

रा.पो.सं. वार्षिक प्रतिवेदन

• NIN

Annual Report

2009-2010





ANNUAL REPORT

2009-2010



सत्यमेव जयते

डॉ. विश्व मोहन कटोच

एम डी, एफ एन एससी, एफ ए एम एस, एफ ए एससी, एफ एन ए

सचिव, भारत सरकार

(स्वास्थ्य अनुसंधान विभाग)

स्वास्थ्य एवं परिवार कल्याण मंत्रालय एवं

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MESSAGE



भारतीय आयुर्विज्ञान अनुसंधान परिषद

(स्वास्थ्य अनुसंधान विभाग)

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Nutrition has come to occupy a pivotal place in several major health programmes being implemented in our country today. The role of effective nutritional interventions in better implementation of the National Nutritional Policy, National Rural Health Mission and ICDS is well recognized. However, there is an urgent need to strengthen the nutritional component of all the health initiatives under implementation at present, so as to improve the overall quality of life of our people. Well-designed research studies in the realm of nutrition would help deal with nutrition related issues in a more effective way. NIN is one of the flagship research Institutes of the country which has always been at the forefront to accept all challenges that spring from time to time. Being adept in basic, applied research and operational research areas and with the support of well trained scientific and technical staff, the NIN is poised to play a significant role in combating hunger and malnutrition in the country. It is heartening to note that it is ably supported by the good work carried out by both FDTRC and NCLAS.

Community-based research has always been the hallmark of the work output of NIN and NNMB. The studies reported in the Report on Food Labelling and Packaged Foods, nutrition education for urban adolescents and complementary feeding and development stimulation in infants indeed point to the significance of community-based research interventions prioritised this year.

HIV-Nutrition interface is yet another new area covered this year and the findings offer interesting sights into the micronutrient status of people living with HIV/AIDS. Also, the studies on importance of calcium during pregnancy to boost the bone mineral density of women belonging to the underprivileged sections of our societies provide new insights. The findings of some of the basic studies carried out this year on cataractogenesis, stem cell research, maternal undernutrition and in determining the phenolic content of certain foods are indeed interesting.

I am pleased to note that FDTRC has prioritised research on several important aspects of food safety during the year. The Total Diet Study has provided useful information on the presence of toxicants like fluorides, toxic metals, pesticide residues and mycotoxins in our foods. Some aggressive measures need to be taken now to ensure food safety and to ward off diseases.

We are looking forward to more landmark contributions from this prestigious institute.

I am sure that the research carried out here will continue to address the national needs in relation to nutrition and food security in India.

(Vishwa Mohan Katoch)

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41. A. Venugopal
42. S.A. Rehman
43. M. Kishan
44. B. Nageswara rao
45. P. Nagulu

46. M. Seenu
47. B. K. Mahadevaiah
48. A. Chandraprakash
49. M. Narasimha
50. M. Jayamma
51. D. Venkatesh
52. M. Satyamma
53. C. Sivaleela
54. G. Satyapaul
55. A. Narsing Rao
56. A. Lakshmi
57. G. Jitender
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59. M. Upender
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63. A. Shanker
64. P. Ravinder
65. D. Madhava Reddy
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67. I. Poshetty
68. G. Yadagiri
69. M. Venkataiah
70. N. Bhasker
71. A. Jangaiah
72. P. Dasharath
73. S. Narahari
74. K. Venkatesh
75. P. Narsimha
76. E. Kondal reddy
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RESEARCH HIGHLIGHTS

1. COMMUNITY STUDIES

1.1 *Assessment of current scenario of food labelling in India*

Consumption of unhealthy foods can be discouraged by effective food labelling, which can help consumers make informed choices. A study was conducted with financial support from WHO-India, employing a combination of systematic review of literature on labelling regulations and community studies, as well as qualitative and quantitative market survey to examine the current scenario of food labelling in the country. The salient observations of the study were that the food labelling regulations in India are on par with those of developed countries; that food labels are not usually read by consumers while making food choices, which may be either due to low literacy rates or lack of nutrition knowledge and awareness. Market survey showed that food packages were 100% in compliance while displaying the basic regulatory requirements like name of the food, net weight, languages used etc.; The labelling information such as 'instructions to use', 'storage conditions', 'use by date', and nutrition information per 100 g or per serving were observed in 77% of pre-packed foods; the nutrition and health claims were indicated on the 10% and 29% of labels respectively. Of the 109 imported and 236 Indian pre-packaged foods, a significantly ($P < 0.05$) higher proportion of Indian foods (79%) consisted nutrient declaration for every 100 g or mL as against 68% for imported foods, which is a mandatory aspect as per PFA. The study clearly highlighted the need to undertake nation-wide studies to understand the consumer knowledge, practices and behaviour related to food labels for formulating strategies to make food labels user-friendly.

1.2 *Multi component health and nutrition interventions on the lifestyles and physical activities among urban adolescents*

The increase in the prevalence of obesity and overweight has been reported to be rapid even among children and adolescents in both developed as well as developing countries. It is very important that the problem of overweight and obesity is addressed early in childhood and adolescence. The school environment is most appropriate to impart health and nutrition education, encourage participation in physical activities and inculcate healthy lifestyle practices. A study examined the effect of multi-component education interventions for 8 months on lifestyles, physical activities of urban school-going adolescents. The following are salient observations of the study. The educational intervention resulted in a significant increase in the health and nutrition knowledge of the adolescents in the perceptions, behaviours and practices among adolescents of intervention group. The mean fat fold thickness at triceps, biceps, sub-scapular, supra-iliac regions and sum of the skin folds significantly declined, with no change in the weight and BMI.

There was an improvement in the healthy lifestyle practices which included physical exercises (intervention: 57.5% Vs 81.7%; control: 48.7% Vs 71.2%), participation in household activities (intervention: 86.9% Vs 99.1%; control: 81.2% Vs 91.2%), playing out door sports and games (intervention: 37.7% Vs 72.4%; control: 33.7% Vs 58.7%) were improved.

2. BEHAVIOURAL SCIENCES

2.1 *The efficacy of an integrated feeding and care intervention among 3-15 months old infants in Andhra Pradesh, India*

The intervention messages, which were developed in the study through a comprehensive formative research were culturally appropriate and could lead to behavior change that was demonstrated through improved dietary intake of energy, protein, vitamin A, calcium, iron and zinc; improvement in growth

(height for age) and development among the infants of the intervened groups. The added benefit of responsive complementary feeding and developmental stimulation was seen in higher mental development scores but not on growth.

3. MICROBIOLOGY AND IMMUNOLOGY

3.1 Evaluation of allergenicity potential of an indigenous anti-HPV vaccine in BALB/c mice

The indigenously developed HPV vaccine (HPV 16 and HPV 18 VLPs) does not seem to have allergenicity potential.

3.2 Micronutrient status in HIV infected population in India

A sub-sample of serum samples collected during an earlier project entitled “Integrated Biological and Behavioral Assessment (IBBA)”, sponsored by Bill and Melinda Gates Foundation; were used to evaluate nutrients such as Vitamin D, Zinc, magnesium, copper and iron in HIV positive and HIV free female sex worker (FSW) population.

The samples were categorized into 4 groups of 100 samples each - Group1 : HIV negative and STI negative. Group2: HIV positive and STI positive, Group 3: HIV negative and STI positive, Group 4: HIV positive and STI negative. The samples were analyzed for Vitamin D, Zinc, magnesium, copper, iron and albumin.

Roughly about 90% of the FSW's were deficient in zinc and iron. FSW with HIV infected asymptomatic population had similar serum concentrations of Zinc, magnesium, copper and iron as FSW without HIV infection. The mean \pm SE of vitamin D and serum albumin were 23.64 ± 0.83 ng/mL and 3.52 ± 0.06 g/dL respectively in HIV infected asymptomatic FSW population. About 80% of the HIV infected FSW population had vitamin D deficiency, when compared to normal women of low socioeconomic status. Iron and zinc deficiency appears to be widely prevalent amongst FSWs with or without HIV infection. Vitamin D deficiency could also be a major problem in this population.

4. CLINICAL STUDIES

4.1 Pregnancy related changes in bone mass in women from low socio-economic group

Calcium requirement increases during pregnancy to support the fetal skeletal mineralization and a few studies from the west have indicated that the maternal bone mineral density (BMD) may transiently reduce during pregnancy due to mobilization of calcium. This study was conducted to investigate the effect of pregnancy on the bone mass of women from low income group consuming low calcium diets.

Baseline DXA was done for 80 women. Of them, 35 women conceived within 6 months of baseline DXA and were recruited as pregnant group. Their BMD was repeated after delivery. Women who did not conceive within 6 months /12 months of baseline DXA served as controls. Mean spinal BMD decreased by 4% in the pregnant group. While, it increased at all sites at 6 and 12 months in control women. The study thus indicates that the undernourished young women from low socio-economic group failed to increase their bone densities as expected during growth phase probably due to the extra demands imposed by fetal skeletal mineralization.

4.2 Vitamin D status in common infections in childhood –A hospital based study

Vitamin D stimulates the expression of potent anti-microbial peptides of host defense system and in epithelial cells lining the respiratory tract where they play a major role in protecting the lung from infection. Studies done in India have shown subclinical vitamin D deficiency to be wide spread in all age groups.

one hundred pre-school children between ages of 1-5 years with different infections were investigated for vitamin D status. Among the infections, diarrhea was 29.3%; fever 13.1%; URTI 19.2% and LRTI 18.4%.

As regards, vitamin D status among the children with infections, it was less than 12ng/mL in 22%, between 12-20 ng/mL in 28% and above 20ng/mL in 50% children. Overall prevalence of vitamin D deficiency was 45%.

4.3 Evaluation of the routine management of anaemia with hemoglobin < 8g in pregnancy and effect on outcome.

Iron deficiency anaemia is one of the most common nutritional deficiencies all over the world. About half of the world's anaemic women live in India and 88% of them develop anaemia during pregnancy. Dietary deficiency of iron and inadequate absorption of dietary iron are the important causes of high prevalence of anaemia. The iron requirement for pregnant women is around 38 mg/day as against the dietary intake of 18 mg/day. Severe anaemia during pregnancy increases the risk of maternal mortality. Daily supplementation was most effective in preventing severe anaemias.

Over 1500 cases were screened to identify pregnant women below 8g both in early and late pregnancies. Pregnant women with hemoglobin less than 8g/dL between 12 to 20 weeks and 24-26 weeks of gestation were recruited for oral supplementation of 120 mg of iron and 500 µg of folic acid and for parenteral iron therapy (iron sorbitol citrate 1000 mg) respectively.

The overall prevalence of anaemia with Hb < 8g in pregnant women was 18.7% and in early pregnancy it was 16.2% and in late pregnancy it was 22.5%. About 65% of the women in early pregnancy and 46.2% in late pregnancy showed increase in haemoglobin above 8g within 3-6 weeks of supplementation. However, 44.1% in early pregnancy and 27.7% in late pregnancy showed increase in haemoglobin above 11g at term. This finding highlights that early supplementation of parenteral iron may increase haemoglobin by term.

4.4 Insulin resistance and TNF α levels in normal and high risk pregnant women

Chronic energy deficiency in women at reproductive age will have impact on the fetal programming in early pregnancy, fetal growth and birth weight of infants. Insulin resistance during pregnancy has been shown to be associated with many complications in addition to impact on pregnancy outcome and birth weight of infants.

The study was carried out to investigate the insulin resistance (IR) and TNF α level at different trimesters of gestation in normal and high risk pregnant women in relation to maternal nutritional status in low socio-economic group.

- ✍ This study indicates the prevalence of insulin resistance around 50% right from early pregnancy.
- ✍ The prevalence of insulin resistance was present right from 12 weeks of gestation.
- ✍ There was significant difference in mean antenatal weight and body fat between insulin resistant group and normal pregnant women at 12, 20, 28 and 36 weeks.

The prevalence of insulin resistance during pregnancy is associated with adverse pregnancy outcome. So it is essential to carry out a prospective study on effect of insulin resistance in early pregnancy on course, complications and outcome of pregnancy.

5. BASIC STUDIES

5.1 Immunoassay for quantitation of metallothionein for assessing zinc bioavailability in Caco-2 cell model.

Inadequate intake and poor bioavailability of zinc are the two major etiological factors for the widespread deficiency of zinc seen in vulnerable segments of the population. Several strategies have been developed to increase the zinc status in vulnerable segments of the population. An important strategy is

dietary diversification. This requires screening and identification of foods with high bioavailable zinc. Induction of mineral specific responsive protein in Caco-2 cell model, a human enterocyte cell line, is a widely accepted screening tool to assess mineral bioavailability in humans. In this context, metallothionein (MT), a zinc inducible cytosolic protein, was considered as a surrogate marker of zinc bioavailability in Caco-2 cells. An indirect competitive ELISA method and an immunoblot for quantitation of MT were developed using an antiserum produced against recombinant human MT. The methods were validated by simultaneously measuring ⁶⁵Zn uptake in Caco-2 cells exposed to varying concentrations of zinc along with quantitation of MT by ELISA and immunoblot. Though zinc uptake increased, there was no concomitant induction in MT levels estimated by both the methods. Thus, the immunoassay method developed may not be suitable as screening test for zinc bioavailability in Caco-2.

5.2 Iron, folate and vitamin B₁₂ levels in pregnant women and the effect of intramuscular iron, folate and vitamin B₁₂ therapy on iron folate and B₁₂ levels

Recent studies have indicated that vitamin B₁₂ deficiency may play an important role in anemia along with iron and folic acid deficiencies. Therefore, a study was conducted with the objective to investigate iron, folate and vitamin B₁₂ status in pregnant women with different grades of anemia and to assess the impact of intramuscular dose of 150 mg iron, 1500 µg folic acid and 150µg hydroxocobalamin acetate on biochemical indicators of these three nutrients in pregnant women with moderate anemia. The study has shown a higher prevalence of iron and folic acid deficiency and relatively lower prevalence of vitamin B₁₂ deficiency. Only 6% had deficiency of all the three micronutrients in the group with hemoglobin less than 8g/dL. Surprisingly, one-fourth of the women with moderate anemia and one half of the women with mild anemia showed no biochemical evidence of deficiency of these three nutrients. Following IM therapy, there was improvement in mean hemoglobin and serum ferritin but no change in vitamin B₁₂ status. There was also a steep increase in folate deficiency from 8.3 to 35.6%. In view of the findings, it is important to assess the prevalence of folate and vitamin B₁₂ deficiency in different parts of the country.

5.3 Studies on the response and interactions of iron and zinc in Caco-2 cells: protein expression

Iron and zinc interact at the enterocyte and influence the absorption of each other. These interactions could be modulated by various factors, particularly the pre-existing iron and zinc status and other pathological conditions such as inflammation. The study presents evidence for the iron and zinc interactions in Caco-2 cells during depletion or repletion of individual nutrients and the effect of inflammatory stimuli on such interactions (i.e. nitrosative stress). The results demonstrate that under simulated inflammatory conditions, iron and zinc uptake decreased in mineral deficient cells, while a similar effect of iron on zinc uptake is conspicuously absent. The observed decrease in iron is mirrored by a decrease in iron influx (DMT-1) and efflux transporter (FPN-1) expression. This was accompanied by changes in iron responsive protein-2 (IRP-2) expression, rather than IRP-1. However, no significant change in zinc influx (Zip-1, -14; except upon NO+zinc) was observed, whereas, efflux transporter (ZnT-1, -4) levels were decreased in mineral deficient cells. It is concluded that zinc selectively affects iron uptake and its interactions under normal conditions, depletion-repletion and simulated inflammation. Inflammation associated hypoferrremia and hypozincemia may be host-protective.

5.4 Maternal magnesium restriction induced increase in the adiposity of WNIN rat offspring may be due to increased stress and fatty acid synthesis

It was reported earlier that maternal magnesium (Mg) restriction irreversibly increased body fat percent, specially the visceral adiposity. In the current year, studies assessed whether or not increased stress was associated with / responsible for this increased adiposity. The results indicated that increased glucocorticoid stress and fatty acid synthesis (suggested by the up-regulation of 11βHSD 1 and FAS) could probably underlie the increased visceral adiposity in the offspring of Mg restricted rat dams. The fact that

rehabilitation could correct the change in 11 β HSD 1 gene expression in adipose tissue but not the changes in FAS expression (gene and protein) or visceral adiposity seem to suggest that maternal Mg restriction induced stress in the offspring may be corrected by rehabilitation but not the changes induced by this stress in the offspring (eg. increased visceral adiposity).

5.5 Health beneficial effects of foods commonly consumed in India: milk, milk products, oil and sugars

Continuing the efforts to generate a database on phenolic content of foods commonly consumed in India and their contribution to the antioxidant activity (AOA), the total phenolic content and AOA (by two different methods: FRAP and DPPH – radical scavenging activity) of milk, milk products, oil and sugar was determined. Although the foods analysed belonged to different classes and their antioxidant activity and total phenolic content showed a wide range of distribution, it was interesting to note that a significant correlation was observed between AOA and TPC in all of them. Among the foods studied, jaggery had the highest AOA, whole milk, milk products and oils were in general poor in their AOA and phenolic content.

5.6 The role of specific nutrients on islet cell generation from adult tissue stem cells - in vitro and in vivo

During regeneration, ductal epithelial cells act as progenitors for the generation of new pancreatic cells. Understanding the modulation rendered by nutrients on the pancreatic endocrine cells and their transcriptional regulation will open intriguing possibilities to basic research. Hence, studies are being conducted to understand the ability of pancreatic progenitors such as ductal epithelial cells (DEC) to proliferate and differentiate into insulin secreting cells regulated by specific nutrients & growth factors. Studies have been undertaken to understand the interplay between nutrient and pancreatic progenitors (PP) towards their expansion and differentiation to neoislets.

The significant findings are: (i) Pyridoxal phosphate (PLP –a vitamin B₆ cofactor) in combination with growth factors, showed increased BrdU incorporation; (ii) stimulated the proliferation of the DEC/CK-19+ve/ABCG-2 (pancreatic progenitors); (iii) increase in the transition from epithelial to mesenchymal phenotype (CK-19 to vimentin positive) before differentiation into the neoislets. Taurine, Nicotinamide and NEAA (differentiation factors) resulted in the formation of islet like cell clusters (iLC) and the matured iLC stained for insulin. The *in vitro* generated neoislets were functionally viable when challenged with high glucose. The transplanted animals showed normalization in their glucose profiles, and cytoarchitecture of the pancreatic tissue. This study gives a scope for understanding the nature of the populating cells either the residual beta cells/progenitor cells of the pancreatic tissue with a diabetic insult.

5.7 Characterization and proliferation of pancreatic progenitor cells/stem cells to insulin secreting cells –Role of nutrients

The present work was focused on inducing differentiation of the pancreatic progenitors such as nestin positive cells (NPC) to insulin secreting cells (ISC) in presence of all trans retinoic acid (RA) with the combination of other mature factors in two weeks and its *in vivo* efficacy in reversing the diabetes in STZ induced mice model. Age dependent characterization and localization of NPC were carried out in the mice pancreatic tissue by immunolocalization technique.

The data showed an increased localization of NPC in the endocrine as compared to exocrine fraction. *In vitro*, RA in combination with growth factors increased the proliferation of Nestin/ ABCG2/ BrdU cells and upregulated nestin to Abcg-2 expression by about 2.5 fold (communicated). Combination of RA and Zn during differentiation showed increased C-peptide content by 3.5-4 times and the neoislets were insulin, Glut2 and Pdx1 positive. *In vivo* transplantation of neoislets in diabetic mice restored the body weights, blood glucose and plasma insulin values shown IPGTT response similar to control animals. The data suggests its potential in the management of diabetes.

5.8 Role of recombinant epidermal growth factor (REGF) factor in cell proliferation / differentiation using drug-induced diabetes, liver damage and in gastric ulcers

The therapeutic modality of rhEGF (developed by Bharat Biotech International limited) has been well documented in the treatment of diabetic foot ulcer, burns and skin grafts *in vitro*. Naproxene was used as the ulceration causing NSAID as it is used more frequently than other NSAIDs by arthritic patients and also because the naproxen-induced gastric antral ulcer model is suitable in the human situation where NSAID-induced gastric ulceration occurs mainly in gastric antrum and there are no reports available documenting the effects of rhEGF against the drug induced gastric ulcers. In this experimental model, an attempt was made to correlate the ulcer healing process by histopathology, Cox-2 immunolocalisation, TBARS assay and expression of the Cox-2 and TGF-Beta genes.

The protective effects of rhEGF have demonstrated as: (i) normalization of cyto-architecture of the gastric mucosa by 14 days; (ii) down regulation of the Cox-2 and TGF-beta genes; (iii) rhEGF negated the increased TBARS levels.

The present study forms the basis for reporting for the first time, the beneficial effects of rhEGF in the management of gastric ulcer healing induced with the use of anti-inflammatory drugs such as NSAID.

5.9 Inhibition of aldose reductase by curcumin

Accumulation of intracellular sorbitol due to increased aldose reductase (ALR2, AKR1B1) activity has been implicated in the development of various secondary complications of diabetes. In this study, it has been described that curcumin, a major active principle present in turmeric, inhibits ALR2 with an IC_{50} of 10 μ M in a non-competitive manner. Further, curcumin was able to suppress sorbitol accumulation in human erythrocytes under high glucose conditions. These results suggest that curcumin holds promise as an agent to prevent or treat diabetic complications.

5.10 Cataract and WNIN-Obese rat

NIN's studies have shown that WNIN-Ob and WNIN-GR/Ob rats are more sensitive to streptozotocin and galactose-induced cataract due to remarkable accumulation of sorbitol levels in the eye lens of these rats. Increased susceptibility of WNIN-Ob and WNIN-GR/Ob rats to galactose- and streptozotocin-induced cataract indicates that WNIN-Ob and WNIN-GR/Ob rats could be employed as osmotic stress-induced cataract models.

5.11 Importance of α -crystallin heteropolymer

Together with previous studies, rationale was provided for the existence of α -crystallin as a heteropolymer with 3:1 α A to α B ratio in the eye lens in terms of chaperone function, structural stability and susceptibility to post-translational modifications. Hence, heteropolymer with 3:1 α A to α B ratio might be vital for eye lens transparency under diverse conditions to prevent cataract.

5.12 Impact of polyunsaturated fatty acids (PUFA) on physical and molecular parameters associated with obesity using WNIN/GR-Ob rats

Compared to the diet with safflower oil (n-6 PUFA) alone, a blend of safflower and soybean oils (n-6/n-3 PUFA at 13/1) effectively reduced the condition of hepatic steatosis, while it enhanced skeletal muscle glucose uptake and increased the formation of functional HDL particles and thereby improving insulin-resistant condition of genetically obese glucose-intolerant rats of WNIN/GR-Ob strain. However, both the diets did not bring down the condition of obesity/adiposity.

Oral glucose tolerance test (OGTT) showed a significant decrease in the AUC glucose concomitant with a significant increase in AUC insulin levels in obese rats fed on a diet with a blend of n-6 & n-3 PUFA containing oils compared to obese rats fed on control diet.

6. EXTENSION AND TRAINING

6.1 *Assessment of knowledge, food preferences and practices among urban slum dwelling adolescent girls*

A school-based nutrition education programme involving 370 adolescent girls living in slums was carried out in two government schools in Hyderabad. A multimedia kit comprising print and audio-visual educational materials was used to educate the girls on matters relating to infant and young child feeding, balanced diet, importance of micronutrients like iodine, iron and vitamin A; family life education etc. after an exhaustive formative research. The study showed significant improvement in the knowledge levels of the adolescents. The multimedia kit is also used in the training programmes for health functionaries.

6.2 *MSc. Course in Applied Nutrition*

A full-time two year M.Sc. course in Applied Nutrition had been started at the institute in the year 2009 under the affiliation of NTR Health University, Vijayawada. The eligibility criteria for the course include MBBS /B.Sc. (Home Science/ Applied Nutrition/Biochemistry/Nursing)/ B.Sc with Nutrition as one of the major subjects.

A batch of 16 students were recruited in the programme. On the batch of an All India Entrance Examination, 16 students are being selected every year. It is proposed to increase the total intake to 32 from next academic year.

7. FOOD AND DRUG TOXICOLOGY

7.1 *Total Diet Study-Andhra Pradesh*

Diet is a source of toxicants, as well as nutrients. Availability of safe food is one of the essential public health functions of any country. It is not possible to totally eliminate contaminants in food supply, which passes through various stages in food chain. However, it is possible to compare their levels present in food in the manner they are consumed with their corresponding toxicological reference intakes such as the acceptable daily intake (ADI) or provisional tolerable weekly intake (PTWI).

Twenty two types of most commonly consumed foods in Andhra Pradesh belonging to eleven food categories were selected for the study based on National Nutrition Monitoring Bureau Report of 2004-06. The food samples were processed as they are consumed and analysed for fluoride, toxic metals, pesticide residues and mycotoxins. Fluoride was estimated in water, sorghum, rice, red gram dhal and spinach.

The estimated levels of fluoride in food composite were within the safe limits for any of the groups or category of individuals. Highest contributor of fluoride among the food items tested appeared to be water. Twelve food items were analysed for 19 pesticide residues. All the samples including water had one or the other of the 19 pesticides analysed. Children in the age group of 7-12 years were more at risk to Aldrin due to high intake of milk and rice. Analysis for mycotoxins revealed that in selected food items (Jowar, groundnut oil, red chillies and milk) they were present at significantly low levels or below detectable levels. Toxic metals namely lead and cadmium were analysed in 22 selected food items and water. Sorghum had highest concentration of lead and amaranth had highest level of cadmium. The estimated dietary intake of contaminants based on NNMB diet survey in all age groups, sedentary workers and pregnant women were uniformly much lower than ADI or PTWI.

7.2 *Development of PCR and RT-PCR based diagnostic kits for the detection and species specific identification of food and water borne pathogens*

Food borne illnesses due to microbial contamination are rampant. Sensitive technique using generic DNA sequence analysis is accurate, quick and less time consuming. Primers to *E.coli*, *Vibrio cholera*,

Vibrio parahaemolyticus, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus* were used and PCR based uniplex detection method was developed.

7.3 Biomarkers for transapacental genotoxic effects and their chemoprevention

Genotoxic effects of B(a)p exposure in tissues of turmeric fed rats(*In vivo*)

In vivo antigenotoxicity of turmeric feeding through diet was studied in WNIN rats that were treated with a single dose of carcinogen. There was significant reduction of DNA damage in tissues and reduction of malondialdehyde in rats given turmeric indicating protective effect of turmeric against genotoxicity.

7.4 Assessment of environmental lead exposure on infection and immunity

Subclinical toxicity due to low level of lead exposure is known to inhibit basal amino levulenic acid dehydratase (ALAD) activity. In this study, 120 children aged 6 months to 12 years were investigated for blood lead, Hb, serum Fe, Zn, Cu, Mg concentrations and basal ALAD. Majority of the cases were anemic accompanied with low Zn and Fe levels. Lead levels were higher in cases than in controls suggesting lead toxicity to be one of the main causative factors for anemia in children.

7.5 Detoxification of mycotoxins by lactic acid bacteria isolated from fermented sorghum and *Cassia tora*

The project on detoxification of mycotoxins by lactic acid bacteria isolated from fermented sorghum and *Cassia tora* was initiated based on earlier findings of reduction in mycotoxins by natural fermentation of mouldy sorghum with *Cassia tora* seeds. The main objectives of the present study were to isolate and identify lactic acid bacteria species present in fermented sorghum and assess their effect on mould growth and mycotoxin degradation potential using naturally contaminated and spiked sorghum samples.

Analysis of sorghum samples obtained from rural households for aflatoxin and fumonisins indicated that about 63% of the samples had fumonisin at levels ranging from 11-145g/kg and about 47% of the samples had aflatoxin at levels ranging from 1-42g/kg. Mycological examination indicated 64-100% of the seeds were infected with mould species belonging to *Aspergillus*, *Curvularia*, *Alternaria*, *Helminthosporium*, *Penicillium* with a predominance of *Fusarium* species. Out of 12 *Fusarium* isolates from sorghum, one isolate produced fumonisin at a level of 8.562µg/g.

Lactobacillus species isolated from fermented sorghum was observed to decrease growth of fumonisin producing strain of *Fusarium moniliforme* after 48 hours of incubation and reduced aflatoxin B₁ to 88% in liquid medium. Using a known mycotoxin reducing strain *Lactobacillus rhamnosus* GG a reduction of 79% in aflatoxin B₁ was observed in liquid medium and 77% when added to spiked sorghum samples at 0 hours of incubation and 24 respectively.

The present study indicated that *Lactobacillus rhamnosus* strain GG has good potential to reduce aflatoxin levels in contaminated grains such as sorghum. The extent of removal of aflatoxin by lactobacillus species isolated from fermented sorghum was observed to be comparable to that observed with *Lactobacillus rhamnosus* strain GG.

8. OTHERS

Total number of publications by scientists in national and international journals was over 36 with an average impact factor of 2.36.

I. COMMUNITY STUDIES

1. ASSESSMENT OF CURRENT SCENARIO OF FOOD LABELLING IN INDIA

The issues related to 'food labelling' are attracting more public and regulatory attention due to the increasing production and sale of food in pre-packaged form. Food labels are also potentially powerful tools of communication, which are often not considered when traditional channels are discussed to discourage consumption of unhealthy packed foods. In the Indian context, where overweight and obesity are assuming epidemic proportions and the resultant non-communicable diseases such as insulin resistance, diabetes mellitus, cardiovascular diseases and, cancers are on the rise; consumption of unhealthy foods can be discouraged to a large extent by effective food labelling practices, which give ample scope for the consumers to make informed choices.

Various countries are trying out ways and means to make labels more user friendly. Considering that India is no exception to this scenario, there is a felt need to document the current status of food labelling in India and to come up with appropriate approaches to make the food labels more user friendly.

Given this context, a study was carried out with the following objectives.

OBJECTIVES

1. To review the current status of food labelling scenario in India vis-a-vis global scenario and prepare a working paper.
2. To identify the issues to be considered for making the food labels more user-friendly by organising a workshop with all the stakeholders.
3. To assess the labels of various categories of pre-packaged foods in a market survey for examining their compliance with food labelling regulations.

METHODOLOGY

The existing food labelling regulations in India and those of various developed countries like the United States of America (USA), United Kingdom (UK), European Union (EU), Australia and New Zealand were obtained through systematic searches on their websites and published literature.

Scientific studies, reports and reviews relating to various aspects of food labelling (like regulations, consumer studies, consumer attitudes, different methods of labelling etc), published in the last one decade (1998-2008) were collected through a systematic search on the internet on authentic scientific websites like www.google.com, www.google.com, www.google.com, www.google.com, www.google.com and www.google.com using key words such as 'food labels', 'food labelling', 'food labelling practices', 'consumer studies + food labelling', 'food labels + consumer perceptions', 'nutrition labelling', 'mandatory labelling', 'GM Foods + Labelling', 'Organic foods + labelling'.

In addition, a market survey was conducted in Hyderabad to assess the compliance of existing food labelling regulations. For the survey, 815 pre-packaged foods including 109 imported pre-packaged foods from 14 countries were collected from various supermarkets, hypermarkets as well as small and medium food stores. These products were broadly classified into 15 categories such

as ready-to-eat foods, snack foods, confectionary, vegetable products, soups etc. Using a pre-tested proforma the labelling information on the above foods was also assessed.

