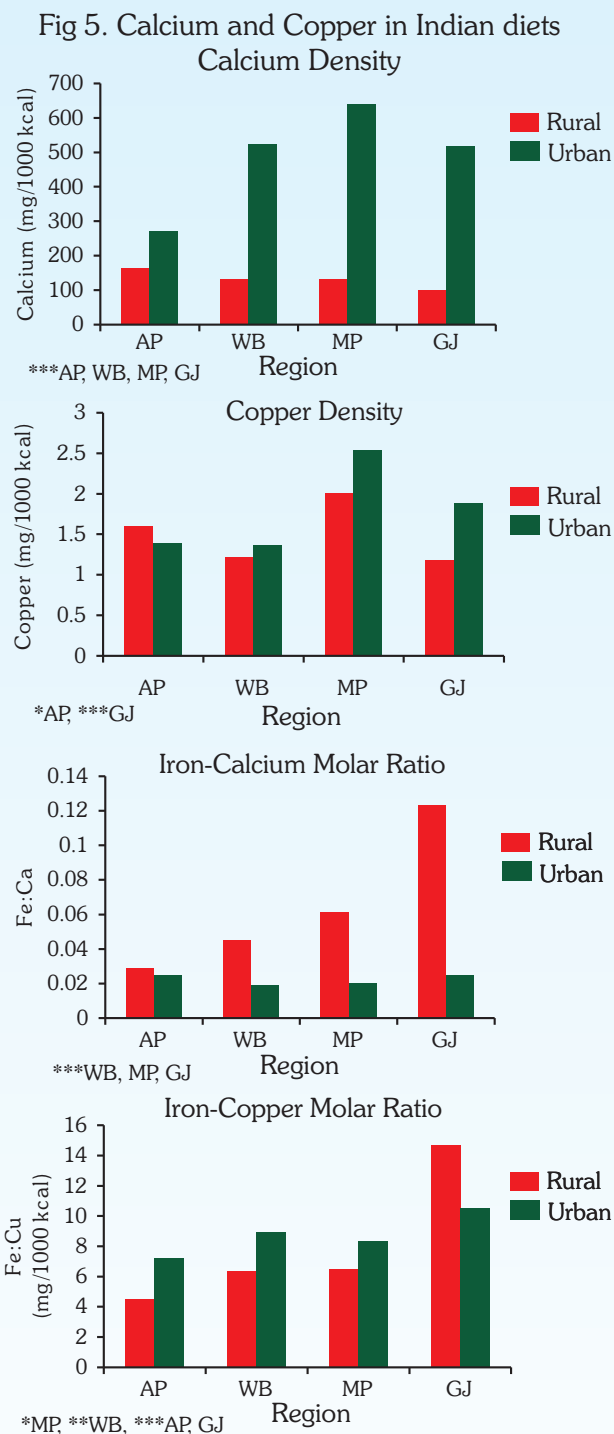
Values are Mean ± SD (n=20), t-test : *p<0.05, **p<0.01, ***p<0.001

In general the diets were not meeting the RDA of iron of 28mg/d for an adult male. Calcium intake and its density was higher in urban than rural households.

Fig 4. Iron and Zinc in Indian diets



Copper intake was significantly higher in rural households but its density was similar in rural and urban diets. As molar ratio, only Fe: Ca was significantly higher in rural diets compared to the urban diet (Figure 5).



Vitamins: Of the vitamins, only mean intake of folate met the RDA among rural and urban households. -carotene, ascorbic acid and vitamin

B₁₂ were lower than the RDA but were similar among rural and urban household diets (Table 6, Figure 6).

Table 6. Vitamins in representative Indian Diets

Parameter	Rural Households	Urban Households
β-Carotene (IU/d)	722 ± 596.7	1970 ± 3022.6
β-Carotene density (IU/1000 kcal)	280 ± 277.1*	1381 ± 2354.9
Folate (mg/d)	0.541 ± 0.291	0.766 ± 0.463
Folate Density (mg/1000 kcal)	0.219 ± 0.122***	0.435 ± 0.229
Ascorbic acid (mg/d)	8.4 ± 6.23	9.2 ± 7.67
Ascorbic acid density (mg/1000 kcal)	3.1 ± 1.63	6.4 ± 8.75
Vitamin B12 (μg/d)	1.70 ± 1.13	0.95 ± 0.44

t-test: *p<0.05, ***p<0.001, Values are mean ±SD (n=20)

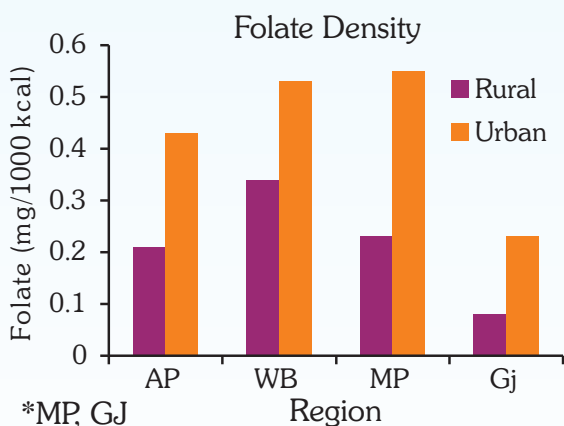
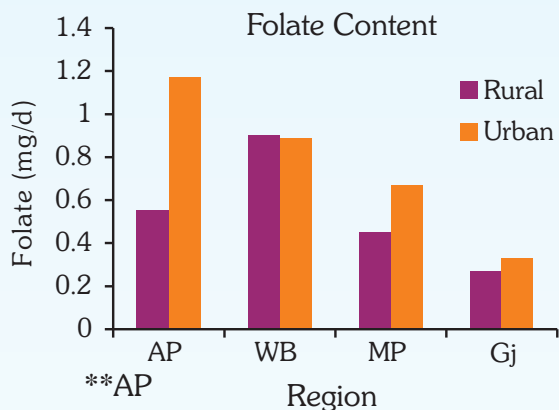
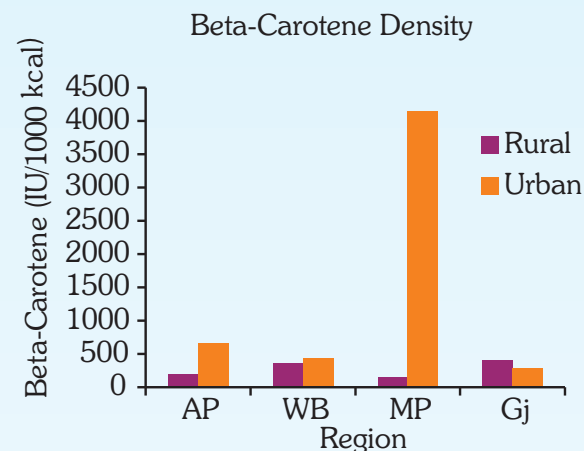
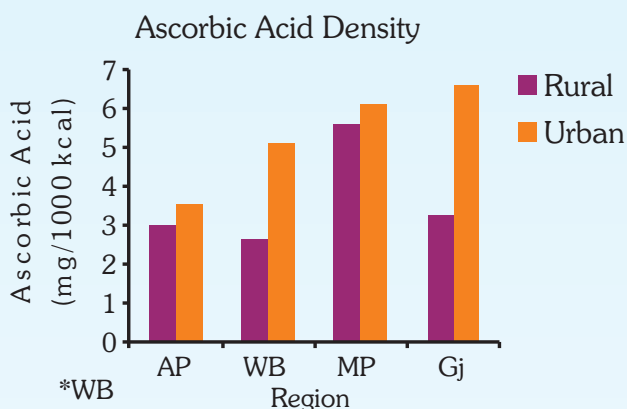
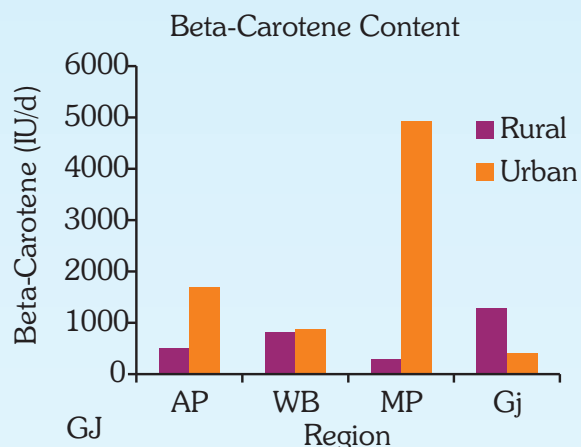
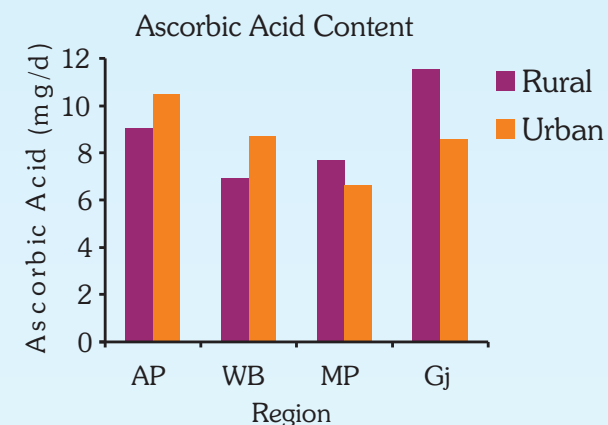
Phytic acid and tannins: Phytic acid and poly phenols in meals are known to inhibit mineral absorption. Intake of phytic acid (1 g/day) and tannins (55 mg/day) in both urban and rural regional diets was similar. Among the regions intake of phytic acid from the rural Gujarat diet was more than 3g/day and was significantly different from urban Gujarat diet. The density of tannin (mg/1000 kcal) was significantly higher in urban diets compared to its rural counterpart (Table 7, Figure 7). The molar ratio of phytate: iron was higher in rural diets.

Table 7. Phytate and tannins in representative Indian Diets

Parameter	Rural Households	Urban Households
Phytate (mg/d)	1392 ± 1268.4	856 ± 286.6
Phytate Density (mg/1000 Kcal)	490 ± 357.6	526 ± 235.6
Tannins (mg/d)	50.9 ± 26.08	63.0 ± 38.21
Tannins density (mg/1000 kcal)	20.2 ± 13.06**	37.3 ± 19.64
Phytate:Iron Molar ratio	4.4 ± 1.53*	3.4 ± 1.01
Phytate:Zinc Molar ratio	11.4 ± 5.46	9.1 ± 2.90

Values are Mean ± SD (n=20), t-test: *p<0.05, **p<0.01

Fig 6. Vitamins in Indian Diets



Iron and Zinc Availability: The pooled mean in vitro availability of iron was similar among the rural (4.3 %) and urban (3.5%) diets. Zinc availability on the other hand was significantly higher in rural (17.3 %) compared to urban (12.4 %) diets (Table 8, Figure 8). Among the regional diets rural south (AP 5.7 vs 2.4%) and urban east (WB 4.8 vs 5.2%) exhibited higher iron availability compared to their counterparts while it was similar among urban and rural MP (3.7%) and Gujarat (2.9%) (Table 9).

Table 8. Iron and zinc availability from representative Indian Diets

Parameter	Rural Households	Urban Households
Iron Availability (%)	4.3 ± 1.89	3.5 ± 1.42
Zinc Availability (%)	17.3 ± 7.48*	12.4 ± 3.89

Values are Mean ± SD (n=20), t-test : *p<0.05

Fig 7. Phytic acid and tannins in Indian Regional Diets

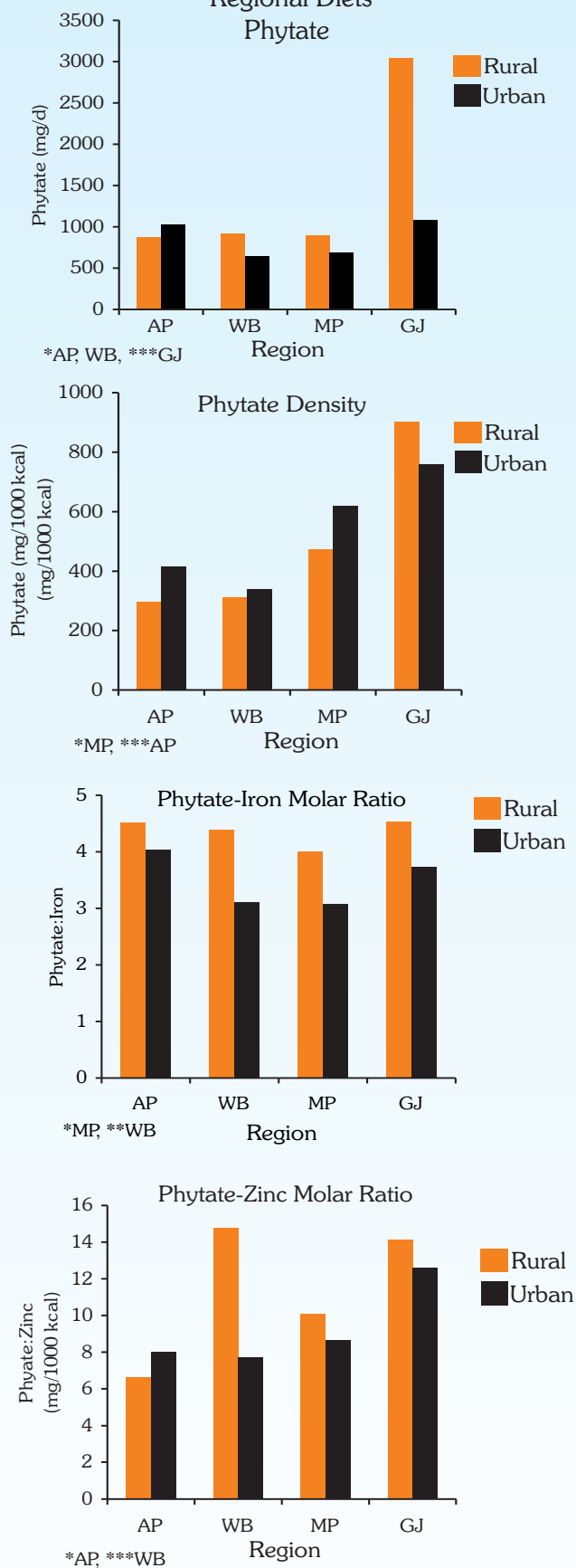


Table 9. Mean iron availability of Indian regional rural and urban household diets

Region	Iron availability (%)					
	Rural Household					
	1	2	3	4	5	Mean
South (Andhra Pradesh)	>5	>5	>5	>5	3-5	5.69 ± 1.07
East (West Bengal)	<3	<3	>5	>5	>5	4.76 ± 2.54
North (Madhya Pradesh)	<3	<3	<3	3-5	>5	3.63 ± 1.54
West (Gujarat)	<3	<3	<3	3-5	3-5	2.97 ± 0.93
Region	Urban Household					
	1	2	3	4	5	Mean
	<3	<3	<3	<3	3-5	2.38 ± 0.90
East (West Bengal)	3-5	3-5	3-5	>5	>5	5.20 ± 0.73
North (Madhya Pradesh)	<3	<3	3-5	3-5	>5	3.64 ± 1.25
West (Gujarat)	<3	<3	<3	3-5	3-5	2.88 ± 0.91

In vitro availability: <3% (1.5-2.9), 3-5% (3.3-4.9) and >5% (5.4-7.9)

Negative Modulators of Iron and Zinc Availability: Role of various dietary components in modulating iron and zinc availability was computed and those having significant correlation are listed in Table 10.

Fig 8. *In Vitro* availability of iron and zinc in Indian diets

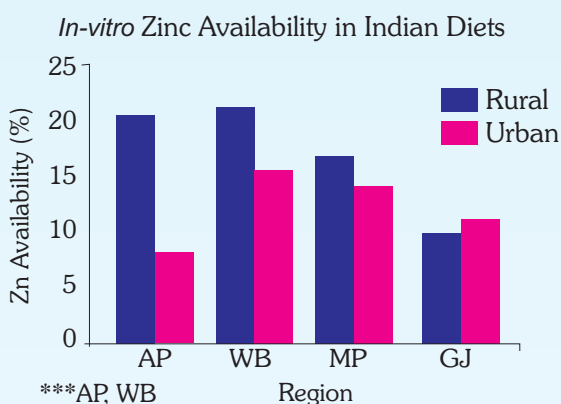
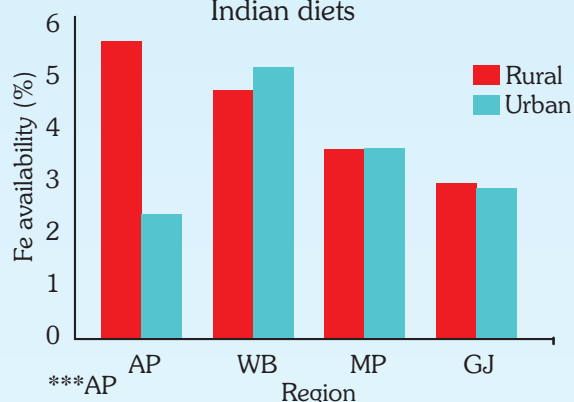


Table 10. Correlation of dietary factors with iron and zinc availability

Parameter	Iron Availability	Zinc Availability
Iron Content (mg/d)	-0.419**	-0.466**
Iron Density (mg/1000 kcal)	-0.489***	-0.508***
Zinc (mg/d)	NS	-0.478**
Zinc Density (mg/1000 kcal)	NS	-0.600***
Iron :Zinc Molar Ratio	-0.408**	NS
Iron :Copper Molar Ratio	-0.412**	-0.370*
Phytate (mg/d)	-0.336*	-0.423**
Phytate Density (mg/1000 kcal)	-0.501***	-0.517***
Phytate :Zinc Molar Ratio	-0.380*	NS

NS- Not significant $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

There are no positive modifiers of iron and zinc availability in the regional diets. Both the mineral content and their density are correlated negatively to their availability. The molar ratios of Fe:Zn, Fe:phytic acid and Zn:phytic acid copper had a

significant negative effect on iron and zinc availability. There was a dose dependent decrease in iron and zinc availability with phytic acid and at higher concentration (> 1.0 g/d) the availability was the lowest. Thus a diet rich in mineral and phytic acid appears to be destined for lower availability (Figure 9).

Classification of Regional Diets based on availabilities of Iron

Thus mineral availability from a diet is dependent on many factors and therefore the composite nature of diet and not an individual factor governs the availability. In an attempt to closely study these governing factors, the diets were further classified on the basis of iron availability; availability $< 3\%$, $3-5\%$ and $> 5\%$. To ascertain the role of various Fe-absorption determinants on availability, multiple comparison was done (Table 11).

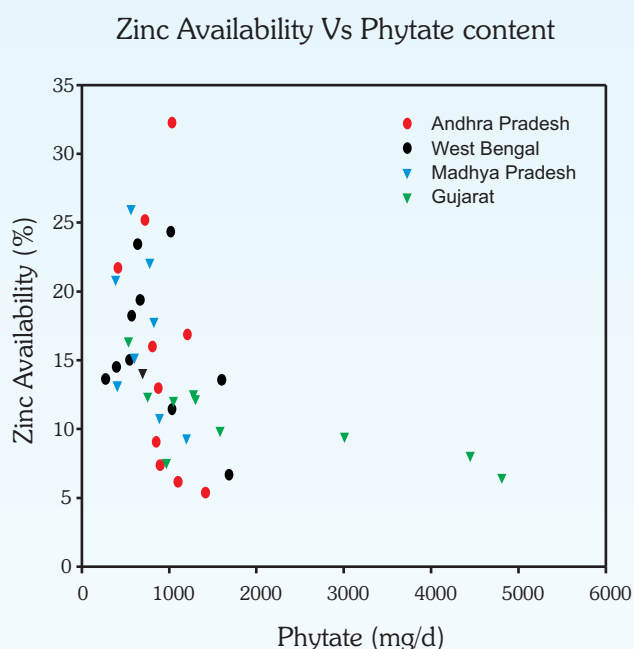
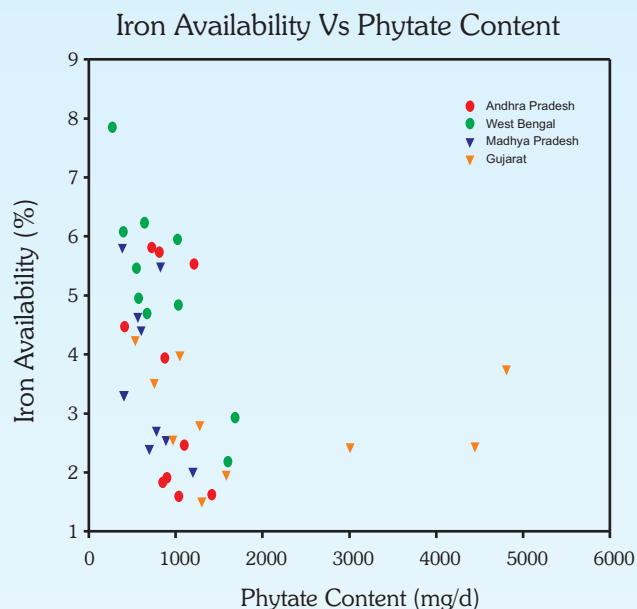
It was observed that the differences in densities of iron, phytate, cereal and milk intake were significantly different between iron availability $< 3\%$ and $> 5\%$. Diets with rice had better availability compared to diets with Bajra (high in iron, phytate, zinc) as staple. Mean iron availability was highest for the rural AP and urban WB diets. While rural WB and urban MP diets showed intermediate and urban AP, rural and urban Gujarat diets were $< 3\%$. Milk intake of households with low Fe-availability was different from high but similar to intermediate Fe-availability diets.

Table 11. Multiple comparison at different levels of iron availability

Factor	Iron Availability		
	< 3.0 n=17	3.0-5.0 n=12	> 5.0 n=11
Dialyzable Iron (mg/100g lyophilized diet)	0.097 ^a	0.12 ^{ab}	0.13 ^b
Zinc Availability (%)	11.58 ^a	15.47 ^{ab}	19.17 ^b
Iron Density (mg/1000 kcal)	14.12 ^a	10.80 ^{ab}	8.04 ^b
Iron : Copper Molar Ratio	9.72 ^a	8.69 ^{ab}	6.06 ^b
Phytate Density (mg/1000 kcal)	657 ^a	488 ^{ab}	301 ^b
Cereal Intake (g/CU/d)	445 ^{ab}	361 ^a	567 ^b
Milk Group Intake (ml/CU/d)	257 ^a	154 ^{ab}	61 ^b

Values with different subscripts are different at $p < 0.05$

Fig. 9 : Relationship between mineral availability and phytate content



Modification of Regional diets: Since there was significant effect of mineral density, cereal intake and phytic acid content on iron availability rice, bajra and wheat based diets were selected for modification. Out of the forty representative diets tested, 11 of them had iron availability more than 5% and 17 less than 3%. Therefore, a regional diet was selected and modified with iron availability less than 3 % from Gujarat and MP with regional diets

with iron availability more than 5 % from West Bengal and AP. Diets of these regions were modified with respect to only cereals of by substituting bajra and wheat with rice at 50% and 100% isocaloric level, keeping other constituents same.

Iron availability in modified diet: It was observed that complete replacement of rice with bajra and vice versa in diets resulted in change in the mineral content. Replacing bajra with rice increased both iron and zinc availability (1.42 to 12.95 %) while rice with bajra decreased availability (Table 12).

Table 12. Mineral content and availability in modified diets

Diet	Fe mg/d	Zn mg/d	Cu mg/d	Ca mg/d	Fe Availability %	Zn Availability %
WBR	20.76	6.4	3.65	866.4	6.53 (1.35)	16.02
WBR+100% Bajra	100.15	28.88	5.26	907.15	1.98 (1.98)	5.94
WBR+50% Bajra	47.48	15.02	4.46	919.84	3.67 (1.74)	11.45
GR	78.79	23.43	9.13	246.71	1.42 (1.1)	6.25
GR+100% Rice	7.40	6.88	3.62	161.67	12.95 (0.96)	15.52
GR+50% Rice	25.95	11.81	6.67	208.48	4.52 (1.17)	22.03
APR	18.24	11.96	4.88	342.68	7.53 (1.37)	23.94
APR+100% Bajra	45.56	23.18	6.74	374.44	3.32 (1.51)	13.19
APR+50% Bajra	33.11	18.67	6.64	319.12	3.31 (1.09)	15.14

Parenthesis indicates accessible iron

CONCLUSIONS

There are regional and rural and urban differences in iron and zinc density and their *in vitro* availability, which are mainly due to the composition of major staple and phytate content in the diet. Modification of diet to improve iron and zinc availability can be achieved by replacing major staple either by improving iron content and minimizing inhibitor phytate. Ironically good sources of minerals are also good in phytates and the intake of absorption promoters such as meat, fish and ascorbic acid is very low.

3. Wheat flour atta food fortification with micronutrients iron, folic acid and vitamin A- Public private partnership

Based on studies carried out on fortification of whole-wheat flour (atta) with micronutrients iron and folic acid (Annual Report 2003), NIN has provided technical assistance to the AP State civil supplies department to fortify and supply wheat flour through fair price shop in the state of Andhra Pradesh on a pilot scale. The fortified atta branded 'VIJAYA ENRICHED ATTA' provides iron - 60 mg, folic acid - 1.5 mg and vitamin A 3300 IU per kilogram of atta (Figure-10). Currently it is produced by two flour millers and supplied to BPL cardholders of the urban areas of Hyderabad, Ranga Reddy and Warangal districts through the fair price shops. The cost of one kilogram of this is Rs.12 and the cost of the premix is 3.2 paise per kg.

The stability of the fortified nutrients was checked and the result is given in Table 13. Stability of iron and folic acid was found to be very good where as that of vitamin A unsatisfactory. However, the premix also contained 50% less than the expected content and that of folic acid 50 % more. Appropriate corrective measures have been suggested to maintain the quality of the product.

Table 13. Stability of micronutrients in fortified atta

Stability	Iron (mg)	Vitamin-A (IU)	Folic acid g
70 days (N=9)	21 0.4	125 38.5	540 183
Expected	20	660	300

*In tune with the premix Vitamin A and folic acid content

Fig. 10. Wheat flour atta fortified with iron, vitamin A and folic acid. The packaging of Vijaya enriched atta carries the name of NIN

4. Effect of sesame lignans on the oxidative stability of edible vegetable oils

The stability of oils during storage or upon heating to frying temperature is an important measure for ensuring good oil performance at elevated temperatures and for maintaining the quality of foods. Antioxidants in foods play a major role in maintaining the quality of oils and foods. Several lines of evidences have documented the vital role of antioxidants in several biological processes and therefore in prevention of diet-related chronic diseases. Synthetic antioxidants approved by PFA are used at permissible levels in edible oil and food industries.

However, as high dietary intake of synthetic antioxidants may have toxic or carcinogenic effects, it is important to identify newer natural components of foods having radical scavenging / antioxidant activity. Sesame (*Sesamum Indicum* Unn) has long been used as a traditional health food in India for its nutritional and medicinal value. Sesame contains substantial amounts of unique components, namely sesamin and sesamol. The higher stability of sesame oil has been attributed to its inherent lignans.

Earlier studies have shown that sesame lignans inhibit oxidative damage in *in vitro* biological systems (Mol Cell Biochem 2004) and iron induced oxidative stress in rats. (Brit J Nutr 2004). Further, blending sesame oil with groundnut oil or palmolein increased the thermal stability as compared to the respective single oils (Annual report 2000). The present study was designed to evaluate the effect of sesamin and sesamol in enhancing the stability of edible vegetable oils.

EXPERIMENT

In the present study, sesamin and sesamol were crystallized as described earlier (J Am Oil Chem Soc 2004) so as to get ~ 10 g sesamin and ~ 5g sesamol. Soyabean (SBO) and sunflower (SFO) oils without added antioxidants were procured from Ruchi Health Foods Ltd, Kannigaipair Village, Thiruvallur Dist, Tamil Nadu and ricebran (RBO) from SSD Oil Mills

Company Ltd. Village Road, Iyyappanthangal, Chennai.

The effect of adding 0.6% or 1.2% purified total lignans (sesamin and sesamol) was studied on (a) thermal stability and b) storage stability of the following oils: (i) SBO (oil containing both linoleic (n-6) and alpha linolenic (n-3) acids or (ii) SFO (oil containing high levels of linoleic acid, (n-6) or (iii) rice bran oil (containing moderate levels of linoleic acid, n-6).

Thermal stability: Each of the above oil either as such (without lignans) or with 0.6% (sesamin 0.4g+sesamol 0.2 g) or 1.2% (sesamin 0.8 g +sesamol 0.4 g) total lignans was heated to frying temperature (FT) for 2hours. The FT (180-200°C) of the oils was maintained by frying purees. Aliquots of the oil before heating and at 30min intervals during heating (0, 30, 60, 90 and 120 min) were taken for determining the thermal stability of the oil.

Storage stability: Each of the above oils as such or with added lignans was stored at room temperature for 60 days. Aliquots were taken initially and at 15 days intervals (0, 15, 30 and 60 days) for determining the storage stability of the oil.

RESULTS

Thermal stability: i) Heating the oils without addition of lignans decreased retention of a) total tocols (SBO 21%; SFO -15% and RBO -46%), b) total PUFA (SBO, SFO or RBO -90%) and c) total radical scavenging activity (RSA). RSA is expressed as g oil / ml required to lower the initial DPPH concentration by 25% as extrapolated from the dose response curve (IC_{25}). The IC_{25} values were as follows: SBO: 0min - 0.012 g, 120 min - 0.04 g, SFO: 0 min - 0.015 g, 120 min - 0.065 g and RBO: 0min - 0.009 g, 120 min - 0.019 g). The higher RSA and retention of total tocols in RBO at the end of 120 min of heating suggests higher thermal stability of RBO as compared to SBO and SFO. ii) Heating SFO, SBO with 0.6 or 1.2% lignans for 120 min increased RSA (SFO +0.6% lignans: 0min - 0.011g; 120 min - 0.04g; SFO+1.2% lignans: 0min-0.012g, 120min-0.043g SBO+ 0.6% lignans: 0min-0.011g, 120min - 0.028g; SBO + 1.2% : lignans: 0min-0.011g, 120min-0.015g).

However, heating RBO with either 0.6% or 1.2% lignans did not affect RSA (RBO +0.6% or 1.2% TL: 0min-0.011g, 120min-0.017g) b) increased retention of total tocopherols only in SBO (0min 21% Vs 120 min 38%, c) decreased % retention of total lignans to about the same extent (SBO+0.6% or 1.2% lignans-77%; SFO+ 0.6% lignans -63%; SFO+ 1.2% lignans-83%; RBO+0.6% or 1.2% lignans 70%) and d) decreased total PUFA to the same extent (90%) in all the oils with or without the addition of lignans.

Storage stability: The data on shelf life of the oil with or without lignans showed that while RSA of SBO and SFO were not altered that of RBO decreased. In all the 3 oils, storage for 60 days did not affect the retention of total tocopherols, total lignans and total PUFA.

CONCLUSIONS

1. The observed higher thermal stability of RBO (irrespective of the presence or absence of sesame lignans) may possibly be due to its unique minor components. However, the decrease in RSA of RBO upon storage for 60 days is not clear.
2. The increase in antioxidant potential and / RSA of SBO or SFO due to addition of lignans may possibly be due to synergism among sesame lignans and non-glyceride components of SBO (soya lignans, isoflavonoids) or SFO (phytosterols).