The following are salient observations of review and market survey:

- ❖ The study indicated that the food labelling regulations in India are on par with those of the developed countries, but there are hardly any studies to examine the extent of compliance.
- ❖ In the Indian context, many studies indicated that food labels are not usually read by consumers while making food choices, this may be either due to low literacy rates or lack of nutrition knowledge and awareness.
- ❖ Market survey showed that food packages were 100% in compliance with displaying the basic regulatory requirements like name of the food, net weight, languages used etc.
- ❖ The labelling information such as instructions to use, storage conditions, 'use by date', and nutrition information per 100 g or per serving were observed in 77% of pre-packed foods.
- ❖ The '*nutrition and health*' claims were indicated on the 10% and 29% of labels respectively.
- ❖ The food labelling information was studied on 109 imported and 236 Indian pre-packaged foods, a significantly ($P < 0.05$) higher proportion of Indian foods (79%) consisted nutrient declaration for every 100 g or ml as against 68% for imported foods, which is a mandatory aspect as per PFA.
- ❖ Similarly, mandatory quality symbols (FPO, MFPO) were shown on more Indian products as against imported foods ($P < 0.001$).

Workshop deliberations and recommendations

The NIN-WHO Joint Workshop on 'Current scenario of Food Labelling in India' was organized during 24-25th June 2009 at National Institute of Nutrition, Hyderabad.

The objectives of the workshop were: i) to disseminate the findings of the literature review and market survey on the food labelling scenario in India and elsewhere, to the participants of the workshop, ii) to arrive at consensus on the strategies for making food labels user friendly in the Indian context.

The workshop was attended by different groups of stakeholders drawn from diverse sectors such as research organizations, industry, consumer societies, regulatory bodies, health functionaries, academics, professionals/scientific associations and media.

Conclusions and recommendations of the workshop

- ❖ Although the food labelling regulations in India are on par with those of the developed countries, there are hardly any studies to examine the extent of compliance.
- ❖ There is an urgent need to initiate such market studies in India and continue to carry them out on a regular basis in order to monitor the compliance by the food manufacturers.
- ❖ All food labelling regulations which are currently guided by many Acts and Orders (such as PFA, FPO, MFPO) need be unified into comprehensive general standards for labelling of pre-packaged foods for the benefit of all stakeholders. At the same time manuals for providing guidance to the industry, regulators and consumers should be prepared for effective implementation, regulation and use.
- ❖ In the Indian context, many studies indicated that food labels are not usually read by consumers while making food choices, this may be either due to low literacy rates or lack of nutrition awareness.

- ❖ In such a scenario perhaps there is a need to evolve and experiment symbol-based labelling of foods in India.
- ❖ There is a need to undertake nation-wide studies to understand the consumer knowledge, practices and behaviour related to food labels for formulating strategies to make food labels user-friendly.
- ❖ This is the time, to evolve permitted 'nutrition and health' claims that can be used by the food industry on the lines of those in countries such as USA, Singapore, Malaysia and Canada.
- ❖ Standardization of food labels and their contents on various categories of foods as well as on different types of packages may also help consumers locate the required information on labels.

2.EFFECT OF HEALTH AND NUTRITION EDUCATION ON THE LIFESTYLES AND PHYSICAL ACTIVITIES AMONG URBAN ADOLESCENTS

The increase in the prevalence of obesity and overweight has been reported to be rapid even among children and adolescents in both developed as well as developing countries. The most significant long-term consequence of childhood / adolescent obesity is the persistence of associated health risks even during adulthood.

It is very important that the problem of overweight and obesity is addressed early in childhood and adolescence for two reasons; firstly, it is relatively easier to inculcate healthy lifestyle practices during this period and secondly, the outcomes are better among those adults who are not obese when they were young. If the children develop healthy lifestyles and nutrition habits, they are likely to become a foundation of their adult behavior. Nutrition education at young age is the key element in promoting healthy lifestyles and eating behaviours. The school environment is most appropriate to impart health and nutrition education, encourage participation in physical activities and inculcate healthy lifestyle practices. Young children and adolescents are more receptive and could act as 'change agents' passing on the messages to other members of the family. Thus, schools form the entry point for initiation of appropriate interventions, where students, teachers and parents can become stakeholders.

Therefore, the present study was carried out to design a multi-component health and nutrition intervention strategy and implement among adolescents for promotion of physical activities and healthy lifestyle practices among adolescents in the twin cities of Hyderabad and Secunderabad. This phase II study was carried out in continuation of the phase I study titled 'Prevalence and determinants of overweight and obesity among urban adolescents in Andhra Pradesh' conducted during 2006-07 with the financial and technical support of WHO with the following objectives:

OBJECTIVES

1. To assess the prevalence of overweight and obesity (BMI) and body fat percentage among 10-15 year school children in select schools of Hyderabad at baseline and end of 8 months of intervention.

2. To assess the knowledge and behaviour of children and their opinion leaders (parents and teachers) using quantitative and qualitative research techniques,
3. To assess the current status of health promotion in schools and the availability of enabling environment (Policies to regulate sale of soft drinks and junk foods in school campus, Imparting nutrition education, promotion of physical activities, etc.), and
4. To develop multi-component health and nutrition intervention strategy and implement and assess its effect on the lifestyles and physical activities among urban adolescents.

METHODOLOGY

It was a prospective, randomized school intervention study. A total of 10 schools were randomly selected from the list of schools that had enabling environment (considering various parameters like presence of play ground, physical education teachers, specific time allocation for outdoor games, willingness of management). Five in each of these ten schools were randomly allocated to intervention and control groups.

A total of about 1362 adolescents from grades VI to IX were recruited for the present study from the selected 10 schools. All the science and physical education teachers who were teaching in the above grades were also included in the study. Data on knowledge, perceptions and practices related to nutrition and lifestyle factors was collected using knowledge and practice assessment questionnaires as well as food frequency questionnaires at baseline and after intervention. Anthropometric measures were also taken both at baseline and after 8 months of interventions. In addition to the above, qualitative methods like Focus Group Discussions (FGDs) and in-depth interviews were also conducted with adolescents and their teachers at baseline.

The baseline study revealed that the socio-demographic profile of the adolescents in both control and intervention schools was almost similar. The FGDs with adolescents revealed that they had basic knowledge of the relationship between nutrition, physical activity and body weight. However, the overweight and obese adolescents were consuming less of fruits and vegetables and liked playing indoor/video games and watch television. The overweight /obese children also had relatively less knowledge on the relationship between overweight/obesity and non-communicable diseases. In-depth interviews with teachers revealed that the science teachers had good knowledge on topics like nutrition during adolescence, need for physical activity and consequences of obesity. However, such knowledge among physical education teachers was limited and scanty.

Various intervention strategies were developed based on the findings of phase I study, baseline data and FGDs and in-depth interviews. Multi-component interventions were prepared in the form of flip charts/folders, education material, an educational film and lectures for the adolescents and their science and physical education teachers. Orientation training of science and physical education and sensitization of other stakeholders (like school managements and parents) were also carried out.

In the control group of schools, only education material (leaflets) on health and nutrition were distributed (Table 1). The intervention was carried out between November 2008 and August 2009 except during summer vacation i.e., March-May 2009. However, take-home educational material was given for the use of students even during the summer vacation but no monitoring was possible during this time.

The following are salient observations of the study:

- ❖ The educational intervention resulted in a significant increase in the health and nutrition knowledge of the adolescents in the intervention group compared to the control group. In

addition favourable change was also observed in the perceptions, behaviours and practices among adolescents of intervention group. In general, use of sunflower oil as cooking oil was almost same in both the groups (intervention group: 76.5%; control group: 70.1%), while the use of groundnut oil was higher among adolescents of control group (22.1%) as compared to intervention group (14.0%)

- ❖ The proportion of adolescents consuming vegetarian or non-vegetarian diet was not significantly different in both the groups at baseline. However, the proportion of adolescents who are consuming non-vegetarian diet was significantly higher at baseline (63.59%) compared to endline (58.2%) in the experimental group.
- ❖ However, no significant changes were observed in the mean weight and BMI of the children in both the groups. The findings are very similar to the earlier studies that reported effects on only knowledge, attitudes and behaviour.
- ❖ The mean fat fold thickness at triceps, biceps, sub-scapular, supra-iliac regions and sum of the skin folds considerably declined among adolescents of intervention group compared to control group after a period of 8 months of intervention.
- ❖ The mean systolic pressure was marginally low at endline (101.3 mm of Hg) compared to baseline (102.5 mm of Hg).
- ❖ There was no change in the mean diastolic pressure from baseline (64.3 mm of Hg) to endline (65.1 mm of Hg).
- ❖ There was no change in the prevalence of hypertension (≥ 140 mm of Hg and/or ≥ 90 mm of Hg) between the two points of time (0.7% Vs 1.0%).
- ❖ The daily consumption of fatty foods by adolescents was reported to be significantly lower after 8 months of intervention (6.3%) as compared to baseline (11.7%).
- ❖ The practice of healthy lifestyles and physical activities among adolescents of intervention group increased significantly after 8 months of intervention. The healthy lifestyle practices included physical exercises (intervention: 57.5% Vs 81.7%; control: 48.7% Vs 71.2%), participation in household activities (intervention: 86.9% Vs 99.1%; control: 81.2% Vs 91.2%), playing out door sports and games (intervention: 37.7% Vs 72.4%; control: 33.7% Vs 58.7%).

Table1: Multi-component H & N Intervention

Control	Experimental
Distributed Health & Nutrition education Material (folders/leaflets) to the adolescents (as per dietary guidelines for Indians) on - Healthy diet - Physical activity - Lifestyles - Overweight/obesity/NCDs	1.Sensitized school authorities 2.Distributed Health & Nutrition education material 3.Oriented the teachers, who in turn educated school children 4.WHO-India Health Promoting Schools framework was provided 5.Helped in the revision of school curriculum for more sports and games periods 6.Discouraged sale of unhealthy foods in school canteens etc. 7.Counselled selected parents in groups

- ❖ The proportion of adolescents with high risk behaviours was very low in both the groups in control as well as in intervention group. However, after 8 months' intervention, the risk behaviours significantly declined in experimental group, while a marginal change was observed in the control group.

CONCLUSIONS AND RECOMMENDATIONS

- ❖ The results provide several leads to encourage and implement multi-component interventions to prevent and control overweight and obesity among adolescents.
- ❖ The present study reiterates the need for nation-wide intervention studies to prevent and control overweight and obesity among adolescents.
- ❖ The role of physical activity, games and sports are to be further emphasized in the schools by teachers and managements.
- ❖ The focus should be given on promotion of healthy food and lifestyle practices and regulated TV viewing.
- ❖ Enabling environment at schools and home should be made available to promote and practice regular physical exercises, while participation in sports and games should be made mandatory in the school curriculum.
- ❖ Health and Nutrition education should be imparted regularly in all the schools and colleges as part of their curricula.
- ❖ Impact on the anthropometric indices and decrease in the prevalence of overweight and obesity could perhaps be observed if the present study is continued for a further period of one year.



II. BEHAVIOURAL SCIENCES

THE EFFICACY OF AN INTEGRATED FEEDING AND CARE INTERVENTION AMONG 3-15 MONTH OLD INFANTS IN ANDHRA PRADESH, INDIA

Despite the vast expenditure in India on programs for improving young children's nutrition and health, recent surveys (NNMB, 2006) indicated poor dietary intakes by under three-year-olds even in families where the adults met their daily dietary requirements. The prevalence of stunting (37.3%) and underweight (40.4%) were found to be high (NHFS-3) in the area where the current study was implemented.

A study on the effectiveness of an educational intervention to promote adequate and sustainable complementary feeding that was undertaken in Haryana indicated that it is possible to improve complementary feeding practices through existing services, but the impact of this on physical growth was limited. This study did not address aspects likely to affect caring such as maternal depression and home environment.

It is not only important to educate caregivers about what kinds of food to give young children but also 'how' to give the nutritious food. The present study included 'responsive feeding', i.e., responding to the cues of the child while feeding, understanding what the child is conveying, so that the feeding situation becomes a happy time where feeding and learning can also take place and leading to a more positive impact on growth and development. Additionally, the present study also included child stimulation for improving cognitive development. This efficacy intervention trial was carried out under the aegis of the Indo-US program on MCHDR with the hypothesis that 'teaching care givers responsive feeding and play strategies through a home visiting intervention will have a greater impact on their children's dietary intake, growth and development than complementary feeding educational intervention or the standard of care'.

The specific objectives were:

- ❖ To develop culture-appropriate home-based nutrition education intervention to improve feeding skills and caring behaviours of care givers through breast-feeding and complementary feeding messages.
- ❖ To develop culture-appropriate home-based behavioural intervention to improve responsive feeding, and caring behaviours and skills of care givers to stimulate psychosocial development of infants.
- ❖ To implement a randomised controlled behavioural intervention trial for assessing the impact of nutrition education on breast feeding and complementary feeding by care givers, and responsive feeding and stimulation of developmental skills on the growth and psychosocial development of infants.
- ❖ To evaluate whether the interventions result in positive changes in caregiver responsiveness and feeding behaviors, and

- ❖ To evaluate whether interventions improve energy intakes and enhance growth and development among young children (3 to 15 months) assigned to the experimental groups.

METHODOLOGY

Cluster randomized educational intervention among 600 mothers from 60 villages in Nalgonda district of Andhra Pradesh, India, aimed to improve feeding, growth and child development through follow-up of children from age 3 through 15 months.

The 3 arms trial had control group (CG) receiving routine care through the Integrated Child Development Services, the Complementary Food Group (CFG), receiving WHO recommendations on IYC foods, and the Complementary Feeding-Responsive Feeding and Play Group (CFRF & PG), receiving Complementary feeding recommendations plus skills on responsive feeding and psychosocial stimulation. The twice-a-month intervention using flip charts was delivered by trained village women to caregivers in their homes.

Variables that were assessed included:

1. Breast milk frequency schedule
2. 24 hour diet recall + food weighed using scales with 2 g accuracy
3. Food frequency schedule for selected micronutrient- rich foods
4. Maternal knowledge, beliefs & behaviors interview schedule on child health & development.
5. Previous week morbidity schedule – every month
6. CES-D maternal depression scale
7. Maternal autonomy interview schedule
8. Maternal self esteem scale & locus of control scale
9. Demographic, household & socio-economic status schedule
10. Home inventory
11. Anthropometry: Infant supine length, weight - monthly from 4 to 15 months
12. Maternal height and weight
13. Maternal & infant hemoglobin
14. Denver developmental screening test
15. Bayley II scales of infant development
16. Videos of feeding styles
17. Developmental stimulation program through play

The main outcomes were dietary intake and infant growth, home stimulation, and assessment of the child's development. Intervention was delivered by trained village mothers twice a month using flip charts and discussion that was based on formative research.

RESULTS

Baseline results indicated no significant differences in outcome variables between Groups.

Dietary Adequacy

- ❖ After 12 months of intervention, the median intake of energy, protein, vitamin A, calcium, iron and zinc were significantly higher in the complementary food group and complementary feeding-responsive feeding and play group children compared to the control group.
- ❖ Mean nutrient intake by stunted and undernourished children ($< -2Z$) was low overall, but with significant differences between groups for protein, energy, fat, vitamin A & C, niacin, iron and folic acid. Lowest intakes were observed by the control group and the highest by the Complementary Food Group (CFG) with no differences between the intervention groups for nutrients such as energy, niacin, vitamin C and iron.

Growth

After adjusting for significant independent variables like maternal height, depression, caste, assets and housing at baseline, only the height for age R2 was significant with a higher coefficient ($B=0.17^*$) among children in CFG compared against the Control Group (Reference). None of the other nutritional indices were significantly different between Groups.

Development

The mean mental development scores were significantly different between the Groups ($p=0.018$) using ANOVA at 15 months. Mental index scores were found to be significantly greater among children in the complementary feeding-responsive feeding and play group compared to the control group, and even to the children in the complementary food group. These differences continued to be significant after controlling for maternal education, assets, and child height for age. However, there were no significant differences in the motor development index scores between the three groups.

CONCLUSION

The 12 month educational intervention through pictorial flip-charts based on the findings of a comprehensive formative research, could improve dietary intakes, growth and development of children. The children in the control group were significantly worse off in all the outcome indicators viz., dietary intake, growth, and mental development. Among the two intervention groups, the dietary intake was similar, but height for age of the complementary group was significantly higher compared even to the complementary feeding-responsive feeding and play group, apart from the control group. Mental index scores were significantly higher among children in the complementary feeding-responsive feeding and play group compared to the CFG apart from the control group.

The study also underlines the importance of studying intervening variables that can independently confound/ modify the effects of any intervention. In the present study, the variables found to be significant were maternal height, depression, caste, type of housing, assets and maternal education.



III. MICROBIOLOGY AND IMMUNOLOGY

1. EVALUATION OF ALLERGENICITY POTENTIAL OF ANTI-HP VACCINE IN BALB/C MICE - INTRA PERITONEAL AND INTRA NASAL

Globally, Cervical Cancer is the second most common cancer amongst women, after breast cancer. In India, 132,000 new cases are diagnosed and 74,000 women die due to this cancer every year. Cervical cancer is caused by Human Papilloma Virus (HPV). Globally, HPV 16, 18, 45 and 31 are the four most common oncogenic HPV types. HPV 16 and 18 together account for 70% of cervical cancer cases while HPV 45 and 31 account for a further 10% of cases. HPV 6 and 11 are not known to cause cancer; but are responsible for benign genital warts.

Infection and vegetative HPV growth are absolutely dependent upon a complete program of Keratinocytes differentiation. Virus infects primitive basal keratinocytes, probably targeting stem cell. It is a replication strategy in which viral DNA replication and virus assembly occurs in a cell that will terminally differentiate and die by natural causes. Thus, there is no viral induced cytolysis and necrosis, and therefore no inflammation.

The most promising means of controlling and reducing the incidence of cervical cancer would be by checking Human papilloma Virus infection. It is well established that, HPV L1 VLP vaccine induced high concentrations of neutralizing Abs to L1, and virtually all subjects in the vaccine trials demonstrated good seroconversion. The L1 VLP is almost identical both morphologically and antigenically to the infectious viral particle.

HPV L1 VLP vaccine induced high concentrations of neutralizing antibodies to the virus. Assembly of capsomeres into VLP is a complex process which requires difficult purification processes, which are both cost and labour intensive.

Dr Denise Nardelli Haefliger at the University of Lausanne, Switzerland, has developed Salmonella vectored vaccine against HPV using oral route that induces mucosal immunity in mice. Indian Immunologicals Limited has further developed the recombinant vaccine with Salmonella typhi Ty21a that expresses the major capsid protein (L1) of HPV16 & 18. These have been shown to induce high titers of neutralizing antibodies in mice after intra-nasal immunization with the live recombinant bacteria.

In view of the efficacy reported in mice model, Indian Immunologicals Ltd. is manufacturing the vaccine for human use for the first time in India. As per the DBT/DCGI (Schedule 'Y') guidelines, the recombinant vaccine is required to undergo Pre-clinical Allergenicity trial. In addition, the vaccine contains live recombinant Salmonella organism that expresses HPV 16 & 18 major capsid protein and hence requires RCGM approval.

METHODOLOGY

Intra Peritoneal

The animals were selected, conditioned, and exposed to the test compound intra peritoneal on 0th day and 7th day (Table 2).

Table 2. IP Dosage

S. No	Test Group	Test Compound	Dose per mice (ml)
1.	VC	PBS	0.25
2.	OVA	2% Ovalbumin	0.25
3.	TD	1x10 ³ CFU/Dose/20gm mice	0.25
4	5X TD AD	5x10 ³ CFU/Dose/20gm mice	0.25

VC=Vehicle Control, TD=Therapeutic dose, AD=Average Dose, PBS-Phosphate buffer solution, OVA=Ovalbumin

Blood samples were collected into microcentrifuge tubes from retro orbital plexus region of mice using capillaries at 0 day (Base line), 14th and 28th day respectively for determination of IgE and IgG levels in serum samples analyzed by ELISA.

Intra Nasal

The animals were selected, conditioned, and exposed to the test compound intra nasal 4 week intervals (3 doses 0th day, 4th week and 8th week) (Table 3).

Table 3. Intra nasal Dosage

S.No	Test Group	Test Compound	Dose per mice (µl)
1	VC	PBS	20
2	OVA	2% Ovalbumin	20
3	TD	1x10 ⁹ CFU/Dose/20gm mice	20
4	5X TD AD	5x10 ⁹ CFU/Dose/20gm mice	20

Blood samples were collected into microcentrifuge tubes from retro orbital plexus region of mice using capillaries at 0 day (Base line), 28th, 56th, 63rd and 78th day respectively for determination of IgE and IgG levels in serum samples analyzed by ELISA.

The administration of test compound was done after anesthetizing the animals with 0.1 ml of ketamine hydrochloride, xylaxine hydrochloride and Diazepam mixture.

RESULTS

- ❖ No pre-terminal deaths were recorded during the test compound exposure.
- ❖ All the animals were alive and no abnormal clinical signs were reported in the animals exposed to test material till the end of the experiment.

- ❖ Signs of allergenicity, that is hair loss, lacrimation, nasal excretion etc. were not observed in the animals that received test material in various concentrations.
- ❖ There was no serum IgG and IgE antibody response to intra peritoneal HPV 16 and HPV 18 antigens at all the time points (Tables 4 to 7 and Figures 1 to 4).

Anti-HPV Allergenicity HPV 16 IgG- Intra Peritoneal

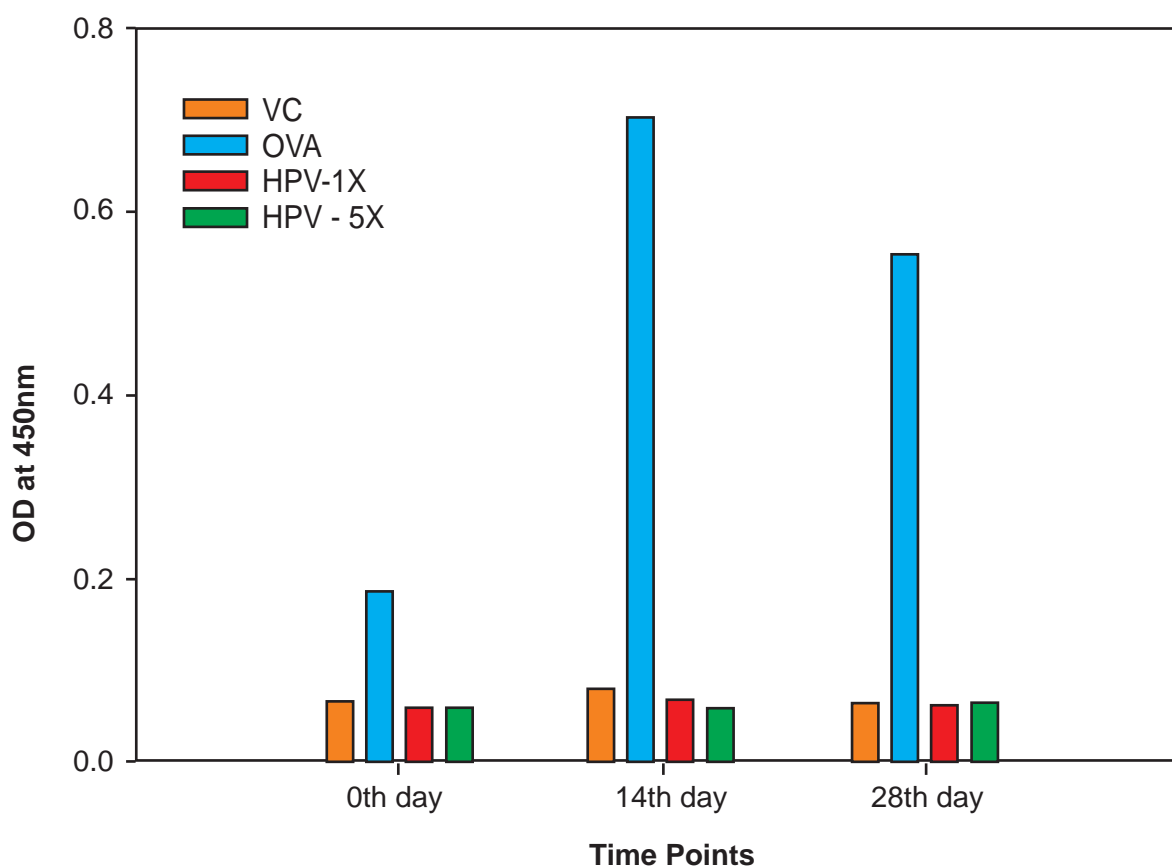
All values are in 1: 100 dilutions

Table 4. Optical density at 450 nm – HPV 16 IgG - IP

Groups	0th day	14th day	28th day
VC	0.066 (5)	0.078 (6)	0.066 (3)
OVA	0.187 (3)	0.701 (5)	0.55 (3)
HPV 1X	0.061 (6)	0.069 (6)	0.063 (3)
HPV 5X	0.061 (6)	0.059 (6)	0.066 (3)

() No of samples

Figure 1. HPV 16 IgG IP



Anti-HPV Allergenicity HPV 18 IgG – Intra Peritoneal

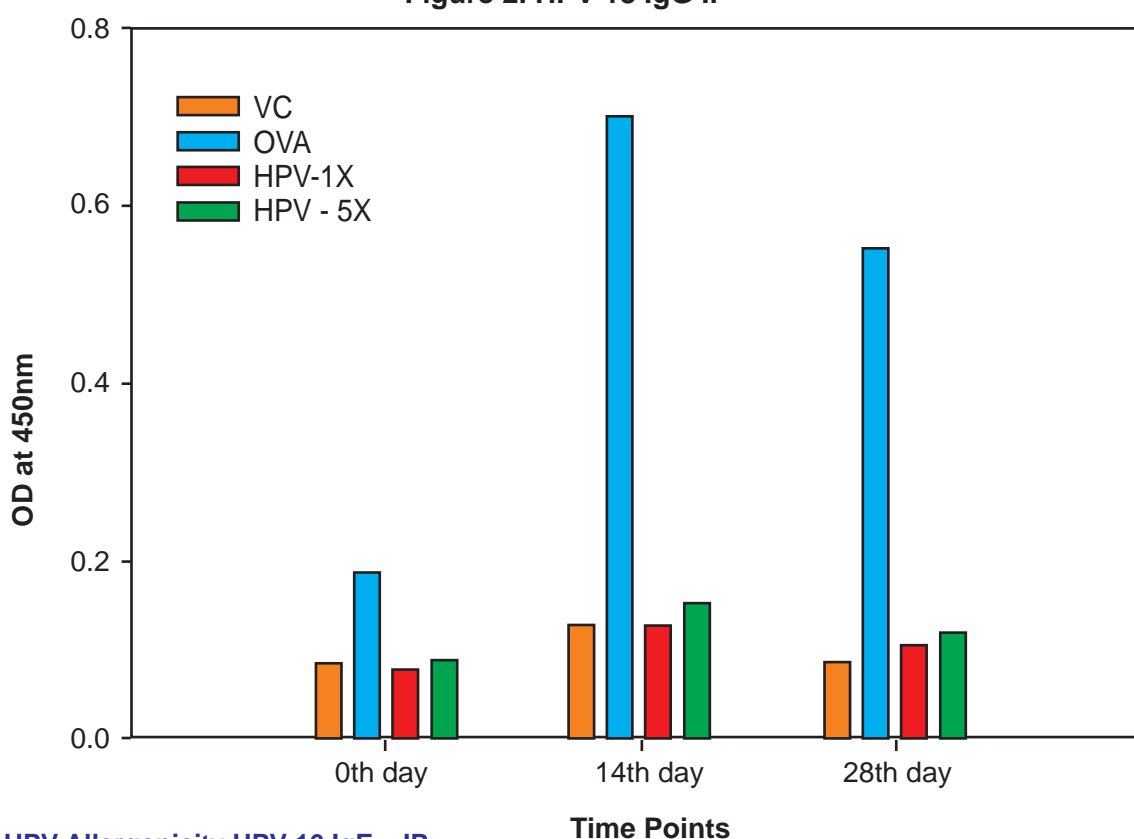
All values are in 1: 100 dilutions

Table 5. OD at 450 nm – HPV18 IgG - IP

Groups	0th day	14th day	28th day
VC	0.084 (5)	0.128 (6)	0.084 (3)
OVA	0.187 (3)	0.701 (5)	0.55 (3)
HPV 1X	0.077 (6)	0.128 (6)	0.105 (3)
HPV 5X	0.087 (6)	0.151 (6)	0.12 (3)

() No of samples

Figure 2. HPV 18 IgG IP



Anti-HPV Allergenicity HPV 16 IgE – IP

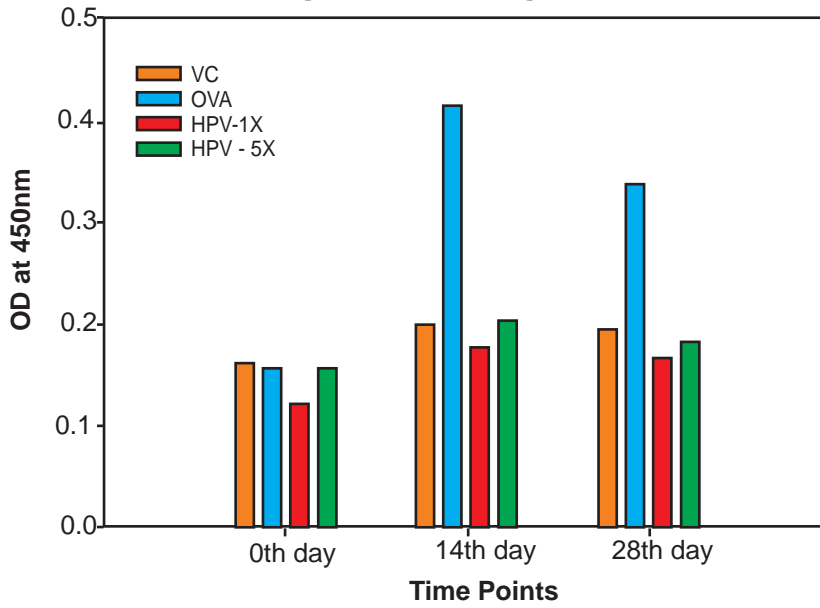
All values are in 1: 50 dilution

Table 6. OD at 450 nm – HPV 16 IgE - IP

Groups	0th day	14th day	28th day
VC	0.162 (2)	0.199 (2)	0.195 (2)
OVA	0.156 (3)	0.416 (5)	0.339 (3)
HPV 1X	0.122 (2)	0.177 (2)	0.167 (2)
HPV 5X	0.157 (2)	0.204 (2)	0.183 (2)

() No of samples

Figure 3. HPV 16 IgE IP



Anti-HPV Allergenicity HPV 18 IgE-Intra Peritoneal

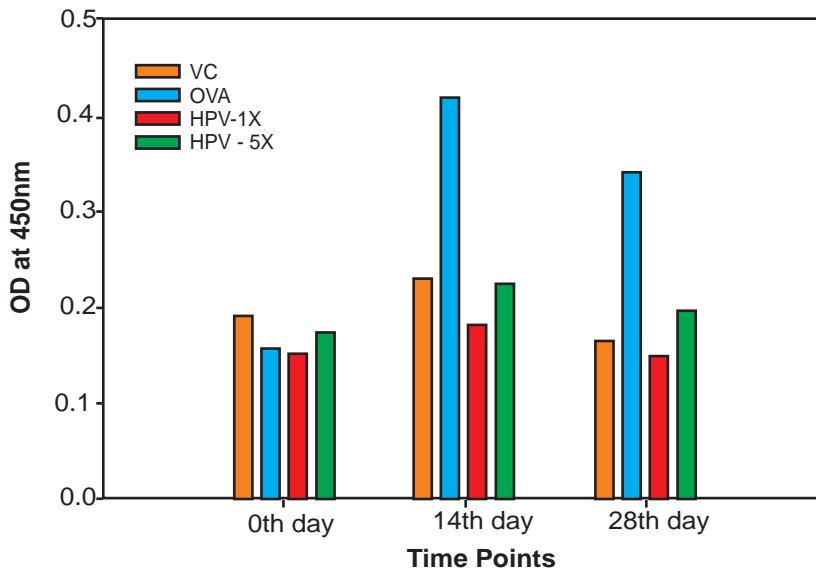
All values are in 1: 50 dilution

Table 7. OD at 450 nm – HPV 18 IgE - IP

Groups	0th day	14th day	28th day
VC	0.191 (2)	0.229 (2)	0.164 (2)
OVA	0.156 (3)	0.416 (5)	0.339 (3)
HPV 1X	0.15 (2)	0.181 (2)	0.148 (2)
HPV 5X	0.172 (2)	0.223 (2)	0.196 (2)

() No of samples

Figure 4. HPV 18 IgE IP



CONCLUSION

No symptoms of allergenicity were observed in animals that received the recombinant HPV vaccine and does not seem to have allergenicity potential when administered by intra peritoneal route.

Anti-HPV Allergenicity HPV 16 IgG-Intra Nasal

(All values are in 1:100 dilutions)

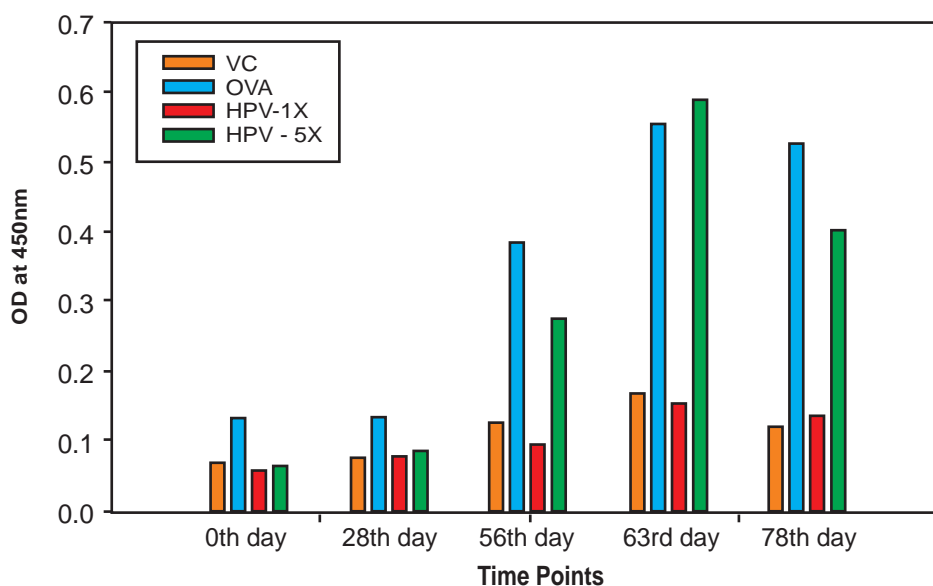
Table 8. OD at 450 nm – HPV 16 IgG - IN

Groups	0th day	28th day	56th day	63rd day	78th day
VC	0.067 (6)	0.075 (5)	0.127 (5)	0.168 (5)	0.121 (2)
OVA	0.135 (3)	0.135 (3)	0.385 (6)	0.555 (5)	0.528 (3)
HPV 1X	0.059 (6)	0.078 (6)	0.094 (6)	0.153 (6)	0.137 (2)
HPV 5X	0.063 (6)	0.085 (6)	0.277 (6)	0.589 (6)	0.403 (2)

() No of samples

VC =Vehicle Control, OVA=Ovalbumin

Figure 5. HPV 16 IgG IN



Anti-HPV Allergenicity HPV 18 IgG-Intra Nasal

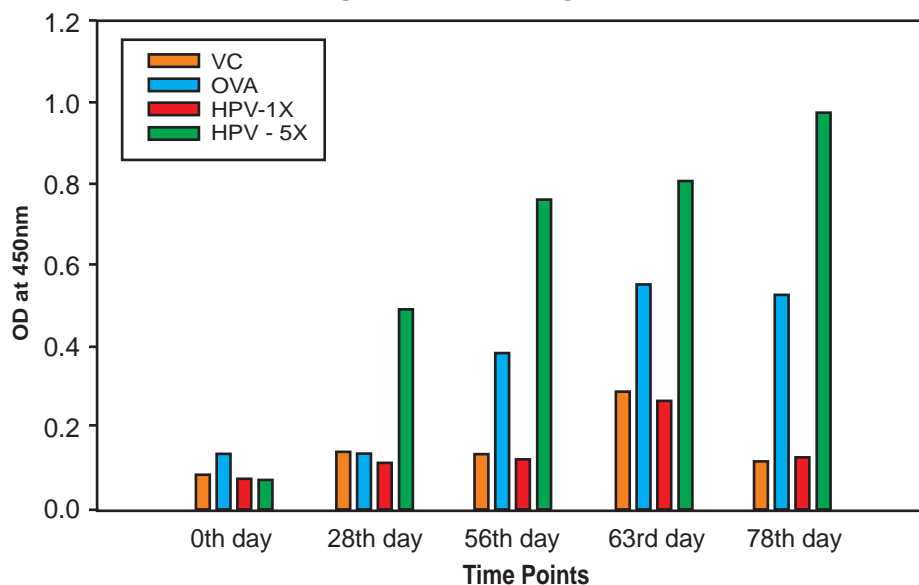
(All values are in 1:100 dilutions)

Table 9. OD at 450 nm – HPV 18 IgG - IN

Groups	0th day	28th day	56th day	63rd day	78th day
VC	0.083 (5)	0.14 (6)	0.137 (5)	0.22 (5)	0.121 (2)
OVA	0.135 (3)	0.135 (3)	0.385 (6)	0.555 (5)	0.528 (3)
HPV 1X	0.077 (6)	0.117 (6)	0.122 (6)	0.267 (6)	0.128 (3)
HPV 5X	0.074 (6)	0.492 (6)	0.761 (6)	0.808 (6)	0.975 (2)

() No of samples

Figure 6. HPV 18 IgG IN



Anti-HPV Allergenicity HPV 16 IgE-Intra Nasal

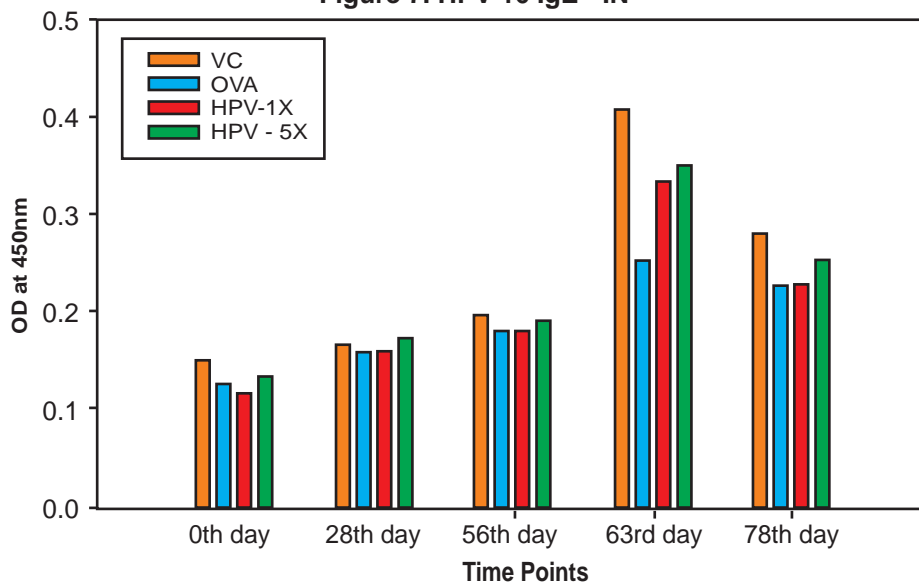
(All values are in 1:50 dilutions)

Table 10. OD at 450 nm – HPV 16 IgE - IN

Groups	0th day	28th day	56th day	63rd day	78th day
VC	0.149 (2)	0.165 (2)	0.197 (2)	0.407 (2)	0.28 (1)
OVA	0.126 (3)	0.159 (3)	0.18 (6)	0.253 (5)	0.227 (3)
HPV 1X	0.117 (2)	0.158 (2)	0.18 (3)	0.332 (2)	0.227 (2)
HPV 5X	0.134 (2)	0.173 (2)	0.191 (2)	0.351 (2)	0.252 (2)

() No of samples

Figure 7. HPV 16 IgE - IN



RESULTS

- ❖ Three (2 animals from 5XHPV and 1 animal from VC groups) pre-terminal deaths were recorded during the test compound exposure.
- ❖ All the animals were alive and no abnormal clinical signs were reported in the animals exposed to test material till the end of the experiment.
- ❖ Signs of allergenicity, i.e., lacrimation, nasal excretion etc. were not observed in the animals that received test material in various concentrations.
- ❖ Alopecia or hair loss was observed in animals treated with Ovalbumin at the end of the experiment.
- ❖ *Antibody Titers:* There was an increase in IgG antibody titers to intra nasal HPV 16 and HPV 18 antigens at 5x dose after the primary and secondary boosters (Table 8 and 9, Figs.5 and 6). The levels of IgE antibody titers to intra nasal HPV 16 and HPV 18 antigens increased non-significantly after the 2nd booster but were comparable with VC at all the time points and it declined during the recovery period (Tables 10 and 11, Figs. 7 & 8).

Anti-HPV Allergenicity HPV 18 IgE-Intra Nasal

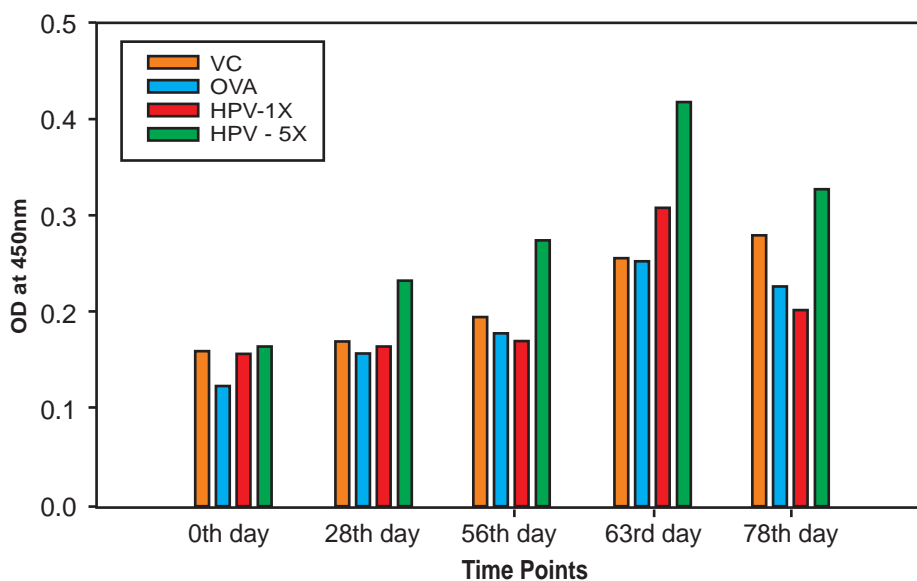
(All values are in 1:50 dilutions)

Table 11. OD at 450 nm – HPV 18 IgE - IN

Groups	0th day	28th day	56th day	63rd day	78th day
VC	0.16 (2)	0.171 (2)	0.196 (2)	0.256 (2)	0.279 (1)
OVA	0.126 (3)	0.159 (3)	0.18 (6)	0.253 (5)	0.227 (3)
HPV 1X	0.156 (2)	0.165 (2)	0.17 (3)	0.307 (2)	0.203 (2)
HPV 5X	0.166 (2)	0.233 (2)	0.275 (2)	0.416 (2)	0.327 (2)

() No of samples

Figure 8. HPV 18 IgE IN



CONCLUSIONS

In this study, 3 pre-terminal deaths were observed. One mouse died within 3 hours of drug exposure and a second one died on the 3rd day of the 1st dose in the 5X group. One mouse died in vehicle control group on the 24th day of 1st dose.

There was significant decrease in body weights of HPV 5X group animals on the 10th day and 21st day of drug exposure compared to VC group ($P < 0.05$). There was no significant difference in live phase, physical activity, and allergenicity potential between the control and test groups throughout the study period among the survived animals.

The given HPV vaccine (HPV 16 and HPV 18 VLPs) is immunogenic only at 5x concentration. At 1x concentration the vaccine failed to stimulate any IgG response when given intra nasally. The vaccine does not seem to have allergenicity potential at 1x concentration. At 5x concentration the IgE response was comparable to vehicle control.

2. MICRONUTRIENT STATUS IN HIV INFECTED POPULATION IN INDIA

In HIV-AIDS, nutritional status or better nutrition cannot protect against infection, however, the initial nutritional status if compromised would result in greater viral load and probably promote disease progression. There would be a greater nutritional need during the acute phases and also during inter current infections and febrile phases. Deficiency of micronutrients like vitamin A, iron, zinc, selenium all play significant role in disease progression and also during anti-retroviral treatments. Several studies have favored specific nutrient supplementation to reduce mother to child transmission. Micronutrients play a critical role in the proper functioning of the immune system. Changes in lipid profiles have been described in literature in asymptomatic HIV infected population.

The serum samples were collected for a project entitled Integrated Biological and Behavioral Assessment (IBBA), sponsored by Bill and Melinda Gates Foundation; on the prevalence of HIV and STI's in six high HIV prevalence states in India. In a subsample nutrients such as vitamin D, zinc, magnesium, copper and iron in HIV positive and HIV free Female sex workers (FSW) population were evaluated.

MATERIALS AND METHODS

The serum samples were collected in the IBBA project were categorized into 4 groups with 100 samples in each group. Group1 : HIV negative and STI negative. Group2: HIV positive and STI positive, Group 3: HIV negative and STI positive, Group 4: HIV positive and STI negative. The serum samples were analyzed for vitamin D, zinc, magnesium, copper, iron and albumin. Vitamin D and serum albumin were estimated in fresh samples. Zinc, magnesium, copper and iron were analyzed by AAS. Vitamin D was estimated by using RIA kit. Serum albumin was estimated by colorimetric method.

RESULTS

Roughly about 90% of the FSW's were deficient in zinc and iron. FSW HIV infected asymptomatic population had similar serum concentrations of Zinc, magnesium, copper and iron as FSW without HIV infection (table 12). The mean \pm SE of vitamin D and serum albumin were 23.64 ± 0.83 ng/mL and 3.52 ± 0.06 g/dL respectively in HIV infected asymptomatic FSW population. About 80% of the HIV infected FSW population had vitamin D deficiency, when compared to normal women of low socioeconomic status who had a mean vitamin D of 33.8 ± 9.1 ng/ml.

Table 12. Nutrients in FSW

Elements	FSW	HIV & STI +ve	HIV +ve	STI +ve
	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Magnesium (1.5-2.5 mg/dL)	2.28 ± 0.040	2.40 ± 0.034	2.06 ± 0.024	2.51 ± 0.038
Copper (85-155 μ g/dL)	146.8 ± 3.865	146.6 ± 4.636	135.3 ± 3.61	149.9 ± 5.94
Zinc (70-150 μ g/dL)	34.82 ± 1.609	44.59 ± 2.646	57.99 ± 1.408	49.87 ± 1.561
Iron (133-180 μ g/dL)	82.56 ± 5.12	62.15 ± 3.818	57.98 ± 5.164	93.72 ± 9.384

CONCLUSIONS

Iron and zinc deficiency appears to be widely prevalent amongst FSW's with or without HIV infection. Vitamin D deficiency could also be a major problem in this population. Iron, zinc and Vitamin D are very critical for optimal immune response and intact immune response reduces HIV transmission. Deficiency of these micronutrients might increase transmission of HIV amongst FSW's.

3. INTEGRATED BEHAVIORAL AND BIOLOGICAL ASSESSMENT – ROUND II

The project was implemented by the FHI, ICMR and research partners in close collaboration with National AIDS Control Organization (NACO) and State AIDS Control Societies (SACS). The second phase of the survey was initiated in February 2009. Eight districts were selected as study areas in A.P based on socio-cultural region and size of the high-risk population. High risk population include female sex workers (FSW), men who have sex with men (MSM) or Hijra and male clients of female sex workers.

AIMS AND OBJECTIVES

1. To assess outcomes and impact of HIV interventions in Avahan project districts.
2. To measure major outcomes and impact of interventions funded by the BMGF under the Avahan India AIDS Initiative by collecting behavioural and biological trend data in populations targeted by the interventions.

3. To make available data for estimating size of populations targeted by the project.
4. To make available information for modeling the impact of the intervention.

MATERIALS AND METHODS

Participants were assessed for Syphilis, HIV prevalence, HSV2, NG and CT. Serum samples were analyzed for Syphilis by RPR and TPHA; HIV prevalence and HSV2 by ELISA. Urine samples for NG and CT were analyzed using Transcription Mediated Amplification method. Genital ulcer swabs were tested by mPCR test for TP, HD and HSV.

WORK DONE DURING THE YEAR

Blood and urine samples (n=6900) and genital ulcer swabs (n=26) were collected from the above study participants and were analyzed for Syphilis, HIV prevalence, HSV2, as per GLP. Conducted RPR test for syphilis, performed ELISA's for HIV and HSV-2; TPHA was carried out to confirm syphilis. Conducted training programmes for field and district laboratory technicians and maintained stock inventory. Made monitoring visits to the field and district laboratories. Extended external proficiency services to all district laboratories and given the feed back to them with appreciation. Maintained QC (56 serum samples for each category per each district) in the laboratory. Transported all serum, urine samples and ulcer swabs collected from study participants to NARI, Pune. Successfully completed the survey and analysis of biological samples.



IV. CLINICAL STUDIES

1. VITAMIN D STATUS IN COMMON INFECTIONS IN CHILDHOOD – A HOSPITAL BASED STUDY

Several studies have shown wide range of benefits of vitamin D, in addition to its well established role in bone health. These include infection and immunity, insulin sensitivity, some cancers, hypertension etc. Its role as an immuno modulator has been investigated in recent years. *In vitro* studies and animal experiments have shown that $1,25(\text{OH})_2\text{D}_3$ modulates the immune system by inhibiting T cell proliferation and decreases the production of Th 1 cytokines.

Vitamin D stimulates the expression of potent anti-microbial peptides of host defense system and in epithelial cells lining the respiratory tract where they play a major role in protecting the lung from infection. Some studies carried out in Sri Lanka and India have shown low vitamin D levels in patients with pneumonias and also seasonal variation, with low levels of vitamin D in winter months.

Studies carried out in India have shown subclinical vitamin D deficiency to be wide spread in all age groups. Dietary intakes of both calcium and vitamin D are very low in majority of Indian population except in high socio-economic groups. In India the sub-clinical vitamin D deficiency and nonexclusive breast-feeding in the first 4 months of life were significant risk factors for severe acute lower respiratory infection (ALRI) in Indian children. Higher maternal intake of vitamin D was associated with decreased incidence of recurrent wheeze in children up to 3 years.

A study was planned to determine the vitamin D levels of children below 5 years, hospitalized for respiratory and other infections, with age and sex matched controls attending the out patient department, and to relate the severity, duration of hospitalization and out come with the serum vitamin D levels.

HYPOTHESIS

Vitamin D levels differ in children hospitalized for various infections with special reference to lower respiratory tract infections (LRTI) compared to diarrheal and other infections.

OBJECTIVES

To determine serum vitamin D (25, OH vitamin D) levels of all children admitted for ALRI to the pediatric ward, relate this with proinflammatory cytokine levels, severity and out come of infections and compare with matched controls who do not have ALRI.

METHODOLOGY

1. To recruit children admitted for diarrheal and lower respiratory tract infections admitted to the pediatric ward along with matched controls attending outpatient department.
2. To determine the Vitamin D levels, Th1 and Th2 cytokine levels (IL2, TNF, IFN and IL10).

3. To determine their nutritional status by anthropometry.
4. To determine their hemoglobin, serum vitamin A levels.
5. To study the association of serum vitamin D & A and cytokine levels with severity, outcome and type of infection and also relate with the other micronutrient status.

RESULTS

Altogether 99 children aged 1- 5 years were recruited for the study. Mean age of the children was 23.99 months and mean weight on admission was 9.5 kg, 57.6% of children were males, 78.8% of children were from urban area.

Among the children recruited for the study, 29.3% had diarrhea, 32.3% had fever /upper respiratory tract infection (URTI) and 38.4% had LRTI/ bronchiolitis/ pneumonia. Mean hemoglobin was 10.08g/dl (SD 1.5) and mean vitamin D levels were 20.3 ng/ml (SD 10.23) and 50.5% children had vitamin D >20 ng/ml and 16.9% children had vitamin A <20 µg/dL. Children with LRTI had significantly higher values of vitamin D (mean 26.4, CI 23.4 -29.4 and p=0.001) compared to children with either diarrhea or URTI or viral fevers (16.1, 16.6 and 17.2 respectively). Cytokine profile of these children with various infections and in relation to vitamins A & D status is similar to some of the earlier reports.

2. EFFECT OF RUTF (READY TO USE THERAPEUTIC FOOD) ON WEIGHT GAIN AND BODY COMPOSITION OF CHILDREN WITH SEVERE ACUTE MALNUTRITION (SAM)

Severe Acute Malnutrition (SAM) defined by severe wasting (Weight for height <-3 Z scores) and/or the presence of nutritional edema is a life threatening condition requiring urgent attention. The prevalence of severe malnutrition is estimated at around 2% in least developed countries and 1% in developing countries translating to 10 million severely malnourished children at any point in time. In the Indian context, as per the NFHS 3 data, 26.2% of children less than five years of age are malnourished (weight for height). Of these children, about 6.4% are severely malnourished (<-3SD). This figure is higher than the estimated prevalence in other developing countries.

This is attributed mainly to inadequate dietary intakes both in terms of quantity and quality, which is further compounded by recurrent infections and infestations. Infant and young child feeding practices in India are grossly inadequate both in rural and urban areas. Surveys carried out in the rural areas by National Nutrition Monitoring Bureau (NNMB) in 9 States in India show a deficit intake of around 600 kcal per day among children in the age group of 1-6 years. The foods are mostly cereal based, and are marginally deficient in proteins which are of low quality in terms of essential amino acid content.

Feeding with an energy dense food with good quality proteins supplying all the essential amino acids and adequate amounts of vitamins and minerals is essential in the management of children with SAM. Indian diets are mainly cereal based foods and may have certain limiting amino acids which are essential for growth. In a study it was demonstrated that an increase in weight, height along with increase in fat free mass (FFM) by feeding children with diets rich in animal protein (15% of energy from protein Vs 7.5% in controls). In an unpublished study from NIN (2007) involving 66 children of 1-5 years with severe malnutrition, when fed *ad lib* for over one month on diets containing bread, milk, eggs, khichri and bananas, a mean weight gain of 6.2g /kg/day (SD 3.7) was observed and the children with highest weight gain had a higher increase in FFM but not in fat mass.

A ready-to-use food (RTUF) designed to be a possible substitute for hospital therapeutic diets (F-75/F-100) has been developed. This food has an energy density that is > 5 times that of these diets, but it has a similar ratio of nutrients to energy. This food is obtained by replacing part of the dried skim milk used in the F100 formula with peanut butter. RTUF is at least as well accepted by children as is F100 and can be eaten directly by the child without the addition of water, which eliminates the risk of bacterial contamination from the added water. RUTF is energy dense food (5.5cal/g) and provides almost 55-60% of calories from fat, has sufficient protein (contributing 10-12% of calories) from skimmed milk which provides most of the required essential amino acids. Studies carried out in Africa with RUTF have demonstrated significant weight gain among children with severe acute malnutrition (SAM).

Though, there is evidence available from emergency and non-emergency situations on the usage of RUTF for treatment of severe acute malnutrition, the same is lacking in the Indian context. It was therefore, proposed to carry out a hospital based study in Indian context to assess the efficacy of RUTF in promoting growth among children with SAM.

HYPOTHESIS

Children with SAM fed with RUTF will have significant weight gain as compared to those fed on traditional diets.

OBJECTIVES

1. To study the efficacy of RUTF on weight gain of children with SAM.
2. To study the association of RUTF consumption in terms of quantity and duration with extent of weight gain.
3. To determine the changes in body composition by DEXA, during rehabilitation with RUTF and compare with body compositional changes with routine nutritional rehabilitation foods.

The study was stopped in December 2009 as per the request from the funding agency, WHO. Till December end, 31 children were recruited in the study. Out of which, 23 children have completed one month period of rehabilitation in the nutrition ward.

3. PREGNANCY RELATED CHANGES IN BONE MASS IN WOMEN FROM LOW SOCIO-ECONOMIC GROUP

Calcium economy is strained during pregnancy to support the fetal skeletal mineralization. Apart from this, during pregnancy, changes occur in hormonal milieu, body weight, body composition, food intake and physical activity, all of which may have an influence on bone density. Data from prospective studies addressing the effect of pregnancy on bone mass are scarce and there is no consensus as to whether Bone Mineral Density (BMD) changes during pregnancy and if so whether the losses are reversible.

Evidence suggests that much of the calcium drain for fetal skeletal mineralization is met by increase in dietary calcium absorption during pregnancy. Maternal skeleton acts as a reservoir of minerals that potentially can be mobilized to buffer shortfalls in mineral supply. A study from NIN using a wedge of standard thickness on an X-ray film of the arm has shown that calcium supplementation in pregnant women from the low socio-economic group resulted in significant increase in the bone density of the newborns.

Studies from this Institute in young lactating women from low income group subsisting on 300-400mg of calcium and who may not have achieved peak bone mass showed that these women had mineral depletion to provide for lactation at an age when there is accrual of bone mass. However, studies from the West have indicated that well-nourished young women can even increase their heights during pregnancy suggesting mineral accrual in the presence of abundance during the growth phase.

Pregnant women in their mid 20s from low income group consume diets that are deficient in calcium and other nutrients. Since the minerals absorbed need to be deployed for the maternal peak bone mass and the rapidly growing fetal skeletal needs, it is possible that both the compartments of the maternal-fetal unit undergo sub-optimal mineralization.

This study was therefore initiated to investigate the effect of pregnancy on the bone mass of women from low income group, and the possible impact on the mineral deposition in the fetus.

HYPOTHESIS

Young women from low income group lose BMD during pregnancy to support fetal skeletal mineralization.

OBJECTIVES

1. To estimate the BMD and whole body bone mineral content (WB-BMC) before conception and soon after delivery in women from low socio-economic group and to compare these changes with that of non-pregnant women in the same age and socio-economic group.
2. To determine the relationship of
 - a) Nutritional status
 - b) Dietary calcium intake
 - c) Weight gain during pregnancy to the pregnancy related changes in BMD and WB-BMC.

METHODOLOGY

Sample size: Based on the BMD values of previous studies at NIN, the sample size required to appreciate the differences of 3-5% in bone mineral density was estimated as 25. It was therefore decided to recruit 25 women who conceived within 6 months after the baseline DXA scan, had term delivery and the postpartum bone density measurements done.

All the mothers of around 1 year old children residing in an urban slum who were not using any contraception and wished to conceive were recruited for this study. Background information was collected and routine anthropometric measurements like weight, height and skin folds were measured.

Baseline bone densitometry study (including BMD at lumbar spine, hip, forearm and whole body bone mineral content) by Dual Energy X-ray Absorptiometry (DXA) was performed soon after recruitment. Fasting blood samples were collected at the same time to study the biochemical parameters like - Hb, serum calcium, serum phosphorus, serum zinc, serum alkaline bone specific phosphatase, serum bone specific acid phosphatase, serum vitamin D and serum PTH. Urine samples were collected to estimate calcium and creatinine.

Subjects were followed up every 6 months and those who reported pregnancy within 6 months after baseline DXA formed the 'pregnant group'. The subjects who did not report pregnancy at 6 months after the baseline densitometry scan, had a second densitometry scan and were followed up again after every 6 months. If they failed to conceive during the study period, they were considered as controls.

Pregnant women received routine antenatal care. Their anthropometric measurements such as weight, height, skinfold thickness were measured in each trimester. Fasting blood and urine samples were collected during second trimester of pregnancy to study the biochemical parameters.

The subjects were contacted again after delivery and birth weight of the baby was recorded. DXA scan and biochemical parameters of the mothers were repeated as soon as possible after delivery usually within 15 days postpartum. Their dietary intakes of calcium and other nutrients were assessed by 24 hour recall method at baseline as well as during each trimester of pregnancy.

RESULTS

A total of 80 women were enrolled in the study and their baseline bone density measurements were carried out using DXA. A total of 36 women reported pregnancy within 6 months of baseline DXA scan. These women were followed up during pregnancy and the repeat bone density measurements were completed in 34 women within one month of delivery. Two women could not have their DXA measurements within a month postpartum. Remaining women who did not report pregnancy were followed up and their bone density assessment was repeated at 6 and 12 months. A total of 30 women completed their follow up DXA scan after 6 months and 20 women underwent a follow up bone density examination after 1 year of baseline scan.

Background information

The women reside in an urban slum (Addagutta) and consume diets that are cereal based. Intake of all the major nutrients was below the RDA. At baseline, their mean \pm SD age, height, weight and BMI were 21.2 ± 2.4 years, 153.2 ± 3.3 cm, 42.6 ± 6.7 kg and 19.2 ± 3.4 respectively. All the women except one in the pregnant group had singleton full term delivery. One woman had

preterm delivery at 34 weeks gestation. Twenty eight women had vaginal deliveries and 6 had LSCS. The mean \pm SD birth weight of their newborns was 2.95 ± 0.57 kg.

Pregnancy related changes in bone densities at different skeletal sites and body composition parameters

The overall values of baseline bone densities of the total study group were significantly lower than those of well nourished women from the high income group indicating poor bone health of the study group. The bone density values at different skeletal sites and body composition parameters at baseline and postpartum follow up are presented in Table 13. Figure 9 indicates these changes in bone parameters in terms of percentages. Table 13 and figure 9 also indicate baseline and follow up values of bone density and body composition parameters in non-pregnant women who were followed up at 6 and 12 months.

It was observed that there was significant loss of bone density at lumbar spine as well as whole body during pregnancy. The BMD at femoral neck and total hip did not have significant changes during pregnancy. There was mean accretion of 2.7 kg fat and 0.6 kg lean body mass during pregnancy.