Earlier studies (Ann. Rep. 2000) showed that i) sesame oil blended with either groundnut oil or palmolein had higher thermal stability as compared to single oils ii) sesame lignans enhanced the antioxidant activity of Vitamin E in *in vitro* biological systems and in rats fed diets containing purified sesame lignans.

Therefore, it may be concluded that: a) sesame lignans can be used as natural antioxidants in edible oil and food industry b) blends of sesame oil with preferred oil (s) may increase the antioxidant potential of oils c) use of sesame seeds in snacks/ready to eat foods/pickles and for fortification of foods (food-food fortification) can contribute to improving the antioxidant potential of Indian diets.

5. Role of n-3 PUFA in fetal programming of insulin resistance in offspring: Biochemical and molecular mechanisms

During development of fetus there are critical and restricted periods which are often coincident with periods of rapid cell division during which individual tissues/organs differentiate and mature. According to theory of 'fetal origins' of chronic adult disease, nutritional deprivations/ imbalances in utero and early postnatal growth and development alter physiology and metabolism of developing tissues/organs and increase the risk of chronic adult diseases.

Long chain polyunsaturated fatty acids (LC-PUFA) of both n-6 and n-3 series are integral components of cell membrane and are important determinants of fetal growth and development. Docosahexaenoic acid (22:6n-3) is the predominant fatty acid present in brain, retina and other nerve tissues. Earlier studies in rats showed that partial substitution of dietary linoleic acid (18:2 n-6) with either alpha - linolenic acid (18:3n-3) (Ghafoorunissa et al, BBA 2005) or LC n-3 PUFA (Ghafoorunissa, et al. J. Nutr 2005) in casein-sucrose based diets decreased insulin resistance by improving peripheral insulin sensitivity (adipose tissue and muscle). However, partial substitution of total dietary saturated fatty acids (SFA) with trans fatty acids (TFA) in casein-starch based diets decreased peripheral insulin sensitivity (Ahamed et al, Metabolism 2005 and Saravanan et al, Br. J. Nutr. 2005).

Hypothesis: Diets providing TFA or low levels of n-3 PUFA during intrauterine and postnatal growth and development predispose to insulin resistance in adult life.

STUDY DESIGN:

Phase 1 (preconception, pregnancy and lactation):

WNIN female weanling rats (n=32) were randomly divided into 4 groups and fed cereal-pulse based diets containing (energy %) : fat ~22, SFA ~ 4.6 , MUFA ~5.3, PUFA ~10.2 (18:2n-6+18:3n-3).

The levels of either 18:3 n-3 or LCn-3 PUFA or TFA were as follows:

Group I (low 18:3 n-3): ~ 0.3 en% 18:3n-3 (n-6/n-3 ratio ~28, n=12)

Group II (18:3 n-3 suppl): ~2.9 en% 18:3n-3 (n-6/n-3 ratio ~2.5, n=6)

Group III (LCn-3PUFA suppl): ~0.37 en% 18:3n-3 + 0.5 en% L C n-3 PUFA (n-6/n-3 ratio ~11, n=6)

Group IV (TFA): ~0.3 en% 18:3n-3 + 1 en% TFA (n-6/n-3 ratio ~28, n=8)

WNIN male weanling rats were fed stock colony diet (n=16)

The diets were fed *ad libitum*, daily food intake and weekly body weight were recorded throughout the period of study.

Phase 1a : (Preconception)

After 90 days of feeding the above diets, blood was collected after 18 hours fasting from rats in groups I to IV. Plasma was analyzed for glucose, total triglycerides and total cholesterol.

Phase 1b : (Pregnancy and lactation)

After 5 days of bleeding (95 days feeding), rats in groups I to IV were mated with males fed stock colony diet (2 females + 1 male). The pregnant rats were continued on the respective diets throughout the period of pregnancy. After delivery, the litter weight was recorded, the litter size for each mother was equalized to 8, and the mothers were continued on the respective diets. At the end of 30 days of delivery, blood was collected after 18 hours fasting. Plasma was analyzed for glucose, total cholesterol, total triglycerides and oral glucose tolerance (OGT) test was done.

Phase 2: (Post weaning):

At the age of 21 days, male rats in groups I and IV were randomly grouped and fed the following diets:

Group Ia (n=13): were continued on group I diet (low 18:3 n-3)

Group I b (n=13): were fed group II diet (18:3n-3 suppl)

Group I c (n=13): were fed group III diet (LC n-3 PUFA suppl)

Groups II (n=13): were continued on group II diet (18:3 n-3 suppl)

Group III (n=13): were continued on group III diet (LC n-3 PUFA suppl)

Group IVa (n=9): were continued on group IV diet (TFA)

Group IVb. (n=9): were fed the diet fed to group Ia (low 18: n-3)

At the end of and 90 days, blood was collected after 18 hour fast for analysis of the following parameters:

1. Plasma: glucose, insulin, and total free fatty acids
2. OGT: Following oral glucose load, plasma glucose and insulin at 30, 60 and 90 minutes.
3. At the end of 105 days of feeding blood was collected after 18 hours fasting and animals were sacrificed by CO₂ asphyxia. Liver, pancreas, epididymal and retroperitoneal fat pads, muscle (diaphragm and soleus) and retina were dissected and the following analysis were done:

Plasma: Total cholesterol and total triglycerides

Epididymal fat: Adipocyte lipolysis, antilipolytic effect of insulin and insulin stimulated glucose transport.

From 2 rats in each group epididymal fats were pooled for mRNA expression of lipoprotein lipase (LPL), adiponectin, Glut 4, tissue necrosis factor (TNF) and steroid regulatory element binding protein (SREBP-1c).

From 2 rats in each group soleus were pooled for m RNA expression of LPL, Glut4 and pyruvate kinase (PK). The relative m RNA expression of individual genes (mean of 3 assay) was calculated according to the beta actin levels (internal control). The percentage of relative expression of candidate genes in different groups was calculated by considering gene expression observed in group Ia.

Diaphragm: Intramyocellular triglycerides and fatty acid composition of phospholipids

Soleus: Intramyocellular triglycerides.

Liver: Antioxidant enzymes (GSH-px, SOD Catalase), TBARS and total tocopherols

Retina: For histology study eyeballs of 2 rats in each group were dissected and fixed in 10 % neutral buffered formalin overnight and processed in Thermoshandon-Citedal 2000. The tissues were later paraffin embedded and blocks were

prepared. Hematoxylin and Eosin staining was done on 4 paraffin sections using Leica autostainer X1 and retinal morphology was examined under light microscopy.

For ELOVL4 expression, RNA was extracted from pooled rat retinas from each rat (n=6 from each group) using TRI reagent (Sigma) according to manufacturer's instructions. 1 g of RNA was transcribed to cDNA using Biorad iscript cDNA synthesis kit. Real Time PCR was performed using 20 ng of the above cDNA of each sample in triplicates using SYBR Green supermix and gene specific primers. GAPDH, used as a house keeping control, was also amplified in separate tubes using same cDNA in duplicates. Expression levels of ELOVL4 were normalized with that of house keeping gene GAPDH. Total lipids were extracted from retina of 4 rats in each group and fatty acid composition was determined by gas liquid chromatography.

RESULTS

- ✎ The data were analyzed by one-way ANOVA and the results were as follows:
- ✎ Phase 1a (in female rats during post weaning / adult / preconception): Food intake, gain in body weight, plasma glucose, cholesterol and triglycerides were essentially similar in all groups.
- ✎ Phase 1b (pregnancy and lactation): Daily food intake, gain in body weight was similar in all groups. The % conception rate in group IV was lower (~60%) as compared to groups I, II and III (90, 80 and 80 % respectively) and one rat in group IV delivered malformed and dead litter. However, litter weight and size was similar in all groups.
- ✎ Plasma glucose, total triglycerides total cholesterol and area under the curve of insulin (AUC) was similar in all the groups.

Phase 2 (post weaning -adult): Comparison of the following groups evaluates the effects of dietary fatty acids throughout life cycle (fetal and postnatal growth and development)

1. Effects of dietary: a) 18:3n-3 n-3, Ia vs. II b) LC n-3 PUFA, Ic vs. III c) TFA, IVa vs. Ia

2. Fetal programming on low n-3 PUFA diets and: after weaning effects of dietary a) 18:3 n-3, Ia vs Ib or b) LC n-3 PUFA, Ia vs Ic.
3. Fetal programming on diets providing TFA: IVa vs IV b

At the end of 105 days of feeding, gain in body weight, total body fat (omentum + retroperitoneal + epididymal) were similar.

Plasma: Glucose, insulin, AUC of insulin and AUC of glucose after oral glucose load were essentially similar in all groups. Plasma total cholesterol, free fatty acids were high in group fed TFA diet (group I Va) as compared to group Ia (81 ± 4.7 Vs. 65 ± 4.9 mg / dl, $p < 0.05$; 0.65 ± 0.06 Vs 0.48 ± 0.05 , $p < 0.05$ respectively). After weaning, switching to diets devoid of TFA (group IVb) did not normalize the increase in plasma cholesterol (IV b 71 ± 3.4) and free fatty acids (group IV b 0.58 ± 0.05). Neither 18:3n-3, nor LCn-3 PUFA altered plasma cholesterol and free fatty acid levels.

Adipocyte: Lipolysis, antilipolytic effect of insulin were similar in all groups. In group IVa, though insulin stimulated glucose uptake decreased at all concentrations of insulin, the decrease was not statistically significant. However, in group IV b significant decrease was observed (0.05 nM insulin Ia $17.6 \pm$ vs. 9.0 ± 1.3 0.1 nM insulin 25.9 ± 5.4 vs 10.5 ± 2.0 , 1 nM insulin 45.4 ± 9.6 vs. 15.1 ± 2.9 , 10 nM insulin 56.9 ± 11 vs. 28.0 ± 7.4 $p < 0.05$). However, the reason for greater reduction of glucose uptake in adipocytes following withdrawal of dietary TFA during postnatal period is not clear. The mRNA expression of adiponectin decreased in TFA group (IV a). Switching to diet devoid of TFA after weaning (group IV b) did not normalize the TFA induced decrease in mRNA expression of adiponectin. Dietary 18:3 n-3 or LC n-3 PUFA did not affect insulin stimulated glucose uptake and mRNA expression of adiponectin. mRNA expression of LPL, Glut4, TNF, SREBPI in adipocytes and LPL, Glut4 and PK in soleus were similar in all groups.

Soleus and diaphragm: Intramyocellular triglyceride levels in both soleus and diaphragm were

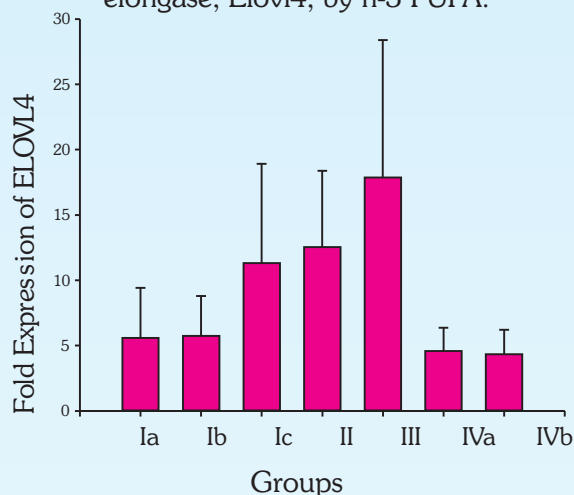
similar in all groups. The data on fatty acid composition of phospholipids showed that dietary 18:3 n-3 increased 18:3 n-3 (group 1a -non detectable levels , groups 1b and II 0.4 ± 0.06 and 0.37 ± 0.02 respectively),decreased arachidonic (Ia 14.4 ± 0.6 , Ib 10.6 ± 0.6 , II 10.7 ± 0.7 , Ia vs. Ib $p < 0.05$, Ia vs. II $p < 0.05$), docosatetraenoic (22:4 n-6) and docosapentaenoic acid (22:5 n-6) and increased LC n-3 PUFA (22:5n-3 Ia 1.1 ± 0.1 , Ib 2.6 ± 0.3 , II 2.3 ± 0.2 Ia vs Ib $p < 0.05$, Ia vs II $p < 0.05$;22:6n-3 Ia 4.4 ± 0.23 Ib 6.9 ± 0.8 II 7.8 ± 0.5 Ia vs. Ib $p < 0.05$ Ia vs. II $p < 0.05$) . Dietary LC n-3 PUFA (Ic and III) decreased 20:4n-6, 22:4 n-6 and 22:5n-5 and increased LC n- 3 PUFA to the same extent as 18:3 n-3 (Ib and II). In TFA fed groups, except for increase in 18:1 trans ,the fatty acid compositions were essentially same as in group Ia.

Liver: The data on lipid peroxides and antioxidant enzyme activities showed that dietary 18: n - 3 as well as LC n-3PUFA increased the activities of hepatic glutathione peroxidase (GSHpx) and catalase. However, the magnitude of increase was greater in groups II and III (groups continued after weaning on 18:3 n-3 or LCn-3 PUFA supplemented diets respectively). Dietary TFA did not alter lipid peroxidation and antioxidant enzymes.

Retina: The integrity of photoreceptor layers in retina were comparable in groups Ia, Ib, Ic, II and III. In groups IVa and IV b, the photoreceptor and outer nuclear layers were severely damaged. The relative expression of ELOVL4 was calculated by comparative Ct method (Figure 11). The expression of ELOVL4 was highest in groups III followed by groups II and Ic as compared to groups fed low 18:3 n-3 (Ia) or group supplemented with 18:3 n-3 from postweaning stage (Ib).

These observations suggest that ELOVL4 expression may be related to n-3 PUFA nutritional status. Dietary TFA exposure during fetal growth and development decreased ELOVL4 expression (group IVa) and withdrawing TFA after postweaning stage (group IV b) did not normalize the ELOVL4 levels. The retinal total fatty acid composition was essentially similar in all groups except for a decrease in 20:4 n-6 in group III.

Fig. 11: Modulation of expression of fatty acid elongase, Elov14, by n-3 PUFA.



CONCLUSIONS

This study has shown that increasing dietary 18:3 n-3 or LCn-3 PUFA throughout life (fetal, postnatal growth upto adult) elicits beneficial effects with respect to n-3 PUFA and antioxidant nutritional status and relative expression of ELOVL4 in retina. The data on effects of n-3 PUFA on fetal programming of biochemical and molecular parameters associated with insulin resistance suggests that increasing 18:3 n-3 above 0.3 en% (n-6/n-3 =28) only during fetal and postnatal growth and development may not provide beneficial effects for preventing insulin resistance in adults.

Maternal TFA intake may increase the susceptibility to insulin resistance as evidenced by increase in plasma free fatty acids and cholesterol, decrease in relative mRNA expression of adiponectin. These observations suggest that maternal intake of TFA (from hydrogenated vegetable oils) may increase the susceptibility to biochemical/ metabolic alterations known to be associated with increase in risk of chronic diseases.

ELOVL4 is a novel member of family of human fatty acid elongases involved in long chain fatty acids and whose function is essential of photoreceptor maintenance. These observations suggest that ELOVL4 expression may be related to n-3 PUFA nutritional status. Further, the decrease in retinal ELOVL4 expression associated with abnormality in retinal morphology in TFA fed groups suggests that TFA may affect retinal function and metabolism of long chain PUFA.

6. Health beneficial effects of fruits and vegetables : Total phenolic content and antioxidant activity of dry fruits

Epidemiological studies have reported a significant negative correlation between the intake of fruits and vegetables and mortality due to chronic diseases such as cancer, heart disease and stroke. Phenolic compounds present in fruits and vegetables are reported to have multiple biological effects including antioxidant activity (AOA). Natural antioxidants have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic values. Literature on the health beneficial effects of Indian plant foods is scanty. The phenolic content and antioxidant activity of some commonly consumed plant foods of India including some preliminary data on the antioxidant activity of a few fresh fruits was earlier reported. Dry fruits, some of which are consumed commonly, have longer shelf life and are relatively non-perishable and these could be the added advantages for dry fruits as natural sources of antioxidants. The present study was carried out to generate a data base on the phenolic content and antioxidant activity of some commonly consumed dry fruits in India (Tables 14 & 15).

Table 14 : Antioxidant activity of few commonly consumed dry fruits

Name of the dry fruit	Scientific names	Phenolic content mg/100g	DPPH (% inhibition of control)	FRAP μ mol/g	Reducing power mg/g
Almond	<i>Prunus amygdalus</i>	109.07 15.96	21.15 0.08	56.38 5.96	0.56 0.04
Apricot	<i>Prunus armenidca</i>	304.63 38.84	26.69 4.67	163.60 14.06	5.75 0.67
Brown raisins	<i>Vitis vinifera</i>	749.37 24.62	70.48 11.99	286.15 3.30	6.59 1.01
Cashew nuts	<i>Anacradium occidentole</i>	153.27 3.98	16.66 0.57	42.26 6.28	0.91 0.04
Dry dates	<i>Phoenix dactylifera</i>	241.61 50.48	33.02 3.24	172.01 22.50	4.00 0.61
Fresh dates	<i>Phoenix dactylifera</i>	193.08 33.63	34.88 4.77	107.61 13.32	6.30 1.24
Figs(Anjeer)	<i>Ficus carica</i>	331.93 51.19	35.80 4.62	128.73 15.22	8.40 2.01
Ground Nut	<i>Arachis hypogeo</i>	323.55 42.47	38.89 5.78	146.27 6.76	0.78 1.03
Piyal seeds	<i>Pistaciavera</i>	99.06 2.00	14.11 2.01	51.08 2.72	1.06 0.11
Walnuts	<i>Juglans regia</i>	959.79 78.13	80.17 5.93	1166.07 236.81	15.1 1.30

Values are mean \pm SD

MATERIAL AND METHODS

In this pilot study, ten varieties of most commonly consumed dry fruits were purchased from three different local markets of Hyderabad and Secunderabad. Edible portions were extracted with 60% methanol according to standard procedures and the extracts were used for the determination of total phenolic content (using Folin Ciocalteu reagent) and antioxidant activity by three different methods : FRAP (Ferric Reducing Antioxidant Power at pH 3.9), DPPH radical scavenging activity and reducing power(to reduce ferric to ferrous at pH 7).

Table 15: Correlation between Phenolic Content & AOA

PC Vs	r	r ² %
DPPH	0.97	94.4
FRAP	0.87	75.6
REDUCING POWER	0.81	66.4

The salient findings of the study are as follows:

✍️ Antioxidant activity of dry fruits expressed as FRAP showed a wide range and the values ranged from 56.38 to 1166.07 μ mol/g. The highest activity (1166.07 \pm 236.81) was found in the walnuts.

- ✍ DPPH radical scavenging activity ranged from 14.11 to 80.17 (% inhibition) with the highest activity seen in walnuts followed by brown raisins.
- ✍ Reducing power of the dry fruits ranged from 0.56 to 15.1 mg of vitamin C equivalents / g and the walnuts had the highest reducing power (15.1 mg Vit C equivalents / g).
- ✍ Phenolic content of dry fruits ranged from 109.07 to 959.70 mg of Gallic acid equivalent / 100g and the highest amount of 959.79 mg Gallic acid equivalent / 100g was found in walnuts.
- ✍ Among the dry fruits studied walnuts had the highest and pival seeds the lowest phenolic content. While walnuts had the highest antioxidant activity estimated by the three methods, cashew nuts, almonds and pival seeds had the lowest activities of FRAP, reducing power and DPPH scavenging activity respectively
- ✍ Correlation analysis between the PC and AOA showed that phenolics may contribute maximally to DPPH ($r^2\% = 94.4$) but only around 75-66% respectively to FRAP and reducing power of the dry fruits studied.
- ✍ Dry fruits are rich in antioxidant activity and phenolic compounds appear to be significant contributors to their antioxidant activity. Consumption of dry fruits may therefore augment the antioxidant status and protect against the chronic diseases.
- ✍ Iron chelating capacity of the extracts is currently being determined.

7. Development of antioxidant rich recipes utilizing legumes as the base

Free radicals generated during normal metabolism, are neutralized by the endogenous antioxidant system. However, external environmental conditions like stress, chemical carcinogens, irradiation and smoking generate more free radicals, which the endogenous antioxidant system may not be able to cope up with. Plant foods are good sources of antioxidants. Phenolic compounds are the potent antioxidant substances ubiquitous in plant foods. This study is an attempt to generate the data base on the antioxidant activity (AOA) and phenolic content (PC)

of plant foods commonly consumed by the Indian population and assess the effects of different types of domestic processing on these parameters (Annual report 2004-2005). It also involves formulating AOA rich recipes based on the data generated and assessing the acute effect of the consumption of these recipes on AO status in human volunteers.

OBJECTIVES

Formulate antioxidant rich recipes using the data generated.

Effect of different kinds of domestic processing on AOA and PC of chosen plant foods (Completed project 2004-2005) showed that AOA was higher in the sprouts of green gram, Bengal gram and moth beans compared to all other types of processing done, which had variable but not generally significant effect on AOA.

As the sprouted legumes had the highest AOA among all the processed foods studied, a number of salads were prepared using the sprouts of green gram, Bengal gram and moth beans in an attempt to develop recipes rich in AOA. In all, seventeen different kinds of sprouted legume salads were prepared using different permutations and combinations of different sprouts and vegetables. The sensory evaluation of these recipes was done by forty normal, healthy, adult human volunteers. In general all the recipes had comparable scores for appearance, colour, flavour, texture and taste and their overall quality and acceptability were good. Traditional legume sprout salad recipes had significantly lower AOA than the legume sprouts *per se* and this was observed to be due to the presence in them of low AOA foods such as capsicum, cucumber and carrots. Removal of these low AOA foods from the recipes improved the AOA of the recipe, but the AOA of such recipes were still lower than that of legume sprouts. However, addition of more of relatively AOA rich foods such as sprouts of groundnuts and mustard seeds did not improve the AOA of the recipe any further. It appeared that this could be due to the very small quantities of these unconventional sprouts present in these recipes, although the possible effects of interaction among various constituents of the ingredients could not be ruled out. Nevertheless it was interesting to observe from these attempts to develop AOA rich recipes that, addition of lemon juice and powdered black pepper (i.e. foods at least

Table 16 Effect of salt, pepper and lemon on AOA and PC of green gram sprouts

Ingredients	AOA+		PC#	
	Without salt	With salt	Without salt	With salt
Green gram	0.43± 0.02	0.35± 0.07	112± 5.2	113± 7.8
Green gram + Lemon	0.41± 0.06	0.41± 0.05	101± 6.5	111± 8.4
Green gram + Pepper	0.52± 0.11	0.53± 0.03	118± 9.2	118± 6.3
Green gram + lemon+ pepper	0.51± 0.07	0.52± 0.05	121± 6.6	128± 7.7

Values given are Mean ± S.D. (n=3)

+ AOA expressed as mg of food required for 50% inhibition of auto-oxidation of β -carotene in the β -carotene and linoleic acid mixture.

PC expressed as mg of gallic acid equivalent in 100g of raw food stuff

Table 17. Effect of pepper and lemon on AOA and PC of Bengal gram sprouts with salt

Ingredient	AOA+	PC#
Bengal gram + Salt	0.93± 0.11	65.5± 1.35
Bengal gram + Salt + Lemon	0.81± 0.00	67.4± 2.78
Bengal gram + Salt + Pepper	0.93± 0.05	68.4± 4.44
Bengal gram + Salt + Lemon+ Pepper	0.64± 0.10	74.6± 3.39

Values given are Mean ± S.D. (n=3)

+ AOA expressed as mg of food required for 50% inhibition of auto-oxidation of β -carotene in the β -carotene and linoleic acid mixture.

PC expressed as mg of gallic acid equivalent in 100g of raw food stuff

as good as legume sprouts in their AOA) had no adverse effect on the AOA of the legume sprouts. In fact the recipes developed had at least as much AOA as the legume sprout *per se*. Indeed, the AOA of salad prepared with green gram sprouts with lemon, salt and pepper was the highest among the different salad recipes tested and the one prepared from Bengal gram sprouts was the next best. (Tables 16, 17)

8. B-crystallin assisted reactivation of glucose-6-phosphate dehydrogenase

Small heat shock proteins (sHSP), distributed across prokaryotes and higher eukaryotes, are a special class of molecular chaperones involved in preventing the denaturation and aggregation of proteins, thus giving thermo resistance and aid in maintaining cellular protein homeostasis.

α -Crystallin, a prominent member of sHSP family constitutes the major portion of eye lens cytoplasm, reaching up to 50% of the total soluble protein and contains two polypeptides, A and B. However, in non-lenticular tissues β -crystallin is mainly present as either A- or B-homopolymers. Among the two, β -crystallin shows a wider extralenticular distribution and appears to have a physiological function in tissues like heart, kidney, brain and muscle. Like other sHSP, β -crystallin exhibits chaperone-like activity. Several studies have demonstrated that β -crystallin suppresses the aggregation of heat, UV-irradiated and chemically denatured proteins. In this direction, β -crystallin has been shown to facilitate proper refolding upon denaturation and also protection of several enzymes against inactivation. However, it is still debated whether β -crystallin is capable of protecting enzymes from inactivation, and the mechanism of enzyme reactivation by β -crystallin remains incomplete. The objective of present study was to understand the role of β -crystallin in the reactivation of denaturant (guanidinium hydrochloride, GdmCl) induced inactivation of enzymes using glucose - 6 - phosphate dehydrogenase (G6PD) as a model enzyme.

METHODOLOGY

Human β -crystallin was expressed and purified as reported previously (FEBS Lett 2002, 522, 59-64). Bovine β -crystallin was purified from calf lenses as described earlier (Exp. Eye. Res 2004, 79, 577-583). Unfolding of G6PD was performed by mixing stock solutions of G6PD and GdmCl (8 M) with 50 mM Tris-Cl buffer, pH 7.4 to give the desired concentration of protein and denaturant. Solutions were incubated at room temperature (25°C) overnight, and tryptophan fluorescence as a function of GdmCl was recorded by excitation at 295nm. Fluorescence of 1-anilino naphthalene-8-

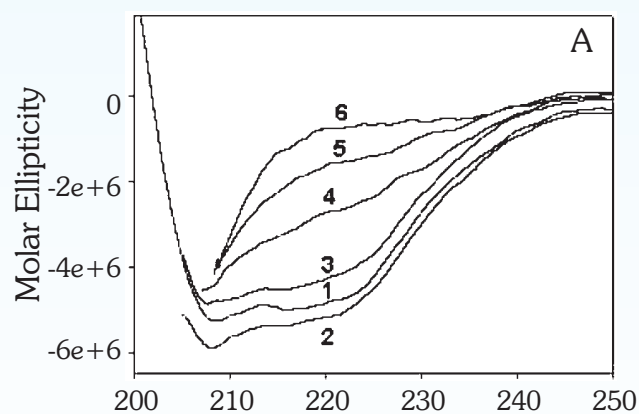
sulfonic acid (ANS) in the presence of native and GdmCl denatured G6PD was measured by excitation at 390nm and following the emission between 450 and 550nm. Far-and near-UV CD spectra were recorded at room temperature using a JASCO J-810 spectro polarimeter. Dilution-induced refolding and renaturation was studied at room temperature in 50 mM Tris-Cl buffer, pH 7.4, in the presence and absence of β -crystallin. G6PD activity was assayed by a spectrophotometric method, where reduction in NADP was monitored as measure of increase in absorbance at 340nm. The interaction of β -crystallin with G6PD during its refolding was studied by gel filtration chromatography. To study the effect of modifications on the ability of β -crystallin to refold and reactive G6PD, bovine β -crystallin was modified with 10mM methylglyoxal as described previously (Biochem J, 2004, 379, 273-282).

RESULTS

- Denaturant induced unfolding studies indicate that unfolding occurs at as low as 0.4 M GdmCl and proceeds through a molten globule-like state, which is characterized by elevated hydrophobic surfaces.
- However, even at 2.0 M GdmCl concentration, G6PD retained substantial amount of secondary structure but lost its secondary structural elements completely beyond 3 M GdmCl as assessed by far-UV CD (Figure 12A).

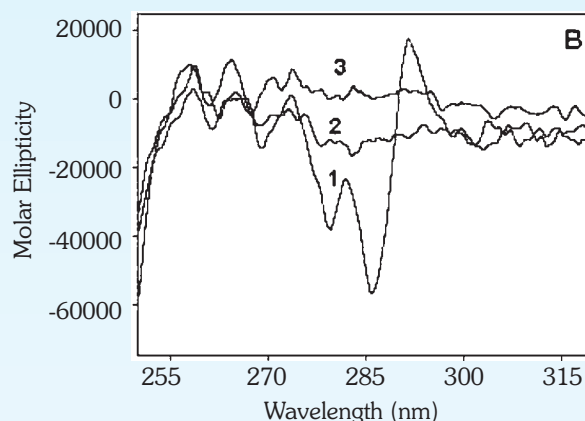
Figure 12. Secondary and tertiary structure of G6PD upon unfolding.

12A: Far-UV CD spectra of G6PD in different concentrations of GdmCl. Curves 1-6 represent the spectra of the protein in 0, 0.9, 1.2, 2.0, 3.0 and 4.0 M GdmCl respectively



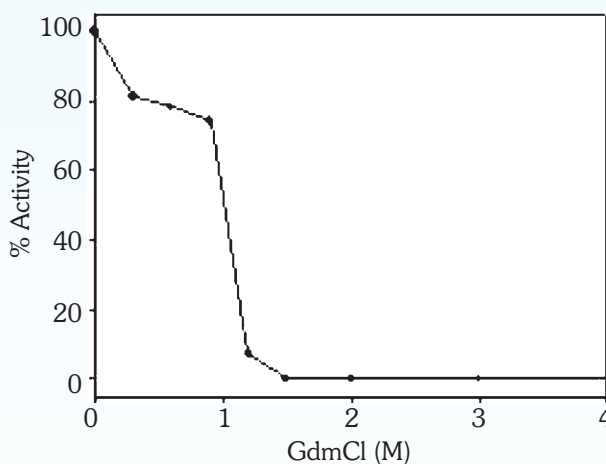
- In contrast to changes observed in secondary structure, G6PD lost its tertiary structure at 1.2 M GdmCl as protein exhibited altered signal in near-UV region (Figure 12B), suggesting that the intermediate(s) exhibit substantially loosened side-chain packing, substantiating the existence of a molten-globule like intermediate.

12B: Near-UV CD spectra of G6PD in different concentrations of GdmCl. Curves 1-3 represent the spectra of the protein in 0, 1.2, and 3.0 M GdmCl respectively



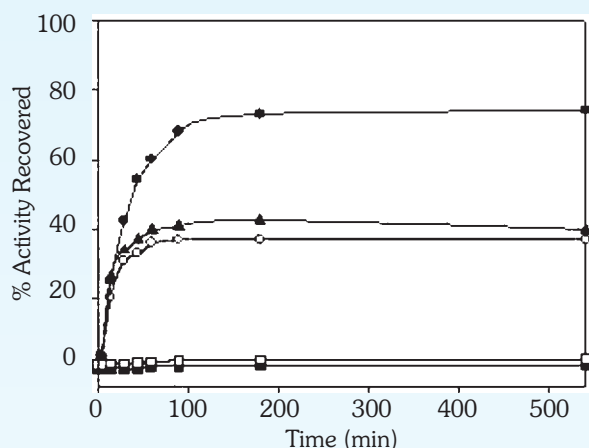
- There was a marginal decrease in the enzyme activity up to 0.9 M GdmCl but a sharp decrease and complete loss of activity thereafter. However, it should be noted that molten globule-like state (1.2 M GdmCl) was associated with the loss of enzyme activity (Figure 13).

Fig. 13. Enzyme activity of G6PD at different concentrations of GdmCl. Data are average of three independent assays



✎ Refolding of G6PD from completely unfolded state resulted in regain of 35% original activity but no activity could be achieved when it was refolded from molten globule like state (Fig. 14).

Fig. 14. Reactivation of G6PD in the absence and presence of β -crystallin following enzyme denaturation in 1.2 (molten globule-like) and 4.0 M GdmCl (completely unfolded). G6PD was refolded from completely unfolded state in the absence (open circle) and in the presence of either 0.5 M β -crystallin (closed circles) or 1 M lysozyme (triangles). Like wise, G6PD was refolded from molten globule-like state in the absence (open squares) and in the presence of 0.5 M β -crystallin (closed squares)



✎ Interestingly, refolding of completely unfolded G6PD in the presence of β -crystallin yielded 70% of original activity. However, β -crystallin was unable to reactivate the G6PD when refolded from its molten globule like state (Figure 15).

✎ Size exclusion chromatography data indicate β -crystallin assisted reactivation of unfolded G6PD is associated with the restoration of native G6PD but not with the complex of chaperone bound substrate (Table 18).

CONCLUSIONS

These results show that β -crystallin assists in the reactivation of G6PD and prevents misfolding/aggregation upon refolding. However, β -crystallin appears to reactivate conformational specific unfolding/ refolding intermediates, probably by providing the necessary energetics to these

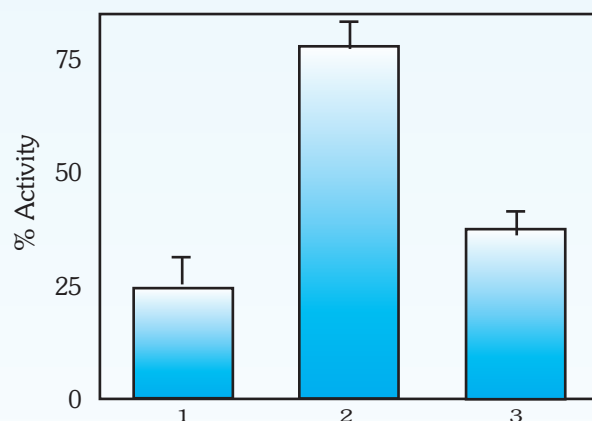
Table 18: G6PD activity of the HPLC fractions corresponding to chaperone-substrate complex and native G6PD. G6PD was refolded in the absence and presence of β -crystallin from molten globule-like state and completely unfolded state. Activity of native G6PD that was refolded from completely unfolded state in the absence of β -crystallin was considered as 100%

HPLC Peak	Refolded from molten globule-like state		Refolded from completely unfolded state	
	(-) β -crystallin	(+) β -crystallin	(-) β -crystallin	(+) β -crystallin
Chaperone-substrate complex	N.A.	N.A.	N.A.	N.A.
Native G6PD	N.A.	N.A.	100	187%

✎ However, β -crystallin did not solubilize the preformed aggregates

✎ Methylglyoxal- modified β -crystallin, which is a major modification in diabetic lenses, was unable to restore the activity of completely unfolded G6PD upon refolding (Figure 15).

Fig.15: Reactivation of G6PD from completely unfolded state by native and MGO-modified β -crystallin. Bar 1- G6PD refolded alone, Bar 2 and 3, G6PD refolded in the presence of native and 10mM MGO modified β -crystallin, respectively. Data are mean (\pm SE) of three independent experiments



Intermediates for crossing the kinetic barriers in achieving native state. β -Crystallin may reversibly interact with the unfolding intermediates of G6PD, favoring the formation of active G6PD through a native-like intermediate. Thus, chaperoning of

denaturant inactivated G6PD by B-crystallin involves two distinct mechanisms; (i) formation of a complex with partially or completely unfolded substrates, thus preventing the aggregation of G6PD and (ii) favoring the refolding of specific intermediates (completely unfolded) to active G6PD. More importantly, posttranslational modifications decrease B-crystallin ability to refold and reactivate enzymes, which may have a bearing on lens transparency.

9. Effect of hyperglycemia on the expression of A- and B-crystallins under diabetic conditions

B-Crystallin, a major lenticular protein, consists of two subunits A and B of each 20 kDa. A- and B-Crystallins belong to the small heat shock protein (sHSP) family and they share around 57% sequence homology. Interestingly, the presence of these proteins has also been demonstrated in a variety of non-lenticular and non-ocular tissues.

B-crystallin is shown to be present in several non-lenticular tissues such as cornea, optic nerve, retinal glia, astrocytes and muller cells. Furthermore, it is also present in non-ocular tissues such as cardiac and skeletal muscle and, to a lesser extent, in brain, kidney, skin and lungs. In contrast to B-crystallin,

A-crystallin is believed to be largely lens-specific. In addition, increased expression of B-crystallin is associated with a multitude of pathologies in non-ocular tissues such as desmin-related cardio myopathy, and most notably with the neuronal diseases. Although, the function of B-crystallin in non-lenticular tissues has not been demonstrated, it is believed that B-crystallin is associated with a variety of pathological conditions. It has been reported that B expression is found to be elevated under oxidative and other stress conditions. Oxidative stress induced expression of B-crystallin as well as the presence of B-crystallin in tissues with high oxidative potential (such as skeletal muscle, cardiac tissue and lung) suggests that the expression of B-crystallin gene is related to oxidative stress.

Diabetes is known to be associated with various metabolic stresses including oxidative stress. Being, a stress induced protein, it is important to study the

expression pattern of B-crystallin in diabetic conditions. In this report, the expression pattern of A and B-crystallins in various tissues of diabetic rats was studied.

OBJECTIVES

To investigate the expression of A- and B-crystallins in the lens and in non-lenticular tissues under diabetic conditions

METHODOLOGY

Three-month-old male WNIN rats were used in this study. The control group rats (n=6) received only 0.1 M citrate buffer, pH 4.5 (vehicle) and were fed AIN-93 diet. The experimental rats (n=6) received a single i.p injection of streptozotocin (STZ; 35 mg/kg). After 72hrs, fasting blood glucose levels were monitored and rats with serum glucose levels less than 250 mg/dL were excluded from the experiment. Fasting blood glucose levels were monitored once in a week.

At the end of 8 weeks, the rats were sacrificed by CO₂ asphyxiation and organs were harvested. Total RNA was extracted from various tissues of control and diabetic rats using Tri reagent. RT-PCR was carried out using single step access RT-PCR system. Amplification products were electrophoresed on agarose gel for the expected RT-PCR products and analyzed densitometrically.

Polyclonal antibodies against human recombinant A- and B-crystallins were raised in New Zealand white rabbits and IgG was purified using DE-52 anion exchange chromatography. To correlate the mRNA expression of A and B to their gene products, expression of A and B was evaluated at protein level under hyperglycemic conditions by immunoblotting with polyclonal IgG for A and B. To study the expression analysis of A- and B-crystallins at protein level tissue extract was prepared by homogenizing tissues in lysis buffer and immuno-blotting was carried out using polyclonal antibodies against human recombinant A- and B-crystallins.

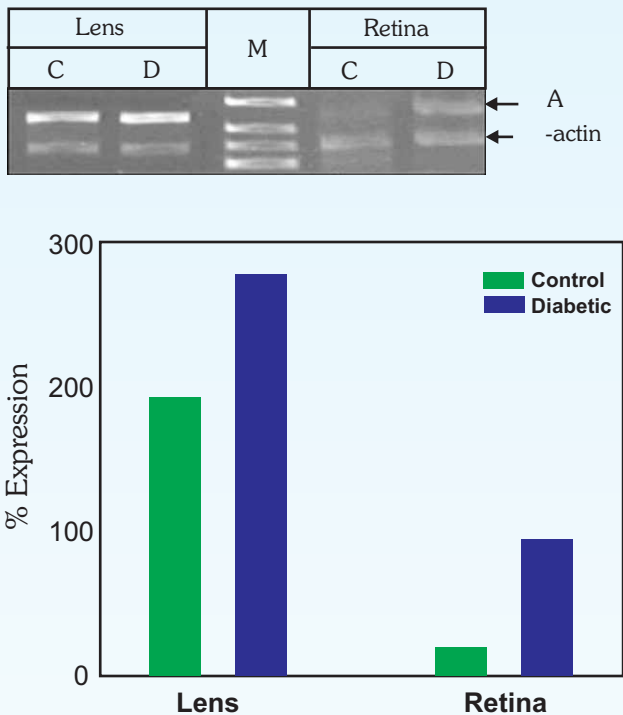
RESULTS

1. Diabetic rats showed a significant elevation in blood glucose levels throughout the

experimental period: 250 mg/dL for diabetic rats and 85 mg/dL for control rats at the time of perfusion.

2. While A-crystallin expression was observed only in lens and retina, B-crystallin was expressed in all the tissues tested.
3. Interestingly there was an increased expression of A in both lens (1.5 fold) and retina (4 folds) of diabetic animals compared to control rats (Figure 16).

Fig.16. Expression (upper panel) and quantification (lower panel) of A-crystallin in control (C) and diabetic rat (D) tissues by RT-PCR. Results (lower panel) are average of three independent experiments. M in upper panel denotes DNA ladder



4. Expression of B was found to be elevated in lens, heart, muscle and brain of diabetic rats (Figure 17).
5. Expression of B marginally decreased in retina and kidney and significantly decreased in adipose tissue in diabetic rats when compared to control rats (Figure 17)
6. Immunoblotting was performed with respective antibodies and found that A protein (both A

and A^{ins}) levels were higher in diabetic retina (Figure 18).

7. A protein decreased in diabetic lens. This may be due to increased degradation of A in diabetic rat lens (Figure19).
8. B-crystallin levels were appreciably higher in retina, heart, muscle, lens and brain, but low in adipose and unaltered in kidney under diabetic conditions (Figure 19).
9. Though, B protein was increased in diabetic retina and lens, it was degraded considerably.

Fig. 17. Expression (upper panel) and quantification (lower panel) of B-crystallin in control (C) and diabetic rat (D) tissues by RT-PCR. Results (lower panel) are average of three independent experiments

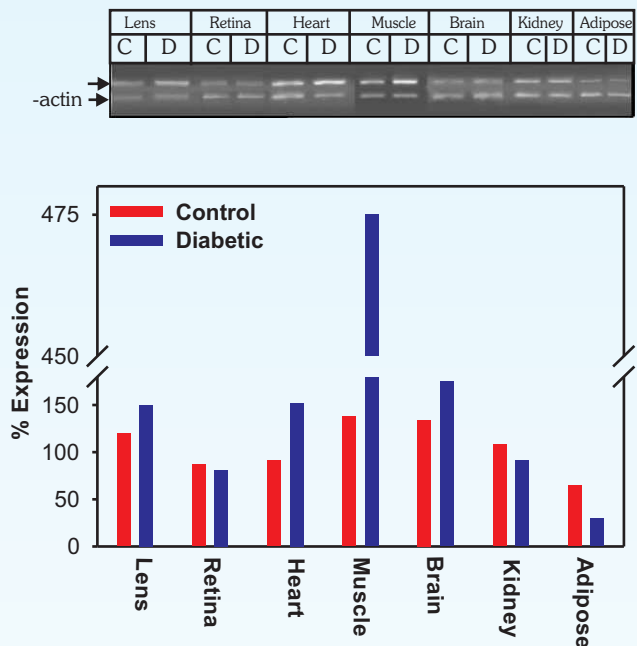


Fig. 18. Immunodetection of A-crystallin in lens and retina of control (C) and diabetic (D) rats. 30 g of protein loaded on SDS-PAGE. Lower band in diabetic retina and lens is a degradation product of A-crystallin

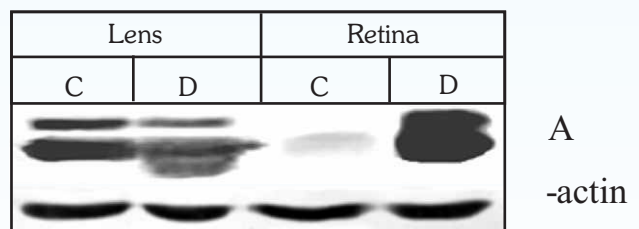
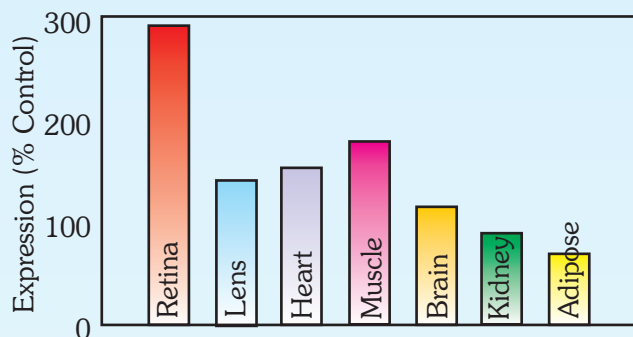


Fig. 19. Quantification of β -crystallin in tissues of diabetic rats. Expression of β -crystallin in tissues of control rat is considered as 100%.



CONCLUSIONS

These observations suggest for the first time, that small heat shock protein, β -crystallin expression is elevated in diabetic conditions particularly in lens, retina, heart, muscle and brain and α -crystallin in lens and retina. It is of interest to know the physiological or pathophysiological significance of increased expression of β -crystallin. Studying modulation of dietary antioxidants on the expression of β -crystallin under diabetic conditions may help in identifying the molecular targets in diabetic complications. Studies are under way to investigate the mechanism of increased expression of β -crystallin under diabetic stress condition and possible role of heat shock factors, if any.

10. Effect of non-enzymatic glycation on structure and molecular chaperone function of β -crystallin

β -Crystallin, the prominent member of small heat shock protein family of the eye lens has been shown to function like a molecular chaperone to protect the other lens proteins by preventing their aggregation from various denaturing and stress conditions. β -Crystallin is believed to be instrumental in maintaining the transparency of eye lens. Being a long-lived protein with negligible turnover, β -crystallin undergoes various post-translational modifications with age such as glycation, oxidation, truncation, mixed disulphide formation, deamidation etc. resulting in loss of chaperone

activity which eventually may result into the opacification of the lens. Some of these modifications particularly non-enzymatic glycation and oxidation are accelerated in diabetic conditions. Hence effect of these posttranslational modifications on β -crystallin chaperone activity in diabetic conditions is of great concern.

In addition, β -crystallin chaperone activity has been shown to be compromised in diabetic human and rat lens. Aldose sugars react non-enzymatically with amino group of β -crystallin to form Schiff's-base intermediates, which rearrange to form advanced glycation end (AGE) products. These AGEs cross-link the proteins to high molecular weight light scattering aggregates and AGEs mediated cross-linking may affect β -crystallin chaperone activity. Therefore, in the present study the effect of non-enzymatic glycation on structure and function of β -crystallin was investigated.

OBJECTIVE

To investigate the effect of non-enzymatic glycation on structure and function of β -crystallin by various sugars.

METHODOLOGY

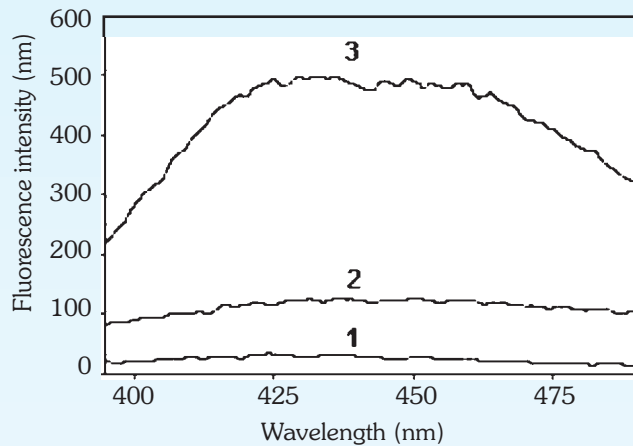
β -Crystallin was isolated from 6-month-old goat eye lens. Lenses were homogenized and centrifuged as previously reported (Kumar et al Mol Vis, 2005) to separate water-soluble and water insoluble fractions. Water soluble fraction was applied onto a 90 cm X 2.5 cm Sephacryl S-300 column. Fractions corresponding to β -crystallin were pooled. The purity of pooled crystallins was assessed by SDS-PAGE.

The effect of sugars and sugar derivatives on chaperone like activity of β -crystallin was monitored *in vitro* by non-enzymatic glycation. In non-enzymatic glycation β -crystallin was incubated with fructose (0.1 M) or glucose-6-phosphate (0.05 M) in dark at 37°C for 4 & 2 weeks. At the end incubation mixture was dialysed extensively to remove unbound glycating agent. Extent of non-enzymatic glycation of β -crystallin and protein cross-linking due to glycation was monitored. Chaperone activity and physicochemical properties like hydrophobicity and secondary and tertiary structure was assessed.

RESULTS

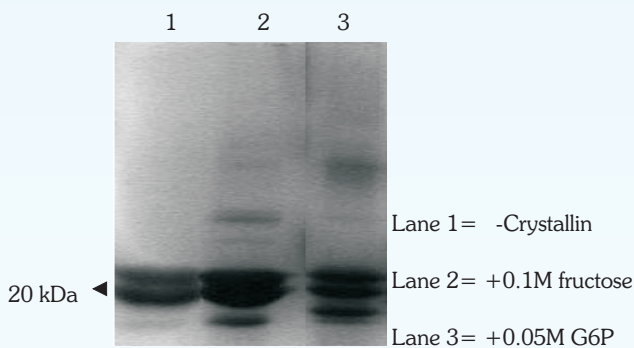
1. Incubation of α -crystallin with 0.1 M fructose and 0.05 M glucose-6-phosphate resulted in non-enzymatic glycation as monitored by AGE fluorescence (Figure 20).

Fig 20. AGE fluorescence of α -crystallin on non-enzymatic glycation: Control α -crystallin (trace1), α -crystallin incubated with Glucose-6-phosphate (trace2), α -crystallin incubated with fructose (trace3)



2. High molecular weight protein aggregates were formed on incubation with sugars *in vitro* as monitored by SDS-PAGE (Figure 21)

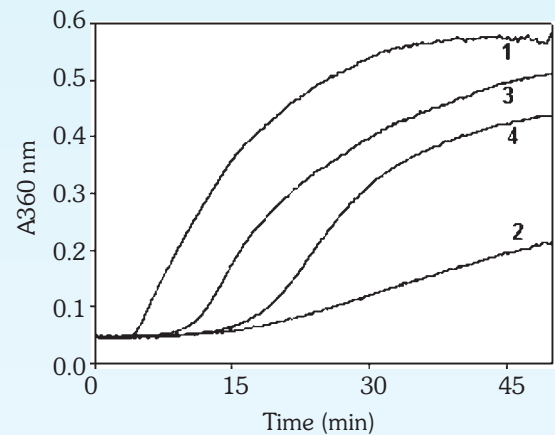
Fig 21. SDS-PAGE profile of α -crystallin upon glycation with fructose and glucose-6-phosphate



3. *In vitro* glycation of α -crystallin with fructose resulted in the formation of carboxy methyl lysine (CML) as a predominant AGE and incubation with G6P into the formation of Glucose AGE.
4. *In vitro* glycation of α -crystallin caused oxidative damage to the protein as assessed by the formation of protein carbonyls.

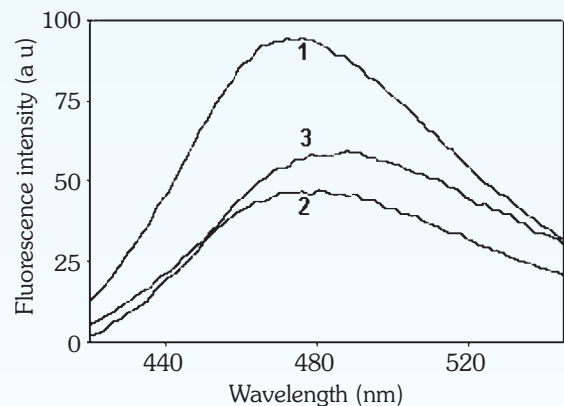
5. Non-enzymatic glycation of α -crystallin by fructose and glucose-6-phosphate has led to decreased chaperone activity (Figure 22)

Fig. 22. Chaperone activity of α -crystallin as assessed by the suppression of heat-induced aggregation of α -crystallin. α -Crystallin (0.2 mg/ml in 50 mM phosphate buffer, pH 7.4) was incubated at 65°C in the absence (trace 1) or in the presence of either control α -crystallin (0.025 mg/ml) (trace 2), α -crystallin glycated with fructose (trace 3) or glucose-6-phosphate (trace 4).



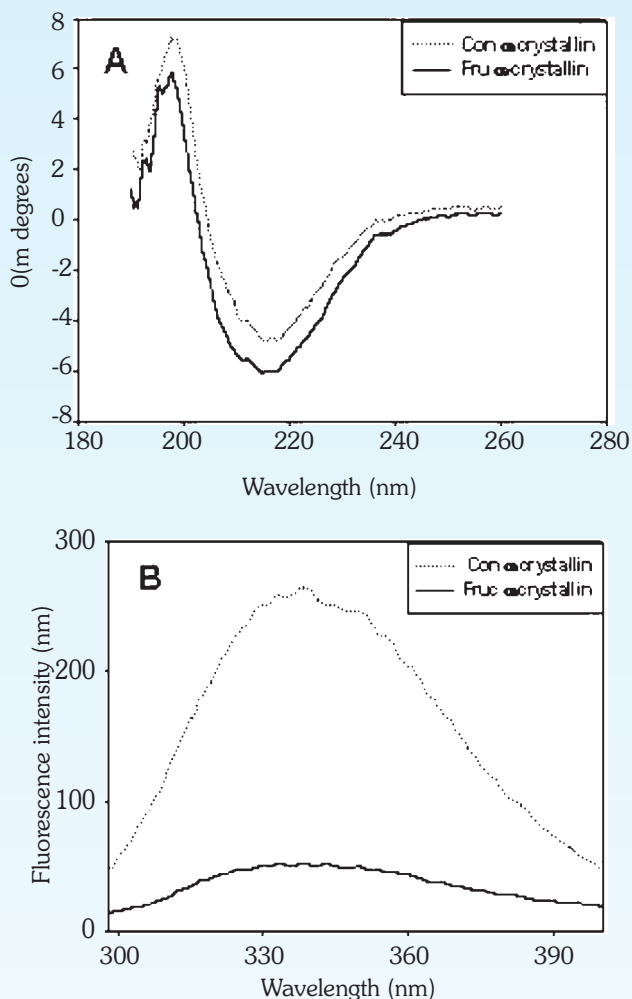
6. The decreased chaperone activity of α -crystallin during *in vitro* glycation was associated with decreased hydrophobicity as shown by ANS binding (Figure 23)

Fig. 23. ANS fluorescence of control (trace1), glucose-6-phosphate (trace2) and fructose (trace3) glycated α -crystallin



In vitro glycation by fructose resulted in significant change in secondary and tertiary structure of α -crystallin as monitored by far-UV CD spectrum and tryptophan fluorescence (Figure 24).

Fig. 24. Far UV- CD spectrum (A) tryptophan fluorescence (B) of control α -crystallin and fructose glycosylated α -crystallin



CONCLUSION

Studies on non-enzymatic glycation of α -crystallin suggest that on glycation there is formation of specific AGEs and cross-linking of α -crystallin to high molecular weight aggregates. There is a considerable loss of protein native structure. Chaperone activity of α -crystallin is decreased significantly on glycation and this decrease is associated with decreased surface hydrophobicity. Therefore, non-enzymatic glycation of α -crystallin under hyperglycemic conditions may have deleterious effects on the structure and function of α -crystallin. Studies are underway to investigate the effects of dietary agents with antiglycating potential to prevent changes to α -crystallin due to non-enzymatic glycation.

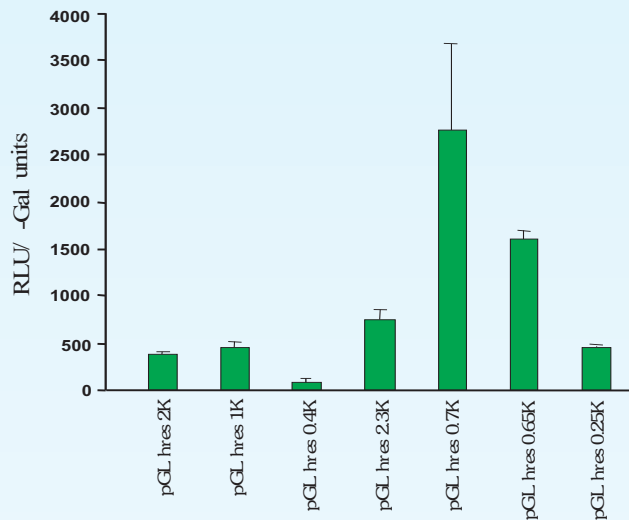
11. Transcriptional analysis of resistin and identification of cis- and trans acting factors regulating resistin expression

The adipocytokine resistin, a member of a family of cysteine-rich proteins known as resistin-like molecules (RELM) is also shown to be involved in inflammatory processes. Previous studies have however highlighted that resistin impairs glucose tolerance and insulin action in mice. In addition, resistin also inhibits adipogenesis in murine 3T3-L1 cells. Therefore resistin, an adipocyte-secreted factor was proposed as a link between obesity and T2DM. Subsequently, rodent and human studies have pointed towards the conflicting role of resistin. It was shown earlier that mouse resistin is regulated by C/EBP and PPAR- (Ghosh et al., 2003). In humans resistin is primarily secreted by monocytes/macrophages. The transcriptional regulation of human resistin varies greatly from that of the mouse gene. Therefore, human resistin may have different trans-activating factors regulating its expression in macrophages and thereby implicating its function.

In silico analysis of upstream sequences of resistin ORF was performed to identify the probable factors involved in the regulation of human resistin gene transcription. The regulatory elements detected include AP-1, NF- κ B, C/EBP- and c-Rel. To analyze the role of each transactivating factor, 2.3 Kb promoter sequence was amplified and cloned into pGL Basic vector. Several deletion constructs of the promoter region were made in pGL Basic vector. These constructs were transiently transfected using electroporation in U937 cells and luciferase enzyme activity was scored to identify the promoter. The study indicated that 700 bp upstream region of human resistin showed maximal expression of luciferase gene in the reporter vector and is sufficient to drive its expression (Figure. 25). This region includes the binding sites for AP-1, c-Rel, PPAR- as well as C/EBP. pGL 650 clone which does not contain the AP-1 binding site shows a significant decrease in expression thus confirming the importance of AP-1 transcription factor in resistin regulation. Also the construct that contains only the AP-1 site, 400 bp clone, is not able to drive the luciferase gene expression. This suggests that

AP-1 is necessary but alone is not sufficient to transcribe and it requires the presence of the other transcription factors for the optimal expression of human resistin. This is suggestive of a cross-talk of AP-1 with these transcription factor(s).

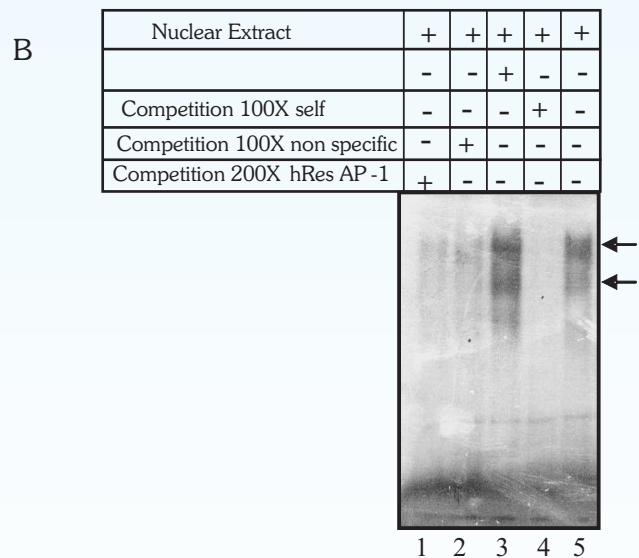
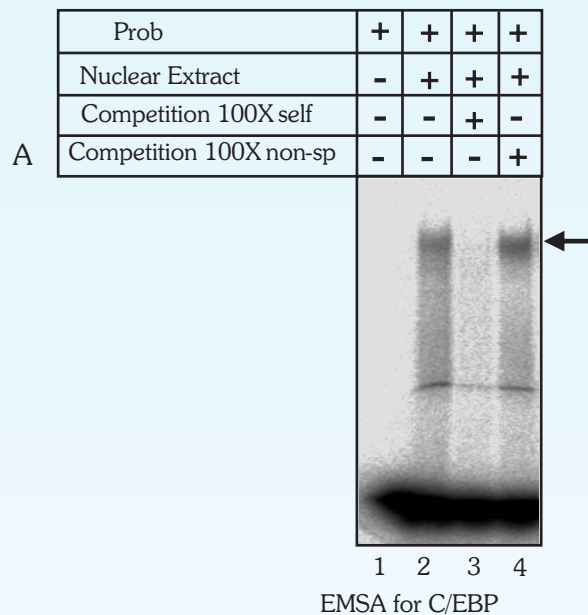
Fig 25. Defining the minimal promoter of human resistin. The -673 to +32 (pGL hres 0.7K) region of the human resistin promoter was able to drive the expression of luciferase gene maximally indicating that the minimal promoter lies within this region.



In order to further evaluate the role of these transcription factors in the expression of human resistin, an electrophoretic mobility shift assay was performed wherein the binding of AP-1, C/EBP and c-Rel to their respective cognate oligonucleotides was characterized. Resistin promoter sequences containing the binding sites for C/EBP, AP-1 and c-Rel shows binding with nuclear extracts prepared from corresponding cells (Figure. 26A, 26B, 26C). These experiments clearly demonstrate that AP-1, C/EBP and c-Rel present in the nucleus bind to the resistin promoter and could thereby modulate the expression of human resistin.

To further characterize the effect of various stimulators and inhibitors of AP-1 (PMA, PDTC) and NF-kB (LPS, PMA, PDTC) on resistin expression. It was found that the stimulators of AP-1 and NF-kB also stimulate resistin gene expression and vice versa (Figure. 27), strengthening the important role of these transcription factors in resistin regulation.

Fig 26. EMSA for C/EBP, AP-1 and c-Rel. A. lane 1 radiolabeled human resistin specific C/EBP oligonucleotide, lane 2 is reaction of the probe with nuclear proteins, lane 3 is competition with the non-radioactive oligonucleotide and lane 4 is competition with a non-specific oligonucleotide. B. lane 1 and 2 represents competition with non-radioactive human resistin specific AP-1 oligonucleotide. Lane 3 is competition with a non-specific oligonucleotide, lane 4 is self-competition and lane 5 is reaction of the probe with nuclear proteins. C. lane 1 is competition with consensus NF-kB oligoneucleotide, lane 2 is no-specific competition, lane 3 is competition with human resistin specific c-Rel motif, lane 4 is reaction of the probe with nuclear proteins and lane 5 is free probe.