Table 13. Changes in bone parameters during pregnancy and during follow up of non-pregnant women at 6 and 12 months

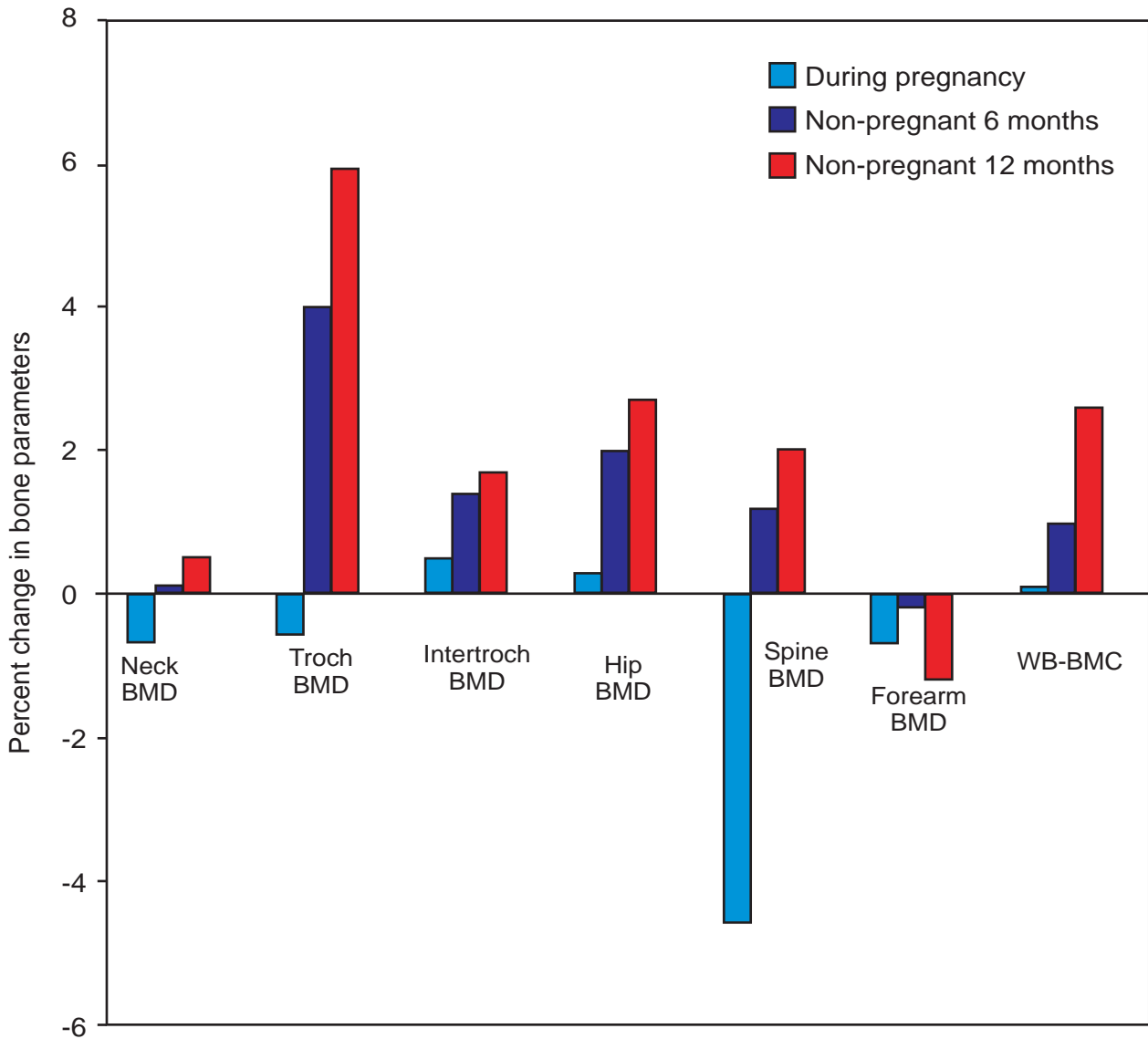
Bone parameter	Pregnant women (n=34)		Non-pregnant women followed up for 6 months (n=30)		Non-pregnant women followed up for 12 months (n=20)	
	Baseline	After delivery	Baseline	After 6 months	Baseline	After 12 months
Neck BMD	0.732 \pm 0.103	0.726 \pm 0.111	0.745 \pm 0.100	0.745 \pm 0.100	0.754 \pm 0.100	0.759 \pm 0.111
Hip BMD	0.798 \pm 0.103	0.799 \pm 0.095	0.815 \pm 0.106	0.830 \pm 0.106***	0.837 \pm 0.100	0.858 \pm 0.093**
Forearm BMD	0.503 \pm 0.034	0.499 \pm 0.037	0.514 \pm 0.034	0.513 \pm 0.038	0.514 \pm 0.031	0.508 \pm 0.033
Spine BMD	0.835 \pm 0.102	0.797 \pm 0.106***	0.863 \pm 0.097	0.873 \pm 0.100*	0.864 \pm 0.084	0.881 \pm 0.086**
WB-BMC	1504 \pm 203	1506 \pm 204	1582 \pm 234	1597 \pm 239	1613 \pm 235	1653 \pm 241*
WB-BMD	0.975 \pm 0.075	0.956 \pm 0.080**	1.015 \pm 0.092	1.011 \pm 0.100	1.022 \pm 0.081	1.013 \pm 0.098
WB-fat	11.4 \pm 5.5	14.1 \pm 5.1***	13.3 \pm 6.1	14.2 \pm 7.0**	14.9 \pm 7.0	16.8 \pm 8.6**
WB-lean	29.9 \pm 3.2	30.5 \pm 3.9 ***	30.2 \pm 4.0	30.7 \pm 4.6*	31.8 \pm 4.1	32.8 \pm 5.0**
WB-% fat	25.4 \pm 7.5	29.8 \pm 5.4 ***	28.3 \pm 7.0	29.0 \pm 7.3	29.4 \pm 8.0	31.2 \pm 8.2**

*** - p <0.001; ** p<0.01; * p<0.05

Changes in bone density and body composition parameters during 6 and 12 months follow up of non-pregnant women

There was significant increase in BMD at the femoral neck, hip and lumbar spine as well as whole body BMC during 6 and 12 months follow up periods. There was also significant increase in lean mass as well as fat mass indicating growth of these tissues during this age.

Figure 9. Percent change in bone parameters during pregnancy and during follow up of non-pregnant women at 6 and 12 months



CONCLUSION

Undernourished young women who had not attained their peak bone mass failed to increase their bone densities as expected during growth phase probably due to the extra demands imposed by fetal skeletal mineralization. This may have a negative influence on bone mass during later life and it is necessary to examine whether calcium rich food supplementation helps achieving normal growth of bone mass during pregnancy.

4. EVALUATION OF THE ROUTINE MANAGEMENT OF ANAEMIA (Hb <8g/dL) IN PREGNANCY AND EFFECT ON OUTCOME

Iron deficiency anaemia is one of the most common nutritional deficiencies all over the world. Women and preschool children are most vulnerable segment of the population to iron deficiency anaemia. About half of the world's anaemic women live in India and 88% of them develop anaemia during pregnancy. Dietary deficiency of iron and inadequate absorption of dietary iron are the important causes of high prevalence of anaemia. The iron requirement for pregnant women is around 38 mg/day as against the dietary intake of 18 mg/day. Severe anaemia during pregnancy increases the risk of maternal mortality. Daily supplementation were most effective in preventing severe anaemias.

Oral and parenteral iron supplementations are the treatment of choice for iron deficiency anaemia in pregnancy. Oral iron (120mg of elemental iron and 500ng folic acid) is routinely supplemented in severe early pregnancy anaemias. The parenteral iron therapy is opted in severe anaemias of pregnancy around 26-28 weeks of gestation or when oral iron is not tolerated. Blood transfusion is routinely given for severe anaemias in late pregnancy. This is the regimen followed in Niloufer Hospital, Hyderabad where the study was planned. Iron sorbitol citrate (Jectofer) injections did not show side effects compared to iron dextran complex. Therefore, a study was initiated to evaluate the effect of oral and parenteral iron supplementation in severe anaemias on haemoglobin, maternal and fetal pregnancy outcomes.

HYPOTHESIS

Iron supplementation in severe anaemic pregnant women improves maternal hemoglobin and has impact on maternal and fetal outcomes.

AIMS AND OBJECTIVES

To study the effect of oral and parenteral iron supplementation on hemoglobin, in pregnant women with Hb <8 g/dL.

MATERIALS AND METHODS

Pregnant women with hemoglobin <8g/dL between 12 to 20 weeks of gestation were recruited for oral supplementation (120 mg of elemental iron and 500 µg of folic acid). Pregnant women around 24 –26 weeks of gestation with hemoglobin <8 g/dL were enrolled for parenteral iron therapy (iron sorbitol citrate 1000 mg). All the pregnant women were informed and written consent was taken. Demographic, socio-economic, obstetric and nutritional data were collected on a pre-coded proforma. Blood samples were collected from each subject before initiation of therapy for estimating hemoglobin. Follow-up blood samples were collected for hemoglobin estimation every month for three months in oral supplementation and at the end of 3 weeks and 6 weeks in parenteral therapy.

All pregnant women were followed up regularly during antenatal period to monitor for fetal growth and pregnancy complications. Intrapartum complications were recorded and birth weight of infant were recorded.

RESULTS

Pregnant women between 14 to 20 weeks of gestation and 24-28 weeks of gestation were screened to identify women with severe anaemia (<8g/dL) for enrollment for oral and parental supplementation respectively. Fifteen hundred pregnant women were screened. The prevalence of severe anaemia (<8g/dL) was around 18.7%. The prevalence of severe anaemia in early pregnancy was 19.5% and in late pregnancy 22.5%. Eighty women with hemoglobin <8g/dL in early pregnancy and 120 pregnant women with hemoglobin <8g/dL in late pregnancy were recruited as the study subjects. About 64.7% of the pregnant women in the early pregnancy and 46.2% in late pregnancy showed improvement in haemoglobin in >8g/dL between 3-6 weeks of supplementation. About 44.1% of pregnant women in early pregnancy and 27.7% in late pregnancy showed haemoglobin rise above 11g/dL at 34-36 weeks of gestation (Table 14).

Table 14. Profile of subjects with haemoglobin <8g/dL

Details	Early pregnancy Oral iron 14 – 20 weeks	Late pregnancy Parenteral iron 24 – 26 weeks
Total number	779	721
Hb <8g/dl	19.5% (152)	22.5% (162)
Cases recruited as study subjects	80	120
Data analyzed	68	108
No. of cases crossed 8g/dL between 3- 6 weeks suppl.	44 (64.7%)	50 (46.2%)
No. of cases >11g/dL at 34-36 weeks of gestation	30 (44.1%)	30 (27.7%)
Not tolerated oral iron reaction to injection	10 (14.7%)	20 (18.5%)
No. response to therapy	10 (14.7%)	23 (21.2%)
No. received blood transfusion	4 (5.8%)	15 (13.9%)

The mean haemoglobin at 3-6 weeks of gestation was better in early pregnancy (10.1 ± 1.37 g/dL) compared to the late pregnancy (9.0 ± 1.35 g/dL). However, there was no difference in mean hemoglobin at 34-36 weeks of gestation between the two groups (10.8 ± 1.56 , 10.0 ± 1.52 g/dL). There was no difference in mean birth weight and percentage of low birth weight infants between both the groups (Table 15).

Table 15. Mean \pm SD of haemoglobin (g/dL) rise with supplementation

Groups	Initial	3 to 6 weeks after supplementation	At 34 to 36 weeks of gestation
Oral iron during 14 to 20 weeks	7.5 ± 0.47	10.1 ± 1.37	10.8 ± 1.56
Parenteral iron during 28 to 30 weeks	7.3 ± 0.81	9.0 ± 1.35	10.0 ± 1.52

CONCLUSION

The effect of iron supplementation in pregnant women with Hb<8g/dL (oral therapy in early pregnancy and parenteral therapy in late pregnancy) showed more or less similar rise in hemoglobin by term in both the groups. However, in early pregnancy (<20 weeks) with Hb <8g showed better improvement in hemoglobin by term (44.1%) compared to late pregnancy group (24-26 weeks of gestation – 27.7%). This indicated the need for early initiation of parenteral by 20 weeks to improve Hb above 11g by term.

5. INSULIN RESISTANCE AND TNF α LEVELS IN NORMAL AND HIGH RISK PREGNANT WOMEN

Pregnant women and preschool children are vulnerable segment of population. In spite of various national prophylaxis programmes in action, the prevalence and trends in maternal malnutrition has not changed over decades. Reported studies demonstrated that chronic energy deficiency was prevalent in women at reproductive age and it will have impact on the fetal programming in early pregnancy, fetal growth and birth weight of infants. The relationship between fetal-placental unit and pregnancy malnutrition has already been established. Data (from west) on Insulin resistance during pregnancy has been shown to be associated with various complications, in addition to impact on pregnancy outcome and birth weight of infants.

Studies done on body composition in pregnancy demonstrated that low birth weight infants had higher percentage fat compared to normal birth weight of infants (*Annual Report, 2002*). The mothers' who delivered low birth weight were malnourished and their gestational weight gain and fat gain were low. Studies from NIN on placental development in early pregnancy in relation to maternal nutritional status and socio-economic status demonstrated the significant differences in VEGF expression in placental tissues in pregnant women from LSG with low BMI (*unpublished data*). It is necessary to investigate when exactly insulin resistance occurs in relation to gestational age and maternal nutritional status and what could be the gestational outcome in low income pregnant women.

Data is not available on prevalence of insulin resistance in different gestational age in relation to nutritional status and pregnancy outcome. The study is initiated to investigate the insulin resistance and TNF α level at different trimester of gestation in normal and high risk pregnant women in relation to maternal nutritional status in low socio-economic group. It has been reported that TNF α could be used as a marker for insulin resistance. The present study assessed if TNF α could be used as a marker of IR.

HYPOTHESIS

Insulin resistance during pregnancy leads to adverse pregnancy outcomes.

OBJECTIVES

1. To detect prevalence of insulin resistance in different gestational ages (<12weeks, 20 weeks, 28 weeks, 34-36 weeks).

2. Can TNF α used as a marker for detection of insulin resistance.
3. What is the difference in prevalence of insulin resistance in normal and high risk pregnancies in low income group at different gestational ages in relation to maternal nutritional status.

Sample size:

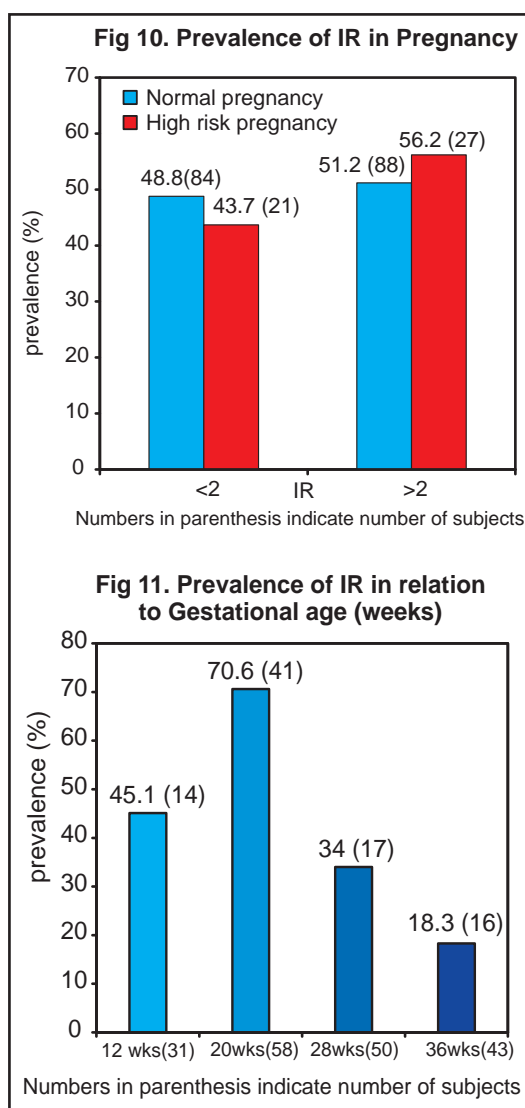
To calculate the sample size initially, 25 subjects in different gestational ages between normal and high risk pregnant women were investigated.

MATERIALS AND METHODS

1. Pregnant women attending antenatal clinic at Niloufer Hospital were enrolled.
2. Pregnant women were recruited at around 12 weeks, 20 weeks, 28 weeks and 34-36 weeks in normal pregnant women and high risk pregnant women.
3. Fasting blood sample were collected for glucose, insulin, TNF α and hemoglobin, leptin and adiponectin. Insulin resistance was calculated by HOMA Index.
4. The percentage of women showing insulin resistance and TNF α levels at different gestational ages were investigated.
5. Hundred normal pregnant women and 50 pregnant women with history of adverse pregnancy outcomes from low socio-economic group attending Niloufer hospital in different gestational age were recruited.
6. Fasting blood samples were collected for glucose, insulin, TNF α , leptin, adiponectin and haemoglobin. Recruitment of pregnant women of different gestational ages in normal and high risk group was completed.

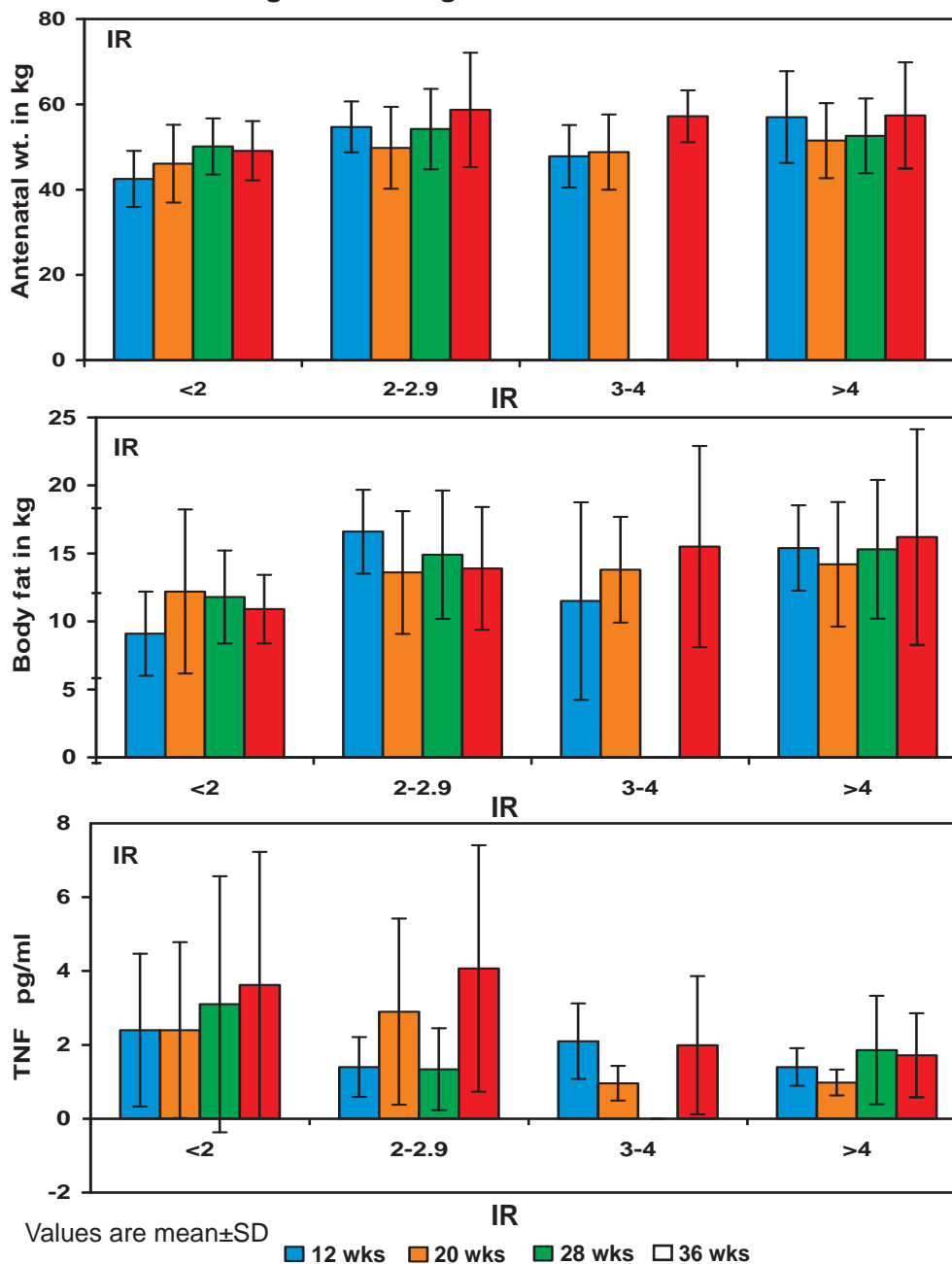
RESULTS

- ❖ The prevalence of IR (HOMA index >2) was 51.2% in normal pregnant women and 56.2% in high risk pregnant women (Fig. 10).
- ❖ The prevalence of IR (HOMA index > 2) in different gestational ages are as follows – 45.1% (12 weeks), 70.6% (20 weeks), 34% (28 weeks) and 18.3% (36 weeks). The insulin resistance was present right from 12 weeks of gestation which is an important finding and requires indepth investigation by a prospective study (Fig. 11).
- ❖ There was significant difference in mean antenatal weight and body fat at 12 weeks, 20 wks, 28 wks and 36 wks between insulin resistant group (HOMA index 2-2.9, 3-4, >4 and normal pregnant women) (Fig. 12).



- ❖ TNF α levels did not show significant difference in relation to insulin resistance and gestational age (Fig. 12).

Fig 12. Antenatal weight, body fat and TNF levels in relation to gestational age and insulin resistance



CONCLUSIONS

This study indicates the prevalence of insulin resistance around 50% right from early pregnancy. The prevalence of insulin resistance during pregnancy is associated with adverse pregnancy outcome. So, it is essential to carryout a prospective study on effect of insulin resistance in early pregnancy on course, complications and outcome of pregnancy.

V. BASIC STUDIES

1. IMMUNOASSAY FOR QUANTITATION OF METALLOTHIONEIN FOR ASSESSING ZINC BIOAVAILABILITY IN Caco-2 CELL MODEL

Zinc bioavailability studies using Caco-2 cell line requires either an established surrogate marker or a sensitive probe which reflects the cellular zinc status. Uptake of radioactive zinc has been used to assess absorption of zinc from food matrices in Caco-2 cells. However, non-availability of high specific activity radio isotope of zinc limits its application as a screening tool for bioavailability. Moreover, it is assumed that radioactive zinc may not completely equilibrate with the endogenous zinc present in the foods to reliably estimate availability. Therefore, identification of a surrogate marker and developing an immunoassay for the same would help in quantifying cellular zinc status, and thereby assessing bioavailability of zinc from food matrices. In this context, metallothionein (MT), a low molecular weight (6-7 kDa) protein which selectively binds metal ions such as zinc, cadmium and copper is considered. MT has been shown to reflect tissue and cellular zinc status. The objective, therefore was to develop an immunoassay method for quantitation of MT and validate the same for assessing zinc bioavailability in Caco-2 cells.

Initial attempts to develop a sandwich ELISA method failed, as there was no binding of MT with immobilized antiserum or to the indicator antibody. Subsequently, a competitive ELISA was tested with MT labeled with biotin. However, it was observed that biotinylation of MT leads to loss of binding of MT to antibody.

AIMS AND OBJECTIVES

To develop and validate an indirect immunoassay method for quantitation of MT for assessing zinc bioavailability in Caco-2 cells.

METHODS

Human recombinant (hr) MT was over expressed and purified from E.coli BL-21 cells. Purified hr MT was conjugated to Keyhole Limpet Hemocyanin (KLH) by carbo-di-imide coupling method to produce an antiserum in New Zealand albino rabbits. IgG was isolated and conjugated to biotin using commercially available kit (Genei, Bangalore).

Indirect ELISA of MT

An indirect competitive ELISA method for metallothionein was developed. To obtain the coating MT concentration and the dilution of biotinylated MT (BT-MT) antiserum, 96 well plates were coated with varying concentrations of hr MT (0-10 $\mu\text{g/mL}$) and incubated with 1:500, 1:1000, 1: 2000 and 1:5000 dilutions of BT-MT antiserum. The binding of BT- MT antiserum to the immobilized MT was quantitated using streptavidin-biotin peroxidase complex (Genei, Bangalore). For the indirect ELISA, 96 well plate was coated with MT standards (0-10 ng/mL) or sample (lysate of different concentrations) in 10 mM phosphate buffer saline, pH 7.2 over night. Non-specific binding was assessed in uncoated wells. On the day of the assay the wells were blocked with 1% BSA and added biotinylated anti MT IgG (1:1000) and incubated for 3 h at room temperature. The wells were

washed thrice and incubated with 200 μ L/well of streptavidin-biotin peroxidase complex (Genei, Bangalore) for 30 min at room temperature. The wells were then washed three times and enzyme activity measured using H₂O₂-OPD substrate at 492 nm (Bioteck plate reader). The linearity with hr MT was assessed by plotting log concentrations on X-axis versus OD on Y- axis. The concentration of MT in unknown was determined by linear regression using Sigma plot 10.0.

Validation

Validation of indirect ELISA of MT was performed by comparing ⁶⁵Zn uptake and densitometry scanning of the western blot of the induction of MT in Caco-2 cells. Uptake experiment was carried out in 6-well plates. The plates were seeded at an initial seeding density of 50,000 cells/cm² and the cells were used 12-14 days of post seeding.

⁶⁵Zinc uptake in Caco-2 cells

Dose dependent uptake of zinc was monitored by incubating Caco-2 cells with 0-200 μ mole/L ZnSO₄ traced with ⁶⁵Zn for a period of 2 h. Non-specific binding of the tracer was assessed with 100-fold molar excess of non-radioactive zinc in the form of ZnSO₄. At the end of incubation, cells were washed thrice with 10 mM HEPES containing 1 mM EDTA and 140 mM NaCl and cell associated radioactivity was counted in auto-counter (Perkin Elmer wizard, 1480).

Induction of MT in Caco-2 cells

MT expression in Caco-2 cells exposed to various concentrations of zinc (0-200 μ M) for 12 h was assessed by resolving 100 μ g of cell lysate on 12 % SDS-PAGE followed by immunoblotting with MT antibody. Same lysate was also used for quantitation of MT by the indirect ELISA method.

RESULTS

The indirect ELISA method developed had the following characteristics. The optimal dilution of biotinylated anti MT was 1:2000 as this dilution yielded low non specific reaction and an OD of 1 for 1000 ng/mL (Fig. 13A). The binding of BT-MT antibody to the immobilized MT was linear with different dilutions of MT. The assay was linear in the range of 50 ng-10 μ g/mL (Fig. 13 B) with an R²=0.95. (Fig 13 B).

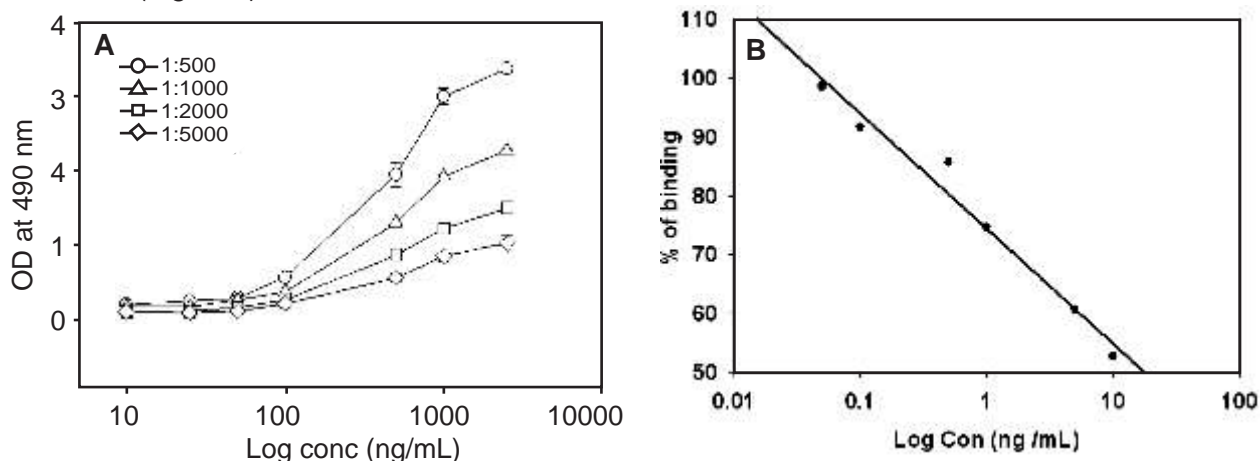


Figure 13: Indirect ELISA for metallothionein. Immobilized MT (10-5000ng/mL) in microtitre plate incubated with various concentrations of MT and biotinylated anti MT antibody (1:500-1:5000). Determination of the optimal concentrations of r MT and MT-IgG-biotin (A). Standard graph of concentration Vs % binding (B). The % of displacement with standards 50 to 10000 ng/mL with a coating of 2.5 μ g/mL of r MT and detection with 1:1000 dilution of biotinylated IgG followed by streptavidine biotin peroxidase complex. The R² = 0.95 (B).

Validation of MT as surrogate marker of Caco-2 cell zinc status

Zinc uptake increased with media zinc concentration. MT induction assessed by immunoblotting plateaued off at about 5µM concentration of zinc (Fig. 14 A). Induction of MT level assessed by indirect ELISA showed similar results and plateaued off at the same concentration of 5µM of zinc (Fig.14 B). These results suggest that MT expression measured in Caco-2 cells by indirect ELISA and immunoblotting may not be suitable to assess bioavailability of zinc at higher concentrations of zinc.

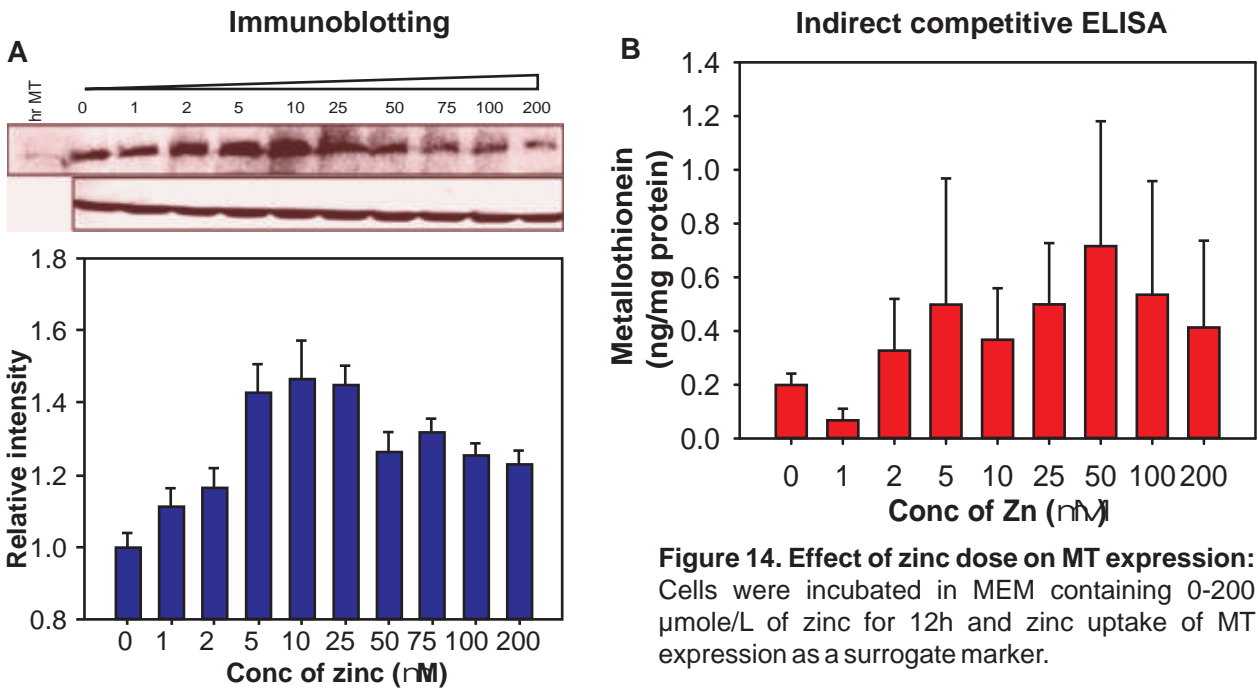


Figure 14. Effect of zinc dose on MT expression: Cells were incubated in MEM containing 0-200 µmole/L of zinc for 12h and zinc uptake of MT expression as a surrogate marker.