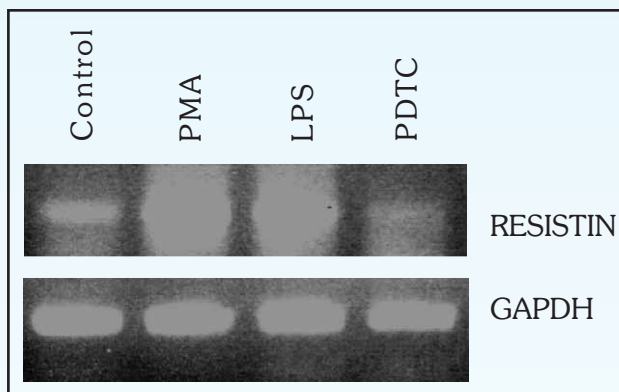


Nuclear Extract (293T cells)	+	+	+	+	-
Competition 100X consensus NF-kB	+	-	-	-	-
Competition 100X self (hRes c-rel 1)	-	-	+	-	-
Competition 100 X nonself	-	+	-	-	-



EMSA for c-Rel

Fig. 27. Effect of stimulators and inhibitors of AP-1 and NF-kB on the expression of resistin transcript U937 cells were treated with 100nM PMA, 5 g/ml LPS or 1 mM PDTC for 3 hours and total RNA isolated. Quantitative RT-PCR was performed to analyze the effect on resistin expression. Both PMA and LPS stimulated the expression of human resistin in U937 cells while PDTC suppressed it.



12. Comparative structural analyses of human, mouse and rat resistin

Insulin resistance is one of the major contributing factors in the development of metabolic syndrome.

Resistin, a hormone secreted by adipocytes in mice was suggested to be an important link between obesity and insulin resistance. However, there have

been conflicting reports regarding the function of resistin in humans. Several studies have demonstrated a lack of correlation between levels of resistin and obesity, whereas in other studies increased level of resistin was found in visceral obesity. In a very recent study conducted at NIN as well as other labs, it was observed that resistin mRNA level in white blood cells increases upon treatment with lipopolysaccharide (LPS). Also, resistin was initially identified as a homolog of FIZZ1 (found in inflammatory zone), a secretory protein found in the bronchoalveolar lavage fluid from mice with experimentally induced pulmonary inflammation. Hence, its role in inflammation apart from causing insulin resistance in the obese subjects was proposed.

Resistin belongs to a family of cysteine rich secretory proteins called the RELMs. There is only 58% homology between human and mouse resistin proteins although the C-terminus of the resistin family proteins is highly conserved with a consensus sequence of CX11CX8CX4CX3CX10CX7CX8CX9CC. A study to reveal the structural differences in the human, mouse and rat proteins was carried out.

In order to assess the stability of human, mouse and rat resistin proteins, the effect of salts and temperature on the secondary as well as the tertiary structure of the recombinant proteins expressed and purified from E.coli cells were performed.

Earlier experiments showed that human resistin protein is resistant to denaturation by urea and it could be completely unfolded only under strong reducing conditions. This unusual behavior could be attributed to inter- and intra-molecular disulfide bond formation within the resistin protein. Interestingly, resistin also undergoes a reversible concentration-dependent α -helical to β -sheet conformational change (Aruna et. al., 2003, Biochemistry).

Furthermore the effect of ionic strength on the secondary and tertiary structure of the proteins using circular dichroism and intrinsic protein fluorescence was studied. CD results reveal that at secondary structural level, even 500 mM NaCl did not alter the structure of the human and rat proteins whereas, mouse resistin showed conformational

changes (Figure. 28). 50 mM NaCl was able to disrupt the tertiary structure of mouse resistin whereas human and rat proteins showed no effect. It was also seen that human resistin showed changes in secondary structure in the presence of NaF having high electronegativity (Figure. 29). These results demonstrate the importance of ionic interaction in stabilization of human resistin.

Fig 28: Effect of ionic strength on the secondary structure of resistin

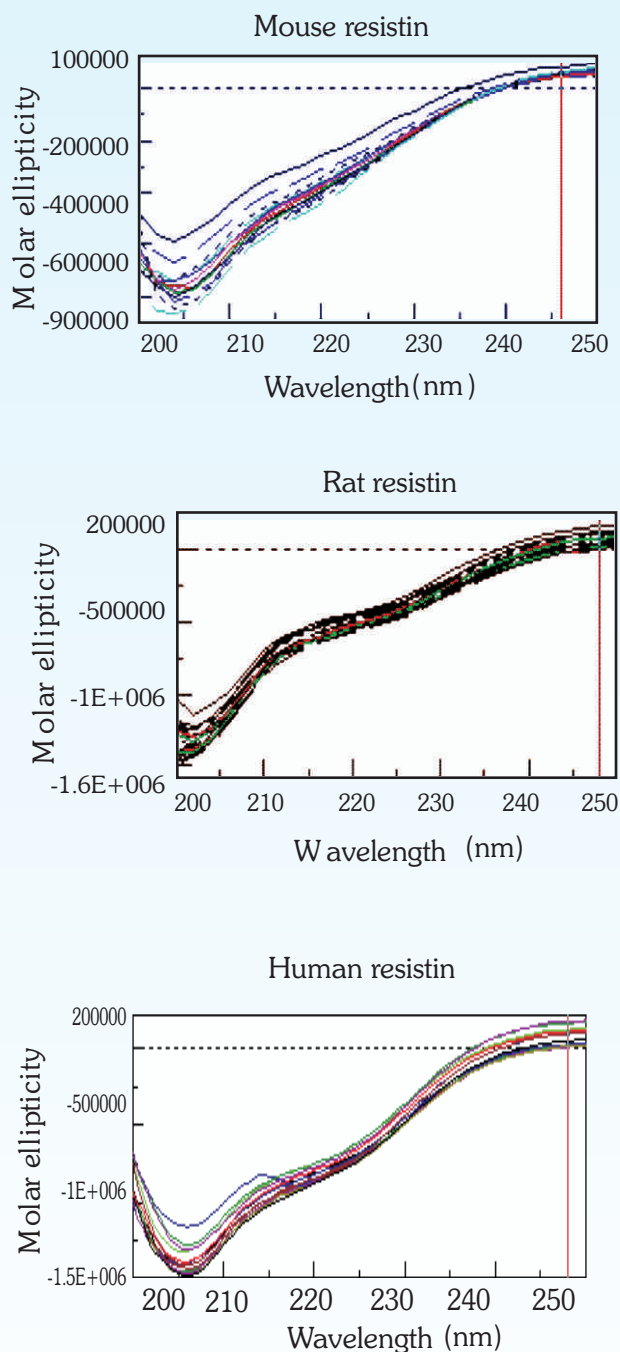
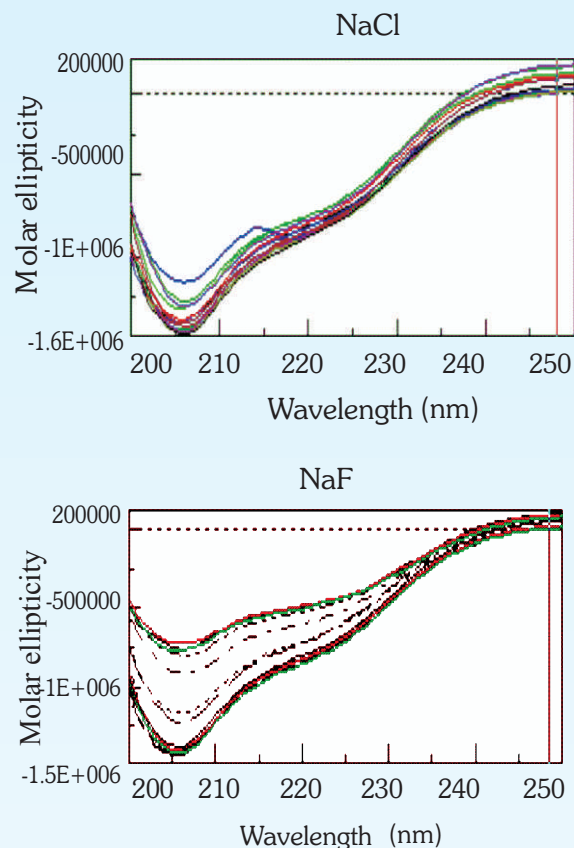


Fig 29: Loss of secondary structure of human resistin is dependent on the electronegativity of the ions present



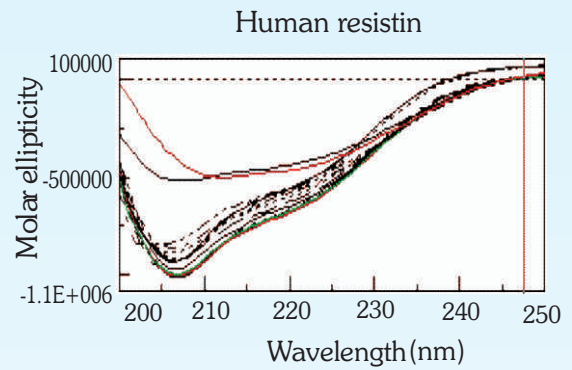
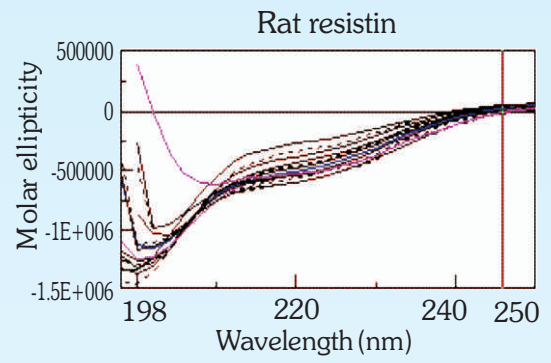
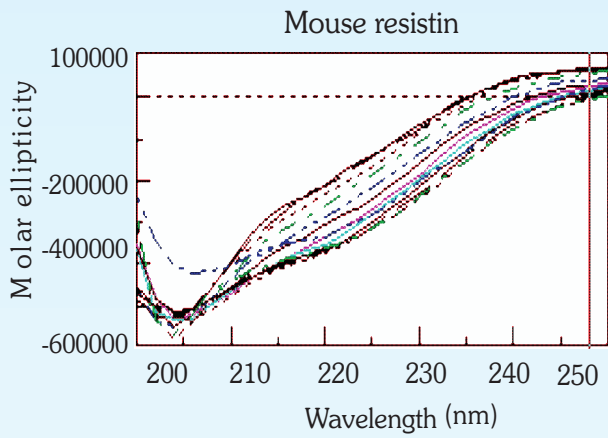
Effect of temperature on the stability of human, mouse and rat resistin proteins was studied. Although slight conformational changes were evident with the increase in temperature, it was observed that human resistin was most stable, retaining its secondary structure even at 90°C.

Complete loss of secondary structure in human resistin protein was observed only at 110 °C. The mouse resistin protein was found to be more sensitive to thermal changes. Loss of secondary structure in the mouse protein was seen at 80 °C (Figure 30).

These studies show that the human resistin protein is the most stable of the three proteins. This stability could be attributed to the inter and intra molecular disulfide linkages as well as the greater ionic interactions present in the human protein as deduced from the conformational changes brought about in the presence of increasing salt concentrations.

These differences in the structural attributes of the proteins might have profound implications by defining varying functions for resistin in the different species.

Fig.30. Effect of temperature on the secondary structure of resistin



IV. PATHOLOGY

Cytokines profile and micronutrients in plasmodium vivax infection

Malaria is one of the leading causes of morbidity and mortality worldwide, with an estimated 350 to 500 million new cases each year. Despite wide reputation as the benign parasite, *P.vivax* is nevertheless associated with severe complication and death. Relapses due to *P.vivax* can occur even after 3 to 5 Y and may cause death as a result of high parasitemia (2%) after anemia or ruptured spleen and thrombocytopenia or rarely cerebral malaria. There is no information about cytokine profile in *P.vivax* though it is more commonly prevalent infection in India, hence the present study was carried out with the following objectives.

OBJECTIVES

To assess circulating IL-2, IFN , IL12, IL10, IL-4 and TNF concentrations in patients with mild and severe *P. Vivax* infection and correlate with serum, zinc and vitamin A.

SUBJECTS & METHODS

Patients attending the out patients clinic, Hospital for tropical diseases, Hyderabad, during the month of August and September, who were diagnosed to be suffering from malaria on the basis of peripheral blood smear examination, aged between 20 to 40Y were enrolled for the study. Patients recruited for the study were classified into 2 groups based on the density of parasites in blood. Patients with 1-2 parasites in occasional high power field (HPF) and those with more than 2-10 parasites in all HPF were classified as mild/moderate and those with high density of parasitemia with poor clinical conditions (vomiting, dehydration and temperature, $>39^{\circ}\text{C}$) were classified as severe. Finally, there were 15 mild/moderate and 16 severe cases of malaria. Ten apparently normal subjects were selected randomly for the control group. Patients who received recent anti malarial treatment and those with other systemic infections were excluded from the study. After collecting a venous

blood sample, the patients received treatment as outpatients with sulfadoxin and pyrimethamin. The Institute's Scientific Advisory Committee approved the study.

Work done during the year 2004-05

Five ml of venous blood samples from each were collected into sterile EDTA-containing vacutainer, tubes. Plasma was separated immediately and frozen in -70°C to avoid denaturation of cytokines, which were quantified by sandwich ELISA. Sensitivity of detection for the cytokine assay was as follows: IL-2, 5 pg/ml; IFN , 5 pg/ml; and IL-4 0.5 pg/ml. As IL12 and IL10 are known to be regulatory cytokines and TNF is suggested to be associated with anemia in malaria, these three cytokines were determined again in the same patients after recovery from malaria.

Serum zinc was determined by atomic absorption spectrophotometry and vitamin A was assayed by HPLC. Log transformed data were compared between groups using students t test. When data remained skewed after log transformation the Mann Whitney U test was used.

The mean age of patients was similar in the three groups. The mean number of days the patients had pyrexia before collection of blood was similar in mild/moderate ($4.2 \pm 0.5\text{d}$), and severe ($4.4 + 0.4\text{d}$) malaria. The results were as follows.

1. The initial hemoglobin concentration was significantly lower in mild and severe malaria (12.23 g/dl) compared to normal population and decreased to 10.63 g/dl within 1 week. Packed cell volume (PCV) percent was significantly lower in patients compared to controls and decreased significantly from the initial level in both mild and severe malaria.
2. Serum zinc ranged from 40 to 100 g/dl with a mean \pm SE of 63.8 ± 2.6 g/dl, which was much less than the reported normal range (70-120 g/dl). Of the 36 cases of malaria 61% had

zinc deficiency and the rest (14) had equal to or more than 70 g/dl. The mean zinc levels were comparable between the groups in malaria though mild malaria group had low zinc concentrations (Table 19).

Table 19. P.vivax and Micronutrients

P.vivax	Hb gms/dL	Zn µg/dl	Vitamin A µg/dl
Mild	12.3± 0.88	59.09± 2.73	25.10± 3.52
Severe	12.26± 0.5	68.83± 8.88	21.24± 5.01

Values are Mean±SE

3. Serum Vitamin A concentration ranged from 8 to 55 g/dl and was less than the observed range (20 to 70 g/dl) in normal adults. The total mean ± SE of vitamin A was 29.3±2.41 g/dl. Of the 36 patients of malaria, 25 had normal vitamin A (>20 g/dl), while 11 had less than 20 g/dl. Vitamin A levels did not correlate with severity of malaria.
4. The mean concentration of IL2 was higher in the mild (16.9±5.19 pg/ml) malaria compared to severe (11.6±3.33 pg/ml), while IFN was significantly higher in the severe malaria (619.7±230.97). IL4 was 2.4±1.0 and 3.8±1.99 pg/ml in the mild and severe malaria respectively (Table 20).
5. The IL12 ranged from 85.2 to 464 pg/ml and the mean was 289.5±67.94 pg/ml in mild

malaria and was comparable to severe malaria (240.2±46.24). After one week with the clearance of parasites the IL12 decreased by fifty percent in the mild malaria, while same level was maintained in the severe malaria thus showing the association of serum IL12 with parasite clearance.

Table 20. Cytokine profile in P.vivax infection

Cytokines	Mild	Severe
IL-2 pg/ml	16.9 5.19(14)	11.6 3.33(6)
IFN pg/ml	177.3 40.98(14)	619.7 230.7(6)
IL4 pg/ml	2.8 1.0(14)	3.8 1.99(6)
IL-12 pg/ml	289.5 67.94(5)	240.2 46.24(10)
IL-10 pg/ml	452.76 334.33(5)	279.1 121.8(10)
TNF pg/ml	32.64 3.65(5)	44.3 19.3(10)

Values are mean SE Numbers in parenthesis

6. The initial plasma level of IL10 was 452.7±334.33 in the mild malaria that was significantly higher than the severe malaria (279.1±121.8). However, after 1 week there was a 50% increase in IL10 in the severe malaria while it remained the same in mild malaria. The plasma level of TNF was 32.6 ±3.65pg/ml, which was maintained even after 1week in the mild malaria. However, TNF increased 3 fold from the initial value of 44.3±19.36 pg/ml in the severe malaria.

V. 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)”, Diet and Diabetes (English), Low Cost Nutritious Supplement (English), Menus for South India (English) and Some Common Indian Recipes.

2. TRAINING PROGRAMMES

2.1. Regular Training Programmes

Post Graduate Certificate Course in Nutrition and Annual Training Course in Endocrinological Techniques were conducted. Eleven candidates attended these training programmes. Out of the eleven candidates nine members were in-service candidates, while two were private candidates.

2.2 Adhoc Training Programmes

An ad-hoc training programme was organized for two participants from Srilanka in the field of Research Methodology and Nutrition Communication. (Nov. 21-25, 2005)

Basic course in “Nutrition and Dietetics” for the trainers of “Amway India Enterprises” between 14th and 25th December 2005, About 25 trainers were imparted training on various aspects of nutrition and health.

An off - campus “Nutrition Orientation Programme” for the college students of different colleges in around Kanyakumari as part of a National Seminar on Nutrition and Dietetics held at Malankara Catholic College between 3rd and 4th February 2006.

Nutrition Orientation Programme for Health and ICDS Functionaries of State of Maharashtra, organized by NIN in association with Rajmata Jijau Mother-Child Health & Nutrition Mission, Aurangabad, Maharashtra. About 20 participants attended the training programme (March 20-24, 2006).



Participants receiving certificate from the Director, NIN

About 50 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

Participated in the Science & Technology Exhibition, “Pride of India Science Expo”, and the posters related to Health and Nutrition were displayed in the ICMR Pavilion, organized as part of 93rd Session of Indian Science Congress held at Hyderabad during January 3 7, 2006. About 500 people visited the stall from all walks of life.

3.2 Popular Lectures/ Awareness Camps

1. Organized an orientation programme on “Low cost Nutritious food and its health benefits” in coordination with an NGO - Confederation of Voluntary Associations (COVA) for Adolescent girls on 22nd May 2005. About 120 girls participated in the programme.

2. An extension lecture was delivered on “Nutrition and Health” on 26th June 2005 for the members of Walkers Club of Hyderabad Centre, Bagh Lingampally. During the programme, handouts on “Tips to Stay Healthy and Fit” were distributed as gratis to the participants. About 100 people from different walks of life including women participated in the programme.
 3. Organized an interactive programme on “Nutrition and Health” on 27th July 05 for the women students of Singareni College for Women at Kothagudem. About 200 students participated in the programme.
 4. Participated in the Employees' Welfare Programmes of Singareni Collieries and delivered extension lectures on “Nutrition and Health” between 28th and 29th July 05. About 250 employees including housewives attended the programme.
 5. A popular lecture on “Nutrition and Health” was delivered to the members of the Rotary Club of Hyderabad North on 23rd September 05. About 30 members participated in the programme.
 6. Organized a "Nutrition Awareness Camp" for the executives and workers of Singareni Collieries on 26th, 27th and 28th October 05 at Ramagundam, Bommalaramam and Kothagudem. About 400 employees including housewives participated in the programmes.
 7. A popular talk was delivered on “Nutrition and Health” for the college students in a function organized by the City College, Hyderabad on 17th November 05. About 150 students participated in the programme.
 8. Conducted a nutrition awareness programme for members of a Women's Self Help Group at a Training Centre in the old city of Hyderabad on 7th December 2005, in association with COVA, Hyderabad. Over 200 women members of SHG participated in the programme.
 9. An Interactive session on “Nutrition and Health” was organized for the PG Science students of Muslim Arts College, Nagercoil on 2nd February, 06. About 150 students participated in the programme.
 10. Delivered an extension lecture on “Diet and Nutrition” with the support of audiovisuals in a half day session organized for the Indian Airlines Cabin Crew on 16th February 2006. About 40 in-flight service personnel attended the programme
- ### 3.3 Radio talks/ TV Interviews
- A radio talk was given on the importance of “Breast Milk for infants” and Colostrum in local vernacular language ie. Telugu on 3rd August, which was broadcast on 5th August, 05.
- In connection with the National Nutrition Week Celebration, an interview with the Head of the Extension & Training Division covering various aspects of nutrition was telecast by Doordarshan Kendra, Hyderabad (Sept.6, 2005).
- ## 4. SPECIAL EVENTS
- ### 4.1 National Technology Day (11th May 2005)
- An interactive nutrition session was organized for adolescents in association with COVA in the old city of Hyderabad. About 60 adolescents including boys, girls and other members of the COVA participated in the programme.
- ### 4.2 World Breast Feeding Week (7th August, 05)
- Organised a “State Level Workshop on Breast Feeding and Family Foods: Loving and Healthy”, in association with Dept. of Women Development and Child Welfare, UNICEF and Food and Nutrition Board (FNB), Hyderabad, on 6th August 2005.
- ### 4.3 National Nutrition Week Celebrations (1- 7th September, 05)
- Nutrition Orientation Programme on 'Adolescent Nutrition' for the Heads of the Schools in association with the Private Schools Managements Association of Hyderabad was organized on 1st Sept. 2005.
- Conducted awareness camp on Nutrition & Health by the scientists of E&T Division in a “Nutrition Programme” for the house wives at Jamatkhana premises, Abids, Hyderabad on 3rd September, 05 organized by Aga Khan Health Service, Hyderabad. About 30 members participated in the programme.

A State Level Workshop on “Micronutrient Deficiencies A drain on Indian Economy” was organized by the FNB in association with our Institute on 5th September, 05.

Nutrition awareness camp for NSS students of Bharat Degree College for Women, Hyderabad on “Nutrition and Balanced diet” on Sept. 8, 2005.

4.4 World Food Day Celebrations (16th October, 05)

An awareness camp was conducted for Self Help Group women in the old city of Hyderabad in association with COVA on the occasion of the World Food Day. About 80 SHG women took part.

A Symposium on “Culture Food Interface”, was organized at the institute on Oct. 14, 2005.



5. MEDIA AND PUBLIC RELATIONS

5.1. Portable Exhibition Set: Developed the Telugu version of the flex sheet as an add-on to the existing state-of-the-art portable exhibition set.

5.2. Coverage of Institute's Activities in Media: The Division acted as an interface between the Institute and the Media. Cordial relations were maintained with the media in order to ensure coverage of scientific activities of the Institute from time to time and gaining visibility to the institute as well as ICMR. The staff took active role in preparation of curtain raisers, press releases and follow-up reports for obtaining coverage for various special events and conferences like NSI, EMSI etc held at the Institute.

5.3. Electronic Media: Facilitated the production of news stories / TV Programmes on various topics like women's bone health, food safety, oils, diet, WNIN Obese Rat etc., TV Channels like ETV2, TV9 and NDTV.

5.4. Preparation of Reports - The staff of Extension & Training Division rendered editorial and design assistance to bring out the proceedings of a State Level Workshop on “Micronutrient Deficiencies A drain on Indian Economy”, Proceedings of the “National Seminar on Pesticide Residues and their Risk assessment”, report on “Double Fortified Salt NIN's Perspective” and reports of the “WHO-FAO Inter-country Workshop on Updating and Implementing Food and Nutrition Plans and Policies” and WHO-NIN Workshop on “Dietary Fats and Non-Communicable Diseases”.

5.5. NIN's Website: The Institute's website, www.ninindia.org was updated from time to time. Employment opportunities at NIN were also put up regularly on the website. Efforts to revamp some web pages have been initiated.

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

Staff of the Extension & Training Division were involved in carrying out the day-to-day activities and all correspondence related to the secretariat of the WHO South East Asia Nutrition Research-cum-Action Network.



The first issue of the network's bi-annual newsletter 'SEA NETWORK' newsletter was

brought out in December 2005, after NIN took over the secretariat from Mahidol University, Thailand.

This newsletter was for the first time brought out in multi-colour and it received wide acclaim from all quarters.

B. RESEARCH ACTIVITIES

Development of communication strategies to improve nutrition and health related knowledge of NSS volunteers.

Nutrition education is an important tool to bring awareness about good nutrition practices among the public. In the present education system students are exposed to nutrition related information to some extent as part of their curriculum, mostly only up to high school level. Apart from the curriculum mass media and interpersonal communication are the other sources available for the students to obtain information on health and nutrition. The National Service Scheme (NSS) is one of the major programmes run by the Ministry of Human Resource Development, to train and encourage the college going youth to take up some community welfare program. The NSS student volunteers spend about 120 hours per year to carry out various social service activities in community level. It is important to impart nutrition information among the NSS volunteers. Hence NSS volunteers (degree level) were selected as respondents.

OBJECTIVES

- ✍ To assess the knowledge, behavior and practices of NSS volunteers vis-à-vis food and nutrition.
- ✍ To assess the health and nutrition knowledge levels of NSS volunteers.
- ✍ To develop a set of nutrition education material comprising of a set of folders, color slides and charts. To carry out intervention with NSS volunteers.
- ✍ To conduct training program to orient NSS functionaries (program officer and volunteers) on nutrition themes to enable them to be better nutrition educators in the community.
- ✍ To develop innovative training methods (roll play, brain storming, group discussion) to NSS

volunteers who in turn will educate the community.

Development of research tools

Based on the discussions held with NSS programme officers during the camp 3 different research tools were developed.

- ✍ An interview schedule was developed to assess the socio-economic background, food practices and behaviour.
- ✍ A multiple choice questionnaire was developed to measure the nutrition and health related knowledge of the NSS volunteers
- ✍ A questionnaire was developed to find the media using habits of the NSS volunteers

Pilot study

A pilot study was carried out in three different colleges of Hyderabad City, namely Railway Degree College and Kasthurba Degree College. After the pilot study necessary modifications were made in the research tools.

Data Collection

Base line data among 660 NSS volunteers from ten affiliated colleges of Osmania University was collected.

Communication material development

One set of colour folders was developed on different nutritional themes such as energy, protein, vitamins and minerals, fat, nutrition during adolescent age, nutrition during pregnancy, and obesity. The folders were used in the first intervention while for the second intervention a CD with a folk song was developed on the nutritional themes, which were already identified. It is proposed to educate the women of reproductive age group (18-45) about the importance of micronutrients like iron, vitamin A, and iodine. In addition, a color flipchart was developed.

First intervention

The intervention was carried out in the classroom situation. During the intervention the sequence of methods involved in conducting the intervention was similar for all the colleges. Since it is a follow up study the same set of students who were involved at the baseline data collection were included for the intervention. A lecture covering all

the major themes was given to the NSS volunteers in addition to the folders. NSS volunteers were given adequate time to go through the folders.

After the intervention knowledge assessment questionnaire was administered to the students to find out the knowledge improvement.

Orientation programme

As part of this project an orientation program to NSS program officers on Feb. 7th 2005 was organized at the National Institute of Nutrition. In the orientation programme, various important topics such as micronutrient deficiency disorders, dietary guidelines, nutrition and infection, recommended dietary allowances for collage students, fast food and health implication, interpersonal communication and attitudinal change, intersectoral cooperation and social mobility, food safety issues and concern were covered. Thirty four Program Officers from different colleges affiliated to Osmania University participated in the program.

RESULTS

Food consumption pattern of NSS volunteers

Analysis of the data indicated that 50.3% of the NSS volunteers reported regular intake of meals and 20% of them reported to have the habit of consuming snacks between the meals. As regards the cereal intake 75% of them were consuming more than 1 cereal and remaining 25 % of them were consuming only rice as the staple diet. Regarding the intake of pulses, 33% of them were not consuming pulses daily. Most of them belong to agricultural family only 50% of them consumed milk and milk products daily.

The daily intake of fruits, vegetables and green leafy vegetables was very minimum. Only 9% of the NSS volunteers reported consumption of green leafy vegetables daily while another 9% of the volunteers did not consume GLV. About 54% of them consumed GLV weekly twice. Similarly 49% consumed fruits twice a week and 21% never had fruit intake.

Regarding the processed food intake the results indicated that 20% of them consumed processed food weekly twice and 40% of them reported to consume aerated cool drinks once a week.

Nutrition knowledge improvement levels of NSS volunteers.

Table 21. Nutrition knowledge improvement levels of NSS volunteers

Region	Baseline	After intervention
Urban	30.91 ± 9.58	45.06 ± 5.5
Rural	33.92 ± 5.83	46.48 ± 6.3
District Head-quarters	35.58 ± 5.5	44.07 ± 5.92

*p<0.001

*p<0.005

Table 22. Knowledge difference between control and experimental group (District headquarters)

Sl.No	Baseline	After intervention	Increment
Experimental (80)	35.56 ± 6.67	44.4 ± 5.9	9.25 ± 8.7
Control (37)	36.43 ± 6.0	37.04 ± 6.7	-0.2 ± 8.3

P<0.001

The NSS volunteers were selected from the colleges of urban, rural and district headquarters. The score (0 for wrong and 2 for correct) was assigned for all the 32 questions. The rural and district headquarters students scored better than their urban counter part (30.93 ± 9.67 urban; 33.93 ± 5.83 rural; 35.57 ± 6.67 district head quarters). The knowledge levels were significantly different among the students of rural, urban and district headquarters (ANOVA, p<0.001). Paired T test showed significant improvement in nutrition knowledge levels before and after intervention in all the three areas. A subgroup analysis of the data (district head quarters) indicates that increment in nutrition knowledge scores were significantly different between experimental and control group. Better nutrition education and suitable communication materials will improve the nutrition knowledge and food consumption practices of NSS volunteers of degree colleges. Since the NSS volunteers are involved in community education programmes, educating the students may in turn help to educate the community on health and nutrition aspects (Tables 21, 22).

VI. FOOD & DRUG TOXICOLOGY RESEARCH CENTRE

A. FOOD SAFETY

Effect of magnesium compounds on mobilization of deposited fluoride in rabbits

Fluorosis is a major public health problem all over the world including India. While dental fluorosis results in mottling and pitting of teeth, skeletal fluorosis results in severe crippling deformities of bones. Endemic genu valgum has been identified in some parts of India affecting a number of adolescents and children. Epidemiological studies show that prevalence of genu valgum in fluorotic areas is significantly higher in subjects whose staple is sorghum (4%) as compared to those whose staple is rice (1%). Genu valgum and sometimes paraplegia caused due to endemic fluorosis make people totally handicapped. Medical intervention has not been very effective and hence alternative strategies have to be developed to treat this malady. It is reported that calcium supplementation interferes with fluoride absorption in animals. Aluminium sulphate and boron have also been tried for this purpose. There have also been some reports on the protective effect of intravenous/oral administration of magnesium salts ($MgCl_2$, MgO , $Mg(OH)_2$ and serpentine) against fluoride toxicity and intravenous injection of $Mg(OH)_2$, increases fluoride excretion through urine (eleven fold).

However, none of these compounds are currently under use as public health measures, due to non-availability of data on their efficacy as well as toxicological effects on organ systems, if any.

The present study was done to assess the possible benefits of magnesium compound administration in fluorosis and its capacity to mobilize already deposited fluoride from the bones as well as prevent new fluoride deposition and, toxicological potential of Mg salts on various organs.

AIMS AND OBJECTIVES

To assess the beneficial effects of magnesium compound on fluoride toxicity *in vivo*.

Specific parameters

1. To monitor urinary fluoride excretion
2. Bone fluoride levels
3. Evaluation of bone density

Toxicological evaluation of all vital organs by histopathological and haematological studies.