Figure 15. Zinc uptake

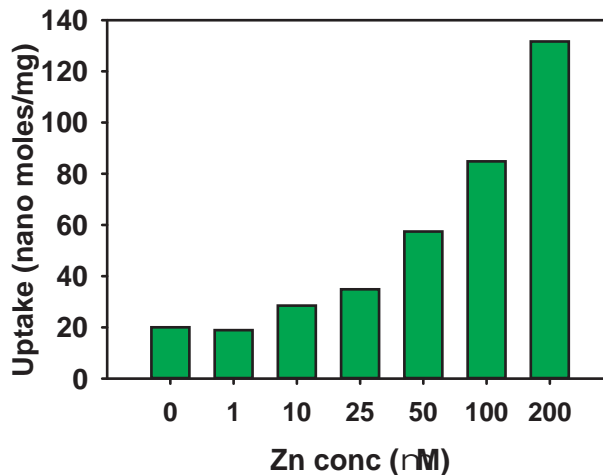


Figure 15. Dose dependent zinc uptake in Caco-2 cells. Caco-2 cells were incubated in MEM containing 0-200 µM traced with 1µCi of ⁶⁵Z for a period of 2h and uptake was measured. The bar represents mean ± SD.

CONCLUSION

Thus, it is concluded that quantitation of MT may not be possible with the immunoassay developed due to poor assay sensitivity. The choice of method appears to be ^{65}Zn uptake in Caco-2 cells to assess absorption of zinc from food matrices.

2. STUDIES ON THE RESPONSE AND INTERACTIONS OF IRON AND ZINC IN Caco-2 CELLS: EFFECT OF SIMULATED INFLAMMATORY CONDITIONS

It is now widely recognized that iron and zinc interact at the site of absorption (intestine) and influence the uptake of one another. Earlier studies at NIN have established that increased oxidative stress induced damage during iron repletion is alleviated by natural antioxidants such as ascorbic acid, α -tocopherol and also by zinc. Also, a negative interaction between iron and zinc, as evidenced by the decreased uptake of mineral in the presence of the other and induction of ferritin and metallothionein, under conditions of a zinc depletion-repletion study in rats has been recently reported. The aim was therefore to understand how iron and zinc interact during uptake at the enterocyte.

The Caco-2 cell line is an *in vitro* model of the absorptive enterocyte that has been extensively used for bioavailability screening. The aim was therefore to grow Caco-2 cells in iron and zinc deficient media (SFM), in order to simulate chronic combined deficiency of iron and zinc seen in human populations and to study iron-zinc interactions during uptake in such cells. In the presence of a model that recapitulates physiological changes occurring *in vivo* during chronic iron-zinc depletion, an effort was made to understand the effect of iron and zinc deficiency alone or in combination, effect of repletion prior to inflammation or during inflammation on iron-zinc uptake and interactions. Nitric oxide is a key effector molecule of the inflammatory response and was used to simulate inflammatory conditions.

AIMS AND OBJECTIVES

To assess the effect of repletion on uptake, interactions and responsive proteins under simulated inflammatory conditions in chronically iron-zinc deficient Caco-2 cells.

MATERIALS AND METHODS

Serum-free medium (SFM)

Serum-free growth medium deficient in iron and zinc was custom-made and procured from M/s. Biowhitakker (Vervier, Belgium) as pre-sterilized liquid media. Antibiotic-antimycotic solution and NEAA were added at a final concentration of 1X prior to use. Iron and zinc content of normal media with 10% FBS (CM) and SFM was 370 $\mu\text{g/L}$ and 68 $\mu\text{g/L}$, respectively. The zinc content of CM and SFM was 130 $\mu\text{g/L}$ and 11.8 $\mu\text{g/L}$, respectively.

Culture of Caco-2 cells in SFM

Cells were routinely maintained in T-75 flasks and seeded into 6-well or 12-well plates as necessary in normal media (CM) containing 10% serum. They were shifted to SFM two days post-seeding and grown in SFM for 12 days. Medium was changed once in 72 hr.

Diethylenetriamine-nitric oxide adduct (DETA/NO): DETA/NO, is a NONOate with a half-life of 20.5hr and was used to generate NO in the medium which enables simulation of inflammatory conditions.

Selective repletion of iron and/or zinc prior to NO exposure: Cells maintained in SFM were selectively repleted with 50 μ M iron (zinc deficient) or 50 μ M zinc (iron deficient) or with 50 μ M each iron+zinc for 24 hr prior to 8 hr incubation with 100 μ M DETA/NO. This group of cells is denoted (P+D). Cells that were left untreated served as control and that incubated with 50 μ M iron, 50 μ M zinc or iron+zinc for 24 hr, are denoted (P) and served as cognate controls.

Simultaneous repletion with iron and/or zinc during NO exposure: Cells in SFM and CM were left untreated, exposed to 100 μ M DETA/NO alone or treated with 50 μ M iron, 50 μ M zinc or 50 μ M each (iron+zinc) in the presence of 100 μ M DETA/NO for 8 hr. Groups are labeled SFM and CM respectively. All simultaneous repletion experiments were run together to enable comparison between groups.

Uptake: Cells seeded in 12-well plates were used for uptake experiments. In cells that were selectively repleted or simultaneously repleted during exposure to DETA/NO, uptake of iron, zinc individually or together in equimolar ratio was quantitated.

Protein expression: For protein expression, cells were seeded in 6-well plates and maintained in respective media. After respective treatments, cells were washed in ice-cold PBS, lysed in RIPA buffer and protein estimated. Expression of the following proteins was studied by immunoblotting, viz, iron: DcytB, DMT-1, FPN-1, IRP-1, IRP 2, zinc: ZnT-1, ZnT-4, MT, and GAPDH to ensure equal loading. All selective repletion experiments were run together; similarly, all simultaneous repletion experiments were run together to enable comparison between groups. Experiments were performed in duplicate and repeated once to generate 4 observations per data point.

Transcript levels: Cells were maintained in T-75 flasks. After the respective treatments, cells were washed and stored at -70°C until they were processed for RNA isolation and RT-PCR.

Statistical analyses: Results are presented as mean \pm SD. Comparisons were made only between groups P and P+D or between SFM and CM for a given treatment and between treatments for a given group to understand the effect of CM (repleted) or SFM (chronic depletion) with or without added NO-stress. Means within groups were compared using one-way ANOVA followed by post-hoc 'LSD' and means between groups for a given repletion were compared using student's 't' test. P<0.05 was considered significant.

RESULTS

Uptake of iron and zinc under simulated inflammatory conditions

Iron uptake

Selective repletion: Iron uptake decreased in mineral repleted and NO exposed (P+D) SFM cells compared to SFM cells (P), except in zinc repleted cells (Fig 16A).

Simultaneous repletion: In SFM cells, NO significantly decreased iron uptake compared to CM cells under identical conditions (Fig. 16B).

Zinc uptake

Selective repletion: In SFM cells NO per se reduced Zn uptake, except the cells that were Zn repleted.(Fig. 16C).

Simultaneous repletion: Between SFM and CM cells that were identically treated, zinc uptake was significantly higher in SFM cells (Fig.16D).

Changes in responsive proteins and transporters

Ferritin

Selective repletion: NO per se did not increase ferritin concentration in SFM cells (Fig 17A). Ferritin increased significantly upon iron+NO, iron+zinc+NO treatment compared to respective controls, except in the case of zinc only repleted cells.

Simultaneous repletion: NO per se decreased ferritin content in both SFM and CM cells. In SFM cells, ferritin content decreased further with iron, zinc and iron+zinc when compared to untreated cells (Fig 17B). Between cells in SFM and CM, significant decrease in ferritin in SFM cells was observed.

Metallothionein

Selective repletion: NO per se significantly reduced MT levels, which were further reduced upon prior repletion with iron, or iron+ zinc (Fig 17C).

Simultaneous repletion: In both SFM and CM cells, NO decreased MT expression irrespective of the presence of iron or zinc (Fig. 17D). Between SFM and CM cells, SFM cells had lower MT levels, which decreased further in the presence of NO and iron/zinc.

DMT-1

Selective repletion: In cells that were treated with iron+NO, zinc+NO or iron+zinc+NO, DMT-1 expression was higher compared to NO per se (Fig.18A).

Simultaneous repletion: In CM cells, NO+iron, NO+zinc or NO+iron+zinc significantly decreased DMT-1 where as SFM cells showed significantly decreased DMT-1 expression upon NO and NO with mineral treatment (Fig. 18B).

Ferroportin-1

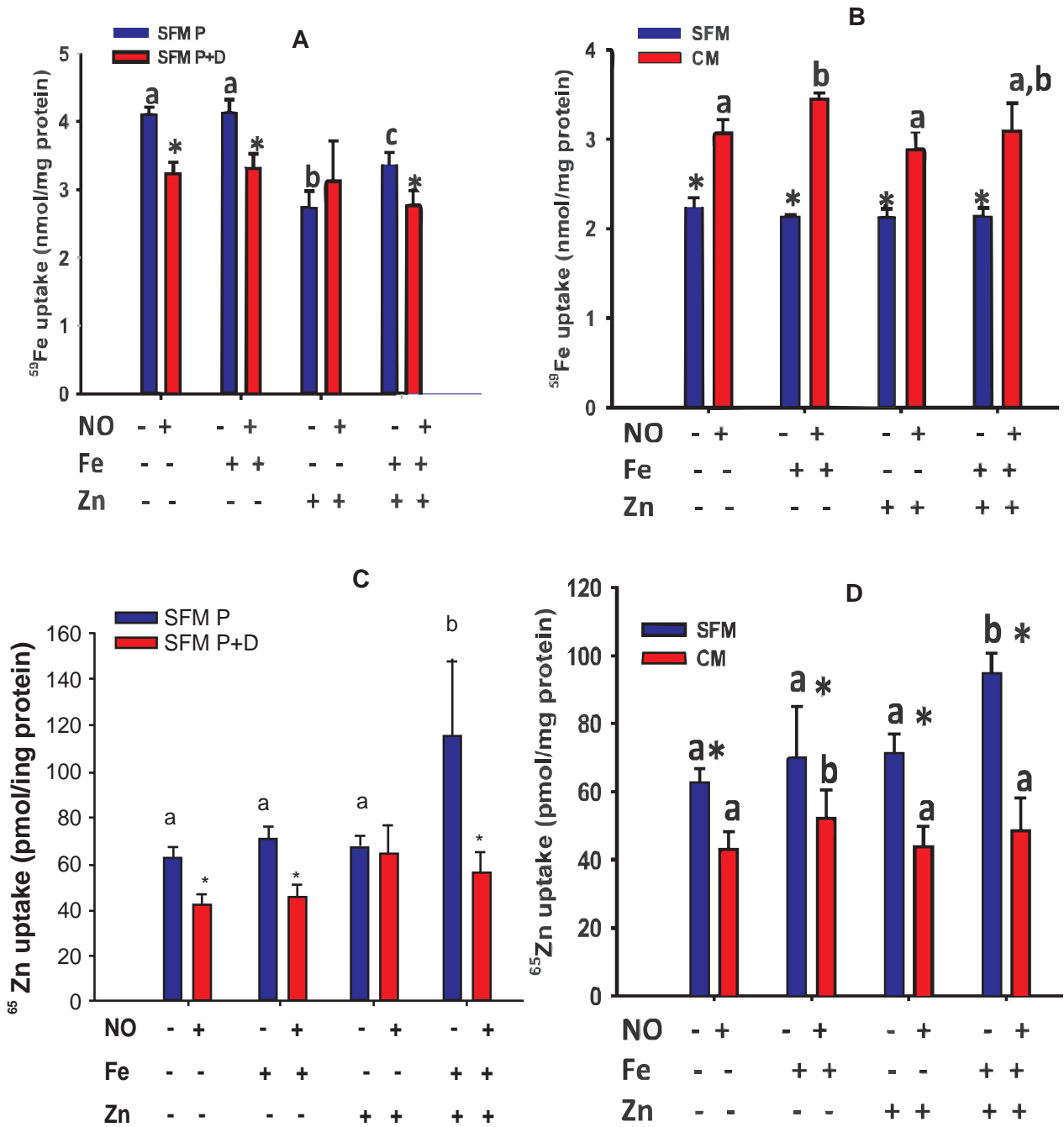
Selective repletion: In cells that were only repleted, zinc, iron+zinc significantly increased FPN-1 expression. Between cells that were only repleted and repleted+ NO treated, NO per se and iron+zinc+NO significantly decreased expression (Fig 18C).

Simultaneous repletion: In SFM cells, NO per se decreased expression and NO+iron significantly increased FPN-1 expression. In CM cells, NO per se, NO+zinc and NO+iron+zinc significantly increased FPN-1 expression. Between SFM and CM cells, SFM cells showed higher FPN-1 expression irrespective of repletion or NO treatment (Fig 18D).

IRP-1

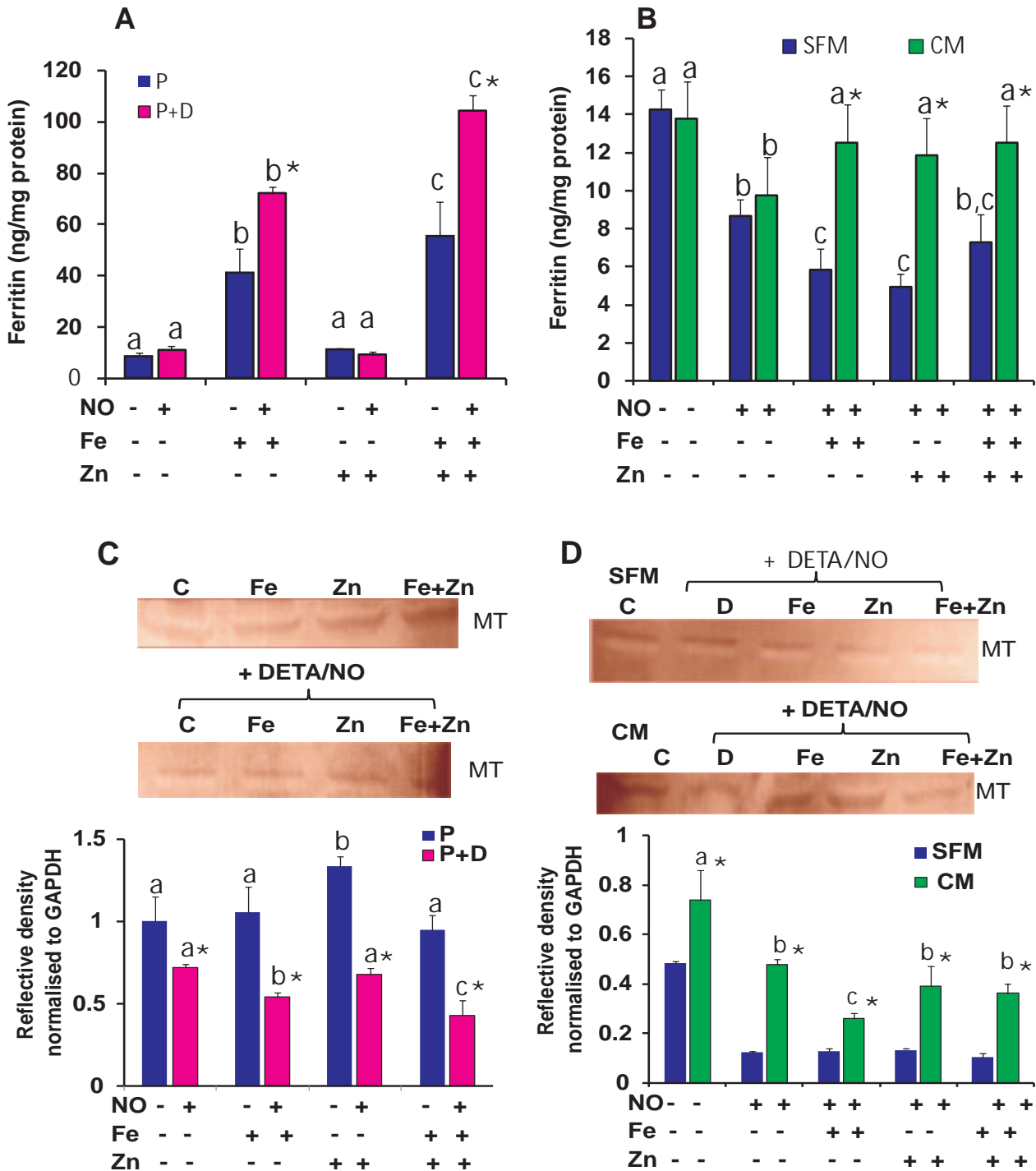
Selective repletion: The cells that were only repleted did not significantly affect total IRP-1 expression (Fig 19A, black bars). In cells that were repleted+NO treated, NO per se did not decrease IRP-1 expression (Fig 4A) but iron+zinc+NO significantly decreased IRP-1 expression.

Figure 16. Effect of NO on iron, zinc uptake during selective and simultaneous repletion in deficient cells



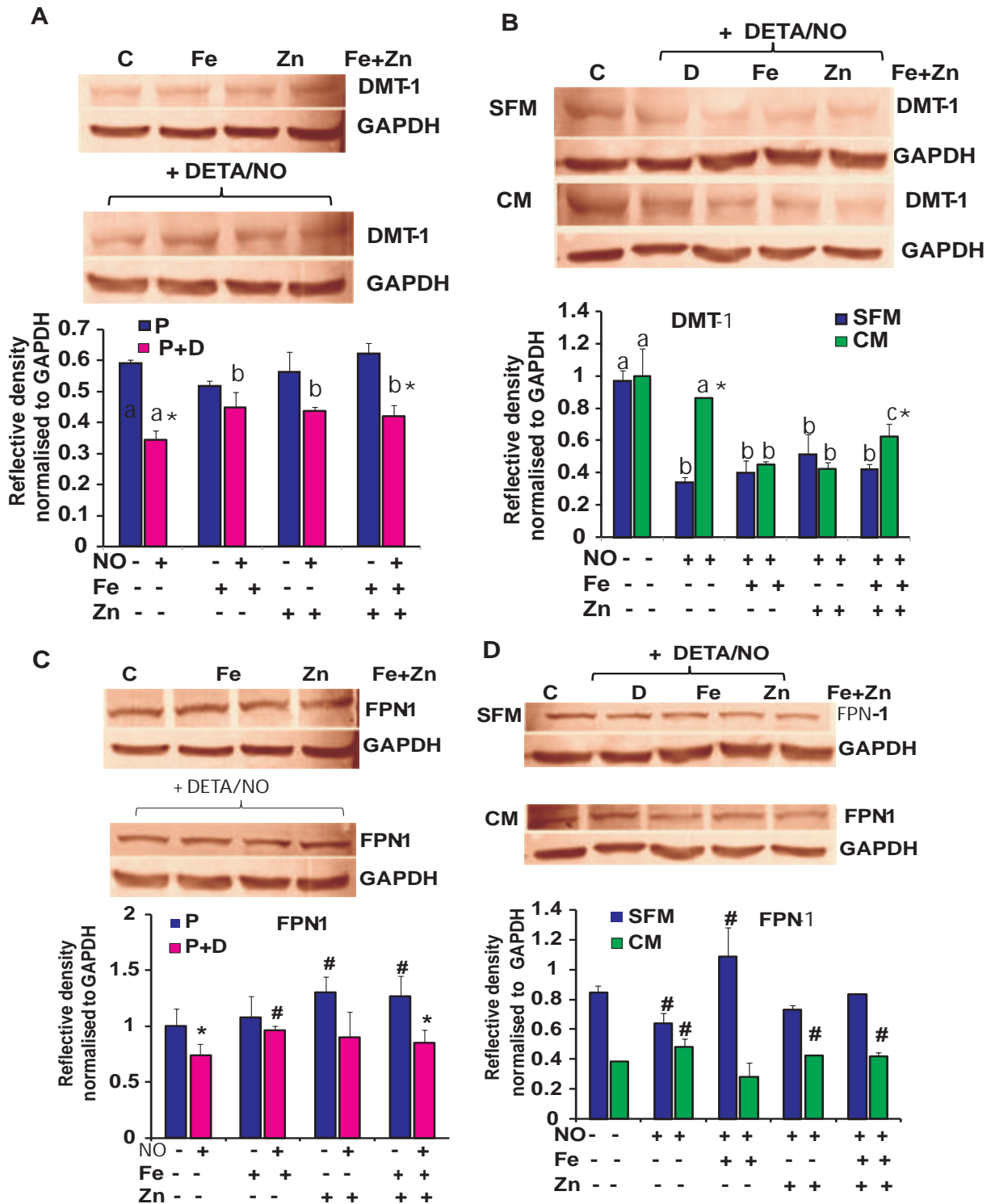
Deficient cells (SFM) were selectively repleted and treated with or without NO (A, iron; C, zinc), control cells (CM) and deficient cells (SFM) were simultaneously repleted with NO (B, iron; D, zinc) and iron and zinc uptake quantitated. Bars represent mean \pm SD (n=9), * indicates significant difference between groups and bars that do not share a common superscript are significantly different at P<0.05.

Figure 17. Effect of repletion regimens on ferritin and MT



Cells were subjected to selective repletion in SFM (A) or simultaneous repletion in SFM and CM (B). Ferritin was estimated using an in-house sandwich ELISA (top panel A&B) and metallothionein expression assessed by immunoblotting and densitometrically quantitated (bottom panel C&D). Data are mean \pm SD of experiments in triplicate. * indicates significant difference between groups and bars that do not share a common superscript are significantly different at P<0.05

Figure 18. Effect of repletion regimens on DMT-1 and FPN-1 expression



Cells were subjected to selective repletion in SFM (A&C) or simultaneous repletion (B&D) in SFM and CM. DMT-1 (top panel A&B) and FPN-1 expression (bottom panel, C&D) was assessed by immunoblotting and densitometrically quantitated. n=4, * indicates significant difference between groups at P<0.05. # indicates significantly different from control within group, P<0.05.

Simultaneous repletion: In SFM cells, IRP-1 expression significantly decreased with NO+zinc, NO+iron+zinc compared to untreated cells and CM cells (Fig 19B). In CM cells, IRP-1 expression decreased significantly only with NO+iron (Fig 19B).

IRP-2

Selective repletion: In cells that were repleted+NO treated, NO *per se* decreased expression and was not significantly altered due to repletion (Fig. 19C). In cells that were only repleted, zinc and iron+zinc repletion significantly increased IRP-2 expression. Between cells that were repleted and repleted+ NO treated, there was a significant decrease in IRP-2 expression except for iron+zinc+NO treated cells.

Simultaneous repletion: Between SFM and CM cells, IRP-2 expression was significantly higher only in untreated SFM cells. However, in SFM and CM cells, NO, *per se*, significantly increased IRP-2 expression (Fig. 19D).

Zip-1 and Zip-14 transcript levels

Selective repletion: Between cells that were repleted and repleted+NO treated, only NO *per se* and iron+NO significantly decreased Zip-1 and Zip 14 transcript levels (Figs 20A and 20C)

Simultaneous repletion: No significant change was observed in SFM or CM cells or between SFM and CM cells with NO or iron, zinc or iron+zinc, except for a significant decrease in Zip4 in CM cells treated with NO+zinc (Figs. 20B & 20D).

ZnT-1 and ZnT-4

Selective repletion: In cells that were only repleted, only iron significantly increased ZnT-1 expression. In cells that were repleted+NO treated, only zinc+NO significantly increased ZnT-1 expression (Fig 21A). On the other hand, ZnT4 significantly increased in cells that were repleted with zinc and iron+zinc (Fig 21C).

Simultaneous repletion: In SFM cells, NO *per se* significantly decreased ZnT-1 expression, which decreased further upon NO+iron (Fig 21B). In CM cells, NO+iron, NO+zinc or NO+iron+zinc significantly decreased ZnT-1 expression. Between SFM and CM cells, ZnT-1 expression was significantly higher in untreated SFM cells and upon NO+iron.

In both SFM and CM cells, NO+repletion decreased ZnT-4 expression, irrespective of iron, zinc or iron+zinc (Fig 21D). In CM cells, NO+repletion decreased ZnT-4 expression.

Redox status

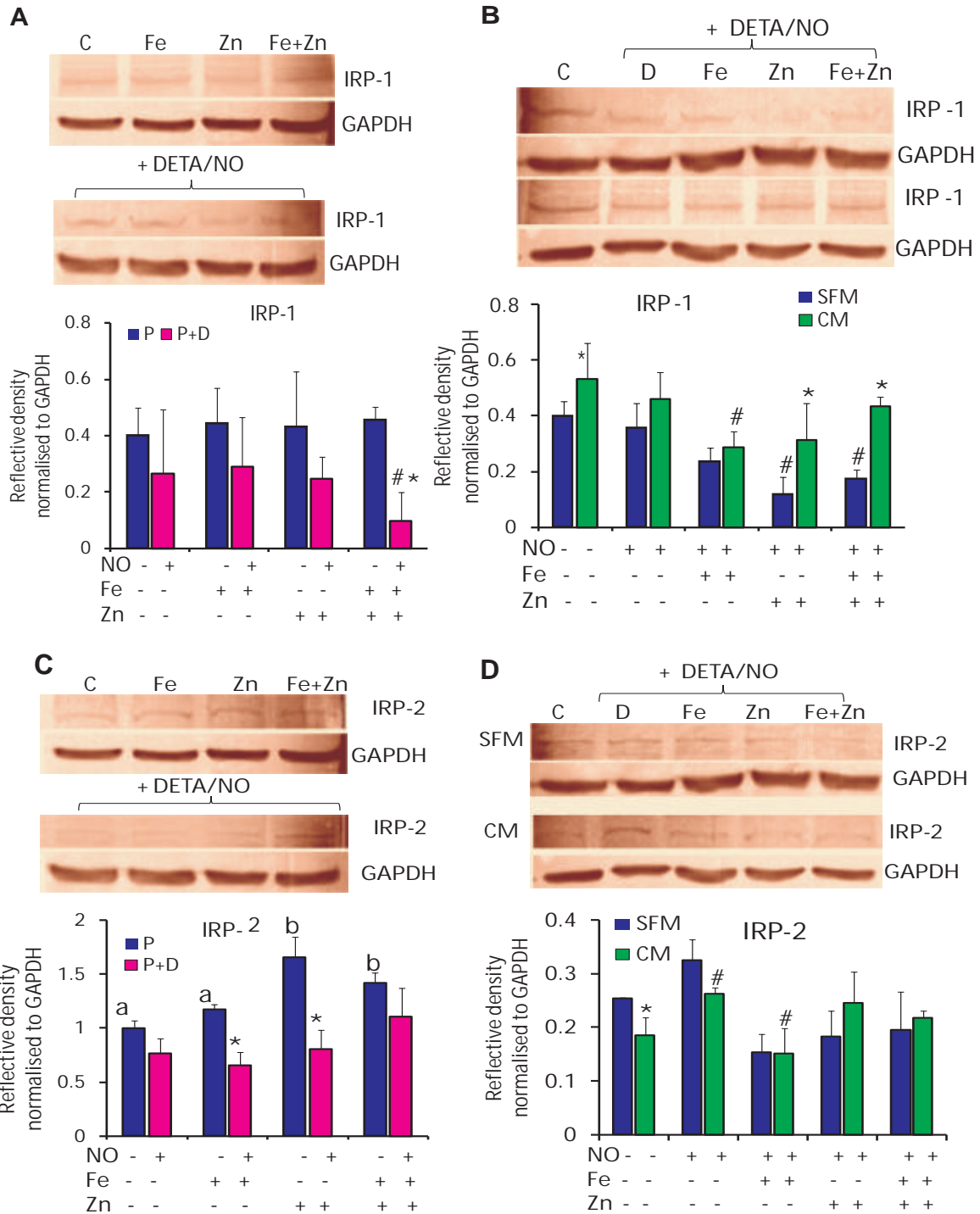
Selective repletion: In the presence of NO, the GSH/GSSG ratio increased due to either zinc and with iron+zinc (Fig. 22A).

Simultaneous repletion: In SFM the GSH/GSSG ratio was not different between treatments, compared to control untreated cells (Fig. 22B). Between SFM and CM cells, SFM cells had drastically reduced GSH/GSSG ratio compared to CM cells.

CONCLUSIONS

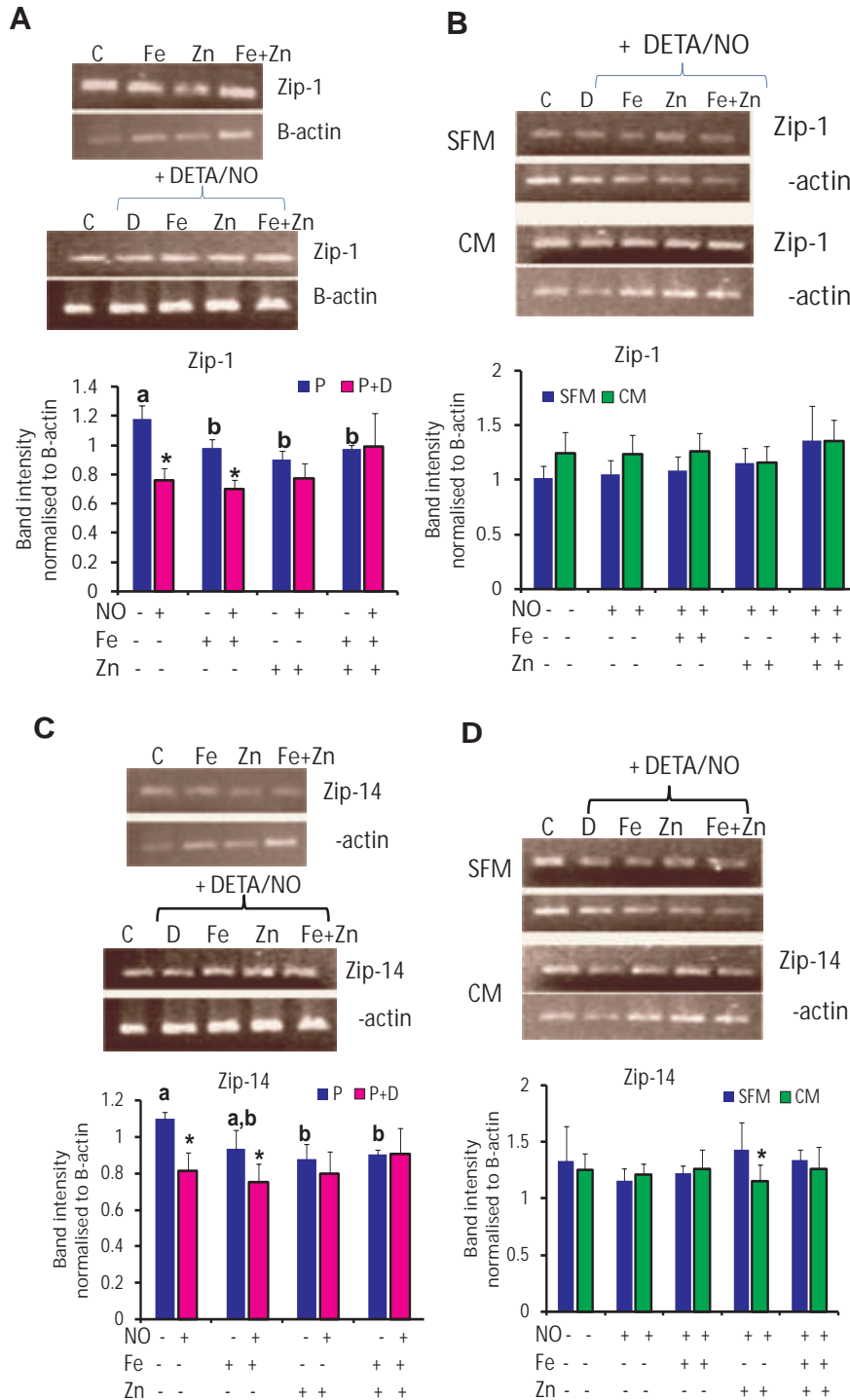
1. Under simulated inflammatory conditions, iron and zinc uptake decreased in deficient (SFM) cells.
2. Cellular zinc status and zinc uptake modulate iron uptake in the presence of inflammatory mediators such as NO, while a similar effect of iron on zinc uptake is conspicuously absent.

Figure 19. Effect of different repletion regimens on IRP-1 and IRP-2 protein expression



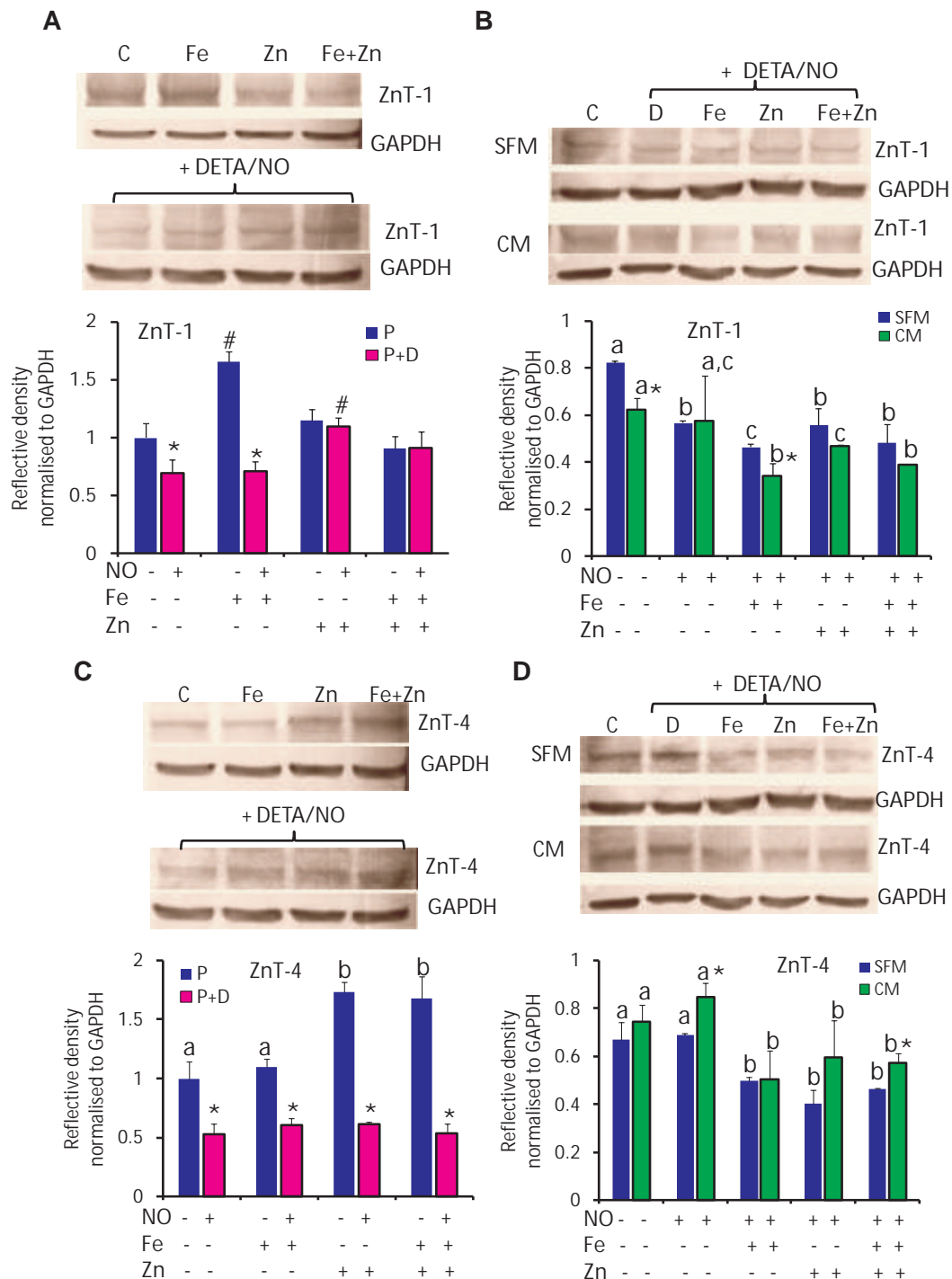
Cells were subjected to selective repletion in SFM (A&C), simultaneous repletion in SFM and CM (B&D) and IRP-1 (top panel) and IRP-2 protein expression (bottom panel) was assessed by immunoblotting and densitometrically quantitated. n=4, * indicates significant difference between groups at P<0.05. # indicates significantly different from control within group at P<0.05. Bars that do not share a common superscript are significantly different at P<0.05

Figure 20. Effect of repletion regimens on Zip-1, Zip-14 transcript levels



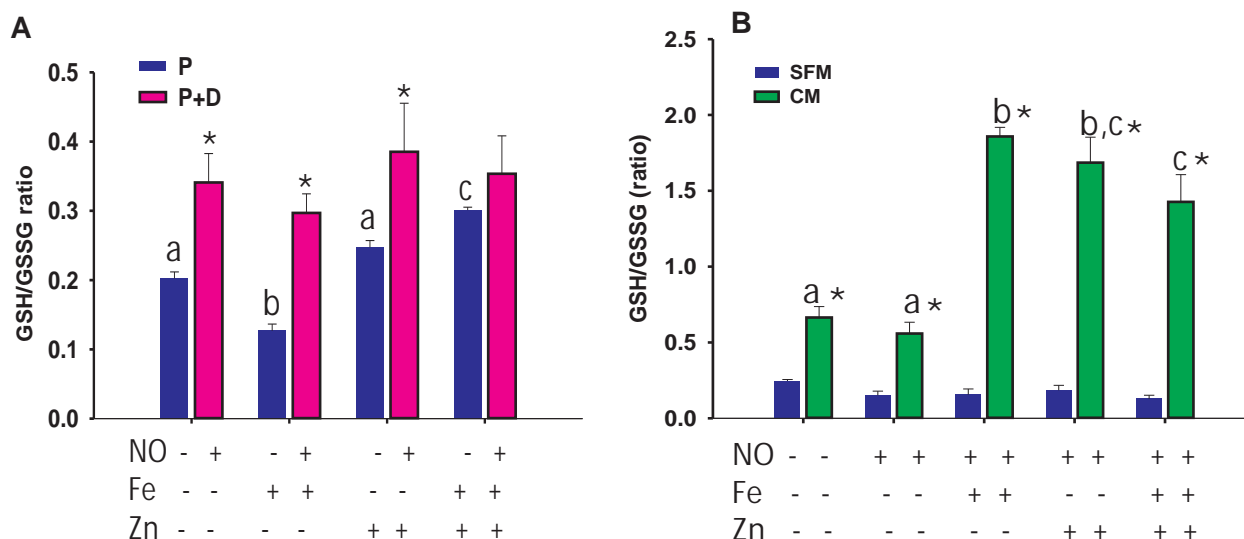
Cells were subjected to selective repletion in SFM (A & C) or simultaneous repletion in SFM and CM (B & D) and Zip-1 and Zip-14 transcript levels were assessed by semi-quantitative RT-PCR and bands densitometrically quantitated. $n=4$, * indicates significant difference between groups. # indicates significantly different from control within group $P<0.05$. Bars that do not share a common superscript are significantly different at $P<0.05$.

Figure 21. Effect of repletion regimens on ZnT-1 and ZnT-4 expression



Cells were subjected to selective repletion in SFM (A&C) or simultaneous repletion in SFM and CM (B&D) and ZnT-1 (top panel) and ZnT-4 (bottom panel) expressions were assessed and densitometrically quantitated. n=4 * indicates significant difference between groups, # indicates significantly different from control within group. Bars that do not share a common superscript are significantly different at P<0.05

Fig 22. Effect of selective repletion on redox status



Cells were subjected to selective repletion in SFM (A) or simultaneous repletion in SFM and CM (B) and GSH/GSSG was derived. n=4, * indicates significant difference between groups, bars that do not share a common superscript are significantly different at P<0.05.

- The observed decrease in iron is mirrored by a decrease in iron influx and efflux transporter (DMT-1, FPN-1) expression. This was accompanied by changes in IRP-2 expression, rather than IRP-1. However, no significant change in zinc influx (Zip-1, -14; except upon NO+zinc) was observed, whereas, efflux transporter (ZnT-1, -4) levels were decreased in SFM cells. These changes probably enabled maintenance of cellular iron, zinc homeostasis under simulated inflammatory conditions.
- Inflammatory stimuli significantly alter mineral uptake and can thus contribute to hypoferrremia and hypozincemia that is observed during enteritis.

Based on the data generated earlier and those presented above, it is concluded that zinc selectively and dose-dependently affects iron uptake and its interactions with zinc under normal conditions, depletion-repletion and simulated inflammation. Inflammation associated hypoferrremia and hypozincemia may be host-protective. Therefore, combined supplementation should take into consideration baseline zinc status and underlying inflammation, and use titrated molar ratios to minimize the adverse effect of zinc on iron status.

3. IRON, FOLATE AND VITAMIN B₁₂ LEVELS IN PREGNANT WOMEN AND THE EFFECT OF INTRAMUSCULAR IRON, FOLATE AND B₁₂ THERAPY ON IRON FOLATE AND B₁₂ LEVELS

Anaemia in pregnancy is shown to be mainly due to iron and folic acid deficiency. Studies carried out in Chennai, Vellore and Delhi in the sixties and seventies showed that anemia in pregnancy is mainly due to iron and folate deficiency. NIN carried out supplementation studies in which pregnant women were given iron alone, iron and folic acid and iron and folic acid and vitamin B₁₂. The response was best with iron and folic acid. NIN studies also showed that IFA supplementation is associated with improvement in birth weight of the offspring. Based on these observations the National Anaemia Prophylaxis Programme (NAPP) of iron and folic acid supplementation to all pregnant women was initiated.

Recent nationwide surveys show that the high prevalence of anaemia during pregnancy has not declined for the last 5 decades. However, this is mainly due to the fact that screening for and appropriate treatment of anaemia envisaged in the National Anaemia Control Programme had not been operationalised and coverage and compliance with oral iron folic acid supplementation is poor. Numerous studies including ICMR task force studies have shown that 90 tablets of iron and folic acid intake results in prevention of the deterioration of Hb but do not result in significant improvement in Hb levels. Studies carried out by NFI had indicated that even after IM injection of 1500 mg iron as iron sorbital citric acid complex majority of the women had Hb levels below 11 g/dL. Some of the recent studies have indicated that vitamin B₁₂ deficiency may play an important role in anemia.

AIMS AND OBJECTIVES

1. To investigate iron, folate and vitamin B₁₂ status in pregnant women with different grades of anaemia
2. To assess the impact of intra-muscular iron, folic acid and vitamin B₁₂ injections on indicators of iron, folic acid and vitamin B₁₂ status in pregnant women with moderate anaemia

METHODS

Pregnant women (N=508) during second trimester were recruited by NFI from the antenatal OPD clinic of Defence Colony Maternity Center, New Delhi. Blood samples were collected from 150 women with Hb above 10 g/dL (as there were very few women with Hb more than 11 g/dL). About 192 women with mild anaemia (8 – 9.9 g/dL) and 166 women with moderate anaemia (5 – 7.9 g/dL).

All women who had Hb between 5.0 and 7.9 g/dL, but had no other obstetric or systemic problems and could come for daily IM iron therapy were counselled about the need for outpatient IM therapy and given ten injections each consisting of iron sorbitol citric acid complex containing 150 mg iron, 1500 µg folic acid, 150 µg hydroxocobalamine acetate (vitamin B₁₂). Blood samples were available from 73 women before and after IM iron therapy.

NIN carried out the biochemical investigation for iron, ferritin, folic acid, vitamin B₁₂ status and c-reactive protein using immunoassay.

RESULTS

Majority of women in all the three groups weighed more than 45 kg in the mid trimester. About 48% were primes and the remaining 36% of parity one and 14% with parity two.

Iron folate and B₁₂ status in relation to Hb levels

Mean serum iron, ferritin, folic acid and B₁₂ levels in the three groups is shown in Table 16 .

Serum iron levels were significantly lower in the group with Hb less than 8/gdL.

Nearly half of the women with Hb less than 8 g/dL were iron and /or folate deficient. In contrast, among women with Hb more than or equal to 10 g/dL, less than one fifth had iron deficiency and only 14.2% had folate deficiency. In women with Hb less than 10g/dL about 1/5th had vitamin B₁₂ deficiency and in the group with Hb \geq 10g/dL only 12 % had vitamin B₁₂ deficiency (Table 17) . These data indicate that iron and folate deficiency are major factors responsible for anaemia in pregnancy in the population studied. Vitamin B₁₂ deficiency does exist in this population but the magnitude of vitamin B₁₂ deficiency is much lower than the iron and folate deficiency .

Table 16. Mean Hb, serum iron, ferritin, vitamin B12 and folic acid in pregnant women

	Hb	Hb $\mu\text{g/dl}$	Serum Iron $\mu\text{g/dL}$	Ferritin $\mu\text{g/L}$	Vitamin B ₁₂ pg/ml	Folic acid ng/ml
I	5.0-7.9	7.2 \pm 0.77 (166)	119.4 \pm 113.8 (166)	16.3 \pm 14.8 (165)	409.4 \pm 485.4 (166)	5.0 \pm 7.0 (163)
II	8.0-9.9	9.0 \pm 0.42 (192)	96.0 \pm 43.8 (192)	27.5 \pm 17.8 (191)	315.8 \pm 145.5 (192)	4.7 \pm 4.1 (191)
III	\geq 10.0	10.5 \pm 0.44 (150)	104.3 \pm 45.4 (150)	23.0 \pm 16.2 (150)	314.1 \pm 138.8 (149)	8.3 \pm 6.0 (148)
Total		8.9 \pm 1.4 (508)	106.1 \pm 75.1 (508)	22.5 \pm 17.0 (506)	345.9 \pm 304.0 (507)	5.9 \pm 5.9 (502)

Values are mean \pm SD and parenthesis (N)

Table 17. Prevalence of iron, folic acid and vitamin B₁₂ deficiency in relation to Hb status

Parameters	Haemoglobin (g/dL)			Total
	5.0 - 7.9	8.0 – 9.9	\geq 10.0	
Ferritin (less than 12 $\mu\text{g/L}$)	54.2 (90)	10.4 (20)	19.3 (29)	23.9 (139)
Iron (less than 60 $\mu\text{g/dL}$)	33.7 (56)	22.4 (43)	8.7 (13)	22.0 (112)
Folic Acid (less than 3 ng/ml)	47.6 (79)	45.3 (87)	14.2 (18)	36.2 (184)
Vitamin B ₁₂ (less than 200 pg/ml)	19.3 (32)	21.9 (42)	12.6 (16)	15.5 (90)

In the group with Hb less than 8 g/dL about 30 % of women had combined iron and folic acid deficiency; over 12% had combined iron and B₁₂ deficiency and 6% had deficiency of all the three micronutrients (Table 18). Prevalence of combined deficiencies were significantly lower in the group with Hb levels between 8-9.9 g/dL. It was surprising to note that in one-fourth of the women

with moderate anaemia and one half of the women with mild anaemia there was no biochemical evidence of iron, folic acid or B₁₂ deficiency. Some of these women might have haemoglobinopathies but haemoglobinopathies cannot account for 50% of the women with mild anaemia. Obviously, there is a need to investigate for the presence of other micronutrient deficiencies that may contribute to anaemia.

Prevalence of isolated iron, folic acid and B₁₂ deficiencies in relation to Hb levels in these pregnant women was computed and is shown in Table 19. Isolated iron deficiency appeared to be more common in women with Hb <8 g/dL ; while isolated folic acid and B₁₂ was more in pregnant women with moderate anemia. However, it is clear that isolated iron, folic acid and B₁₂ deficiencies exist, but are not common.

Table18. Prevalence of combined deficiencies in pregnant women

Combined Deficiencies	Haemoglobin (g/dL)			Total
	5.0 - 7.9	8.0 – 9.9	≥ 10.0	
Iron and Folic Acid	29.5 (49)	6.8 (13)	1.3 (2)	12.6 (64)
Iron and vitamin B ₁₂	12.7 (21)	3.1 (6)	0	5.3 (27)
Iron, Folic Acid and vitamin B ₁₂	6.0 (10)	1.0 (2)	0	2.1 (12)
Normal (above cut off of defieicny)	24.7 (41)	38.5 (74)	57.3 (86)	39.6 (201)

Parenthesis (N)

Table 19. Isolated iron, folate and B₁₂ deficiencies in pregnant women

Isolated deficiencies	Haemoglobin (g/dL)			Total
	5.0-7.9	8.0-9.9	≥ 10.0	
Iron	17.5 (29)	1.6 (3)	12.7 (19)	8.8 (51)
Folic acid	13.3 (22)	31.8(61)	6.0 (9)	14.1 (82)
Vitamin B ₁₂	1.8 (3)	11.5 (22)	8.7 (13)	6.6 (38)

Parenthesis (N)

Impact of IM injection of iron folic acid and vitamin B₁₂

Following IM therapy there was a dramatic improvement in mean values of hemoglobin and serum ferritin but there was no significant change in mean values of folate and vitamin B₁₂ (Table 20). Over 80% completed the six injections. They were followed up through pregnancy and till delivery. There was a significant rise in Hb levels but even 9 weeks after completion of IM therapy mean Hb was only 9.6g/dL.

Prevalence of combined iron and folate deficiency came down from 34.2 to 6.8% and isolated iron deficiency from 23.3 to 1.4%. There was a steep increase in isolated folate deficiency from 8.3 to 35.6% following IM therapy. There was no change in prevalence of B₁₂ deficiency after IM therapy (Table 21).

These results suggest that IM therapy resulted in differential response to the indicators of iron, folate and vitamin B₁₂ status. This could be due to the following reasons. The improvement in iron status indicators could be due to the slow release of iron from the site of absorption and its utilization

in Hb production and store repletion. Lack of response to the injected folate and vitamin B₁₂ may be due to the faster excretion of these two water soluble vitamins. The poor folate and B₁₂ status might have partly contributed to the suboptimal Hb response. It is well known that iron therapy unmasks latent folate deficiency in moderately anaemic women; folate deficiency might be one of the factors responsible for the relatively sub-optimal response to IM therapy.

Table 20. Impact of IM injection on iron, folic acid and vitamin B₁₂ status of Pregnant women

Hb	Hb g/dL	Serum Iron µg/dL	Ferritin µg/L	Vitamin B ₁₂ pg/ml	Folic acid ng/ml
5.0-7.9 Pre	7.2±0.82 (73)	122.6 ±114.8 (73)	12.8 ±12.2 (72)	435.9 ±431.7 (73)	5.4 ±5.2 (72)
Post	9.2 ±0.86 (73)	120.2 ±44.6 (73)	36.1 ±22.8 (72)	427.7 ±215.8 (73)	5.1± 5.2 (73)

Values are Mean±SD; Parenthesis (N)

Table 21. Impact of IM injection on the prevalence of iron, folate and vitamin B₁₂ deficiencies

Combined Deficiencies	Pre IM	Post IM	Isolated deficiency	Pre IM	Post IM
Iron + Folic Acid	34.2 (25)	6.8 (5)	Iron	23.3 (17)	1.4 (1)
Iron + vitamin B ₁₂	10.9 (8)	0	Folic acid	8.2 (6)	35.6 (26)
Iron + Folic Acid + Vitamin B ₁₂	4.1 (3)	0	Vitamin B ₁₂	1.4 (1)	4.1 (3)

Parenthesis (N)

CONCLUSIONS

The study has shown that prevalence of iron and folic acid deficiency is high and prevalence of vitamin B₁₂ deficiency is relatively low. It is logical that the National programme for control of anaemia in pregnancy has a focus on iron and folic acid medication in appropriate doses and routes in this population. However, Pune maternal nutrition study and the Bangalore study indicate that prevalence of vitamin B₁₂ deficiency was high in their study population. In view of the findings that following IO IM injection of iron, folic acid and vitamin B₁₂ there was no improvement in folic acid and vitamin B₁₂ status, it might be important to estimate the prevalence of folate and vitamin B₁₂ deficiency in different parts of the country. If the prevalence of B₁₂ deficiency is high in many regions, it might be appropriate to undertake studies to find out the impact of B₁₂ given along with the iron and folic acid to pregnant women.

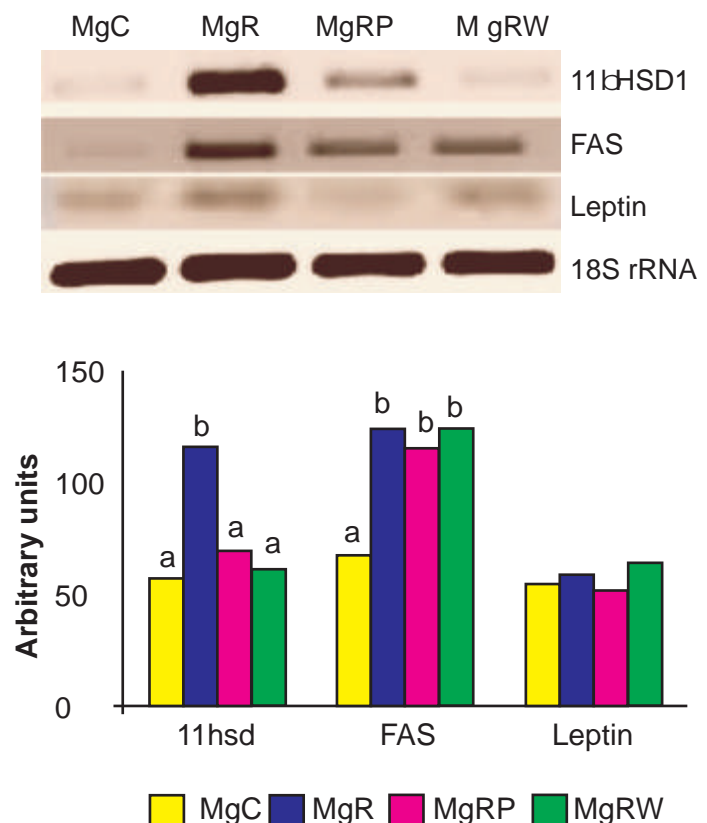
4. MATERNAL MAGNESIUM RESTRICTION INDUCED INCREASE IN THE ADIPOSITY OF WNIN RAT OFFSPRING MAY BE DUE TO INCREASED STRESS AND FATTY ACID SYNTHESIS

It was recently reported by NIN that maternal magnesium restriction irreversibly increased body fat %, specially the visceral adiposity. It was assessed whether altered adipogenesis (expression of fatty acid synthase and/ or adipokine: leptin) underlies this increased adiposity and also whether this could be due to increased gluco-corticoid stress in them. This was achieved by determining the expression of 11 HSD 1, fatty acid synthase (FAS) and leptin genes in the adipose tissue of the offspring of different groups at the time of their sacrifice at 18 months of their age. In addition, plasma leptin levels and FAS (protein) levels in liver were also determined.

RESULTS

Circulating leptin levels were significantly lower in MgR than control offspring. Interestingly, rehabilitation from parturition (MgRP) but not weaning (MgRW) appeared to correct these changes suggesting the importance of Mg during pregnancy and lactation in modulating this parameter. That the expression of leptin gene in adipose tissue was however comparable among the offspring of different groups appears to suggest changes in the plasma leptin levels observed may not be due to changes at gene transcription level. On the other hand, expression of FAS was increased in MgR compared to control offspring and similar changes were observed both at the protein and gene expression levels (Fig.23). It was of interest that rehabilitation in general had no effect on this parameter at both protein and gene expression levels. Taken together, these two observations suggest that maternal Mg restriction induced irreversible changes in adiposity (visceral adiposity) could be due to altered adipogenesis (leptin levels) and or fatty acid synthesis (FAS expression).

Fig 23. Expression of leptin, FAS and 11 HSD 1 genes in the rat offspring of different groups



The letters 'a' & 'b' are statistically different at $p < 0.05$ using one - way ANOVA

That increased stress may be associated with / responsible for the increased body adiposity (visceral adiposity) is evident from the significant increase in the expression of 11 HSD 1 gene in the adipose tissue of the MgR offspring compared to controls. Although both the rehabilitation regimes could correct the change in the expression of 11 HSD 1 gene in the adipose tissue, they could not correct the changes in the expression of FAS (gene as well as protein) or the increased visceral adiposity which appear to suggest that maternal MgR induced stress in the offspring may be correctable by rehabilitation but not the changes induced by this stress in the offspring (eg.increased visceral adiposity).

CONCLUSION

Results suggest that increased stress (gluco-corticoid related) and fatty acid synthesis (up-regulation of 11 HSD 1 and FAS) could probably underlie the increased visceral adiposity in the offspring of Mg restricted rat dams.

5. HEALTH BENEFICIAL EFFECTS OF PLANT FOODS COMMONLY CONSUMED IN INDIA: MILK, MILK PRODUCTS, OILS AND SUGARS

Epidemiological evidence demonstrates that plant foods are rich sources of phenolic compounds with potent antioxidant activity. In recent years much work has been focused on antioxidant properties of natural sources. In this context, NIN is carrying out studies to assess the antioxidant activity (AOA) in foods commonly consumed in India and to find out its correlation with their phenolic content (TPC). So far studies were conducted at NIN reported data on the phenolic content and AOA of cereals, millets, pulses and legumes, fresh and dry fruits, roots, tubers, vegetables, nuts and oil seeds commonly consumed in India (*Annual Reports 2005-09*). In continuation of these studies, the antioxidant activity of milk, milk products, oils and sugars was studied.

MATERIALS AND METHODS

Milk, milk products (curd, butter, ghee, khoa etc), oils and sugars were collected from four different local markets of the twin cities. Standard extraction and estimation protocols were adopted as described earlier (*Annual Reports 2000-2007*). While the total phenolic content (TPC) was determined by the Folin's method, the anti-oxidant activity (AOA) was determined by two different methods. 1. Ferric Reducing Scavenging (FRAP) 2. DPPH Scavenging activity. The salient findings of the study were:

RESULTS

1. Data on AOA and TPC of the foods is given in table 22 and the correlation between AOA and TPC is given in Table 23.
2. DPPH activity ranged from 3.41 – 208.17 mg / 100g with jaggery having the highest DPPH activity followed by ground nut oil (22.14) and lowest activity found in whole milk (3.41).

- FRAP activity ranged from 11 -11674 mg/100g, with the highest activity in jaggery (11674) followed by cane sugar juice (872.64) and lowest activity observed in ground nut oil (11.12 mg/100g).
- Total phenolic content of milk, milk products, edible oils and sugars also showed a wide range: 0.72 – 336 mg/100g. Here again jaggery had the highest TPC (336.48) followed by honey (140.43) and the least phenolic content was in vanaspathi (0.72).

Despite that the foods analysed belonged to different food classes, a significant correlation was observed between AOA (both FRAP and DPPH) and TPC and the “r” value was 0.93 for both the AOA parameters. As expected the antioxidant parameters DPPH and FRAP in different foods showed significant correlation as well $r = 0.999$.

Table 22. Antioxidant activity of milk, milk products, edible oils and sugars commonly consumed in India (mg / 100g or ml)

S. No.	Common name	Phenolic content (Gallic acid equivalent)	Antioxidant activity	
			DPPH (Trolox equivalent)	FRAP (FeSO ₄ equivalent)
1	Toned milk (dairy milk)	3.42±0.46	4.17±0.39	73.94±8.12
2	Whole milk	3.05±0.25	3.41±0.39	38.92±1.69
3	Curds (yoghurt)	7.46±0.67	4.13±0.53	117.04±2.78
4	Ghee (clarified butter)	10.27±1.35	14.71±0.88	333.04±19.47
5	Groundnut oil (unrefined)	3.25±0.30	22.14±0.50	11.12±0.00
6	Groundnut oil (refined)	3.18±0.11	13.27±0.05	36.42±5.56
7	Sunflower oil	1.50±0.24	12.46±0.70	36.14±1.39
8	Vanaspathi (Dalda)	0.72±0.06	13.14±1.88	73.94±3.36
9	Palmolein oil	3.26±0.25	12.10±0.61	132.32±19.74
10	Til (Sesame) oil	5.15±0.67	19.93±1.71	372.79±78.98
11	Honey	140.43±26.6	19.62±3.31	604.92±86.41
12	Jaggery	336.48±12.85	208.17±27.19	11674.88±1133.48
13	Cane sugar juice	27.17±6.00	22.02±6.36	872.64±146.90
14	Sugar	12.28±3.19	15.16±1.48	389.75±24.20
Range		0.72- 336	3.41- 208.17	11-11674

Values are Mean ± SD, n = 3, p < 0.01.

Table 23. AOA Vs TPC correlation of milk, milk products, edible oils and sugars

Correlation*	r	r ² %
TPC vs DPPH	0.93	86.52
TPC vs FRAP	0.93	87.55
DPPH vs FRAP	0.99	99.03

CONCLUSION

Amongst the different foods studied, jaggery appears to be the richest in AOA and the TPC to be an important contributor to its AOA.

6. THE ROLE OF SPECIFIC NUTRIENTS ON ISLET CELL GENERATION FROM ADULT TISSUE STEM CELLS - *IN VITRO* AND *IN VIVO*

Adult ductal cells share some similarities with embryonic primitive ducts and retain the ability to generate endocrine cells in the adult. During regeneration, duct cells act as progenitors for the generation of new pancreatic cells. Nestin positive cells (NPC) are a distinct population of islet progenitor cells residing in the pancreatic islets and in ductal epithelial cells and have been demonstrated to participate in the lineage specific differentiation to Insulin secreting cells (ISC). Understanding the modulation rendered by nutrients on the pancreatic endocrine cells and their transcriptional regulation will open intriguing possibilities to basic research and therapy. Hence, the approach has been to understand the ability of the pancreatic progenitors such as Ductal epithelial cells (DEC), Nestin positive cells (NPC) and Neurogenin positive cells (Ngn3) to differentiate into ISC under regulation of specific nutrients and growth factors.

In fact, dietary modulation to study the pancreatic beta cell mass expansion appears significant due to the participation of the nutrients in wide array of metabolic pathways during the developmental process and this recapitulates the prenatal and early post natal periods of development. NIN primarily investigates to understand the interplay between nutrient and pancreatic progenitors (PP) towards their expansion and differentiation to neoislets.

AIMS AND OBJECTIVES

- a) Isolation, characterization and *in vitro* culture of pancreatic progenitor / stem cells such as Ductal Epithelial Cells (DEC) from the adult mice pancreatic tissue.
- b) Regulation of specific growth factors & micronutrients during *in vitro* propagation, proliferation & differentiation of DEC to ISC – Understanding its microenvironment.
- c) The feasibility of ISC to mitigate hyperglycemia in Type I diabetes using relevant model systems.

METHODOLOGY

Enriching the serum free medium with Growth factors and Nutrients such as PLP for the Pancreatic DECs / ABCG-2 +ve cultures (progenitors)

The primary cultures which contained predominantly DECs were maintained in RPMI 1640 with 10% FBS for a period of 24 hours, then seeded on to collagen coated plates. After 48 hours, the medium was replenished with serum free medium (DMEM /Ham's F12) containing 20ng/ml KGF, 1g/L ITS, 2g/L BSA and EGF (20ng/ml) with or without 5mM PLP. With a third day change of the medium, cultures were followed for two weeks and were subjected for passages (2-3 times) to assess the proliferation and expansion of the cells.

Expansion of pancreatic DEC cultures

After attaining 70% confluency, the cells were trypsinized and replated for the expansion. For passages 2 and 3, the cells were cultured for an additional 5–6 days in the same medium. Later the growth factor medium was removed for the differentiation of the pancreatic ductal epithelial cells. The cells have been characterised by the CK-19 stain, proliferation by Brdu incorporation.