METHODOLOGY

Animal models: New Zealand white rabbits aged 2 to 3 months were provided in two batches 12 each with a gap of two months. Animals were divided into four groups of three each in two batches (Table 23). The animals were fed with

Table 23. Study design

Sl. No	Group	No. of animals	Treatment and duration
1	Control	6	Till end of experiment
2	Fluoride	6	Fluoride(20mg/day/rabbit) 7 months period (fluorosis induction) needed for establishing fluorosis
3	Fluorosis induced	6	Fluoride (20 mg/rabbit/day) followed by Mg supplementation for 6 months followed by Mg compound (40 mg/rabbit /day only) administration for 1 month
4	Fluoride + Mg compound	6	Fluoride + Mg (40 mg/rabbit/day) simultaneous treatment for 8 months

standard pellet diet. After seven months, 24 hr urine and faeces were collected for three consecutive days and blood samples were collected for biochemical parameters. After 7 month of fluoride and Fluoride + Mg compound feeding all animals were stabilized for one month (F and F+Mg was stopped). At the end of one month or total 8 months, 24 hr urine and faeces were collected for 3 consecutive days for biochemical investigations. After stabilization of one month, 11 animals (one lost) of fluoride group were divided into two groups, one group was fed with Mg compound alone and other was kept without Mg compound treatment for comparison. This treatment was given for one month. At the end of the experiment, 24hr. urine and faeces were collected for fluoride and other bone mineral analysis. Fasting blood samples were collected for biochemical parameters as well as haematological analysis. The animals were finally sacrificed by CO₂ inhalation. Femurs and tibia were removed and cleaned of soft tissue and their length was measured. All vital organs were obtained and fixed by routine histopathological methods.

Biochemical investigations

Twenty four hour urinary, faecal and bone fluoride were analyzed by using ion selective electrode. Urinary minerals like Ca, Mg, Zn and Cu were analyzed using Atomic absorption spectrophotometer. Total and bone specific alkaline phosphatase were analyzed by standard methods. Serum iPTH and osteocalcin were analyzed by kit method.

Histopathology

All the organs were collected in 10% neutral buffered formalin after euthanization. Nerves were collected in Flemings solution separately for myelin staining while pancreas and testes were fixed in Bouins fluid.

Histopathology study of various organs was carried out in all different groups to look for any toxicological changes. The organs studied were brain, trachea, gastrointestinal tract, heart, lungs, liver, spleen, kidneys, adrenals, aorta, sciatic nerve, muscle, gall bladder, urinary bladder, skin, pituitary, pancreas, thymus and respective reproductive organs.

Haematology

Total RBC and differential counts, haemoglobin, packed cell volume and peripheral smear examination was undertaken in all animals.

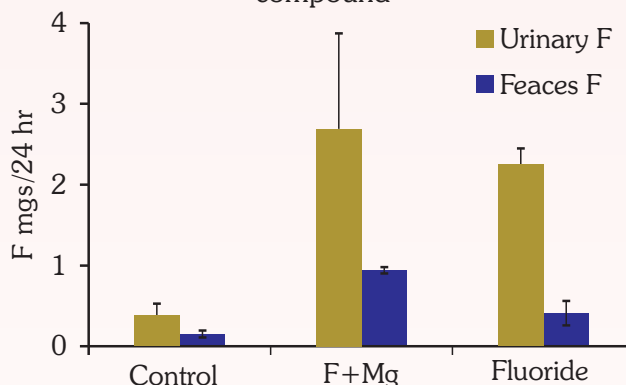
RESULTS

24 hour urine and faeces were collected for three consecutive days at three time points. 1. After 7 months of fluoride and F + Mg feeding, 2. After 1 month of stabilisation and 3. After 9 months.

1) Urinary parameters

✍ After 7 months of fluoride and F + Mg feeding both groups showed, significantly higher 24 hr urinary fluoride as compared to control group. However, urinary F was higher in F group than F + Mg group which was not statistically significant (Figure 31a). There was no significant difference in 24 hr urinary total volume, pH, creatinine, Ca, Mg, Zn and Cu between the groups.

Fig. 31 a: Urinary and Faeces Fluoride after 7 months treatment of Fluoride and F+Mg compound

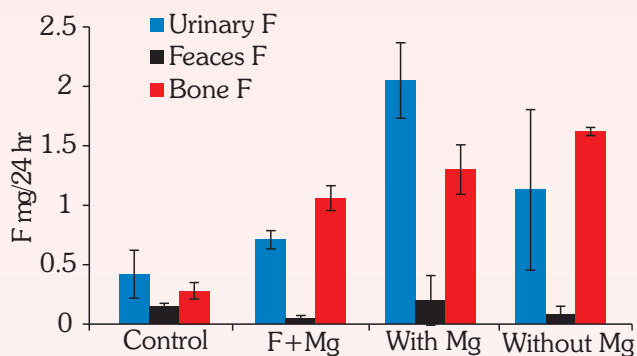


Faecal fluoride was analyzed in three groups, it was significantly higher in F + Mg, and F groups as compared to control group. However, faeces F was significantly higher in F + Mg group than F group (Fig. 31b).

✍ After one month stabilization - One month after discontinued feeding to fluoride to fluoride group, 24 hr urine was collected for three consecutive days and F was analyzed. There was increased urinary F in F + Mg and F groups than control group. There was significant

increase in urinary F in F+Mg group than F group. Urinary creatinine was lower in F + Mg group than control and F groups. However, there was no significant difference in urinary volume, pH, Ca, Mg, Zn and Copper among the groups.

Figure 31 b. Urinary, Feaces and Bone Flouride after 9 months treatment of Fluoride and Fluoride and Magneaium compound



The fluoride treated group after stabilisation for one month (11 animals) were randomly divided into two more sub groups. One group treated with Mg compound and other was kept as control. After one month of Mg compound treatment, 24 hr. urine was collected for 3 consecutive days. There was marginally higher urinary fluoride excretion in Mg treated group than all other groups.

Zinc excretion was higher in Mg treated and Mg untreated group than control and simultaneous Mg treated group. There were no changes in other urinary parameters (Total volume, pH, Ca, Mg and Cu) among the groups. Faeces was also collected for 3 consecutive days at this stage(at the end of 9 month) of experiment and fluoride was estimated. There was no significant difference among the groups at this stage.

2) Serum parameters

After 7 month treatment of fluoride alone and F along with Mg affects total as well as bone specific alkaline phosphatase activity. Total and bone specific alkaline phosphatase activity was significantly higher in Fluoride and F + Mg groups than control group, but not among themselves. Serum fluoride at this stage also showed similar results.

At the end of the experiment (after 9 months) serum total as well as bone specific alkaline phosphatase were not significantly different from control and treatment groups. However, there was higher serum fluoride in treatment group as compared to control group. Serum iPTH and osteocalcin at the end of the experiment, showed no significant difference among the groups.

3) Bone fluoride

At the end of the experiment (9 months treatment) fluoride was analyzed in bone ash. Bone fluoride in experimental groups was higher than control however, there was no significant difference among experimental groups.

4) Organ weights

All the vital organs (liver, kidney, brain, heart, lungs and spleen) obtained after euthanization, showed no significant differences in organ weight ratio among the groups.

5) Histopathology

It was observed that all the changes seen were nonspecific in nature and common to all colony bred animals. Some lungs showed presence of chronic interstitial pneumonitis (grade I) while few others showed peribronchial and perivascular round cell collection.

Medial hypertrophy of pulmonary vessels was prominent in most animals. Livers showed focal areas of necrosis and perivascular / periportal round cell collection. Otherwise the study was unremarkable. Sciatic nerve study did not show any differences in the myelin staining pattern or the fibre counts between the groups.

6) Haematology

The various parameters studied did not show any significant differences between the groups.

CONCLUSION

Simultaneous feeding of magnesium compound (milk of magnesia) reduces fluoride absorption suggesting a beneficial effect of magnesium hydroxide ingestion on fluoride retention and toxicity. Histopathology and haematological study showed there was no adverse effect of magnesium compound in experimental animals.

B. CANCER AND XENOBIOTICS

1. Antimutagenicity of heat processed ginger

Spices are important constituents in the preparation of various foods in Indian culinary practices. Spice mixes are prepared by using either the raw or the roasted ground spices. As interest in the possible relationship between diet and cancer has increased in recent years attempts have been made to determine whether diet contains antimutagens/anticarcinogens. Therefore, a study was planned to understand the mutagenicity of fresh and dry forms of ginger under commonly practiced culinary conditions.

AIMS AND OBJECTIVES

To investigate the antimutagenicity of fresh and dry ginger under normal cooking conditions using

Ames test with two tester strains namely TA 98 and TA 100 in the absence of metabolic activation system.

The extracts of ginger (fresh and dry form) namely 1) unboiled, 2) boiled, 3) fried and 4) unfried has been evaluated and tested for its antimutagenicity.

RESULTS

The antimutagenic effect was not altered in all the extracts irrespective of boiled and unboiled, fried and unfried in both fresh and dried forms of ginger in TA 98 in the absence of metabolic activation system (Tables 24 and 25).

Similar results were observed in the tester strain TA 100 (Tables 26 and 27).

Table 24: Antimutagenic potential of unboiled and boiled ginger paste - TA 98 (- S₉)
No. of histidine revertant colonies

Sample	Unboiled	Boiled	Unfried	Fried
Ginger 1 mg	48 ± 8.4	39 ± 5.6	64 ± 5.6	64 ± 5.6
Ginger 2 mg	43 ± 9.1	46 ± 4.2	56 ± 5.2	56 ± 5.6
Ginger 3 mg	42 ± 5.6	52 ± 5.6	52 ± 5.6	52 ± 5.6
B(a)P 2 g	81 ± 5.6	81 ± 5.6	102 ± 8.4	102 ± 8.4
Ginger 1 mg + 2 g B(a) P	70 ± 1.4	89 ± 9.8	84 ± 8.4	96 ± 11.3
Ginger 2 mg + 2 g B(a) P	57 ± 2.8	69 ± 5.6	88 ± 7.0	78 ± 8.4
Ginger 3 mg + 2 g B(a) P	54 ± 9.8	65 ± 5.6	65 ± 11.3	72 ± 9.9
B(a)P 5µg	124 ± 8.4	124 ± 8.4	148 ± 11.3	148 ± 11.3
Ginger 1 mg + 5µg B(a) P	98 ± 11.3	86 ± 4.2	104 ± 4.2	64 ± 4.9
Ginger 2 mg + 5µg B(a) P	86 ± 2.8	78 ± 2.8	96 ± 7.1	50 ± 7.0.
Ginger 3 mg + 5µg B(a) P	67 ± 5.6	67 ± 5.6	88 ± 7.1	48 ± 4.6

Spontaneous frequency of revertants (- S₉) = 17.8 ± 2.2, Values are mean ± S.D. Each sample was tested in duplicate plate assay. Between group differences were seen by means of Kruskal Wallis test.

Table 25 Antimutagenic potential of unboiled and boiled ginger powder - TA 98 (- S₉)
No. of histidine revertant colonies

Sample	Unboiled	Boiled	Unfried	Fried
Ginger 0.5 mg	50 ± 5.6	49 ± 4.2	34 ± 3.1	43 ± 4.1
Ginger 1.0 mg	49 ± 11.3	46 ± 2.8	22 ± 4.2	39 ± 3.1
Ginger 1.5 mg	27 ± 4.2	52 ± 3.8	20 ± 2.6	30 ± 2.0
B(a)P 2 g	88 ± 5.6	88 ± 5.6	64 ± 3.5	64 ± 5.2
Ginger 0.5 mg + 2 B(a)P	73 ± 7.0	73 ± 8.4	41 ± 2.6	34 ± 6.1
Ginger 1 mg + 2 gB(a)P	82 ± 6.0	71 ± 4.2	29 ± 4.2	33 ± 3.2
Ginger 1.5 mg + 2 gB(a)P	85 ± 3.5	62 ± 7.7	38 ± 4.2	35 ± 5.6
B(a)P 5µg	118 ± 8.4	118 ± 8.4	73 ± 4.7	73 ± 4.7
Ginger 0.5 mg + 5µgB(a)P	94 ± 8.5	86 ± 2.8	36 ± 2.4	41 ± 3.4
Ginger 1.0mg + 5µg B(a)P	96 ± 2.1	78 ± 2.1	34 ± 2.1	39 ± 2.1
Ginger 1.5 mg + 5µg B(a)P	77 ± 4.2	67 ± 4.2	27 ± 4.2	37 ± 3.2

Spontaneous frequency of revertants (- S₉) = 12.2 ± 3.6.

Table 26: Antimutagenic potential of unboiled and boiled ginger paste - TA 100 (- S₉)
No. of histidine revertant colonies

Sample	Unboiled	Boiled	Unfried	Fried
Ginger 1 mg	109 ± 14.1	107 ± 1.4	112 ± 5.6	72 ± 7.0
Ginger 2 mg	98 ± 8.5	85 ± 5.6	88 ± 5.6	78 ± 5.6
Ginger 3 mg	90 ± 4.2	67 ± 7.0	96 ± 5.6	64 ± 4.2
B(a)P 2 g	170 ± 7.0	170 ± 7.0	153 ± 7.0	153 ± 7.0
Ginger 1 mg + 2 g B(a)P	164 ± 8.4	111 ± 1.4	102 ± 8.4	120 ± 9.8
Ginger 2 mg + 2 g B(a)P	166 ± 8.4	107 ± 5.6	94 ± 7.0	100 ± 5.6
Ginger 3 mg + 2 g B(a)P	142 ± 4.2	113 ± 1.4	104 ± 11.3	90 ± 5.6
B(a)P 5µg	281 ± 11.3	281 ± 11.3	230 ± 14.1	230 ± 14.1
Ginger 1 mg + 5µg B(a)P	189 ± 12.7	192 ± 4.2	160 ± 4.2	138 ± 8.4
Ginger 2 mg + 5µg B(a)P	182 ± 4.2	171 ± 1.4	180 ± 7.0	120 ± 9.8
Ginger 3 mg + 5µg B(a)P	176 ± 8.4	180 ± 3.5	156 ± 3.5	126 ± 5.6

Spontaneous frequency of revertants (- S₉) = 12.2 ± 3.6

Table 27: Antimutagenic potential of unboiled and boiled ginger powder - TA 100 (- S₉)
No. of histidine revertant colonies

Sample	Unboiled	Boiled	Unfried	Fried
Ginger 0.5 mg	82 ± 8.4	58 ± 5.6	102 ± 7.0	90 ± 7.0
Ginger 1.0 mg	80 ± 7.0	47 ± 6.4	90 ± 7.0	72 ± 9.0
Ginger 1.5 mg	72 ± 7.0	68 ± 4.2	96 ± 4.2	78 ± 3.8
B(a)P 2 g	190 ± 11.3	190 ± 11.3	122 ± 7.3	122 ± 7.3
Ginger 0.5 mg + 2 g B(a)P	79 ± 5.1	86 ± 7.1	86 ± 8.1	120 ± 9.0
Ginger 1.0 mg + 2 g B(a)P	74 ± 6.1	79 ± 4.2	66 ± 8.2	100 ± 7.0
Ginger 1.5 mg + 2 g B(a)P	64 ± 9.2	65 ± 4.2	60 ± 4.2	90 ± 7.0
B(a)P 5µg	294 ± 5.6	294 ± 5.6	142 ± 4.8	142 ± 4.8
Ginger 0.5 mg + 5µg B(a)P	102 ± 11.3	90 ± 4.2	96 ± 5.6	140 ± 5.0
Ginger 1.0 mg + 5µg B(a)P	114 ± 5.6	86 ± 2.8	110 ± 7.2	100 ± 8.0
Ginger 1.5 mg + 5µg B(a)P	96 ± 8.4	73 ± 5.6	90 ± 8.4	102 ± 7.0

Spontaneous frequency of revertants (- S₉) = 37.8 ± 5.2.

CONCLUSION

The antimutagenic effect of ginger was not altered in the extracts of ginger processed under normal cooking conditions.

2. H pylori infection and *in vivo* nitrosation

H pylori infection is one of the etiological factors in gastric carcinogenesis. Low intake of vit.C as well as low nutriture was associated with H pylori infection as was seen in earlier studies. It is assumed that low antioxidant levels in an individual predisposes to H pylori infection. At the same time

carcinogenic nitrosation of amines in the gastric lumen occurs if antioxidants are low. Thus this study was undertaken to understand the role of nitrosation in H pylori infection.

The aims of the study

- ✍ To assess the intake of vitamin C through diet
- ✍ To correlate H pylori infection with excretion of nitrosated proline

METHODOLOGY

Patients suffering from acid dyspepsia coming to GE OPD were enrolled. In all there were 100 subjects, of whom 50 were positive for H pylori

Table 28: Vitamin C and Nitrosoproline levels in H pylori + ve cases and controls

Type	Vit C FFQ g / d		P.Vit C g/ml		Nitrosoproline g/24h urine		Nitrosoproline g / l	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Cases H pylori +ve (50)	35.5	1.79*	0.39	0.019*	7.68	0.137*	7.02	0.147*
Controls H pylori-ve (50)	62.3	5.57	0.69	0.029	4.99	0.207	4.23	0.254

()- no. of observations * - $p < 0.001$

infection. H pylori were considered present if GJ tested positive based by rapid urease method. A detailed clinical, anthropometrical and diet history were noted. On an overnight fasting condition basal urine was collected. Blood sample was also collected. L-Proline 500mg was then administered orally and 24 h urine was collected for estimating nitrosamines. The nitrosamines were detected using GC-TEA.

RESULTS

- ✎ Fifty cases of H pylori positive; fifty controls negative for H pylori
- ✎ Vitamin C rich foods mean SEM score 35.6 ± 1.79 , 62.3 ± 5.57 in cases and controls mean Plasma Vit. C was 0.39 ± 0.019 , 0.69 ± 0.29 g/ml in cases and controls
- ✎ Mean nitrosoproline in 24 h was 7.7 ± 0.14 g, 5.0 ± 0.21 g/l respectively

The results suggest that both the intake as well as plasma vitamin C is significantly low in H pylori positive cases (Table 28).

Urinary nitrosoproline taken as a marker for *in vivo* nitrosation of ingested proline is highly significant.

3. *In vivo* nitrosation potential of population at high risk for gastric cancer

In vivo formation of nitrosamines can occur in the gastric lumen. The rate of formation depends

upon several factors like pH of gastric juice, presence and concentration of precursors like amines and nitrates, presence of catalysts etc. A safe amine like proline can be used to mimic *in vivo* nitrosation. This method was used to study the differential formation of nitrosamines in individuals at high/low risk for gastric carcinomas.

The study was initiated with the objective of understanding the difference, if any, in nitrosation between high and low risk area residents.

METHODOLOGY

High and low risk areas for gastric cancer were obtained from Population Cancer Registry, Bangalore. They were in turn determined from number of incident cases reported in select geographical area. These areas were randomized and selected 4 (2 males and 2 females) high risk and 4 (2 male and 2 female) low risk areas for the study. Fifty subjects from high and 50 from low risk areas were enrolled.

Essential details were noted and an overnight fasting urine sample was collected. An oral administration of L-Proline at a dose of 500mg was given and 24h urine collected. The samples were transported and stored at 20°C till analysis. Analysis was done on GC-TEA.

RESULTS

- ✎ The study was done in 50 high risk area residents and 50 low risk area residents.
- ✎ Mean urinary nitrosoproline in 24h was 5.89 ± 0.139 g/l; 2.23 ± 0.132 g/l in high and low risk area residents respectively (Table 29).

Table 29. Nitrosoproline excretion in high / low risk areas

Risk Area	Nitrosoproline g/24 h		Net Nitrosoproline g/l	
High Risk Area (50)	5.89	0.139	5.12	0.132
Low risk Area (50)	2.23	0.132	2.17	0.126

The results suggest that the proline test is feasible to be conducted in field situations. However, it was not significant in picking up high-risk subjects. Perhaps a larger study taking into consideration dietary risk factors may give meaningful results.

4. Ethnopharmacological validation of biodynamic compounds in traditional medicine

Inflammatory diseases such as rheumatoid arthritis, asthma, and hepatitis are among the leading causes of death and disability in the world. During inflammation, various factors like oxidative stress; pro-inflammatory cytokines play a key role in mediating inflammatory response. Therefore, intense research is directed toward the discovery of safe/effective therapeutic agents to prevent/reverse these processes. In the recent past the emphasis on developing safe effective therapeutic agents especially from traditional system is being given in view of the additional benefits and economical viability. The results of earlier studies (AR 2002-04) indicate that extracts (coded 4308, 4212, 3107, 3223 & 5322) of plants, which are traditionally used as anti-inflammatory drugs have potential antioxidant activity as evaluated by battery of *in vitro* tests and *ex vivo* test (AR-2002-04). The present investigation was therefore undertaken to validate its anti-inflammatory potential using standard experimental animal models.

Hypothesis

To evaluate the anti-inflammatory and antioxidant potential of aqueous (4212) and aqueous alcoholic (4308) extract of "Rasna

panchaka" a combination medicine used for the treatment of rheumatoid arthritis in Ayurveda (Table 29).

METHODOLOGY

The conventional model (carrageenan induced rat paw edema) is used for standardizing the effective anti-inflammatory dose of extracts. The air pouch model in NIN Wistar strain of rat has been adopted to understand the mechanism of action and anti-inflammatory potential. After establishing the effective dose in rats paw edema model, the air pouch model was established by injecting 20ml of sterile air into the ventral side of rat. The pouches were allowed to stabilize for 3 days and 20ml of sterile air was re-injected into the cavities on 3rd day followed by 2ml of 2% carrageenan solution.

The test compound 4212 and 4308 in a dose of (50 g of 10gm extract/animal) was administered orally and its potential activity has been evaluated by (i) measuring volume of fluid collected in the pouch (ii) lymphocyte infiltration rate (iii) assessment of anti oxidant activity (TBARS, Catalase, SOD) (iv) Pro-inflammatory markers viz. (IL6 & TNF-) and (v) Histopathological observations.

Study design involved, distribution of 126 male rats in five groups viz (i) Control (non-inflammation group; n=6) (ii) Experimental Control (inflammation group; n=30) (iii) Test Compound 1(4212 treated group; n=30) (iv) Test Compound 1(4308 treated group; n=30) (v) Standard (Acetyl salicylic acid treated group; n=30). All the groups except control were further subdivided into five groups (n=6) to form the time course.

All the animals in each group after administration of test drug / standard drug were subjected to euthanasia at 2h, 4h, 8h, 12h and 24h to undertake the investigation for the above-mentioned parameters.

RESULTS

- i) Rat paw edema results indicated that the animals treated with the aqueous extracts of 4212 and 4808 at a dose (0.225 µl/rat (200g)) reduced the degree of inflammation significantly compared to aspirin (3.0 mg/rat) (Table 30).

Table 30 : Antiinflammating activity of 4212 and 4308

S. No	Group	Control	EC	Rx 4212	Rx 4308	ASA
	Parameters					
1	Volume of exudate in ml (4hrs)	-	1.40 0.1924	1.483 0.2317	0.917 0.1633	1.008 0.2375
2	Viable Lymphocyte Infiltration in millions (4hrs)	-	27.55 2.18	21.267 1.90	14.767 2.76	25.4 1.36
3	TBARS nmMDA/mg protein (4hrs)	46 6.26	253.83 31.46	90.67 5.31	92.83 5.74	97.33 5.57
4	SOD in units	-	0.0748 0.026	0.07767 0.04	0.08117 0.005	0.07483 0.002
5	CATALASE in units	-	2.46 0.216	3.5 0.178	3.55 0.151	3.233 0.1751
4	IL-6 in IU (24hrs)	-	729.33 11.676	81.6 60.11	30.97 22.751	215.7 62.52
5	TNF- in IU (4hrs)	-	983.4 7.97	928.93 18.20	961.96 23.55	975.26 13.35
6	% inhibition in rat paw edema model	-	-	30	40	27

Values are Mean \pm SD (n=6).

- ii) There were significant differences in the mean volume of the fluid collected in the untreated and treated groups at all time points except 2hr.
- iii) Water plus methanol treated group was found to have less fluid collection and was significantly different from all the groups.
- iv) It was observed that the mean number of viable lymphocytes infiltrated in the water plus methanol treated group was significantly less compared to other groups at all time points.
- v) Infiltrated cells in water treated group and aspirin treated group also were significantly less compared to untreated group but not to the extent of water plus methanol treated group.
- vi) It was observed that the cell count was increasing with time in all the groups indicating the possible inflammatory response of the body. These parameters support the findings in rat paw edema model regarding anti-inflammatory property.
- vii) Antioxidant markers viz. TBARS raised up significantly in the experimental groups compared to the control. It was also observed that these levels were high in the initial hours i.e. 2hr but were reduced with time.

- viii) The levels of the interleukin 6 for the 12hours time period didn't alter in treated and non-treated groups. But it was observed that at 24th hour the levels of IL6 in all the treatment groups significantly reduced compared to experimental control group values. It was also evident that the water and water plus methanol have significantly reduced the IL6 levels compared to aspirin treatment.
- ix) There was no difference observed in the levels of TNF- between the groups for the first 4hrs. Water extract treated group alone showed significantly reduced TNF- levels at the 4th hour time point. After 4th hour all the groups showed reduction in the TNF- levels.

CONCLUSIONS

The study results suggest that aqueous and alcoholic aqueous extracts of traditional preparations Rasna panchaka has potential anti-inflammatory activity as evident from decreased exudate volume, reduced oxidative stress and modulate levels of TNF- and IL-6.

The biological plausibility as evident from the present study suggest that water and water plus methanol extracts of Rasna panchaka can be considered as potential candidates in the treatment of rheumatoid arthritis disease.

5. Role of nutrients in environmental toxicity

The use of heavy metals like Lead (Pb), Mercury (Hg), Cadmium (Cd), Arsenic (As) etc. has resulted in the rise of their levels in environment resulting in their exposure which is reported to be toxic to human health. This has emerged as a major public health problem. Since the past one decade, reports mostly from developed countries suggest that the heavy metals (Pb, Cd, Hg, As etc.) used in industries, induce slow progressive and most of the times, irreversible damage to the nervous, haemopoietic and renal systems in population. In addition, few reports indicate their interaction with nutrients (Fe, Zn, Cu, Mg, Ca etc.) and alteration in biochemical functions specially at sub-cellular / cellular levels. The important physiological functions of essential metal ions like Iron (Fe), Zinc (Zn), Copper (Cu), Magnesium (Mg) etc. have been well established. Among the various heavy metal toxicities reported, lead toxicity is reported from all parts of the world.

AIMS AND OBJECTIVES

1. To monitor pollutants specially lead and nutrient levels among the vulnerable groups such as children and pregnant women.
2. To document the demographic, socio-economic, clinical profile and nutritional status of the target population in relation to nutrient and pollutant levels.
3. To assess the interactions, if any, between pollutants, nutrients and nutritional status.

SUBJECTS AND METHODS

Two major groups of population viz. children (159no.) aged between 9-19 years belonging to high (34%), moderate (25%), mild (41%) risk areas of lead exposure and pregnant women (54no.) aged between 18-26 years from rural (20.4%) and urban (79.6%) were enrolled, after obtaining their consent. In addition, 54 neonates were also monitored for blood lead levels. All the subjects have been interviewed and screened for the following information in a pre-tested schedule by the trained field investigator.

1. Socioeconomic and demographic profiles: This includes the economic status, household's

condition, play-habits, cooking practices etc. of target population.

2. Nutritional status: data on height, weight and hemoglobin has been collected and BMI was assessed using NCHS standards.
3. Clinical examinations: A qualified physician and gynaecologist have examined the target population, on various clinical parameters viz. specific signs and symptoms related to heavy metal poisoning.
4. Sample collection: 10 ml blood sample was collected by veni puncture from all subjects. These samples were transported to the laboratory to prepare small aliquots of whole blood /serum and stored at -80°C, till they were analyzed. The neonate blood samples (20 μ l) have been collected by heel prick in a capillary tube containing the reagent kit of ESA.

5. Laboratory Investigation:

- i. RBC examination and Hemoglobin estimation: Using Romauowsky stained smears (Leishman stain) morphological changes in RBC was noted. Hemoglobin (Hb%) was estimated by Cyanomethomoglobin method.

- ii. Serum Albumin Estimation:

Serum albumin was estimated by Bromocresol green (BCG) method.

- iii. Estimation of Heavy Metals and Nutrients:

The Lead, Cadmium, Mercury, Arsenic, Selenium and other metal ions were determined by ICPMS (Model VG -elemental Plasma Quad 3) technique at Center for Compositional Characterization of Materials (CCCM)-BARC, Hyderabad, in whole blood samples.

The nutrients viz. iron, copper, zinc and magnesium by Atomic Absorption Spectrophotometer (Model No- VARIAN 220) at National Institute of Nutrition after digesting on microwave system (MARS Xpress, CEM).

RESULTS

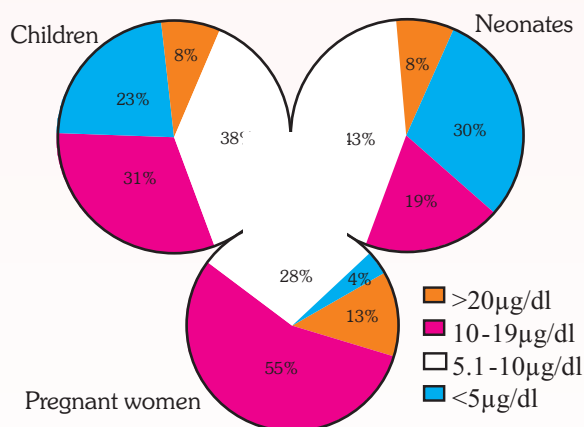
I. Children

- a) The BMI percentiles suggest that 5% children were undernourished, 80% normal and rest

were overweight & obese (15%) based on the standard criteria. The mean hemoglobin levels were found to be $13.0 \pm 2.24\text{gm}\%$.

- b) The demographic profile suggests that approximately 31.5% of subjects were staying in pucca house, 19.5% in semi-pucca and 15.7% in thatched roof houses. The wall coating of the houses was reported to be painted (11.9%), white washed with lime (82.4%), and cow-dung (1.3%) etc. The majority of children (84.3%) were dependent on protected water, where as 10.7% from tube well, 3.7% from pond/tank. The closed drainage system was in 66% of target areas and open drain system in 34%. The dietary pattern indicated that staple food is rice (98.7%) with 87.4% as non-vegetarians. The cooking utensils were stainless steel (25.2%), aluminum (66%), and earthen vessels (8.8%).
- c) The specific clinical symptoms viz. upper respiratory tract infection (11%), pigmented tongue (8.2%), lymph node enlargement (7.4%), knee-jerk (5%), tremors and fatigue (2.5%), pale conjunctiva (2%) were observed in various groups of children.
- d) Microscopic Examination of Peripheral Smear: The differential count of all the subjects was normal. On peripheral smear examination, 21% of the subjects in heavy exposure and 27% in moderate exposure group showed prominent features of hypochromia.
- e) The blood lead levels in children (39%), pregnant women (59%) neonates (27%) was found to be more than $10\mu\text{g}/\text{dl}$ (Figure. 32).

Fig. 32. Trends in Blood Lead Levels of Children, Neonates & Pregnant women



- f) The other heavy metal levels were cadmium $0.55 \pm 0.93\mu\text{g}/\text{dl}$, mercury $26.3 \pm 29.67\mu\text{g}/\text{dl}$ in children were also high.
- g) The mean nutrient levels were iron $296.2 \pm 210.24\mu\text{g}/\text{dl}$, zinc $71.9 \pm 24.39\mu\text{g}/\text{dl}$ copper $93.7 \pm 30.15\mu\text{g}/\text{dl}$ and magnesium $1750.9 \pm 292.98\mu\text{g}/\text{dl}$.