Differentiation of pan DEC to ILCC

After attaining 60-70% confluency, media was depleted of growth factors and replaced by a defined set of differentiation factors. The protocol is defined here: Cells were counted for initial seeding density and re-suspended in growth factor depleted SFM (day zero medium) containing DMEM/F-12 with 1% BSA and 1x ITS. On day four the medium was supplemented with DMEM/F-12 with 1% BSA and 1g/ Lr ITS along with 0.3mM taurine and on day 10 the medium contained DMEM/F-12 with 1.5% BSA, 1g/Lr ITS, 3.0 mM taurine, 100µM NEAA, 1 mM nicotinamide and 100 nM GLP-1. The media was changed every 2 days. The *in vitro* generated neoislets were characterised by DTZ staining. The islets were fixed for immunocytochemistry.

Characterization of the neoislets

The neoislets generated *in vitro* at different time points were characterised for the Insulin secretion potential and C-peptide content by ELISA. The cells were also characterised for the mRNA expression levels of different maturation markers like Insulin, glucagon, Glut2, Pdx-1, Neuro D along with immuno localization of Insulin, Glut2 and Pdx-1 and NeuroD. Dithizone (DTZ) stain (10mg/ml in DMSO) was used to stain neoislets.

RESULTS

- ❖ Enriching the microenvironment of pancreatic ductal epithelial cells with growth factors and PLP stimulated the DEC culture to proliferate which is evidenced by increased BrdU incorporation as compared to the controls.
- ❖ Supportive data has been obtained by using immunohistochemistry for CK-19, BrdU, Vimentin and Insulin and RT-PCR for insulin, pdx-1 and reg-1.
- ❖ The DEC cultures have shown a transition from epithelial to mesenchymal phenotype before differentiation which is evidenced by vimentin staining.
- ❖ Removal of growth factors and addition of differentiation factors (0.3mM taurine) to the medium resulted in formation of islet like cell clusters, which stained for DTZ, a specific dye for the islets. Further addition of 3mM taurine, 1mM nicotinamide and NEAA resulted in the maturation of islet like cell clusters.
- ❖ The 3 step differentiation protocol resulted in increased insulin secretion of neoislets by 2.1x and increased C-peptide levels by 1.5 x (Fig. 24).

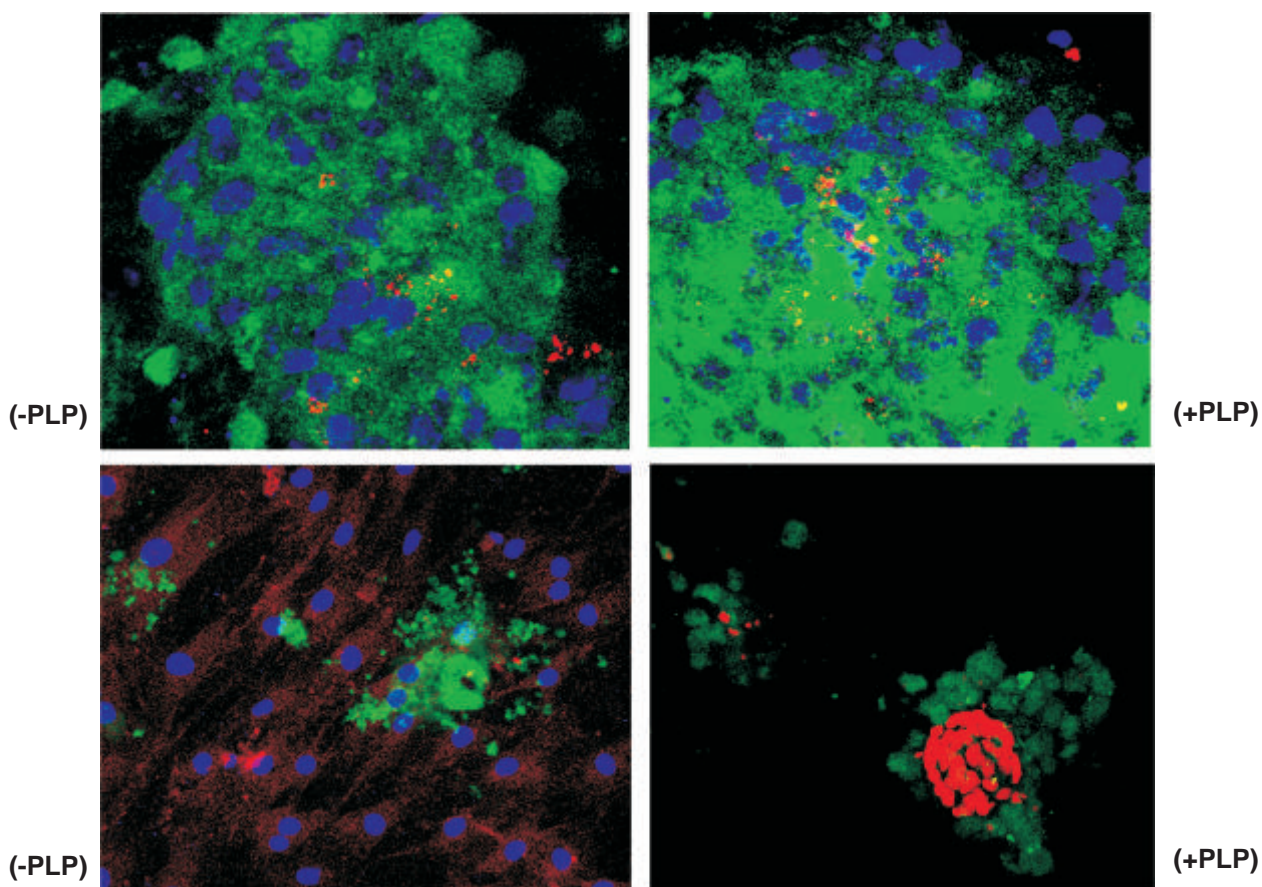
CONCLUSIONS

Addition of EGF, KGF and ITS along with vitamin B6 recreates a physiological environment for epithelial cells (CK-19) and intraislet precursor cells to generate large number of progenitors that can then be allowed to differentiate to endocrine cells.

Functional characterization of neoislets – Transplantation into STZ treated mice

In vivo, functional efficacy of the neoislets were assessed by transplantation into STZ treated mice. For this, adult mice taken from the animal facility at NIN were fasted overnight and injected with freshly prepared STZ (180 mg/kg body weight). Blood glucose concentrations were monitored by tail prick method. The animals were considered diabetic when blood glucose was consistently above 200 mg/dL for more than 4 days.

Fig 24. Ck-19/ Insulin staining of neoislets generated from DEC cultures.



Transplantation of islets into diabetic mice

Mice were randomly divided into three groups ($n=6/\text{group}$). One group (control) of mice received citrate buffer (pH 4.5) and the other two groups received STZ. Animals receiving STZ developed diabetic condition in 4-5 days. These animals were divided into streptozotocin (STZ) group and transplanted (Tx) groups. STZ group mice were treated as experimental controls (sham operated). These animals received vehicle (50 μl of blood from allogenic mice). Tx group animals were transplanted with 400 *in vitro* generated neoislets.

Mice were anaesthetised and after cleaning the left dorsal side an incision was made near the left kidney area. Meanwhile, around 50 μl blood was collected from the same animal (allogenic mouse) and 400 neoislets have been entrapped into them which was subsequently placed under the sub renal capsular space. The STZ group mice received only blood clot whereas Control animals were flushed with saline. The peritoneum was sutured with dissolvable cat guts. The skin was closed using skin punching machine. The animals were allowed to recover under warm lamp. Body weight and blood glucose levels of the animals were monitored on a regular basis. Blood glucose levels were estimated after 24 h and then after every alternate day up to 4 weeks from snipped tail. On the day of sacrifice, IPGTT was performed to the mice from all groups. Animals were sacrificed by CO₂ asphyxiation and tissues were processed for histopathological and immunohistochemical analysis.

Intra peritoneal glucose tolerance test

After an overnight fast, the animals were i.p. injected with 2g/kg body weight of glucose. Blood glucose levels were measured at 0, 15, 30, 60, 90, 120 and 150 min post injection.

RESULTS

The neoislets transplanted into STZ induced diabetic animals (Tx group) have

1. Restored the blood glucose and body weights by the end of two weeks (Fig. 25).
2. Normalised the plasma insulin levels.
3. Shown IPGTT response similar to control and
4. Insulin staining in the renal graft sections (Fig. 26).

Fig. 25 Blood Glucose concentrations (mg/dl) of Control, STZ and Tx group mice

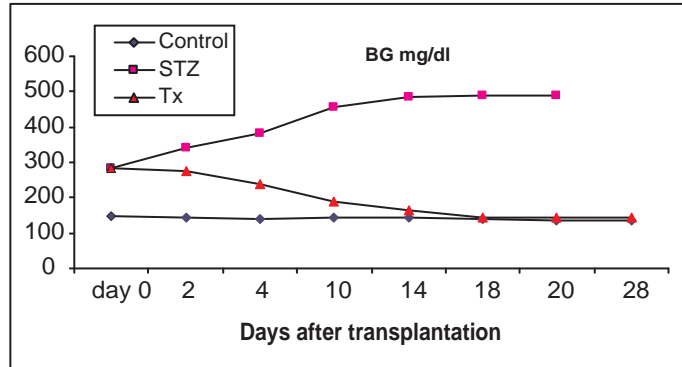
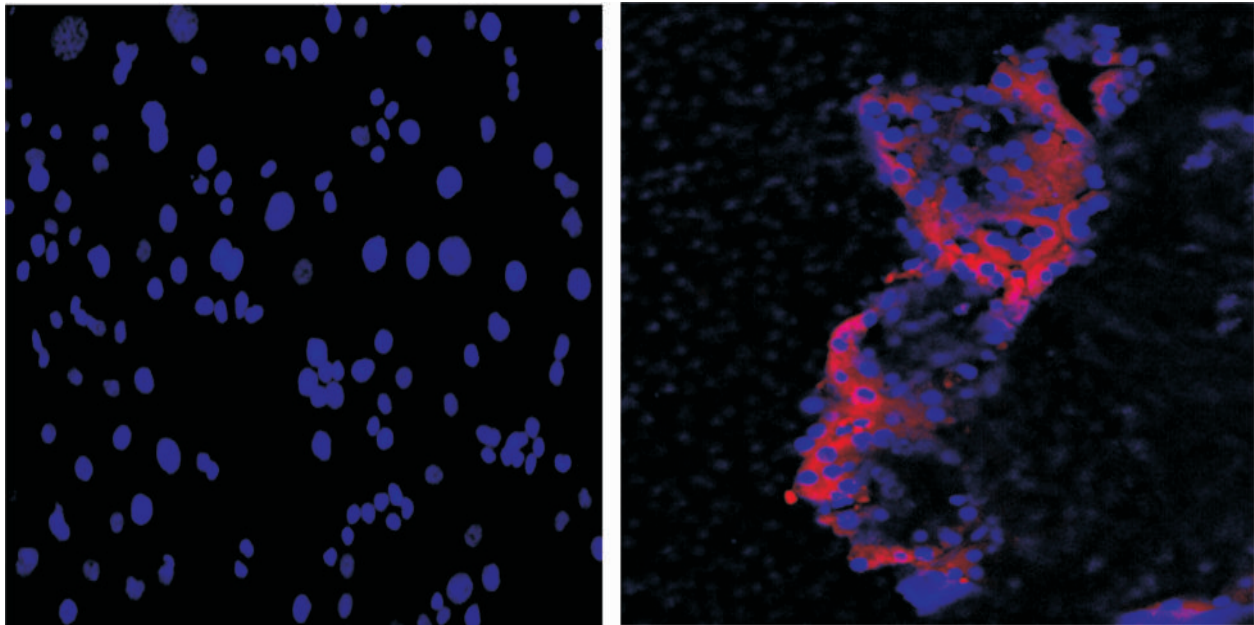


Fig. 26 Insulin staining in kidney grafts of STZ (sham operated) and Tx mice



Salient findings of the study

- ❖ Pyridoxal phosphate (PLP—a vitamin B6 cofactor) in combination with growth Factors, showed increased BrdU incorporation, stimulated the proliferation of the DEC/CK-19+ve/ABCG-2 (pancreatic progenitors).
- ❖ Supportive data has been obtained by using immunohistochemistry for CK-19, BrdU, Vimentin and Insulin and from mRNA expression for Insulin, Pdx-1 and Reg-1.
- ❖ There has been a transition from epithelial to mesenchymal phenotype (CK-19 to vimentin Positive) before differentiation into the neoislets.

- ❖ Addition of Taurine, Nicotinamide and NEAA (differentiation factors) resulted in the formation of islet like cell clusters (iLC) and the matured iLC stained for insulin. The *in vitro* generated neoislets were functionally viable when challenged with high glucose.
- ❖ The transplanted animals showed normalization in their glucose profiles and cytoarchitecture of the pancreatic tissue.

CONCLUSIONS

These observations suggests for the protective and beneficial effects of PLP against the islet cell dysfunction/ beta cell death in the diabetic model as preservation and or cytoprotection of beta cell mass after diabetogenic insult is of utmost importance towards the control and management of diabetes. This study gives a scope for understanding the nature of the populating cells either the residual beta cells/progenitor cells of the pancreatic tissue with a diabetic insult.

7. CHARACTERIZATION AND PROLIFERATION OF PANCREATIC PROGENITOR CELLS/STEM CELLS TO INSULIN SECRETING CELLS – ROLE OF NUTRIENTS

The capacity of self renewal and differentiation of stem cells makes them a potential source of generation of pancreatic beta cells for treating of type 1 diabetes mellitus. Strategies to differentiate progenitor cells into beta cells *in vitro* have been considered as an alternative to increase beta cell availability prior to islet transplantation. Nestin a marker for neural precursor cells has been suggested marker for islet progenitor cells expressed with in the pancreas. NIN primarily investigates to understand the interplay between nutrient and Pancreatic progenitors (PP) towards their expansion and differentiation to neoislets. The present work was primarily focused to induce differentiation of the pancreatic progenitors such as nestin positive cells (NPC) to insulin secreting cells (ISC) in presence of an All Trans Retinoic acid (RA) with the combination of other mature factors in two weeks and its *in vivo* efficacy in reversing the diabetes in STZ induced mice model.

AIMS AND OBJECTIVES

- In vitro* isolation, characterization and culture of pancreatic progenitor /stem cells Nestin positive cells (NPC) in the adult mice pancreatic tissue using appropriate growth factors and modulators.
- Induction of NPC in culture to increase the Nestin positive cells using the appropriate growth factors and the transdifferentiation to ISC (neo islets).
- The effect of nutrients in relation to the proliferation and differentiation of the pancreatic progenitors in cultures at different time points will be studied.
- In vivo* feasibility of the neo islets to mitigate the hyperglycemia in STZ induced diabetes will be assessed using mice as a model.

METHODOLOGY

Concentration of all trans retinoic acid (RA) required for cultures have been standardized, by studying at various cons of RA concentration ranging from 0.5 μ M-10 μ M. The viability and the caspase 3 activity has been assessed. The NPC were cultured with Growth factors(EGF,FGF) and in combination of with and without RA to study the proliferation rate of the cells. At all the time points of the culture different markers by immunocytochemistry and RT PCR were examined. The differentiating medium consisted of agents, RA, Zn in addition to Taurine and Nicotinamide. The generated insulin secreting cells (neo islets) assessed both by immunocytochemistry for insulin, PDX1, GLUT 2, semiquantitative PCR and functionally for Insulin, C-peptide by ELISA and western blot. The neoislets were cryopreserved with DMSO and glycerol.

Transplantation of islets into diabetic mice

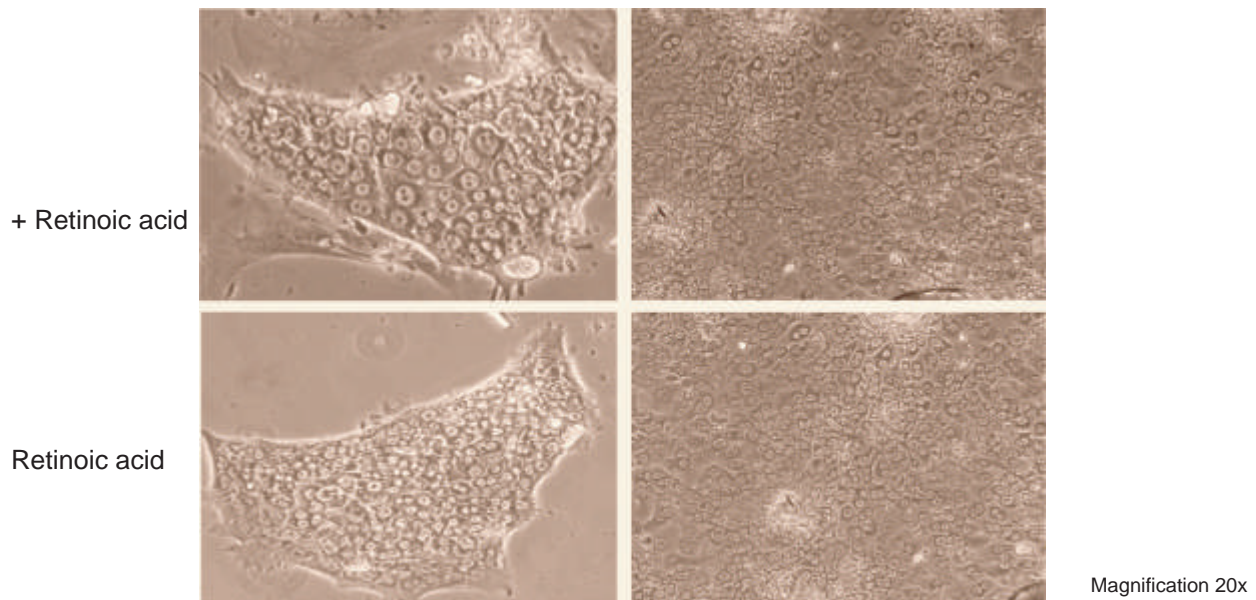
In vivo, functional efficacy of the neoislets were assessed by transplantation into STZ treated mice. For this, adult mice taken from the animal facility were fasted overnight and injected with freshly prepared STZ (180 mg/kg body weight). Blood glucose concentrations were monitored by tail prick method. The animals were considered diabetic when blood glucose was consistently above 200 mg/dL for more than 4 days. The mice were randomly divided into three groups ($n=6$ /group). One group (control) of mice received citrate buffer (pH 4.5) and the other two groups received STZ. Animals receiving STZ developed diabetic condition in 4-5 days. These animals were divided into streptozotocin (STZ) group and transplanted (Tx) groups. STZ group mice were treated as experimental controls (sham operated). These animals received 50 μ l of blood from allogenic mice as vehicle. Tx group animals were transplanted with 400 *in vitro* generated neoislets. The methodology for the transplantation work is similar to the previous work reported above. At the end of the experiment the animals were then sacrificed to look for insulin in the kidney sections and plasma insulin values are determined.

Gene	Forward/Reverse	Size
actin	TGGAATCCTGTGGCATCCA/TAACAGTCCGCTAGAAGCA	450
Nestin	ATACAGGACTCTGCTGGAGG/AGGACACCAGTAGAACTGGG	410
Insulin	TCCGCTACAATCAAAAACCAT/GCTGGGTAGTGGTGGGTCTA	411
Glucagon	ACTCACAGGGCACATTACC/CCAGTTGATGAAGTCCCTGG	353
PDX-1	CCTGCGTGCCTGTACATGGG/TTTCCACGCGTGAGCTTTGG	300
Ngn3	GGTGTCTGCCCACCTTCCC/TGGGAGACTGGGGAGTAGA	212
NeuroD	GCGCTCAGGCAAAAGCCC/GCCATTGATGCTGAGCGGCG	374
Pax4	CTGTGTGAGCAAGATCCTAG/GGAGAAGATAGTCCGATTCC	379
Nkx6.1	TCTTCTGGCCCGGAGTGATG/TCCTGCTTCTTCTGGCCG	313
GLUT2	CGGTGGGACTTGTGCTGCTGG/CTCTGAAGACGCCAGGAATTCCAT	416
ABCG 2	ATGAAACCTGGCCTTAATGCT/AGAAGATGATTGTTGACCCT	423
RAR	CAGTTCCGAAGAGATAGTACC/TACACCATGTTCTTCTGGATTGC	167
PCR conditions		
Initial denaturation 94°C / 3', (35 cycles - denaturation 94°C /30", annealing 56 -58 °C/30", elongation 72°C/45"), final incubation 72°C/3', hold 4 °c		

RESULTS

- ❖ Loss of insulin staining and increased Nestin by 1 week suggests that there is no repopulation of cells, and proliferating cells are progenitor cells.
- ❖ Based on immunocytochemistry cells cultured in the presence of RA showed increased staining for Nestin/ABCG2, BrdU showing the increased number of proliferating NPCs (Fig 27).
- ❖ Based on the RT PCR data upregulation of Nestin, Abcg2 and down regulation of Insulin, Pdx1 confirming the proliferating cells are progenitor cells.
- ❖ Based on the cell cycle data of 3rd passage cultures indicates that the proliferating cells are in either G0/G1, or G2/M (Fig 28).
- ❖ Vimentin positive staining observed during induction suggests the possibility of epithelial to mesenchymal transition during differentiation. Neoislets positive insulin, Glut2, positive DTZ staining suggests the neo islets capable of synthesizing and secreting insulin (Fig. 29).

Fig 27. 3rd passage (14day)- NPC cultures



mRNA for NPC and ABCG-2 was upregulated as indicated.

Fig 28. Neoislets stained with insulin

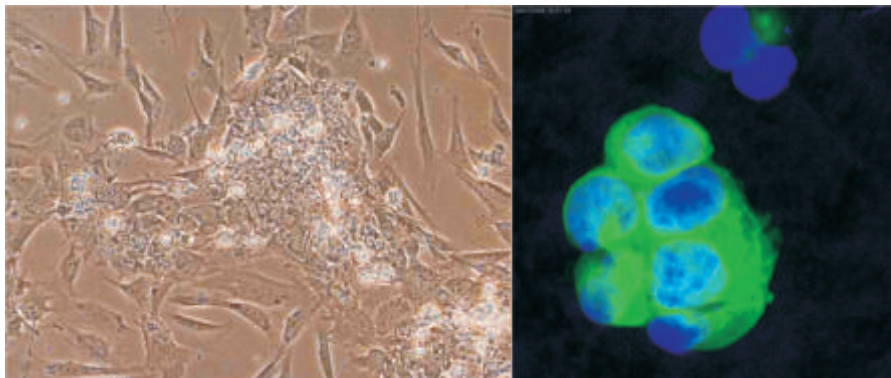
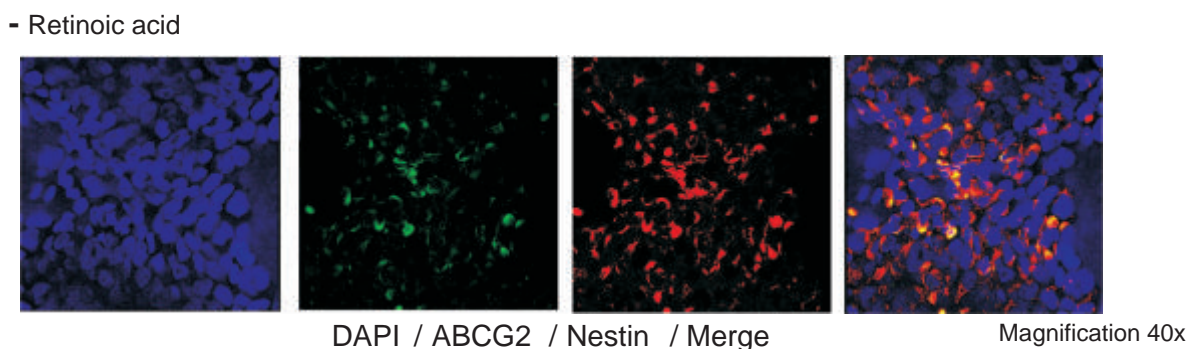
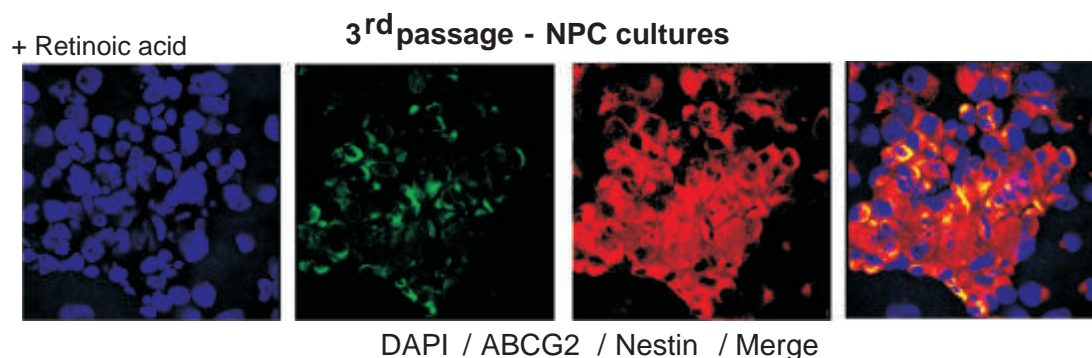
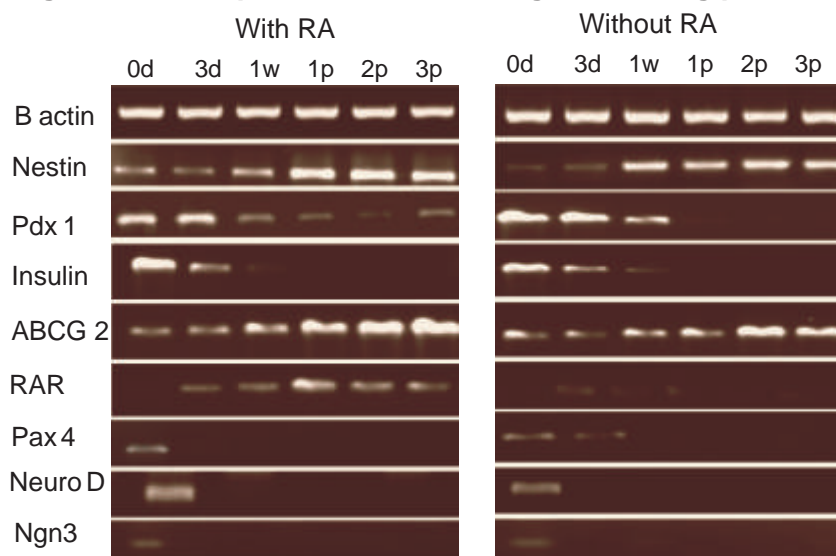


Fig 29 mRNA expression of different genes during proliferation



- ❖ Based on the insulin and c-peptide ELISA values, differentiation media combination (BSA1.5%+ ITS+ Taurine3 mM+ GLP 100mM+Nic1mM+NEAA +100 μ M+ 2 μ M RA+ Zn) neoislets showed better Insulin secretion (7-8 fold).
- ❖ Cryopreservation of neoislets showed no change in viability, and similar insulin values were observed as before.
- ❖ Transplantation of neoislets in the diabetic animals restored body weight and blood glucose close to control, also normalized plasma insulin values and showed IPGTT similar that of control.

- ❖ The presence of insulin positive staining in kidney sections of transplanted animals suggests the neoislets presence and its capability of insulin secretion *in vivo*.

CONCLUSIONS

1. NPC were cultured from adult mice pancreas in SFM containing GFs and 1µM RA on matrigel coated plates.
2. RA in combination with GFs during proliferation:
 - ♦ Increased NPC population and decreased repopulation of cells.
 - ♦ Increased staining for Nestin/ABCG2/BrdU cells.
 - ♦ Upregulated Nestin to Abcg-2 expression by 2.5x.
3. Combination of RA and Zn during differentiation:
 - ♦ PR3 has shown highest Insulin secretion by 7-8x.
 - ♦ Increased C-peptide content by 3.5-4x.
 - ♦ Neoislets were Insulin, Glut2 and Pdx1 positive.
4. Transplantation of neoislets in diabetic mice :
 - ♦ Restored the body weights, blood glucose and plasma insulin values.
 - ♦ Shown IPGTT response similar to control animals.
 - ♦ Insulin staining in renal grafts.

8. ROLE OF RECOMBINANT EPIDERMAL GROWTH FACTOR (REGF) FACTOR IN CELL PROLIFERATION/ DIFFERENTIATION USING DRUG-INDUCED DIABETES, LIVER DAMAGE AND IN GASTRIC ULCERS

Non Steroidal Anti Inflammatory Drugs (NSAIDs), such as naproxen are known to induce gastric antral ulcer, which thereby limit their usage on the long-term basis despite their curative effects. The present study offers a suitable model system to the human situation as naproxen, which is a NSAID is frequently used for the arthritic patients, and also investigated the therapeutic potential of rhEGF against the naproxene induced gastric antral ulcers. Male WNIN rats were randomly divided into Controls, Naproxen treated (80 mg/kg body weight) followed up for 24 hours and either for 7 or 14days and Naproxen + rhEGF treatment either for 7 or 14 days (100 µg/kg body weight).

At the end of the experiment, gastric antral tissues were analyzed for ulcer and it's healing by histopathology, serum MDA levels, Cox- 2 immunoreactivity, Expression of Cox-2 and TGF-βby RT-PCR. Treatment with naproxen induced gastric antral ulcers which were demonstrated by

histopathology as well as with an increase in a) Serum MDA levels, b) COX-2 immuno localization, c) Cox-2 and TGF- β expression. Oral administration of rhEGF to the naproxen treated rats ameliorated all the above changes partially by 7 days, with total normalization by 14 days which was comparable to controls (untreated). These observations are significant as this study forms the basis to be reported for the first time the efficacy of rhEGF in overcoming/ mitigating the gastric-antral ulcer induced with naproxen.

However, the mechanism(s) underlying the efficacy of EGF towards the epithelialization in gastric ulcer is not clearly understood. Hence, the present study was carried out to investigate the biochemical, cellular and molecular mechanism(s) of tissue healing and regeneration with rEGF in Non steroidal anti inflammatory drugs (NSAID) induced gastric ulcer in WNIN rats.

AIMS AND OBJECTIVES

- a) Development of animal models of gastro-intestinal tract, pancreatic and liver ulcers.
- b) *In vivo* observation of the animal tissues before and after the treatment with rEGF will be primarily assessed by histopathology.
- c) *In vitro* culture of the injured tissue and the effects of rEGF will be studied to elucidate the molecular and cellular mechanism(s) of repair.

METHODOLOGY

All experimental protocols were approved by the Animal Ethics Committee at the National Institute of Nutrition. WNIN animals were grouped as:

Groups: Group I- Control untreated, Group II- Naproxen treated, Group III- Naproxen + rEGF (7 days) with their corresponding control and Group IV- Naproxen + rEGF (14 days) with their corresponding control.

Induction of Ulcers: Similar to the earlier reports but repeated in more number of animals.

Treatment and Histopathological Observation

The tissues from antral region were excised, preserved in 10% formaldehyde and stained with hematoxylin and eosin coded specimen were observed and evaluated under light microscope.

Immuno- histochemical Analysis of Cox -2, Bcl-2 and P-53

The IHC was carried out by the standard method using primary antibody Cox-2 1:250 (Santa Cruz, USA), Mouse monoclonal Bcl₂ 1:250 (Santa Cruz, USA) and Goat polyclonal P-53 1:250 (Santa Cruz, USA) with secondary antibody tagged with appropriate fluorescent dye Donkey anti goat-Texas Red (molecular probes), Goat anti mouse-FITC (molecular probes) and donkey anti goat-Texas Red (molecular probes).

Biochemical assay

MDA was measured at 532 nm and were expressed as nanomoles per litre for serum.

Semi - quantitative RT-PCR

Genes for Cox-2, TGF- β and actin were studied using the standard protocol. Briefly, PCR products were separated in a 1% agarose gel, and stained with ethidium bromide. The band intensity was measured using the gel-documentation system (BIO-RAD U.S.A) and quantitated using 1-D analysis software (BIO-RAD U.S.A). Levels of mRNA expression were normalized with those of internal control -Actin.