II. Pregnant women

- a) Pregnant women were staying in pucca (41%), semi-pucca (30%) and in thatched (9%) houses. The wall coating of the houses were reported to be white washed with lime in 78%, painted in 15%, and cow-dung 4% etc. The major source of the drinking water was tap water (65%), tube well (28%) and open well (4), pond/tank water (3%). In 61% of areas there was closed drain system. The staple diet was rice (80%), wheat (11%) and non-vegetarians (89%). The cooking utensils were aluminum 83%, stainless steel 15% and others 2%.
- b) Data on Gestational age at delivery was >37 weeks (70%), <37 weeks (19%) with rest, 37 weeks. The gravida was in the range of 1-5 and para 0-4. The obstetric history was found to be normal in majority of the cases. The hemoglobin levels were found to be $11.5 \pm 2.30\text{gm}\%$.
- c) The clinical symptoms viz pale conjunctiva (35%), abdominal discomfort and pigmented tongue (13%), puffiness of face (11%), nausea & vomiting, oedema (7%), headache, fatigue (6%), irritability, liver enlargement, diarrhea (4%), skin and hair changes were observed.
- d) The mean nutrient levels in the mother blood were iron $165.9 \pm 130.15\mu\text{g}/\text{dl}$, zinc $220.64 \pm 163.83\mu\text{g}/\text{dl}$, copper $156.9 \pm 77.10\mu\text{g}/\text{dl}$, magnesium $2123.4 \pm 1183.02\mu\text{g}/\text{dl}$ and selenium $61.8 \pm 16.94\mu\text{g}/\text{dl}$, where as in cord blood samples the iron level was $237.9 \pm 115.32\mu\text{g}/\text{dl}$, zinc $123.2 \pm 49.62\mu\text{g}/\text{dl}$, copper $91.3 \pm 61.96\mu\text{g}/\text{dl}$, magnesium $1734.5 \pm 390.41\mu\text{g}/\text{dl}$.

The lead level in maternal blood sample $13.5 \pm 7.14\mu\text{g}/\text{dl}$, cord blood $8.5 \pm 2.85\mu\text{g}/\text{dl}$ and placental mean lead levels were $417.6 \pm 359.31\text{ng}/\text{g}$. The mean lead levels in neonates were $7.9 \pm 7.30\mu\text{g}/\text{dl}$

There was a significant correlation between blood lead levels, ALAD activity and serum iron

levels. The haemoglobin and copper levels were also correlated significantly whereas there was negative trends in Zn, Cu, Mg with elevated blood lead levels.

OBSERVATIONS

1. In 57% of the subjects the blood lead levels were elevated as per the recommendations of CDC and WHO. The blood lead levels in 67% among neonates was found $>5\mu\text{g}/\text{dl}$.
2. Interaction of ALAD with Pb and presence of hypochromic cells indicate deranged red cell metabolism.
3. Lead above $10\mu\text{g}/\text{dl}$ correlated with reduced haemoglobin.
4. Placental lead levels were elevated in urban women samples in addition to the significant

correlation between reduced cord blood levels and placental levels.

CONCLUSIONS

The results suggest that 70% of the screened population had lead level above $10\mu\text{g}/\text{dl}$. The hemoglobin was inversely correlated with blood lead levels of $15\mu\text{g}/\text{dl}$. The serum iron levels were found to be high with blood lead levels. The correlation between zinc, Iron and lead levels indicates the interaction of nutrients and pollutants.

The preliminary findings warrant study for establishing the possible interactions between nutrients, pollutants and nutritional status in a large group of population.

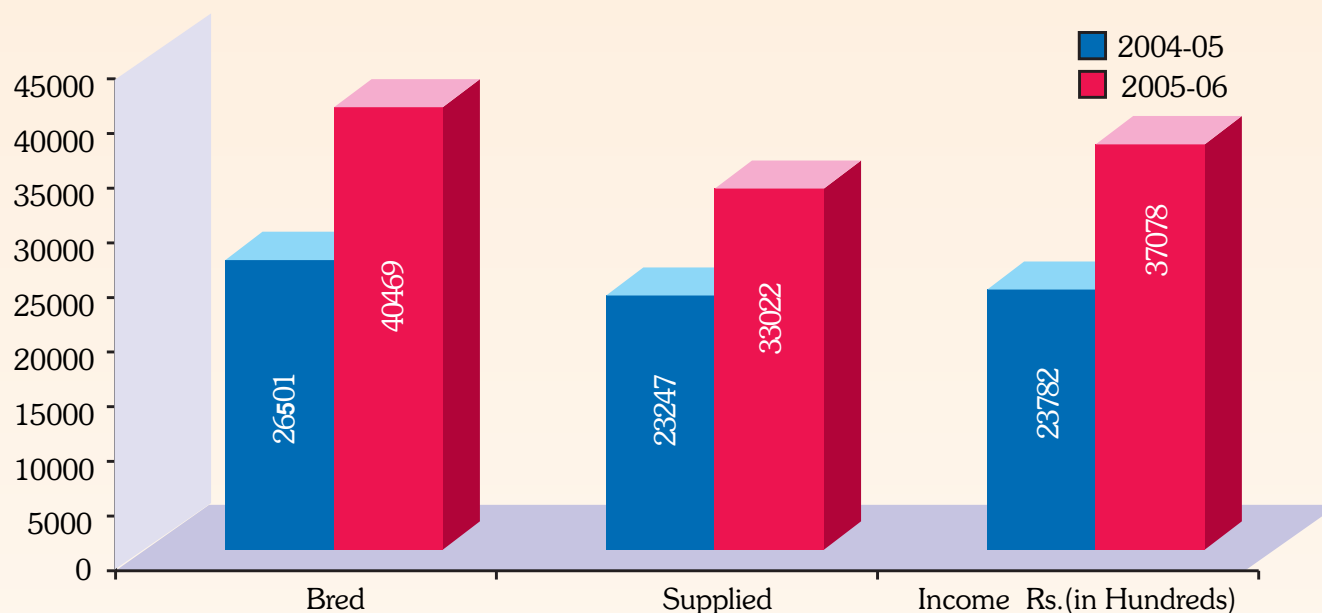
VII. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

A. SERVICE ACTIVITIES

1. Breeding and Supply of Animals

(Figure 33)

Fig. 33 Animals Bred, Supplied and Income-generated



During the year, 40,469 animals of various species and strains were bred, out of which, 33,022 were supplied to government and private commercial institutions. The number of animals bred and supplied were 52% and 42% more than the previous year. The income generated by sale of animals Rs.37.1 lakhs was also 55.9% more than the income generated during the previous year. The details of individual animals bred and supplied are shown in Table 32 & 33. The number of animals died or disposed is 3.45 and 6.15% in the conventional colonies and 2.2 and 2.9% in the barrier maintained colony and are well within the suggested percentage.

Since the SAC (2005) recommended that animals should be kept for life span studies, it has been initiated in the rat strains maintained at the center. The details of the animals kept for life span studies is presented in table 31.

Table 31. Life span study details

Species/ Strain	Date of birth	No. of Animals ♀ + ♂	No. of animals surviving (as on 31-3-2006) ♀ + ♂
Rat/WNIN	9.12.03 10.12.03	24 + 26	8 + 12
Rat/ Fisher 344	23.4.04 24.9.04	4 + 4 8 + 8	4 + 4 8 + 8
Rat/ Wkyoto	2-11-04	12 + 12	6 + 10
Rat/ Holtzman	4-11-04	12 + 12	11 + 2
Rat/CFY	8-11-04	12 + 12	9 + 8

The details of any external abnormalities appearing in the animals like loss of hair, tumor etc. is being recorded. Other species of animals also will be kept for life span studies.

Table. 32. Details of breeding and supply of different species and strains of laboratory animals (Barrier maintained colony) during the period from 01.4.2005 to 31.3.2006.

Sl. No.	Species	Strain or Breed	Stock As on	Total Number of animals							Balance as on 31.3.06	
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disp		
			1.4.2005									
1	Mouse	BALB/c An. N (inbred)	417	5846	6263	6	5637	5643	--	620		
		C57BL/6J (inbred)	394	3753	4147	108	3314	3422	62	172	491	
		N:NIH(S) Nude (athymic) (inbred)	299	832	1131	128	656	784	93	75	169	
2	Rat	Wistar/NIN (inbred)	686	4423	5109	83	3655	3738	79	37	1255	
		SD (Sprague Dawley) (Outbred)	315	2200	2515	176	1524	1700	124	102	591	
		Fischer 344 N (inbred)	416	153	569	10	126	136	30	238	165	
3	G. Pig	N:HART (Hartley)	198	1267	1465	-	1212	1212	63	3	207	
		Dunkin (Hartley)	--	--	--	-	-	-	-	-	--	
		N:NIH (Coloured)	83	696	779	-	651	651	23	7	105	
4	Rabbit	New zealand white	34	224	258	53	52	105	7	1	145	
	TOTAL		2842	19394	22238	564	16827	17391	481 (2.2%)	635 (2.9%)	3723	
Percentage of animals supplied to other Institutions: 75.7%											NIN : 2.5%	

Table 33 Details of breeding and supply of different species and strains of laboratory animals
(Conventional colony) during the period from 1.4.2005 to 31.3.2006

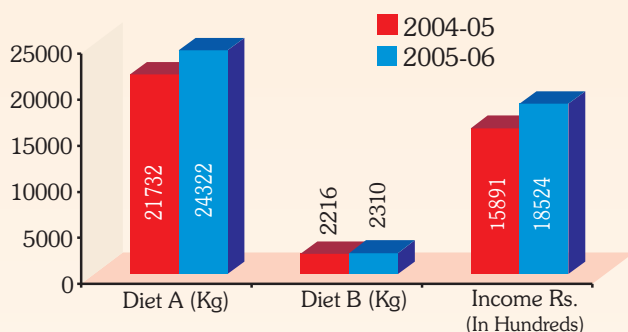
Sl. No	Species	Strain or Breed	Stock as on	Total Number of animals							Balance as on	
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disp. Old age Sick		
1	Mouse	Swiss (inbred)	511	9968	10479	150	7085	7235	175	230	-	2839
		WNIN (inbred)	1204	8431	9635	124	7400	7524	156	70	-	1885
2	Rat	WNIN/Ob-Ob (inbred)	389	895	1284	49	-	49	129	280	-	826
		WNIN/GR-Ob	610	504	1114	121	-	121	140	274	-	579
		Wkyoto (inbred)	184	233	417	-	8	8	33	282	-	94
		CFY/NIN (inbred)	247	150	397	-	-	-	42	256	-	99
3	Hamster	Holtzman (inbred)	104	142	246	-	-	-	44	119	-	83
		Wild White	28	-	28	-	-	-	26	-	-	2
		Golden (inbred)	289	752	1041	-	687	687	106	7	-	241
4	Monkey	Macaca mulatta (Rhesus)	24	-	24	-	-	-	-	-	-	24
5	Sheep		1	-	1	-	-	-	-	-	-	1
6	Rabbit/N.W		13	-	13	7	-	7	-	-	-	6
		TOTAL	3604	21075	24679	451	15180	15631	851 (3.45%)	1518 (6.15%)	-	6679
Percentage of animals supplied to other Institutions: 61.5 %											NIN: 1.8 %	

2. Supply of animal feed

A. Stock animal feed

During the period 24322 kg of rat and 2,310 kg of G.pig feed were supplied to other institutes generating an income of Rs.18.4 lakhs. While the supplies increased by 11.9% for rat/mice feed and 4.2% in G.pig/rabbit feed, the income generated increased by 15.9% (Table 34 and Figure 34).

Fig. 34 Feed Supplied and Income-generated



3. Supply of Blood & Blood products

During the period 280 ml of blood, 212 ml of plasma and 165 ml of sera were supplied to 12 different institutions on 50 occasions. The income generated from this activity was Rs. 30,580/-. Another 270 ml. was used intra institutionally.

4. Health Monitoring Report

During the year 737 samples from animal colonies, feed, water, bedding and equipments were screened bacteriologically. (Rats WNIN 63, SD 53, Mice Balb/C 44, C57BL/6J -94, Nude hetero 60, FVB 65, Rabbits 120, G.pigs 120, Rats WNIN 08 (OAH), Swiss mouse 21, Hamster 01; Nude mice 19, Water 12, Diet 12, Bedding 12 and other experiment 33). Another 115 sick animals (WNIN rats -31, SD rats -39, Rabbits -05, G. pigs -17 and Hamster -01, Nude mice -22 were autopsied and samples taken for microbiology. They have been examined for organ abnormalities. The salient observation were:

- Irrespective of whether the animals were from conventional or barrier maintained colony they all showed the presence of E. Coli, Klesbsiella spp., Bacillus spp., Streptococcus spp, Staphylococcus spp., Listeria monocytogenes, Proteus spp., A.calco.anitrat, Corynebacterium, Serratia spp. Kl. Oxytoca and Citrobacter freundai.
- Old aged rats (WNIN and SD) were found to have liver cysts.
- Both C57BL/6J and BALB/c mice showed the presence of mites (ectoparasites). However, the density of infestation was less when compared to previous year and did not result in loss of hair or erythma which were observed in earlier years.

Table 34. The following feed were supplied to institutions

No	To whom supplied	Type of diet	Qty (kgs)
1	National Brain Research Centre	Iron deficient Low protein High protein	100 100 120
2	Osmania University (Zoology Dept.)	Sucrose diet	5
3	TN Nair Hospital Centre for Ayurveda research	Fructose diet	12
4	Reliance Life Sciences Ltd.	High fat diet	67
5	Indian Institute of Chemical Technology	High cholesterol diet	50
6	Srikrishnadevaraya University, Anantapur	Fructose diet starch based diet	26 26
7	Bharat Biotech Ltd.	6%, 14% & 40% protein diet for rabbits	25 kgs each
8	Dr.Reddy Biologicals	High fat, high cholesterol diet	10

All personnel working with the animals upto the level of supervisors were screened in a super speciality hospital. None of the staff showed any clinical signs attributable to the nature of work.

Screening of Animals for PCT:

Hundred rabbits were procured for PCT of Genpep I and Genpep II. All the animals were quarantined and subjected to health monitoring. First batch of 42 animals were handed over for subchronic studies.

Human Resource Development

The following training programmes were conducted:

- i. 38th Annual Laboratory Animal Technicians' Course in which 11 candidates participated.
- ii. 26th Laboratory Animal Supervisor's Training Course in which 4 participants were awarded certificates.
- iii. Extended the 'Animal care' training to students of Diploma in Medical Laboratory Technology.
- iv. Four students undertook project work in the animal physiology laboratory of NCLAS.

6. Extension of services

During the period the Centre extended help for conducting 6 IAEC approved regular experiments of the Institute. It also extended support to Preclinical Toxicology Centre to complete the studies on WHO sponsored studies on ayurvedic drugs and to initiate studies on Genpep 1 & 2 sponsored by ISSAR pharmaceuticals.

B. RESEARCH ACTIVITIES

1. PCR-based DNA fingerprinting of WNIN strain and its obese mutants

Two mutant obese rat strains, WNIN/Ob and WNIN/GR-Ob were developed from the existing WNIN rat colony, which is being maintained at NCLAS in an inbred status for the past 84 years. Both the mutants are obese, but WNIN/GR-Ob has impaired glucose tolerance additionally. The present project was undertaken to establish genetic identity for these two obese mutant rats.

METHODOLOGY

It was decided to make DNA fingerprint profile for the obese mutants using the RAPD

(Randomly amplified polymorphic DNA) approach using random primers. Three standard rat strains WNIN, WKY and F-344 were used as controls. The two phenotypes of the mutants, lean (+/+) and carrier (+/-) were also included for comparison. Though no unique DNA fingerprint for WNIN/Ob strain was observed a unique sequence in WNIN/GR-Ob, which showed homology with chromosome no.3 and chromosome no.8 and partially to chromosome X was identified. Attempts were made to confirm whether the cloned products were expressed in the rat genome.

1. RNA was isolated from brain, liver, spleen, kidney and adipose tissue of WNIN/GR-Ob and WNIN adult rat using Trizol reagent and the purity was ascertained on agarose gel and also by taking the ratio of A260/A280 nm.
2. 15-20 g of sample from parental strain and WNIN/GR-Ob were subjected to northern blotting using formaldehyde agarose gel. The samples were transferred to nylon membrane by vacuum transfer method and fixed on the membrane by UV cross-linking.
3. The 390 bp cloned insert was labeled using random primer labeling method and used as a probe.

RESULTS

The cloned insert when used as a probe, hybridized to both the control as well as WNIN/GR-Ob tissues (Figure 35).

Fig. 35. DNA finger printing of WNIN/ob mutant rat



Lane 1-6 WNIN rat samples,
B: brain, H; heart, L;lung, K; kidney L; liver, S; spleen,
Lanes 7-11, WNIN/GR-Ob samples A; Adipose tissue,
B; brain, K;kidney, L; Liver, S; spleen

CONCLUSIONS

The cloned product from WNIN/GR-Ob were expressed both in mutant and parental strain and thus not unique to the mutant.

2. Studies on energy metabolism in WNIN obese rat mutants

The basic mechanism leading to obesity is accretion of excess energy either due to increased feed consumption or decreased energy expenditure. WNIN Ob/Ob rats have already been shown to have higher feed consumption and higher feed efficiency ratio. They have also been shown to be lethargic. All the above observations are the outcome of both physiological and biochemical changes, probably genetically transmitted, occurring in them. So it was decided to investigate both carbohydrate and fat metabolism which are responsible for the accretion of body fat.

Earlier studies were done at the age of 90-110 days old animals and the activity levels of key regulatory enzymes involved in glycolytic, gluconeogenic and lipogenic pathways in liver tissue viz. Glyceraldehyde 3 Phosphate Dehydrogenase (GAPDH), Glycerol 3 Phosphate Dehydrogenase (GPDH), Glucose 6 Phosphate Dehydrogenase (G6PDH), 6 PhosphoGluconate Dehydrogenase (6PGDH), Malic enzyme (ME), Citrate synthase (CS), Fatty Acid Synthase (FAS), Pyruvate Kinase (PK), were measured.

These observations showed that except for GPDH and CS all other enzyme activities were elevated in WNIN Ob/Ob animals when compared to their lean or carrier animals.

During the year the activities were determined in both Ob/Ob and GR/Ob mutant rats at 35 days age as well as their lean and carrier animals. Studies on mitochondrial respiration, membrane fluidity were also undertaken.

METHODOLOGY

Colony animals were housed and maintained individually in cages with a daily 12 h light/dark

cycle at a temp. of 23° C and 60% humidity. When the animals were of 35 days age 6 animals from each group were taken and they were kept for an overnight fast. The animals were sacrificed on the next day. Livers were collected as quickly as possible wrapped in aluminium foil and plunged into liquid nitrogen. The tissues were then stored at -40° C till they were used for further study.

Enzyme assays

Parts of the liver were allowed to thaw as slowly as possible on ice. Tissues were homogenized with a loose fitting Teflon pestle and glass homogenizer in 9 vol. Of ice cold homogenizing buffer (250mM sucrose, 50 mM Tris, 1mM EDTA, 1mM DTT pH 7.4) for the assay of enzymes GAPDH, GPDH, G6PDH, 6PGDH and ME.

Oxidative Phosphorylation

Mitochondria was isolated by standard techniques. Measurements of oxidative phosphorylation were carried out at 25° C with a Clark type oxygen electrode. The assay system contained in a total volume of 1.6 ml 225mM sucrose, 5mM Tris-HCl buffer pH 7.4, 20mM KCl, 0.2mM EDTA, 150 g BSA/ml. Glutamate (10mM), Pyruvate (10 mM) + Malate (1mM) Succinate (10mM) and Ascorbate (10mM) + TMPD (0.1mM) were used as substrates. With Succinate and Ascorbate + TMPD, 1.0 um rotenone was also included in the reaction medium. The state 3 respiration rates (initiated by the addition of 200 nmol of ADP in 15-20 l) and state 4 respiratory rates (after the depletion of added ADP) were recorded. ADP/O ratio and the ADP phosphorylation rates were then calculated.

RESULTS

Enzyme studies

All the enzymes screened GAPDH, PK, G6PDH, PGDH, PFK, ACL, FAS showed significantly increased activities in both Ob/Ob and GR/Ob rats in comparison to their respective lean and carrier counterparts. But between lean and carrier of both the mutants the activities were not different. Malic Enzyme (ME) were higher in Gr-Ob rats in comparison to their lean and carrier counterparts. But in WNIN / Ob strain there was no change in the activity (Tables 35 & 36). Ob/Ob did not show statistically significant differences in the activity of GPDH when compared to lean and carriers.

However, the increase in activity was found in GR/Ob when compared to lean and carriers. The pattern was very similar to what was reported earlier in 90-110 day old rats.

Mitochondrial Studies

Oxidative phosphorylation

The pilot study conducted with 35 day old animal liver mitochondria has given good oxygen response with substrates, glutamate, succinate and ascorbate + TMPD in both Ob/Ob as well as Gr/Ob strains. But the Ob/Ob and Gr/Ob group of rats failed to show any response or extended response

was observed with the substrate pyruvate + malate which otherwise has given a very good response with their respective lean and carrier liver mitochondria. Further investigation using Pyruvate + malate has to be done to draw any conclusions.

Membrane fluidity

Mitochondrial membranes were more fluidized in both the Ob/Ob and Gr/Ob rats in comparison to their respective lean and carrier counterparts. There is no difference observed between lean and carrier (Table 37).

Table 35: Activity of enzymes in 35 days WNIN/Ob rats

Name of the enzyme	Lean	Carrier	Obese
Fatty Acid Synthase	17.0±1.50	14.8±1.40	28.7± 2.96***
Glucose Phosphate 6 Dehydrogenase	35.0±5.70	36.0± 4.98	82.6± 6.30***
6 Phosphogluconate	88.8±17.80	71.1±10.50	127.5± 5.70*
Glyderaldehyde 3 Phosphate Dehydrogenase	914±48.4	1104.±100.7	1944± 90.0***
Pyruvate Kinase	733± 17.2	783± 76.1	1110± 31.9
Glycerol phosphate 3 dehydrogenase	507± 26.1	407± 27.0	536± 32.9*
Malic enzyme	15.3±2.85	15.3± 2.77	15.2± 0.58 NS
ATP Citrate Lyase	20.3±2.42	22.2± 3.00	61.0± 11.07**

Activity of GAPDH and GPDH is expressed as μ moles of NADH getting converted to NAD/min/mg protein.
 Activity of FAS is expressed as nmoles of NADPH getting converted to NADP/ min/mg protein
 Activity for G6PDH,6 PGDH, ME is expressed as nmoles of NADP getting converted to NADPH/ min/mg protein
 Activity of PK is expressed as nmoles of NADH getting converted to NADPH/ min/mg protein.

Table 36: Activity of enzymes of 35 days old WNIN/GR-Ob rats

Name of the enzyme	GR-Lean	GR-Carrier	GR-Obese
Fatty Acid Synthase	16.5± 0.86	14.2± 2.20	29.1± 1.93**
Pyruvate kinase	680± 96.0	493± 105.5	1052.67± 75.4**
ATP Citrate Lyase	24.8± 1.98	15.6± 3.11	44.2±1.98***
Glyderaldehyde 3 Phosphate Dehydrogenase	1200± 137.2	1656± 234.9	2181± 148.9**
Pyruvate Kinase	680± 96.0	493± 105.6	1052± 75.4**
Glycerol phosphate 3 dehydrogenase	533 ± 43.4	466± 26.6	695± 25.5**
Malic enzyme	5.80 ± 1.040	7.34± 1.026	11.69±0.820

Activity of GAPDH and GPDH is expressed as μ moles of NADH getting converted to NAD/min/mg protein.
 Activity of FAS is expressed as nmoles of NADPH getting converted to NADP/ min/mg protein
 Activity of PK is expressed as nmoles of NADH getting converted to NADPH/ min/mg protein.
 *p values <0.05, ** pvalues<0.01, *** <0.001 NS- Not significant, Mean ± S>E (n=6)

Table 37 Liver mitochondrial membrane fluidity in 35 day old WNIN/Ob and WNIN-GR-Ob animals

Name of the group	Fluorescence Polarization p	Fluorescence Anisotropy r	Limited Hindered Anisotropy r α	Other parameters
WNIN Lean	0.21 \pm 0.005	0.15 \pm 0.002	0.10 \pm 0.002	0.50 \pm 0.007
Carrier	0.21 \pm 0.003	0.15 \pm 0.002	0.10 \pm 0.003	0.50 \pm 0.009
Obese	0.19 \pm 0.002	0.14 \pm 0.001	0.08 \pm 0.002	0.46 \pm 0.006
WNIN GR Lean	0.20 \pm 0.007	0.15 \pm 0.005	0.10 \pm 0.007	0.49 \pm 0.001
GR Carrier	0.20 \pm 0.002	0.15 \pm 0.001	0.09 \pm 0.002	0.49 \pm 0.001
GR Obese	0.18 \pm 0.003	0.13 \pm 0.002	0.08 \pm 0.003	0.44 \pm 0.001

3. Genetic typing of WNIN/Ob and WNIN/ GR - Ob strains using microsatellite markers

WNIN is an oldest rat strain bred at NIN in inbred status. Two genetically inherited abnormal phenotypes were observed in this strain, one with Obese phenotype (Ob/Ob) and the other with Impaired Glucose Tolerance (IGT). A novel phenotype was developed by crossing the Obese phenotype with IGT rat, which has both Obese and IGT. The two phenotypes identified as WNIN/Ob(Obese) and WNIN/GR-Ob(Obese and IGT) are now maintained as individual strains. The occurrence of above two phenotypes might have been the result of the evolutionary forces i.e, genetic drift/mutation/migration.

The main aim of the study was to determine the evolutionary forces, which might have influenced the development of these abnormal phenotypes.

Microsatellite DNA are 2-4 bp tandem repeats. They may exist as multiple alleles in the population with regard to difference in the number of repeats, hence polymorphic and may also reveal codominance. They are stable over considerable number of generations. They are relatively evenly spread through out the genome. Most of these markers have been mapped on to the genome, defining their location. Polymorphism at a microsatellite DNA locus (within individual or between individuals) may be, mainly due to genetic drift or mutation.

A whole genome scan was planned using upto 100 microsatellite DNA markers in the 5 rat strains (WNIN, WKY, F-344, WNIN/Ob and WNIN/GR-Ob) spanning all the 20 autosomes and X chromosome. A total of 70 markers were screened till the last year, for which a dendrogram was presented in the previous year (Annual Report 2005) showing the relationship among the rat strains used in the study with a report on identification of polymorphic markers which showed inter-strain variation, that could be used for the identification of the individual strains.

OBJECTIVES

1. Genetic typing of WNIN/Ob and WNIN/GR-Ob mutants along with WNIN (parental strain), WKY (related strain), Fischer-344 (unrelated strain) as controls using approximately 100 microsatellite markers, covering all the rat chromosomes with an average of 3-4 markers per chromosome.
2. To perform statistical analysis for studies on genetic variation from the profiles obtained above.
3. Unique DNA markers if identified for the strains, will be cloned and sequenced for using them as specific markers for the strain

Objective 1

Thirty three Microsatellite DNA markers were attempted to screen the 5 rat populations so as to complete the first objective as proposed.

METHODOLOGY

The markers selected for the genome scan were reported as polymorphic among 8 rat strains used in the studies of Serikawa et al, 1992. Some of the markers were obtained from Rat Genome Database

DNA Isolation

DNA was isolated from 6 male rats of each rat strain by the standard protocol, (Sambrook et al. 1989).

PCR and Gel Electrophoresis

PCR conditions

PCR was carried out in a tube type apparatus and the reaction volume was 20ul containing 100ng-1ug of genomic DNA, 125uM each of dNTP's, and 1unit of taq polymerase. (Conditions are as described by Serikawa et al. 1992).

Gel Electrophoresis

The PCR amplified products were resolved on 12% PAGE. Two PAGE gels were run for each marker, one comparing the three standard strains (6

animals in each strain) and one comparing the mutant strains with the standard strains (6 animals each of the two mutant strains and 2 animals each of the three standard strain).

RESULTS

Out of 33 markers, only 17 markers showed amplification in all the 5 populations. Five microsatellite DNA markers were identified as polymorphic. The polymorphic status of these markers in different strains used in the study are reported in the following table (Table 38).

Objective 2

Pooling of all the data, and analysis is in progress and will be completed shortly.

Objective 3

Attempts were made to clone the three unique alleles identified by the primer pair Leukosianin, which could differentiate the three strains, WNIN, WKY, F-344, and which could also show difference between the two mutants, WNIN / Ob and WNIN/GR-Ob. Cloning Experiment was not successful.

Table 38. The polymorphic status of these markers in different strains used in the study

No	Name	Chr. No.	Expected size in bp	Allele size in bp observed for the expected size				
				WNIN	WKY	F-344	Ob/Ob	GR-Ob
1	R35	2	174	140/161	135/152	129/148	135/158 140/161	135/152 140/161
2	R10	10	158	159/188	159/188	159/188 169/200	159/188	159/188
3	D9Bro1	9	166	119/108 125/141	119/108	119/108	119/108 125/141	119/108
4	D16Mgh5	16	148	152/174	160/185	141/160/174	152/174	152/174
5	R15	4	116	116/149	116/149 120/152	120/152	120/152 129/163	120/152 129/163

Summary of the table: Of the five markers, which showed polymorphism, two markers R35, and D16Mgh5 showed inter-strain variation.

R35 & D16Mgh5 could differentiate the three standard strains.

R35 & r15 showed intra-strain variation in the mutant strains.

R10 showed intra-strain variation in F-344

R15 showed intra-strain variation in WKY

D9Bro1 showed intra-strain variation in WNIN

4. Establishment of baseline values of body composition and blood pressure in different species of laboratory animals maintained at NCLAS, NIN - A study in rat strains

National Centre for Laboratory Animal Sciences (NCLAS) is maintaining different species of laboratory animals for biomedical research both for in house use as well as supply to other institutions. As the center is catering to the needs of several institutions including for pre-clinical toxicology testing, it has become necessary to establish normal physiological and biochemical values in the most commonly used strains of laboratory animals. Since rat strains are the most frequently used animals, initial studies were taken up in different strains maintained at the centre viz., Wistar/NIN (WNIN), Sprague Dawely (SD), Fischer-344N (F-344N), Wistar Kyoto (WKY), CFY and Holtzman.

METHODOLOGY

Animals: Weanling rats (12 Male and 12 female) of each WNIN, SD, F-344N, WKY, Holtzman and CFY strains were used for the study. They were housed under standard experimental conditions.

Growth: The animals (both genders) were weighed weekly using an electronic balance (Sartories, 0.1 gm sensitivity) from 21 to 105 days and subsequently every 50 days till 250 days.

Body composition: Body composition of the animals was determined using TOBEC small animal body composition analysis system (EM-SCAN, SA-3000 multi detector model), which measures total body electrical conductivity (E) to arrive at the composition. Rats at different ages (50,100, 150,200 and 250 days) were analyzed for progressive changes in lean body mass (LBM) and fat content of the body.

Spontaneous Loco motor Activity: The detailed activity patterns, which include distance traveled (DT), resting time (RT), stereotypic time (ST), ambulatory time (AT) were quantified in 200 day old animals using a Columbus activity cage. The

activity measurement was made for a period of 4 hours each during "Light" and "Dark" hours of the day (09.00-13.00 hours, 18.00-22.00 hours).

Clinical chemistry: Blood was collected from the animals at 200 days of age following 24 hours fast; plasma was separated from blood and stored at -20°C until analyzed. Glucose, total protein, albumin, urea, creatinine, calcium, phosphorus, total bilirubin, SGOT, SGPT, gama glutamyl transpeptidase, alkaline phosphatase, cholesterol, HDL cholesterol and triglycerides were measured using Schiapparelli biosystems inc. auto analyzer.

Blood pressure (BP): Animals were restrained in the different holders according to their size and body weight and the cuff attached to the tail, which is connected to BP sensor amplifier. Systolic, diastolic, mean BP and heart rate were measured directly from the instrument (IITC).

RESULTS

Growth: Male rats of all the strains had significantly higher body weights than females (Figures 36 & 37).

Fig. 36 .Grwoth in male rats (n=12)

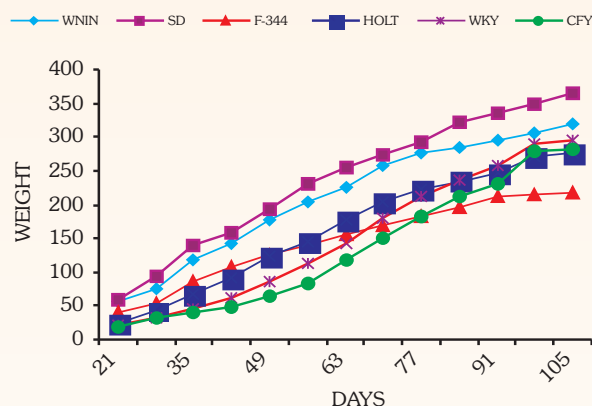
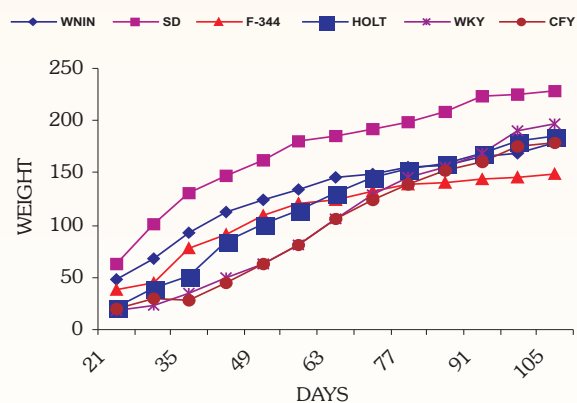


Fig. 37. Growth in female rats



Among the strains both genders of SD rats had significantly higher body weights, followed by both genders of WNIN, Holtzman, WKY, CFY and F-344N rats in that order. F-344N rats male and female initially showed higher body weights compared to Holtzman, WKY, CFY rats and reached a plateau from 11 weeks of age. Till 9 weeks of age both genders of Holtzman rats showed significantly high body weights compared to WKY and CFY rats. Subsequently both WKY males and females showed a significant increase in their body weight gains and by 14 weeks males and by 12 weeks females, caught up with Holtzman strain. ($P < 0.001$).

Body composition: All the strains i.e., WNIN, SD, F-344N, WKY, Holtzman and CFY rats showed progressively higher body weights and LBM with increase in age and males showed more LBM than females (Figures 38 & 39).

Among the strains WKY rats had significantly higher LBM, fat free mass (FFM). Though SD rats had higher body weight than WKY rats, LBM and FFM found to be more in WKY rats followed by SD, Holtzman, WNIN, CFY and F-344N in that order. Similar results were observed in females. Derived parameters like fat, fat%, total body water (TBW), total body potassium (TBK) and total body sodium (TBNa) were also significantly different between the strains and also between sexes ($P < 0.001$). Though LBM and FFM were found to be more in WKY rats, the total body fat content is higher for SD male rats and WNIN, WKY, CFY, F-344N and Holtzman male rats followed them. In contrast to this percentage fat was found to be more for WNIN male rats and followed by SD, F-344N, WKY, CFY and Holtzman. But in females no significant differences were seen in the percentage fat, between WNIN, WKY, SD, F-344N (Figures 40 & 41).

Fig.38. LBM and FFM in male rats(n=12)

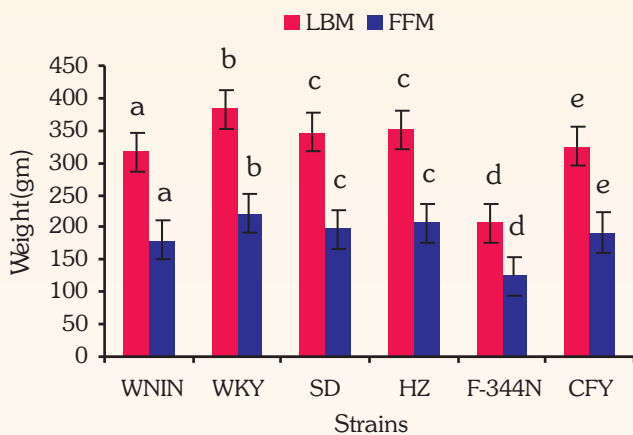


Fig.39. LBM and FFM in female rats(n=12)

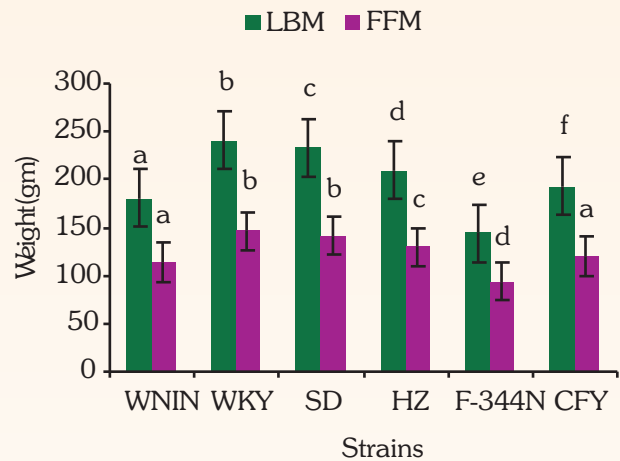


Fig.40. Fat and Fat% in male rats (n=12)

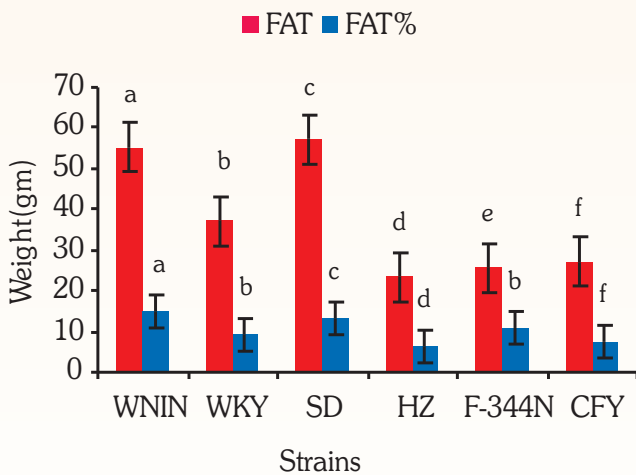
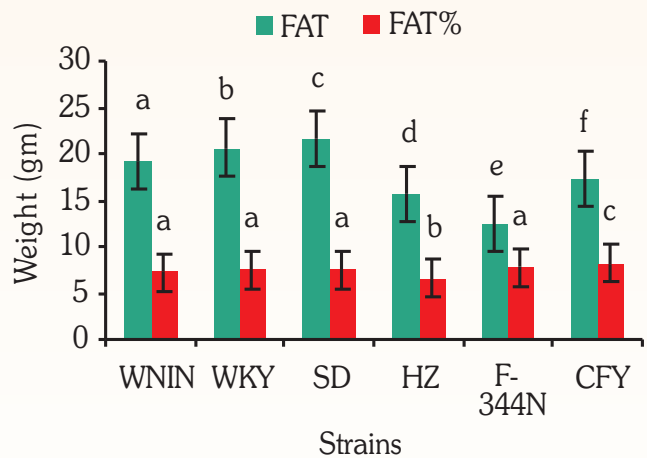
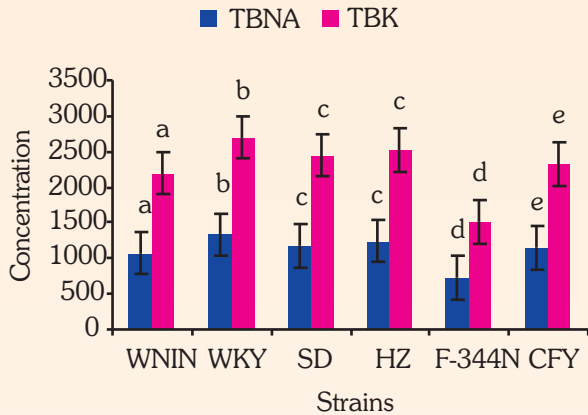


Fig.41. Fat and Fat% in females (n=12)



TBW levels were found to be more in SD rats followed by WKY, Holtzman, CFY, WNIN and F-344N. Between the genders males had significantly higher levels than females (Figure 42).

Fig. 42. TBNa and TBK in male rats (n=12)



Total body sodium, total body potassium was highest in SD rats followed by CFY, F-344N, WKY and WNIN rats in that order (Figures 43 & 44).

Fig. 43. TBNa and TBK in female rats (n=12)

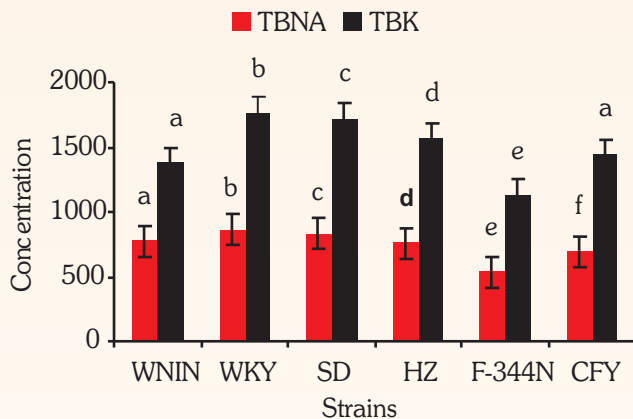
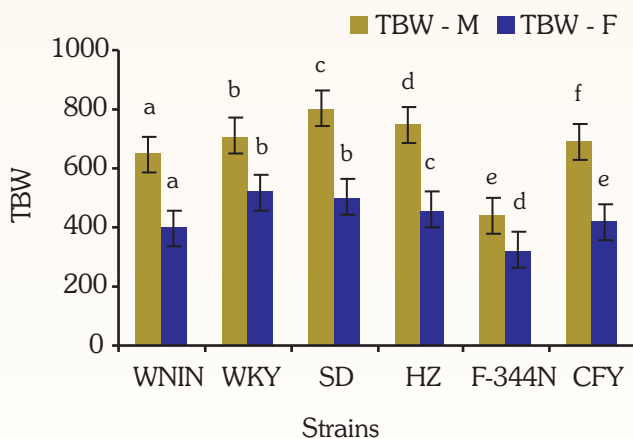


Fig. 44. Total body weight in male and female rats (n=12)



Activity: All strain of rats irrespective of sex showed significantly higher distance traveled (DT) during night time when compared to daytime. Among the strains, the total distance traveled during nighttime is high for females compared to males. In males the nighttime DT was high for SD rats followed by Holtzman, F-344N, WNIN, CFY, WKY rats. In females CFY rats traveled more distance followed by Holtzman, WKY, SD, F-344N and WNIN rats. Resting time (RT) was significantly higher during day compared to nighttime in all the strains and there were significant differences between the sexes. Male rats showed maximum RT and they were higher for WKY and followed by WNIN, SD, Holtzman, F-344N and CFY strains (Figures 45 & 46).

Fig. 45. Daytime activity in males (n=6)

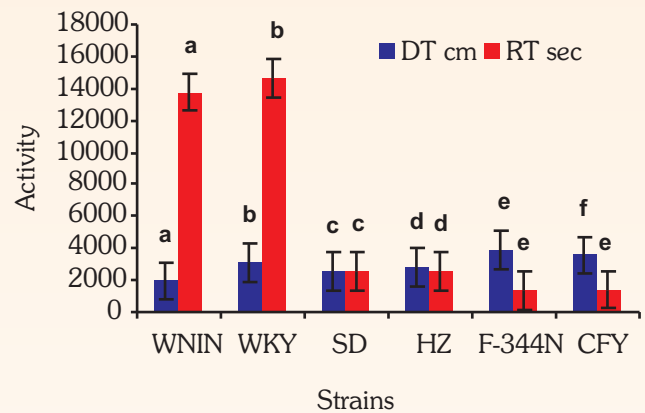
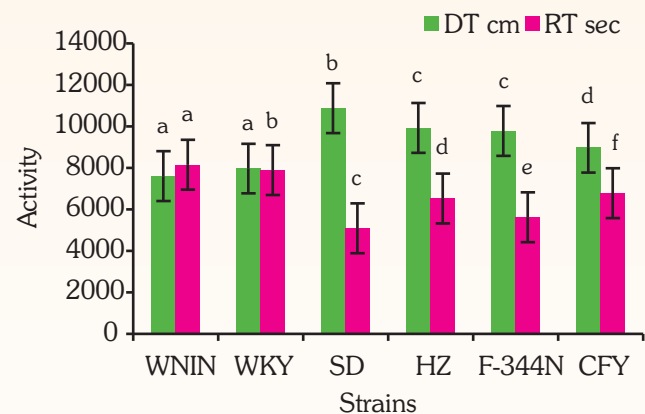


Fig. 46. Nighttime activity in males (n=6)



In females during daytime significant RT was found for WKY and WNIN rats. During nighttime significant differences in RT was found between WNIN, WKY, Holtzman and CFY. However, no significant differences were seen in remaining three strains. Mean ambulatory time was high in F-344N,

CFY strains compared to WKY, WNIN, SD and Holtzman (Figures 47 & 48). Stereotypic activity was higher in Holtzman strains followed by WKY, WNIN, SD, F-344N and CFY strains. The total activity observed among the strains revealed that WNIN rats had highest resting time followed by WKY, CFY, SD, Holtzman and F-344N strains.

Fig. 47. Daytime activity in females(n=6)

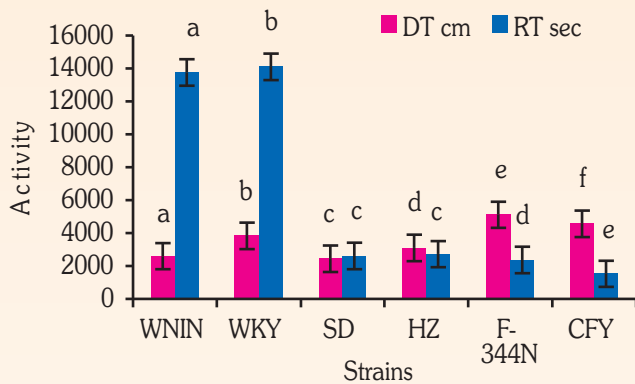
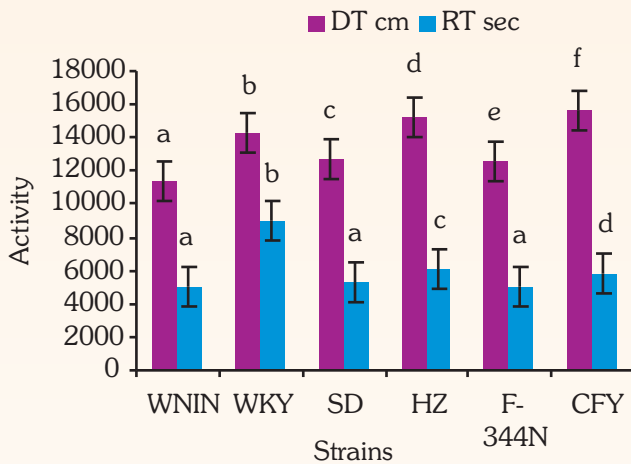


Fig. 48. Nighttime activity in females(n=6)



Clinical chemistry: Glucose levels were significantly higher in SD and Holtzman strains followed by CFY, F-344N, WKY and WNIN rats. Significant differences were seen between the sexes in SD and Holtzman strain and similar differences were not observed in remaining strains. Total protein, albumin and urea levels were higher in CFY and WKY strains, and followed by Holtzman, SD, WNIN and F-344N rats ($P < 0.01$). Plasma triglyceride, total cholesterol and HDL cholesterol values were high in WNIN and WKY in male rats (Figures 49 & 50). No significant changes were found in lipid profile of SD, Holtzman and CFY rats.

Contrast to the F-344N rats had significantly low levels of triglycerides, total cholesterol and HDL cholesterol. There were no significant differences in creatinine, GGT, calcium, phosphorus, SGOT and SGPT levels.

Blood Pressure: Systolic and diastolic blood pressure levels were significantly high in males compared to females (Figures 51 & 52).

Fig. 49. Lipids in male rats (n=12)

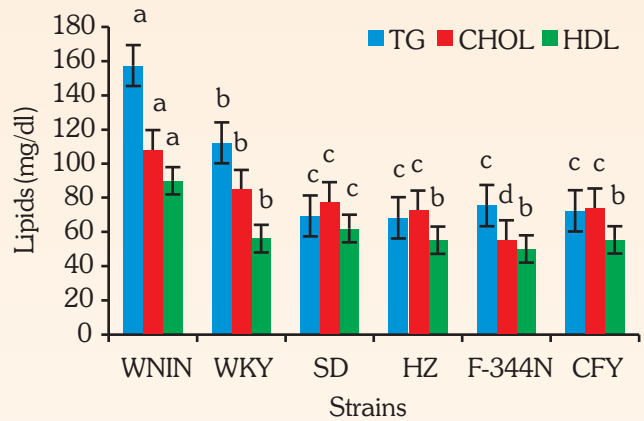
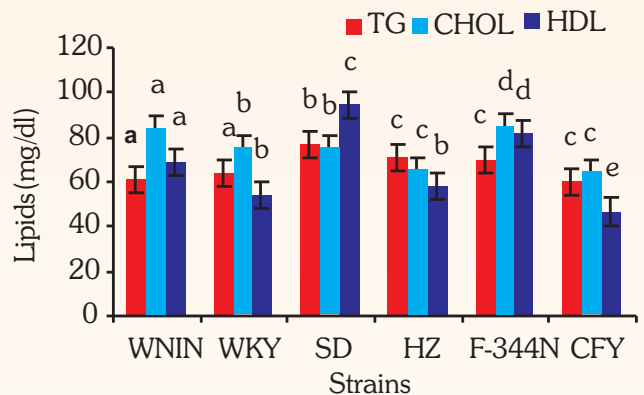


Fig. 50. Lipids in females (n=12)



Among the strains CFY males had significantly high systolic pressure compared to other strains. There was no significant difference between systolic pressure of WNIN, WKY and F-344N rats, though they were high compared to SD and Holtzman strains. Diastolic pressure is high for CFY followed by WNIN, WKY, F-344N, Holtzman and SD rats in that order.

In females, systolic and diastolic pressures are high in Holtzman followed by WNIN, F-344N, CFY, WKY and SD rats. Among the rat strains heart rate was significantly high in WNIN rats of both genders

compared to other strains (Figure 53). The remaining rats strains WKY, CFY, Holtzman, F-344N and SD rats followed in that order.

Fig. 51. Blood pressure in male rats (n=6)

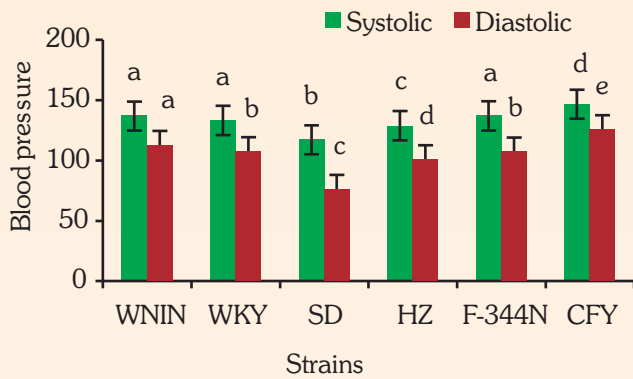


Fig. 52. Blood pressure in female rats (n=6)

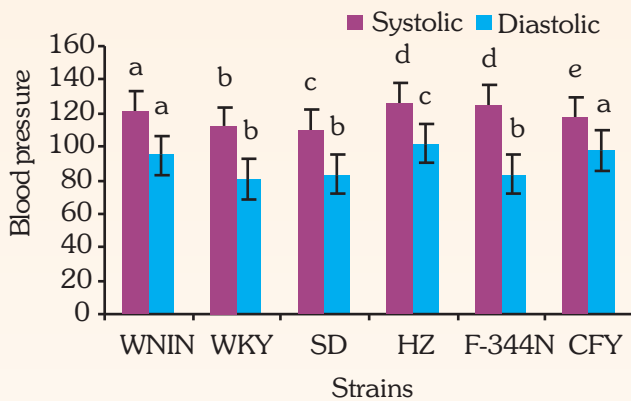
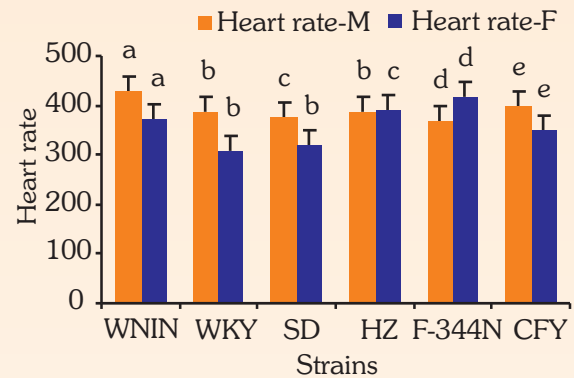


Fig. 53. Heart rate in male and female rats (n=12)



CONCLUSIONS

The study showed that there are significant differences between strains of rats in terms of body composition, physical activity, serum clinical chemistry and blood pressure. By virtue of higher body weight for age in SD rats, their total body fat was also significantly higher than other strain of rats. However, it is WNIN male rats, which had higher percentage of body fat, higher resting time, higher plasma tryglycerides, higher heart rate when compared to other strain of rats. This was followed by Wistar Kyoto strain. The Fischer - 344N rats showed the least growth rate, higher nighttime activity. These studies show that there are differences between strains and between genders in the same strain. Thus this data will be useful to research workers in selecting strain and sex of animals for experimentation.

VIII. PRE-CLINICAL TOXICOLOGY

1. Safety/toxicity studies of Ayurvedic Formulations (a,b,c,d & e) (WHO Biennium Programme)

The traditional use of Ayurvedic formulations is one of the widely accepted therapy especially in chronic diseases viz. arthritis, asthma, and inducing fertility, rejuvenation etc. The data on safety of the Ayurvedic formulations has become important for wider global acceptance.

The coded Ayurvedic formulations developed by CCRAS, MoH & FW are reported to have potential therapeutic activity in chronic diseases and are considered essential for pre-clinical toxicity screening as per WHO guidelines. The present investigations are therefore undertaken to evaluate the safety of five Ayurvedic formulations "a,b,c,d & e" by acute/ sub-acute toxicity tests in mice/rats as per the protocols suggested by sponsor.

OBJECTIVES

To conduct acute and sub-acute toxicity of Ayurvedic Formulation-[a,b,c,d & e] in male and female Swiss Albino Mice and Wistar-NIN Rats.

METHODOLOGY

The acute toxicity test (14 days) and sub-acute toxicity test (28 days) were carried out in the animals which were selected randomly and conditioned to study the safety of Ayurvedic Formulation-[a,b,c,d & e] Table 39. In acute toxicity study, the animals were observed for lethality for 14 days after single exposure to the above mentioned formulations through oral route at 10 times of therapeutic dose. In sub-acute toxicity test the above formulations were exposed at intended clinical dosage schedule in four different dose levels (i) vehicle control (VC) (ii) Therapeutic dose (TD) (iii) Average dose (5 times of TD) (iv) High dose (10 times of TD) and to study the following observations.

Table 39. Dosage details

No	Sample code	Human* /day	Mice* (per kg)	Rat* (per kg)
1	a	3000 mg	390 mg	270 mg
2	b	1500 mg	195 mg	135 mg
3	c	1400 mg	182 mg	126 mg
4	d	3000 mg	390 mg	270 mg
5	e	15000 mg	1950 mg	1350 mg

* Route of administration: Oral

Observations

Food intake, body weight, routine physical, physiological examinations have been recorded at frequent intervals. Hematology, clinical chemistry in blood samples and gross necropsy, histopathology of liver, kidney, lungs and brain has been investigated at the end of experiment. Data is compiled and analyzed for significant difference between treatment groups and vehicle control group by appropriate tests.

RESULTS

1) Acute

No mortality, morbidity, weight loss and abnormal behaviour was recorded after a single exposure of a test compound with ten times of the recommended therapeutic dose after 14 days in swiss albino mice which are exposed to the test formulations.

2) Sub Acute

Formulation a

Pre-terminal deaths occurred in animals receiving (therapeutic dose) 1XTD (10%), 5XTD (30%) and 10XTD (60-70%) between 14th day to 28th day (Table 39). Since pre-terminal deaths (average 27.5%) were high, in view of the high pre-terminal mortality the experiment was repeated where in 50% of pre-terminal deaths were observed by 15th day. In the survived animals, the blood glucose levels, kidney and liver function tests were

found to be within normal range. The hematological parameters were also found to be within normal range. There was no evidence of histopathological changes in organs collected in the survived rats. The statistical analysis could not be undertaken in view of large pre-terminal deaths. However, the autopsy samples from dead animals showed severe dilatation of stomach and intestines suggesting "Paralytic Ileus" and this may be the effect of test formulation.

Formulation b

The test compound exposure of animals did not show any behavioral changes. There were no pre-terminal deaths in animals receiving test formulations at various dose levels. The physical and physiological activities, food intake and gain in body weights were not significantly different between groups exposed to test compound and animals received vehicle. The blood glucose levels, kidney/liver function tests and hematological parameters were found to be in normal range in all groups of animals exposed to the test compound. There were no histopathological changes in all the major organs studied.

Formulation c

The test compound exposure of animals did not show any behavioral changes. There were pre-terminal deaths of males only (10%) in all the groups animals receiving test formulations at various dose levels. The physical and physiological activities, food intake and gain in body weights in survived animals were not significantly different between groups exposed to test compound and animals received vehicle. The blood glucose levels,

kidney/liver function tests and hematological parameters were found to be in normal range in all groups of animals exposed to the test compound. There were no histopathological changes in all the major organs studied.

Formulation d

The test compound exposure of animals did not show any behavioral changes. There were no pre-terminal deaths in animals receiving test formulations at various dose levels. The physical and physiological activities, food intake and gain in body weights were not significantly different between groups exposed to test compound and animals received vehicle. The blood glucose levels, kidney/liver function tests and hematological parameters were found to be in normal range in all groups of animals exposed to the test compound. There were no histopathological changes in all the major organs studied.

Formulation e

The test compound exposure of animals did not show any behavioral changes. There were no pre-terminal deaths in animals receiving test formulations at various dose levels. The physical and physiological activities, food intake and gain in body weights were not significantly different between groups exposed to test compound and animals received vehicle. The blood glucose levels, kidney/liver function tests and hematological parameters were found to be in normal range in all groups of animals exposed to the test compound. There were no histopathological changes in all the major organs studied.

INSTRUMENTATION SERVICES

The instrumentation department caters to the needs of the various divisions of the institute by providing them with valuable technical expertise and prompt services in the use of numerous instruments available at the institute. The department activities include maintenance and repair of Electrical, Electronics, Electro-mechanical, Refrigeration and A/C equipment. Their work involves helping the scientific staff from preparing the specifications for their equipment, floating inquiries, scrutinizing the offers received, preparing comparative statements, ordering the equipment, making prerequisite arrangements for installation, installing the

equipment (Table 40) and finally helping the members of the staff in utilizing the equipment to its optimum level. To top it all the instrumentation staff strives hard to maintain all these instruments in working condition by providing timely repairs, procuring required spares and stocking them to keep the down time to a minimum.

The activities of the instruments department can be broadly categorized into the following divisions:

- I. Procurement and Installation of new equipment.
- II. Maintenance of all existing equipment at the institute.

I. Procurement and installation of new equipment

Table 40. List of new instruments installed

No	Name of the Equipment	Make	Model
1.	Micro Balance Afcoset-4nos.	AND	ER-120
2.	Mini Vertical Electrophoresis-4nos.	OWL	P8DS-2
3.	Midi Vertical Electrophoresis-2nos.	OWL	P9DS-2
4.	Binocular Microscope-1No.	Nikon	YS-100
5.	Thermal Cyler Gene Amp-2nos.	Applied Biosystems	System 9700 PCR
6.	Protein Purification System –1no.	Bio-Rad	Bio Logic LP
7.	Mini Transblot Electrophoresis Transfer cell -3nos	Bio-Rad	Cat. No. 170-3930/35
8.	Sub gel Electrophoresis System-2nos.	Bio-Rad	Cat. No. 170-4401 to 06 & 4481 to 86
9.	Mini protein 3 cell-3nos.	Bio-Rad	Cat. No. 165-3301/02
10.	Dot Blot Micro Filtration Apparatus-2nos.	Bio-Rad	Cat. No. 165-6545/47
11.	HPLC with UV-Visible, Fluorescence, RI Detectors & Auto injector + Degasser –1no.	Shimadzu	Advanced VP series
12.	Thermal Cyler with Vortexer for RNA	MGM instruments	DTS-400 Gene Probe Aptima
13.	Luminescence Analyzer along with IBM PC, Monitor E-54 and HP Desk jet Printer model –6122	MGM Instruments Inc.	Leader 450 HC+
14.	Dry Heat Bath Vortexer – 4 Nos. (PCR machine)	MGM Instruments Inc.	SB-100
15.	Microwave Digestion System	Cem Mars	X-Press (1500Watts)
16.	Atomic Absorption Spectrometer with Hydride Generator Graphite Furnace	GBC-Scientific	Avanta PM
17.	ELSD Low Temperature EvaporativeELSD for HPLC	SEDEXLT-ELSD	SEDEX-75
18.	2D-Gel electrophoresis system	Bio-Rad	2D-Gel-ES
19.	Ref. Centrifuge-2nos.	Eppendorf	5810
20.	Ultrasonic Bath –1no.	Oscar	MIC-103
21.	Auto Stainer-1no.	Sakura	DRS 2000J-D2
22.	Hybridization Oven –1no.	Apollo	Hp-9310
23.	Flash Evaporator-1no.	Buchi	R205V-800

List of new instruments installed (contd..)

No	Name of the Equipment	Make	Model
24.	Rota Rod Treadmill	TSE	337500R/A
25.	Tissue Embedding unit	Thermo	
26.	Blood Lead Analyzer	E.S.A.	
27.	Protein Purification System	Amersham	Akta Prime
28.	Automatic Cages washing machine	IWT,	9LAVE611
29.	TurboVap Evaporator	Zymark	LV 96
30.	Refrigerated Incubator Shaker	New Brunswick Scientific	Innova 4230
31.	Generator-1no.	Kirloskar	SC-90

III. Training programmes.

IV. Audio Visual arrangements.

II. Maintenance of Existing Equipment

Table 41. Details of complaints registered and attended

Name of the Divisions	Complaints Received	Completed	Pending
Electronics	209	202	7
Electromechanical	171	166	5
Refrigeration & Air-Conditioning	321	320	1
Total	701	688	13

The Electrical Department completed the process of Energising the Primate Facility, obtaining all required permissions from the Central Electrical Authority, Chennai.

III. Training Programmes

✍ Conducted Training course on Analytical Equipment's for two trainees of COVA from Govt. Polytechnic College for Minorities, Guntur, from 10-05-2005 to 03-06-2005. The topics covered were on the operating principles and maintenance of pH meters, conductivity meters, Electrophoresis, Spectrophotometers, Gas Chromatography, Liquid Chromatography, Centrifuges, Balances and Practical demonstrations on these instruments.

✍ An in-plant Training programme in the Operation and maintenance of Laboratory equipment in Bio-Medical Engineering was organized for a period of six weeks from 11-07-2005 to 19-08-2005 to two candidates deputed by the Advanced Training Institute, Ramanthapur, Hyderabad, under the aegis of Ministry of

Labour, Govt. of India. This in-plant training is a part of the course and the Ministry of Labour, Govt. of India, considers the training as a nation building activity.

Deputation

✍ Mr. Ramchandra Chaugule, Sr. Technical Officer and Mr. Micheal Fernandes, Technical Assistant, Instrumentation Division, were deputed to the RMRC Bhubaneshwer, from 14-02-2005 to 22-02-2005 to repair and service laboratory electronic analytical instruments and refrigeration equipment. They attended to more than 43 instruments, out of which 29 instruments were brought to working condition. Appropriate suggestions have been provided to RMRC, regarding the maintenance of rest of the equipment.

✍ Mr. Ramchandra Chaugule, Sr. Technical Officer was deputed to RMRC Jabalpur, from 31-08-2005 to 22-09-2005 for the installation of WALLAC make, Model 1409, Liquid Scintillation Counter at the centre. Appropriate reports have been submitted .

Seminar

- 1) Mr. V. Satish Babu, Technical Officer and Mrs. Vijaya Durga, Technician Instrumentation Division has attended a Technical Seminar on HPLC Chemstation Software and HPLC system organized by Agilent Technologies, India , Hyderabad on 7th April 2005 and 12th Sept. 2005, respectively.
- 2) Mr. B. Ramulu, Technical Officer, Instrumentation Division attended a Seminar on Refrigerants HVAC Systems organized by GMP Compliance India at Hyderabad.

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 dissemination services like MEDLINE Searches, Proquest Medical Library Full Text Database of journals and other online retrieval activities using the LAN Network of the Institute. Library continued to participate in exchange of data, journals and information using the URL <[http://Groups.yahoo.com/group/ICMR Librarians](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. 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 2005 and Corporate Membership for "Universities Federation for Animal Welfare, UK" for the year 2005 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) Created the database of Books, Reports and Scientific Articles using ISIS and LIBRIS Software
- B) ICMR has renewed the subscription to Proquest Medical Library Full Text Database of the journals.

During the period total of 4671 Proquest ML Full Text Database Searches were made.

C) 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.

D) The following equipment is procured for the library

- i) PROCOM CD/DVD Server 500 GB Hard Disk
- ii) 5 HP P4 Personnel Computers (HP)
- iii) 6 LAN Nodes have been connected in the library

NEW JOURNALS ADDED

Foreign Journals

1. B.B.A. Molecular Basis of Diseases
2. B.B.A. Molecular & Cell Biology Lipids
3. European Journal of Nutrition
4. Journal of Food Quality
5. Journal of Health, Population & Nutrition
6. Journal of Human Nutrition & Dietetics
7. Molecular Endocrinology
8. Mutagenesis
9. Molecular Nutrition Food Research
10. Maternal & Child Nutrition
11. Nature Methods

Indian Journals

12. Asian Journal of Diabetology
13. Asian Journal of Obstetrics & Gynaecology

14. Asian Journal of Pediatrics Practice
15. Bioinformatics India
16. Indian Journal of Traditional Knowledge
17. Monthly Review of A.P. Economy (CMIE)
18. Pharmabiz
19. Phytopharma
20. Swadeshi Patrika

Deleted Journals

1. Clinical Pharmacokinetics

The following library services were expanded as detailed below:

1. NEW ADDITIONS

Books	303
Reports	363
Journals (New Subs.)	20
Thesis / Dissertations	2
CDROMS (MEDLINE)	75

2. OTHER ACTIVITIES

Journals Bound	465
Visitors using the Library	4037
Circulation of Books/Journals etc	2152

MEDLINE Abstracts provided	3200
No. of E-mails sent outside	375
No. of E-mails received	1195
Photocopying (No.of pages)	363468
Number of Annual Reports mailed	530
No. of Books/Journals received	30
No. of Duplicate Journals sent out.....	250
No. of INTERNET Searches provided.....	110
No. of Reprints sent	300
Proquest Full Text Database searches provided		35

3. TOTAL LIBRARY COLLECTIONS

Books	15521
Journals (Bound Volumes).....	27583
Journals subscribed for 2005.....	267
Journals received (Gratis/Exchange)...	310
Microforms (Microfiche)	1072
Slides	277
Reports	11090
Theses & Dissertations	347
MEDLINE CDROMS Discs.....	243
Current Contents on Diskettes with abstracts	664
Proquest (Full Text E-Journals) on CD ROMS	410

Ph.D. PROGRAMMES

Ph.D Awardees

Research Scholar/staff	Year	University	Title of thesis
1.Jeyakumar S.M.	2005	Osmania	Studies on food intake regulation and obesity in WNIN/Ob and WNIN/GR-Ob rats
2. Rajendraprasad M.P	2005	Osmania	Nitrosamines and its relevance to cancer in India
3. Nirmala K.	2005	Osmania	Plant constituents as chemo preventive agents
4. Satish Kumar M.	2005	Osmania	Molecular chaperone function of alpha crystallin
5. Sreedhar B.	2006	Osmania	Iron and zinc interactions at the site of absorption

Research Scholar/staff	Title of the project	Guide
1. Saravanan N. (2000)	Effects of dietary alteration on n-6 and n-3 polyunsaturated fatty acids on insulin resistance, structure and function of adipocytes	Dr. Ghafoorunissa
2. Rita Saxena (2000)	Role of food processing on antioxidant activity and development of recipes with high antioxidant activity	Dr. Kamala Krishnaswamy
3. Venu L. (2001)	Foetal metabolic programming for insulin resistance syndrome: Role of maternal micronutrient restriction	Dr. Raghunath M
4. Krishna Kumari Menon (2001)	Positive Deviance in child nutrition	Dr.Vijayaraghavan K
5. Manjula T. (2001)	Ethno-pharmacological validation of biodynamic compounds in traditional Medicine	Dr.B.Dinesh Kumar
6. Aruna B. (2002)	Biophysical characetrisation of resistin	Dr. Nasreen Z. Ehtesham
7 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
8. Kiran Kumar B. (2002)	Genetic typing of WNIN/Ob and WNIN/GR-Ob strains using microsatellite markers	Dr. Giridharan N.V

Research Scholar/staff	Title of the project	Guide
9. Anil Kumar (2002)	Molecular chaperone function of alpha crystallin under hyperglycemic condition : modulation by dietary factors	Dr. Bhanuprakash Reddy G
10. Megha Saraswat (2003)	Screening of aldose reductase inhibitors and antiglycating agents from dietary sources and assessing their anticataractogenic potential	Dr.Bhanuprakash Reddy G
11. Mrudula. T (2003)	Characterisation and significance of a novel fatty acid elongase of the eye lens	Dr. Bhanuprakash Reddy G
12. 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.A.Vajreswari
13. Kishore Y.D. (2004)	Role of maternal trace elements in the development of insulin resistance in adult life	Dr. Raghunath M
14. Md.Naseeruddin (2004)	Understanding the role of resistin in inflammatory process leading to type 2 diabetes	Dr.Sudeep Gosh
15. Padmavathi I.J.N. (2004)	Role of maternal chromium status in the development of insulin resistance in the offspring	Dr. Raghunath M
16. Satyanarayana B. (2004)	Biological significance of phytoferritins	Dr.Madhavan Nair K
17. Sreenivasulu K. (2004)	CaCo-2 cell as a model to study bio-availability, mechanism of absorption and cytoprotective effects of zinc	Dr.Madhavan Nair K
18. Vasuprada I. (2005)	Bio-response of a model CaCo-2 cell system of iron and zinc	Dr.Madhavan Nair K
19. Shashikiran G (2005)	In vitro regeneration of the insulin secreting cells from the adult pancreatic ductal epithelial cells (progenitors/stem cells)-The role of specific Nutrients.	Dr. Vijayalakshmi V
20. 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.
21. Rajkumar (2005)	Characterization and differentiation of pancreatic progenitor/stem cells (Nestin Positive cells) to insulin secreting cells-the role of specific micronutrients	Dr. Vijayalakshmi V

Research Scholar/staff	Title of the project	Guide
22. Manisha Ganeshan	Foetal origins of adiposity and insulin resistance: Role of peri/postnatal manganese status	Dr. Raghunath M.
23. Vara Prasad SSS (2005)	Role of 11 β -HSD1 in pathogenesis of obesity and insulin resistance in WNIN/GR-Ob and WNIN/Ob restrains	Dr. Vajreswari A.
24. Sainath P.B (2005)	Insulin, insulin receptor and its signaling mechanism(s) in the brain and insulin sensitive target organs in the WNIN/ob and WNIN/GR-ob rats	Dr. Raghunath M
25. Pratibha B. (2005)	Immune status of WNIN mutant rats with reference to leptin and obesity	Dr. Giridharan N.V.
26. Gitanjali (2005)	Dietary diversification of Indian vegetarian diet to improve iron bioavailability: Studies using Caco-2 cell model	Dr. Madhavan Nair K
27. Sreevani (2005)	Understanding & dissecting the role of resistin in etiology of insulin resistance using obese rat model.	Dr. Nasreen Z.Ehtesham

AWARDS/HONOURS CONFERRED ON SCIENTISTS

Name of the Scientist	Award/Honour
Dr.G.Bhanuprakash Reddy	ICMR International Fellowship for Young Indian Biomedical Scientists for a period of 5 months.
	Dr.V.N.Patwardhan prize – 2002, by ICMR for his significant contributions in biomedical research.
Dr.B.Sivakumar	Vepachedu Gopalkrishna Rao Endowment Award for the year 2005. Delivered endowment lecture in the Department of Biochemistry, Osmania University, Hyderabad.
Mr.G.M.Subba Rao	Young investigators' Grant from the organizers of the 18 th International Congress of Nutrition to attend the Congress held at Durban, South Africa from 18 th – 22 nd September, 2005.
Dr.Nasreen Z. Ehtesham	Elected as Fellow of the National Academy of Sciences, India.
Dr.M.Raghunath	ICMR grant for Excellent Research Output.
Dr. P. Raghu	DBT Overseas Associateship for a period of 3 months
Dr. A. Vajreswari	Excellence Award by Friendship Forum of India for the year 2005

PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS

Date	Scientist	Conference/Meeting/Workshop/Seminar
2005		
April 25-29	Mr.G.M.Subba Rao	WHO sponsored "Asia Pacific Workshop on Raising Profile of Nutrition", at Kuala Lumpur, Malaysia
May 1-5	Dr.G.Bhanuprakash Reddy	Annual Meeting of Association for Research in Vision and Ophthalmology (ARVO) to be held at Florida, USA. Presented a paper on "Delay of diabetic cataract in rats by curcumin: Modulation of α -crystallin chaperone activity"
May 2-6	Dr.B.Sivakumar	FAO/WHO Nutrient Risk Assessment Workshop: A model for establishing upper levels of intake for nutrients & related substances, at WHO Headquarters, Geneva
May 11-14	Dr.Arjun L. Khandare	Third International Conference on Children's Bone Health, held at Sorrento, Italy. Presented a paper on "Severe bone deformities in young children coupled with vitamin D deficiency in fluoride affected village, Bihar, India"
June 6-10	Dr.B.Dinesh Kumar	Final Research Co-ordination Meeting of the International Atomic Energy's Coordinated Research Project on "Application of isotopic nuclear techniques in the study of nutrition-pollution interactions and their impact on the nutritional status of human subjects in developing country populations", held at Vienna
Sept. 19-23	Nutrition Safari 2005, 18 th International Nutrition Congress, organized by International Union of Nutrition Societies, at Durban, South Africa. The following scientists presented the papers:	
	Dr.K.V.Rameshwar Sarma	Low cost nutrition supplement for a National programme – A biotechnological approach
	Mr.T.Longvah	Endemic Goitre in State of Mizoram
	Mr.K.Venkaiah	Assessment of diet and nutritional situation in drought affected areas of India
	Mr.G.M.Subba Rao	Impact of FAO's Global School Based Nutrition Education Programme – Feeding Minds, Fighting Hunger (FMFH)
Oct. 25-30	Dr.V.Vijayalakshmi	Keystone Symposium on "Stem Cells, Senescence and Cancer", held at Singapore. Presented a paper on "Mice pancreatic ductal stem cells/progenitors in culture: Effects of supplementing the medium with human umbilical cord serum and fetal bovine serum".
Nov.21-25	Dr.L.Singotamu	27 th Session of Codex Committee on Nutrition and Food for Special Dietary Uses at Bonn, Germany as Co-Chairperson of the Indian Delegation.
2006		
Jan.30-31	Dr.K.V.Rameshwar Sarma and Mr.G.M.Subba Rao	Review and Evaluation Meeting on the Regional Training Course on Intersectoral Food and Nutrition Plans and Policies, organized by World Health Organisation, at Johannesburg, South Africa. They made a combined presentation on the South-east Asia Regional Experiences in Running the Course and Post-Course Follow-Ups of the Inter-country Workshop on Updating and Implementing Inter-sectoral Food and Nutrition Plans and Policies, which was organized at NIN in April 2005.

WORKSHOPS/CONFERENCES/SEMINARS TRAINING PROGRAMMES HELD AT NIN

1. WHO-FAO Inter-country Workshop for Updating and Implementing Inter-sectoral Food and Nutrition Plans and Policies. About 30 delegates from various South-East countries participated in the Workshop (April 4-8).
2. System Administrators Training for ICMR institutes located in South zone (April 11-15).
3. Project Review Meeting of ICMR Multicentric Study on "Mapping, size estimation and integrated behavioural and biological survey (BBS) in high HIV prevalence settings in India" (May 5).
4. Training of Trainers (ToT) from regional centers under the World Bank, MOHFW assisted "Capacity Building Project for Food Safety" (June 21-22).
5. Training programme for Food Inspectors sponsored by Directorate General of Health services (DGHS), GOI, New Delhi. About 30 food inspectors from Orissa and Madhya Pradesh attended the training programme (June 27- July 1).
6. WHO-NIN Workshop on "Dietary Fats and Non-Communicable Diseases". About 50 delegates from all over the country participated in the Workshop (July 7-8).
7. Meeting of the Pre-SAC and Scientific Advisory Committee of NIN/FDTRC/NCLAS (Aug. 4-6).
8. XXXIV Annual Training Course on Endocrinological Techniques and their Applications (Aug.16-Sept.23).
9. A one-day Workshop on "School Child Nutrition" for the Heads of Schools in association with Recognised Schools Management Association, Hyderabad (Sept. 1).
10. Conference on Nutrition and Self care for Healthy Aging, organized in association with International Life Sciences Institute India (ILSI-India) and ILSI Human Nutrition Institute (Sept.2-3).
11. State-level Workshop on Micronutrient Deficiencies A Drain on Indian Economy in association with Food and Nutrition Board (Govt. of India) and Department of Women Development and Child Welfare (Govt. of A.P) (Sept. 5).
12. Brain storming session on researchable areas in aging, organized by ICMR (Oct.13).
13. In connection with the World Food Day celebrations, a Symposium on "Culture Food Interface", was held at the institute (Oct.14).
14. 37th Annual Conference of Nutrition Society of India (Nov.17-19).
15. An ad-hoc training programme was conducted for two participants from Srilanka in the field of Research Methodology and Nutrition Communication (Nov. 21-25).
16. DGHS-NIN Training Programme for Industrial Personnel on Capacity Building for Food Safety (Nov.22).
17. Meeting of the NNMB Steering Committee (Dec. 20).
18. Nutrition Orientation Programme for Trainers of M/s. Amway India Enterprises, New Delhi (Dec.5-14).
19. Meeting of the DFS Sub-Committee, organized by ICMR (Jan.25-2006).
20. Meeting of the Federation of All India ICMR Employees. A seminar on "Application of modern instruments in biological research" was organized for the Technical staff of all ICMR Institutes (Jan. 28-29).
21. XXXXIII Post-Graduate Certificate Course in Nutrition was held from Jan.2 March 15, 2006.
22. One day training programme of J-Gate Custom Content for Consortia (JCCC) for all ICMR Libraries located in the Southern Region (Feb. 10).
23. XXXI Annual Conference of Environmental Mutagen Society of India and International Symposium on "Mutagenesis and Public Health" (Feb. 23-25).
24. DGHS-NIN Training Programme for Industrial Personnel on Capacity Building for Food Safety (Feb.16-17).
25. National Seminar on "Historical Aspects of Diet and Nutritional Medicine in Ayurveda", organized by Indian Institute of History of Medicine, Hyderabad in collaboration with NIN (March 9-10).
26. One-Day Symposium on "Frontiers of Nutrition Extension and Communication" was held at the institute. Experts from government, academic and NGO sectors participated in the Symposium (March 27).
27. Nutrition Orientation Programme for Health and ICDS Functionaries of State of Maharashtra, organized by NIN in association with Rajmata Jijau Mother-Child Health & Nutrition Mission/UNICEF, Maharashtra. About 20 participants attended the training programme (March 20-24).

SERVICE RENDERED TOWARDS INCOME GENERATION

1. PATHOLOGY SERVICES

During the year, a total income of Rs.3,83,000/- was generated from various projects of Institute's preclinical toxicology and surgical pathology and cytology samples.

SCIENTIFIC PUBLICATIONS 2005

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Ahamed Ibrahim, Saravanan N, Ghafoorunissa : Dietary trans-fatty acids alter adipocyte plasma membrane fatty acid composition and insulin sensitivity in rats. *Metabolism : Clin Exp.* 54 : 240-246, 2005.
2. Anil Kumar P, Abdul Haseeb, Suryanarayana P, Nasreen Z.Ehtesham, Bhanuprakash Reddy G : Elevated expression of alpha A- and alpha B-crystallins in streptozotocin-induced diabetic rat. *Arch Biochem Biophys.* 444 : 77-83, 2005.
3. Anil Kumar P, Suryanarayana P, Yadagiri Reddy P, Bhanuprakash Reddy G: Modulation of α -crystallin chaperone activity in diabetic rat lens by curcumin. *Molecular Vision.* 11 : 561-568, 2005.
4. Arlappa N, Balakrishna N, Brahmam GNV, Vijayaraghavan K : Nutritional status of the tribal elderly in India. *J Nutr Elderly.* 25 : 23-39, 2005.
5. Bhat RV, Vasanthi S : Natural occurrence of ochratoxins in Indian coffee. *Indian J Nutr Dietet.* 42: 106-113, 2005.
6. Chetty CS, Mohan CV, Campbell K, Suresh C : Lead-induced cell death of human neuroblastoma cells involves GSH deprivation. *Cellular & Molecular Biology Letters* 10 : 413-423, 2005.
7. Ghafoorunissa : Dietary (n-3) long chain polysaturated fatty acids prevent sucrose induced insulin resistance in rats. *J Nutrition.* 135 (11) : 2634-38, 2005.
8. Ghafoorunissa, Ahamed Ibrahim, Saravanan N : Substituting dietary linoleic acid with α -linolenic acid improves insulin sensitivity in sucrose fed rats. *Biochimica et Biophysica Acta* 1733 : 67-75, 2005.
9. Ghosh SK, Desai MR, Pandya GL, Bhatia KR, Venkaiah K, Kulkarni PK : Toxicity of agricultural dust extract using microtoxTM. *Bull Environ Contam Toxicol.* 74 (1) : 100-106, 2005.
10. Grace Maria Antony, Visweswara Rao K : Pattern of consumer expenditure and poverty level : a case study of two villages in Kerala. *Ind J Nutr Dietet.* 42 : 357-366, 2005.
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12. Jeyakumar. SM, Vajreswari A, Sesikeran B, Giridharan NV : Vitamin A supplementation induces adipose tissue loss through apoptosis in lean but not in obese rats of the WNIN/Ob strain. *J Mol Endocrinol.* 35(2): 391-398, 2005.
13. Khandare AL, Harikumar R, Sivakumar B : Severe bone deformities in young children from Vitamin D deficiency and fluorosis in Bihar India. *Calcif Tissue Int.* 76 : 412-418, 2005.
14. Khandare AL, Suresh P, Uday Kumar P, Lakshmaiah N, Manjula N, Shanker Rao G : Beneficial effect of copper supplementation on deposition of fluoride in bone in fluoride and molybdenum fed rabbits. *Calcif Tissue Int.* 77 : 233-238, 2005.
15. Moghaddam MS, Anil Kumar P, Bhanuprakash Reddy G, Ghole VS : Effect of diabecon on sugar-induced lens opacity in organ culture: mechanism of action. *J Ethnopharmacology.* 97 : 397-403, 2005.
16. Murthy PVVS, Ramgopal K, Vishnu Vardhana Rao M, Bamji MS : Strategies for augmenting rural sanitation and drinking water supply Impact on environment, health and women's awareness. *J Rural Tech.* 1(6) : 271-275, 2005.
17. Pratima Rao, Bhat RV, Sudershan RV, Prasanna Krishna T : Consumption of synthetic food colours during festivals in Hyderabad, India. *Br Food Journal.* 107 (5) : 276-284, 2005.
18. Qadri SSYH, Uday Kumar P, Sesikeran B: Spontaneous Pathological lesions of liver and

- lungs in preclinical toxicology evaluation studies. *Ind J Vet Pathol.* 29: 102-105, 2005
19. Raju PS, Prasad KVV, Ramana YV, Balakrishna N, Murthy KJR : Influence of socioeconomic status on lung function and prediction equations in Indian children. *Pediatr Pulmonol.* 39: 528-536, 2005.
 20. Ravindranath M, Venkaiah K, Vishnuvardhana Rao M, Arlappa N, Gal Reddy Ch, Mallikharjuna Rao K, Sharad Kumar, Brahmam GNV, Vijayaraghavan K : Effect of drought on nutritional status of rural community in Karnataka. *J Hum Ecol.* 18 (3) : 245-252, 2005.
 21. Saravanan N, Abdul Haseeb, Nasreen Z, Ehtesham, Ghafoorunissa: Differential effects of dietary saturated and trans-fatty acids on expression of genes associated with insulin sensitivity in rat adipose tissue. *Eur J Endocrinology.* 153: 1-8, 2005.
 22. Saravanan N, Ahamed Ibrahim, Ghafoorunissa Dietary trans fatty acids alter diaphragm phospholipid fatty acid composition, triacylglycerol content and glucose transport in rats. *Br J Nutr.* 93 : 829-833, 2005.
 23. Satish Kumar M, Kapoor M, Sinha S, Bhanuprakash Reddy G: Insights into hydrophobicity and the chaperone-like function of A- and B- crystallins. *J Biol Chem.* 280 (23) : 21726-21730, 2005.
 24. Satish Kumar M, Yadagiri Reddy P, Sreedhar B, Bhanuprakash Reddy G: B-crystallin- assisted reactivation of glucose-6-phosphate dehydrogenase upon refolding. *Biochem J.* 391 : 335-341, 2005.
 25. Silswal N, Singh AK, Aruna B, Sangita Mukhopadhyay, Sudip Ghosh, Nasreen Z Ehtesham: Human resistin stimulates the pro-inflammatory cytokines TNF- macrophages and IL-12 in macrophages by NFkB dependent pathway. *Biochem Biophys Res. Commun.* 334 (4) : 1092-1101, 2005.
 26. Singotamu L, Madhusudhana Chary P: Scanning electron microscope studies on genetically modified (GM) crops G.M. cotton fiber (*Gossypium herbaceum*). *Scanning.* 27(3): 160-161, 2005.
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 29. Veena Shatrugna, Bharati Kulkarni, Ajay Kumar P, Usha Rani K, Balakrishna, N : Bone status of Indian women from a low-income group and its relationship to the nutritional status. *Osteoporosis Int.* 16 : 1827-1835, 2005.
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 31. Vijayakumar Reddy C, Vidya Sagar GV, Sreeramulu D, Venu L, Raghunath M : Addition of milk does not alter the antioxidant activity of black tea : *Ann Nutr Metab.* 49 : 189-195, 2005.
 32. Vijaya Lakshmi B, Sesikeran B, Udaykumar P, Kalyanasundaram S, Raghunath M : Chronic low intake of protein or vitamins increases the intestinal epithelial cell apoptosis in Wistar/NIN rats. *Nutrition.* 21 : 949-960, 2005.
 33. Vijaya Lakshmi B, Sesikeran B, Udaykumar P, Kalyanasundaram S, Raghunath, M : Effects of vitamin restriction and supplementation on rat intestinal epithelial cell apoptosis. *Free Radic Biol Med.* 38 : 1614-1624, 2005.
- B. PAPERS PUBLISHED IN PROCEEDINGS OF WORKSHOPS/ CONFERENCES**
1. Bhat RV, Vasanthi S : Food safety assessment issues of transgenic rice in the Indian context. In "Biosafety of transgenic rice" ed. by VL Chopra, S Shantharam, RP Sharma, New Delhi, Natl Acad Agr Sci 2005, 65-74pp.
 2. Bramham GNV, Venkaiah K, Laxmaiah A, Gal Reddy Ch, Sharad Kumar, Sivakumar B: Health & nutritional status of Tsunami affected population. In " 26th Dec'2004 Tsunami : ICMR

- Response” ed. by M.D.Gupte, Natl. Inst. of Epidemiology, ICMR, Chennai, 2005, 57-60pp.
3. Giridharan NV, Kalyanasundaram S : Use of Non-human primates in nutrition research. In “National Centre for Primate breeding and research: Vision challenges and opportunities“, ed. by Chander P. Puri, Nirmal K. Ganguly, National Institute for Research in Reproductive Health, 2005, 515-534pp.
 4. Jayant Kulkarni, Singotamu L, Jaleeli KA, Radhakrishnan T, Adeel Ahmad: Physical properties of human adult and umbilical cord blood a comparative study. Abstract. National symposium on recent trends in molecular & medical biophysics, 22nd 25th January 2005, Organized by Dept. of Physics, School of Basic Medical Sciences, Univ. of Pune, Pune, India & Indian Biophysical Society, 2005, 123-124pp.
 5. Kalpagam Polasa, Sesikeran B : Genetically modified food safety and risk. In “National seminar on food safety and quality control in India“, 2-4 March 2005, organized by Acharya N.G. Ranga Agricultural Univ., ICAR, Hyderabad, 2005, 78-82pp.
 6. Madhavan Nair K : Food supplements and processed foods. In “ Conference on nutrition and self care for healthy aging”, Summaries of presentations and CVs, Sponsored by International Life Sciences Institute India (ILSI India), Co-sponsored by National Institute of Nutrition, held on 2-3 September 2005 at National Institute of Nutrition, Hyderabad, pp.III: 2-1-3.
 7. National Institute of Nutrition : Conference on nutrition and self care for healthy aging: Summaries of presentations and CVs, September 2-3, 2005, NIN, Hyderabad, sponsored by International Life Sciences Institute India (ILSI-India), ILSI-Human Nutrition Institute (ILSI- HNI), Washington DC, Co-sponsored by National Institute of Nutrition (NIN), Hyderabad, 2005.
 8. National Institute of Nutrition : Double fortified common salt (DFS) as a tool to control iodine deficiency disorders and iron deficiency anaemia a report. Hyderabad, NIN, 2005, 12p.
 9. National Institute of Nutrition: Health & nutrition status of tsunami affected population living in the relief camps in Andaman & Nicobar islands a rapid survey by GNV Brahmam, K Venkaiah, A Laxmaiah, Ch Gal Reddy, Sharad Kumar, B Sivakumar. Hyderabad, National Institute of Nutrition, 2005.17p+ Tables.
 10. National Institute of Nutrition: Proceedings of the National seminar on pesticide residues and their risk assessment, January 20-21, 2005. Hyderabad, FDTRC, NIN, 2005, 185p.
 11. National Institute of Nutrition: A Rapid survey on diet and nutritional status of Saharia a primitive tribe in Rajasthan, by GNV Brahmam, K. Mallikharjuna Rao, R. Hari Kumar, K. Venkaiah. Hyderabad, National Institute of Nutrition, 2005. 1-13pp + Tables.
 12. National Institute of Nutrition : Report of WHO-NIN workshop on dietary fats and non-communicable diseases, July 7-8, 2005, Hyd, NIN, 55p.
 13. National Institute of Nutrition : Stability of iodine in double fortified salt (DFS) a short-term study by S Ranganathan, GNV Brahmam, M Vishnu Vardhana Rao, K Vijayaraghavan, B Sivakumar. Hyderabad, National Institute of Nutrition, 2005. 9p+Annexure.
 14. Rameshwar Sarma K V, Balakrishna N, Vijayaraghavan K, Sivakumar B : Low cost nutritious supplement for a National program a biotechnological approach. (Abstract) In “ Proc. of the 18th Int Congr of Nutr “ held at ICC, Durban, South Africa: Nutrition Safari for innovative solutions, from 19-23 September 2005, Durban, ICC, 2005, p.260.
 15. Sesikeran B : Optimizing immunity and gut function in elderly. In “Conference on nutrition and self care for healthy aging”, Summaries of presentations and CVs, Sponsored by International Life Sciences Institute India (ILSI India), Co- sponsored by National Institute of Nutrition, held on 2 -3 September 2005 at National Institute of Nutrition, pp. I:2:2 I:2-4.
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- safety and quality control in India“ 2-4 March 2005, organized by Acharya N.G. Ranga Agri. Univ., ICAR, Hyderabad, 2005, 73-77pp.
17. Sivakumar B : Vitamin A enriched food crops : biosafety and bioavailability. In “Biotechnology for food and nutritional security “ ed. by Vibha Dhawan, New Delhi, TERI Press, 2005, 209-219pp.
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 19. Subba Rao GM, Raghunatha Rao D, Venkaiah K, Dube AK, Rameshwar Sarma KV : Impact of FAO's global school-based nutrition education programme: Feeding minds, fighting hunger (FMFH). In “ Proc. of the 18th Int Congr of Nutr “ held at ICC, Durban, South Africa: Nutrition Safari for innovative solutions, from 19-23 September 2005, Durban, ICC, 2005, 1-6pp.
 20. Suresh P : Infectious diseases of Non-human primates. In “ National Centre for Primate Breeding and Research: Vision challenges and opportunities“ ed. by Chander P. Puri et al. 2005, 195-215pp
 21. Vasanthi S, Bhat RV : Risk assessment of genetically modified foods in the Indian context. In “Biotechnological interventions for dryland agriculture : opportunities and constraints” ed. By G. Pakki Reddy, PS Janakikrishna. Hyderabad, BS Pubs., 2005, 126-135pp.
- C. POPULAR ARTICLES**
- a) NUTRITION NEWS
 - 1) Bhanuprakash Reddy G, Suryanarayana P : Turmeric and curcumin delay cataract in rats. Nutrition News. 26 (1 & 2) : 1-6, Jan - Apr' 2005.
 - 2) Nasreen Z Ehtesham, Vijayalakshmi., Nirupama Silswal : Syndrome X The Next pandemic: Understanding the key players. Nutrition News 26 (3 & 4): 1-6, July/ Oct' 2005.
 - b) OTHERS
 - 1) Vishnuvardhan Rao M, Brahman GNV : Quality of life and sustainable development. Population & Development Education News Bulletin. 12 : 1-4, Mar' 2005.

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