Statistical analysis: All data are expressed as means \pm SE. One-way analysis of variance (ANOVA) was used followed by post hoc LSD test with SPSS software to determine the significance. $p < 0.05$ was considered statistically significant.

RESULTS

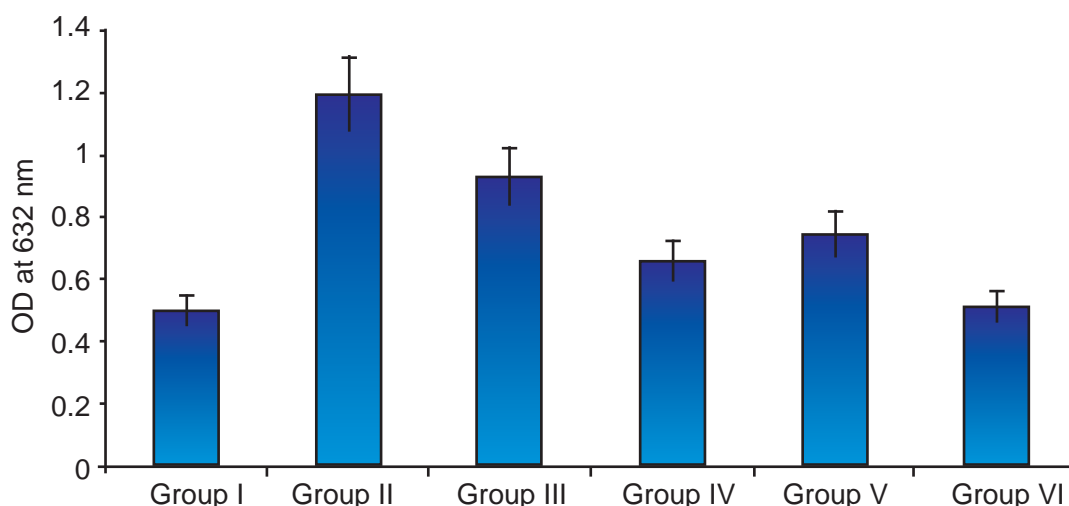
Histopathological examination:

The Group I rats demonstrated normal morphology with mucosal integrity of the gastro-antral region. However, the naproxen treated (Group II) animals showed gastric antral ulcers with denudation of mucosal glands and inflammatory exudates. Administration of rhEGF to the naproxen treated animals ameliorated the pathological changes of gastric mucosa only partially with some inflammatory changes persisting in the gastric mucosa at 7 days (Group III) as compared to total amelioration by 14 days seen in Group V animals. Also, the histopathology of the gastric antral region of the Group V animals was comparable to Group I animals at the end of 14 days. Group IV and Group VI animals which did not receive the oral feeding of rhEGF and which served as the internal controls showed more of denudation of epithelial lining with inflammatory response.

Measurement of Malondialdehyde (MDA)

The concentration of the serum MDA levels were significantly increased in the naproxen treated animals (Group II) as compared to that of the control animals (Group I). With rhEGF treatment the decrease in the concentration of MDA was time dependent with the marginal reduction by 7 days followed by a significant decrease by 14 days (Group V). The Group IV and VI animals which did not receive rhEGF treatment persistently showed an increase in the serum MDA levels similar to that of the Group II animals. Compared with control rats, gastric induced rats had increased lipid peroxide content in serum and rEGF treated rats had decreased lipid peroxide content (Fig. 30).

Fig 30. Serum MDA levels

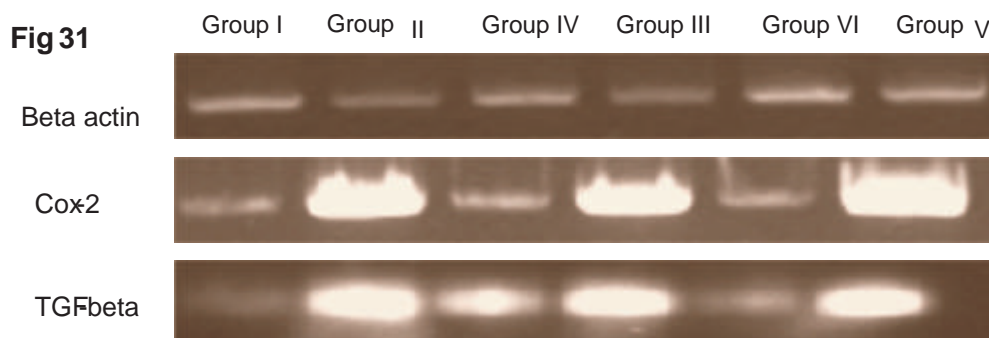


Immuno-histochemical analysis

Group II showed a significant increase in the immunoreactivity for Cox-2 in the ulcerated region of the gastric tissue as compared to the control (Group I). Administration of rhEGF showed a significant reduction in the Cox-2 immunoreactivity, which was more appreciable by 14 days (Group V) as compared to that of 7 days treatment.

RT-PCR analysis

Treatment with naproxen (group II) resulted in an increase in the expression of Cox and TGF- β as compared to the control animals (Group I) where the Cox-2 and TGF- β expression was very weak. Administration of rhEGF reduced the expression of the Cox-2 and TGF- β by 7 days (Group III) with a significant reduction by 14 days (Group V). However, the expression of the Cox-2 and TGF- β of Group IV and VI animals were similar and comparable to the Group II animals which were treated with only naproxene. β -actin was used as the house keeping gene in each assay (Fig 31).



CONCLUSIONS

- ♦ The present study forms the basis to report for the first time the efficacy of rhEGF in overcoming/negating the gastro-antral ulcer induced with naproxen.
- ♦ An attempt was made to correlate the ulcer healing process by histopathology, Cox-2 immunolocalisation, expression of the tissue healing markers such as Cox-2 and TGF β genes and by the serum TBARS levels.
- ♦ It is believed that exploring the potential of rhEGF to understand the mechanism(s) (biochemical, cellular and molecular) of healing in the model system will help in advancing the knowledge towards the management of gastric ulcers.

9. SCREENING AND TESTING OF DIETARY RICH PHYTOESTROGENS IN REDUCING BONE LOSS IN DIET INDUCED RAT MODEL OF OSTEOPOROSIS (OSP)

Osteoporosis (OSP) with its accompanying reduction in bone mass is universally recognized as a major public health problem. The classic etiology of the disease represented by the fractures of the proximal femur, the number increases as age progresses in the population.

Normally, the loss of estrogen after menopause is associated with bone loss leading to fractures, which constitute a battery of bone related problems in many countries. OSP is a

metabolic condition characterized by low bone mass, deterioration of bone tissues and increased risk of fracture. It is a worldwide public health problem that creates significant economic burden on society as well as the families of patients who are suffering from related fractures. Hormone replacement therapy (HRT) used to be the major regimen for prevention and treatment of postmenopausal OSP. However, with the recent discovery that HRT is associated with an increased risk in developing breast, endometrial and ovarian cancers, there is a strong demand for developing alternative approaches for the management of OSP. Nutritional and pharmacological strategies are need of the hour to prevent age related bone loss. Traditional therapies for the OSP have emphasized the use of antiresorptive agents such as estrogen, calcitonin and bisphosphonates. Although these agents may prevent further bone loss in established OSP, they may not restore bone mass that has been lost already.

Thus, it is necessary to develop alternative therapy in the form of naturally occurring compounds with less desirable side effects that can immensely reduce the need for drugs usage. Several studies have suggested that people with a high dietary isoflavone (IF) intake have a lower incidence of OSP related fractures in the Asian population when compared to Western populations. Polyphenolic non-steroidal plant compounds namely Phytoestrogens (PE) are naturally available biological compounds found in a wide variety of sources such as plant foods and are said to exhibit estrogen-like activity because of its structural similarities as that of estrogens. IF are one of the classes of PE and are abundant in plants and have received increasing attention as dietary components that can affect several aspects of human health IF like genistein (Ge) and daidzein (Dz) bind to the ligand binding domain of both Estrogen receptor (ER) isoforms with moderate affinity, but preferentially to ER, in a manner similar to E2. Both PE influence many physiological processes in various estrogen-sensitive tissues and they have been shown to be bone-protective in cultured bone cells and in rat models of OSP. The reports indicate that use of soy isoflavones for protection against or reduction of bone loss is inconsistent.

Further, vitamin D (VD) and its derivatives have an important role in OSP, and the active forms of VD can significantly improve bone mass and reduce vertebral fracture rates in osteoporotic conditions. Investigation related to the role of dietary habits on the development and prevention of osteoporosis has focused mainly on Ca intake and VD repletion. A report showed that bone loss induced by ovariectomy can be prevented by feeding rats with a diet including soybean protein isolates. There is no data available till now in the situations where OSP is been induced by the low Ca and low VD diet and the effects of PE derived from foods. This study was primarily designed to elucidate whether supplementation of PE rich CP is capable of preventing the rapid bone loss occurring after diet induced OSP in the rats.

AIMS AND OBJECTIVES

1. To screen commonly consumed and locally available cereals, pulses and legumes for phytoestrogens: Genistein and Daidzein.
2. To select the food with rich phytoestrogen content and to evaluate the efficacy of the food in reducing bone loss in turn osteoporosis-using diet induced rat model of osteoporosis.

MATERIALS AND METHODS

Animals and diet

Animals

Female weanling WNIN rats (30-35 gm) were maintained under controlled conditions of temperature (20°C ± 2, relative humidity 50–80%) and illumination (12hr light, 12hr dark). All animal

experiments were duly approved by the Institute's Animal Ethics committee and complied with accepted veterinary medical practice.

Study design and animal experimentation

A total of sixty eight WNIN rats were fed a semisynthetic diet with low Ca (0.15%) and low VD (0.1 IU/day/rat). The CPIF mixed diet was fed to the animal (Table 24). Group I of six rats included the Control diet (with normal Ca (0.47 %) and normal VD (25 IU/day/rat), Group II containing thirty eight rats fed with low Ca (0.15%) and 0.1 IU VD, Group III having eight rats were fed with low Ca (0.15%) and 0.1 IU VD and supplemented with low concentrations of CPIF (10 mg/kg diet), Group IV included eight rats and fed with low calcium (0.15%), 0.1 IU VD and supplemented with high concentrations of CPIF (25mg/kg diet) and Group V contains eight rats fed with low Ca (0.15%) and 0.1 IU VD supplemented with 17 β -estradiol (3.2 mg/kg diet).

Table 24. Composition of the animal diet

	Control (g/100g)	Osteoporotic (g/100g)
Glucose	64.04	64.04
Egg Albumin	18.00	18.00
Refined cotton seed oil	10.00	10.00
¹ Salt Mixture (Ca and P free)	2.00	2.00
Calcium Carbonate	–	–
² Equimolar Phosphate	1.17	0.37
³ Vitamin Mixture	1.50	1.50
Cellulose	0.10	0.10
Choline chloride	3.00	3.00
D-Biotin	0.20	0.20
Vitamin D (units/day/rat)	0.003	0.003
Cowpea Isoflavones	25	0.1
Low Conc.(Dz 7.38,Ge 2.7 mg/kg)	–	10
High Conc (Dz18.27,Ge 6.72 mg/kg)	–	25

¹Composition of Salt mixture (Ca and P free g/kg diet)

Potassium chloride,577; Sodium chloride, 209; Magnesium sulphate,179 (anhydrous); Ferrous sulphate, 32.2; Copper sulphate, 0.78;Sodium fluoride,1.13; Ammonium molybdate,0.05; Cobalt chloride,0.04; Potassium Iodide,0.10; Manganous sulphate 0.40; Zinc sulphate, 4.40; All salts are to be weighed and mixed initially. Later they were ground to yield uniform powder of the salt mixture.

²Composition of Equimolar Phosphate (g)

Potassium dihydrogen phosphate, 816.54; Disodium hydrogen phosphate,1045.08 (Salts were mixed and ground thoroughly to a fine powder in a ball mill).

³Composition of Vitamin mixture (g/100g diet)

Thiamin hydrochloride, 0.5; Riboflavin, 0.5; Pyridoxine hydrochloride, 0.5; Calcium D(+) pantothenate, 2.8; Nicotinamide, 2.0; Meso-inositol, 20.0; Folic acid, 0.02; D-Biotin, 0.01, Cyanocobalamin, 0.002; Starch, 73.7; All the vitamins were uniformly mixed using mortar and pestle. The mixture is finally mixed with starch to yield a uniform powder of the vitamin mixture.

Various dietary constituents excluding oil were mixed thoroughly. Then cotton seed oil containing vitamin A acetate, vitamin E and vitamin K at concentrations 6,30, and 0.05 mg/kg diet respectively were added and mixed to a uniform consistency.

After the development of OSP (approximately six weeks) as indicated by BMD, BMC and biochemical analysis, the group II was sub-divided into five subgroups (SG) and supplemented CPIF. Number of rats in all the SGs was maintained with eight except group V which was having only six animals. In the Group II, SG I was continued with low Ca (0.15%) and 0.1 IU VD, SG II was fed with low concentrations CPIF, (10 mg/kg), SG III was fed with high concentrations of CPIF (25mg/kg diet), SG IV was fed with 17 β -estradiol (3.2 mg/kg diet) and SG V was replenished with normal Ca and VD. Rats had free access to deionized water and food intake was recorded every alternative, and body weight was measured once in four days. After a period of ninety days, the animals were sacrificed and the investigations were carried out to find the protective and therapeutic effects of the CP.

Preparation of Vitamin D₃ (VD3) drops

Crystalline VD3 is procured from Sigma chemical company. A pinch of crystalline VD3 was dissolved in 3ml of 95% ethanol and OD is measured at 265 nm. The exact concentration of VD3 is calculated based on its molar extinction coefficient and OD at 265 nm. For the preparation of VD3 drops, an aliquot containing a known amount of VD3 is evaporated to dryness under a stream of nitrogen and re-suspended in refined cotton seed oil, so as to get 50 IU of VD3 in 0.1 ml aliquot. This is further diluted to get different concentrations of VD3.

Extraction of CPIF

Extraction

Five grams of each powdered sample was defatted in duplicates and extracted twice into 70 ml of 70% methanol. The extract was hydrolyzed with 2.0M HCl and then concentrated in a flash evaporator, and made up to known volume and then filtered through 0.45 filters and used for HPLC analysis. C18 Sep-pack cartridges were preconditioned with methanol followed by water for Solid-phase extraction within 24 hours. One ml of concentrated extract was loaded on the column and washed with water to remove sugars. Retained IF were eluted with ethyl acetate and then analyzed by HPLC.

HPLC-DAD Analysis

Lichrosphere- ODS 2, C18 column (250 x 4.6mm), was used for chromatographic analysis on Shimadzu LC- 2010A equipped with auto injector, binary pump and diode array detector (Shimadzu SPDM10A). Linear gradient of mobile phase 0.1% phosphoric acid (A) and Acetonitrile (B) with a flow of 0.8ml/min. The linear gradient was started at 10% acetonitrile and increased up to 100% and brought back to 10% in 35 minutes of run time. A wavelength range of 200-800nm was used in the DAD detector.

Bone Mineral Density (BMD) and Mineral Content (BMC)

BMD (g/cm²) and BMC (g) of the whole body, other regions of the bone arrangement were assessed by dual-energy X-ray absorptiometry (DXA; QDR-2000, Hologic Inc., Waltham, MA) equipped with appropriate software for use with small laboratory animals. The regions analyzed were divided in to four parts (Fig. 34) such as right hind limb (R1), left hind limb (R2), thoracolumbosacral spine (R3) and both fore limbs, a part of basal skull and cervical spine (R4). The results are expressed as the net values for both BMD and BMC.

Quantification of Serum Vitamin D

Serum 25 hydroxy vitamin D (25-OH-D) was quantified by the RIA method and procured from DiaSorin (Stillwater, Minnesota 55082-0285, U.S.A). Briefly the assay consists of a two-step

procedure. The first procedure involves a rapid extraction of 25-OH-D and other hydroxylated metabolites from serum with acetonitrile. Following extraction, the treated sample was then assayed using an equilibrium RIA procedure. The RIA method is based on an antibody with specificity to 25-OH-D. The sample, antibody and tracer are incubated for 90 minutes at 20-25°C. Phase separation is accomplished after a 20 minute incubation at 20-25°C with a second antibody precipitating complex. A NSB/Addition buffer is added after this incubation prior to centrifugation to aid in reducing non-specific binding in terms of average CPM of NSB Tube/Average CPM of Total Count Tubes. The values of 25-OH-D are expressed in ng/ml of the sample.

Determination of Serum alkaline phosphatase (ALP)

Serum ALP was determined by the method of Walter and Schutt. Enzyme activity was expressed as mmol of *p*-nitrophenol liberated/ minute/ milligram of protein. Protein concentration was determined by the method of Lowry *et al.*

Determination of Serum calcium (Ca) and phosphorus (P)

Serum Ca was measured using atomic absorption spectrophotometry by appropriately diluting with 0.1% lanthanum chloride solution as per the modified method of Zetner and Seligson. Serum P was determined by the modified method of Taussky and Shorr.

Statistical analysis

Data were expressed as mean \pm standard error (SE). The comparison between the groups was done by Student's two-tailed *t* test. Comparisons among treated groups were statistically processed by one-way analysis of variance (ANOVA) with Tukey's post-hoc analysis by use of a SAS software package. *P* values of less than 0.05 were considered significant.

RESULTS

Animal diet was designed to develop OSP with Ca and P free salt mixture and incorporating calcium carbonate with variation in the composition in the control (1.17 gm/100gm diet) and experimental osteoporotic diet (0.37 gm/100 gm diet) with low VD (0.1U/ rat/day) levels (Table 25). The important constituents of the CPIF as identified are Dz (18.07 mg/100gm dry weight) and Ge (6.6 mg/100gm dry weight) were utilized to formulate the experimental diet with low concentration (10mg/kg diet) and high concentration (25 mg/kg diet) and incorporated in the diet appropriately.

In the data, initial always indicates the point where the completion of feeding of the osteoporotic diet and initiation of the CPIF diet for the G II animals and the final always indicates the point where the study was completed unless mentioned specifically.

Food intakes of the animals are more or less the same through out the experiment and there were no significant differences between the initial and final groups and the average consumption was found to be in the range of 12-14 g/day (Table 26). Body weights were significantly high in the G I animals (Control diet) when compared with other groups. The weight of the SG V in the G II was significantly increased when compared to the diet given to develop OSP in the same group, except this there is no visible change in the body weights in the groups. G II maintained low body weights till the end of the experimentation (Table 26). The fat deposition percentage is also found to be the similar as that of body weights, except in the SG V of G II, where the animals are replenished with the control diet with normal Ca and VD (Table 26).

BMD and BMC were found to be significantly increased in the SG II, III, IV and V of the G II ($p < 0.05$). There is no significant difference observed in the other groups when compared to the initial and final status of the supplementation of CPIF (Fig 32 and 33).

Table 25. Effects of supplementation of CPIF on food intake, body weight and total body fat %

Parameter	G I	G II					G III	G IV	G V
		SG I	SG II	SG III	SG IV	SG V			
Food Intake g/day									
Initial	15.4 ± 0.2	12.2 ± 0.2	12.3 ± 0.21	11.9 ± 0.3	12.9 ± 0.3	12.3 ± 0.3	13.7 ± 0.4	13.4 ± 0.3	14.7 ± 0.3
Final	14.9 ± 0.2	12.6 ± 0.3	12.9 ± 0.3	11.4 ± 0.2	12.7 ± 0.2	14.6 ± 0.2*	13.9 ± 0.3	13.7 ± 0.2	14.2 ± 0.3
Body weights									
Initial	92.3 ± 0.9	64.2 ± 0.8	73.2 ± 0.7	68.2 ± 0.8	67.2 ± 0.9	65.2 ± 0.9	74.2 ± 0.7	71.3 ± 0.8	70.2 ± 0.9
Final	99.3 ± 0.9	62.3 ± 0.7	69.2 ± 0.6	69.3 ± 0.8	66.2 ± 0.7	88.3 ± 0.8*	72.2 ± 0.76	73.1 ± 0.8	71.8 ± 0.7
Fat (%)									
Initial	26.9 ± 0.1	13.4 ± 0.2	12.6 ± 0.2	11.2 ± 0.1	12.7 ± 0.2	12.2 ± 0.2	11.7 ± 0.3	13.3 ± 0.2	14.3 ± 0.3
Final	28.9 ± 0.3	14.3 ± 0.2	14.3 ± 0.2*	9.8 ± 0.2*	11.2 ± 0.2	23.4 ± 0.2*	12.4 ± 0.3	12.8 ± 0.2	13.7 ± 0.2

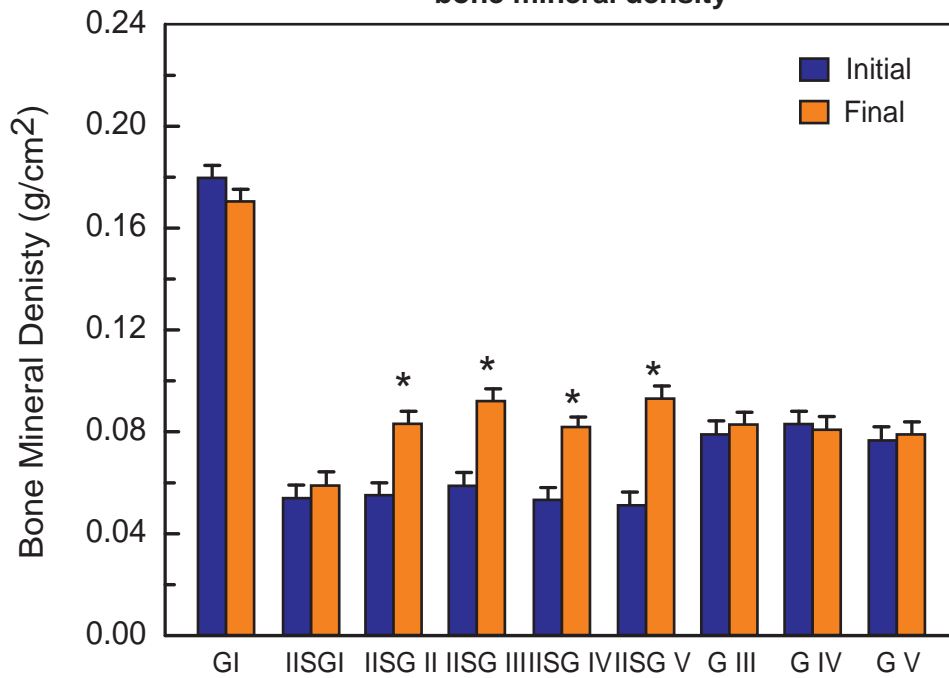
Values are expressed means ± SE . * The significant differences are expressed with respective initial values when compared with their respective final values at p<0.05

Table 26. Effects of supplementation of CPIF on the serum levels of Calcium, Phosphorus and alkaline phosphatase

Parameter	G I	G II					G III	G IV	G V
		SG I	SG II	SG III	SG IV	SG V			
Calcium									
Initial	10.4 ± 0.2	5.75 ± 0.4	5.83 ± 0.3	5.89 ± 0.3	5.36 ± 0.3	5.67 ± 0.3	6.99 ± 0.28	7.34 ± 0.3	6.78 ± 0.3
Final	10.5 ± 0.21	5.82 ± 0.32	7.33 ± 0.32*	7.89 ± 0.34*	6.33 ± 0.5*	8.32 ± 0.3*	7.34 ± 0.36	7.56 ± 0.31	6.97 ± 0.18
Phosphorus									
Initial	6.97 ± 0.33	9.76 ± 0.29	9.66 ± 0.31	9.87 ± 0.3	9.56 ± 0.3	9.67 ± 0.2	7.33 ± 0.3	7.53 ± 0.26	8.01 ± 0.2
Final	7.23 ± 0.33	9.87 ± 0.23	7.83 ± 0.23*	7.89 ± 0.31*	8.3 ± 0.3*	7.66 ± 0.24*	7.42 ± 0.3	7.49 ± 0.21	7.99 ± 0.11
Alkaline Phosphatase (U/l)									
Initial	115.2 ± 3.2	216.2 ± 6.2	221.3 ± 3.2	216.3 ± 4.2	224.7 ± 3.8	216.2 ± 3.2	142.4 ± 2.8	136.3 ± 2.7	163.2 ± 2.4
Final	112.2 ± 2.9	221.3 ± 5.3	152.6 ± 3.8*	138.3 ± 3.2*	183.2 ± 4.2*	128.1 ± 4.2*	137.3 ± 2.6	138 ± 2.6	158.3 ± 2.9

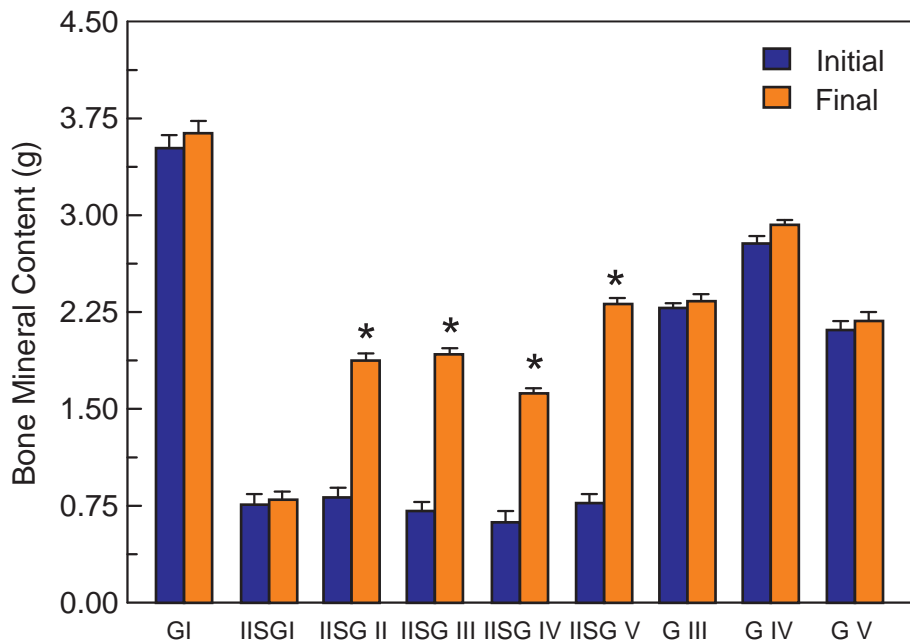
Values are expressed means ± SE. The significant differences are expressed wrt initial values when compared with their respective final values at p<0.05 for table 25 & 26

Fig 32. Effects of dietary supplementation of cowpea extract on bone mineral density



Values are expressed means \pm SE . * The significant differences are expressed with respective initial values when compared with their respective final values at $p < 0.05$.

Figure 33. Effects of dietary supplementation of cowpea extract on bone mineral content



Values are expressed means \pm SE . * The significant differences are expressed with respective initial values when compared with their respective final values at $p < 0.05$

The DXA report indicates that the hind limbs of the rat had low BMD and BMC in the osteoporotic group (Fig. 34) and was partially but significantly reversed with the supplementation of CPIF (data not reported). The status of Ca and P levels in the final point significantly altered in the SG II,III and IV of the G II when compared with the initial point. Even though, the levels were altered in the G III, but there was no significance changes when compared with their corresponding comparative group. The changes in the ALP levels were significant particularly in the SG II,III,IV and V ($p < 0.05$) and difference of 65-80 U/l was observed. Similarly the 25-hydroxy VD levels were significantly altered in all the SG of G II ($p < 0.05$) and other groups almost remains the same with their respective initial and final points of the experimentation (Fig 35).

Fig 34. DXA showing the different regions of the rat such as Right Hind Limb (R1), Left Hind limb (R2), Thoracolumbo-sacral spine (R3) and Both Fore limbs, a part of basal skull and cervical spine (R4).

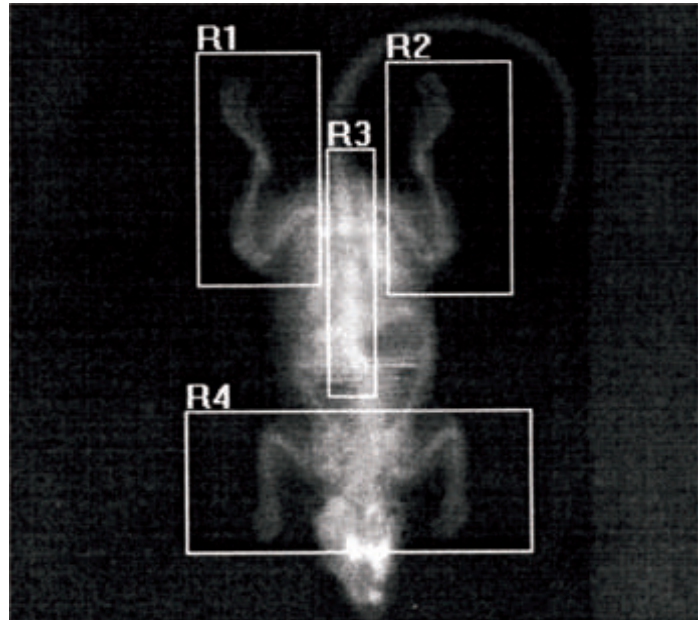
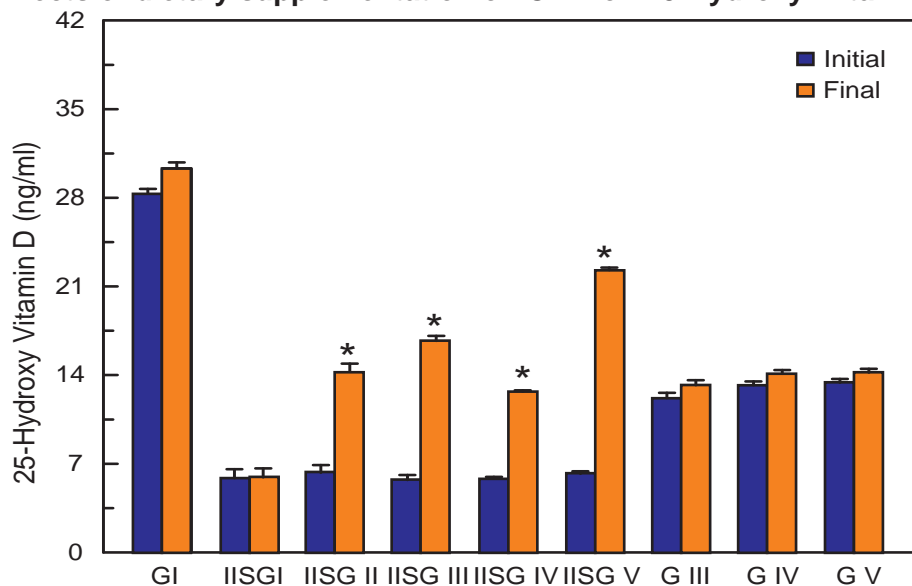


Fig 35. Effects of dietary supplementation of CPIF on 25-Hydroxy Vitamin D levels



Values are expressed means \pm SE . * The significant differences are expressed with respective initial values when compared with their respective final values at $p < 0.05$.

CONCLUSIONS

CPIF had a convincing effect in protecting the bone mineralization in the critical situations like OSP interms of improving the BMD, BMC, Ca, ALP and VD levels, it is inconclusive whether these IF had a therapeutic effect and needs to be further investigated.

10. CHARACTERIZATION OF ACTIVE PRINCIPLES AND MECHANISM OF ACTION OF ALDOSE REDUCTASE INHIBITORS AND ANTIGLYCATING AGENTS FROM DIETARY SOURCES: SPECIFICITY, SIGNIFICANCE AND MECHANISM OF INHIBITION OF ALDOSE REDUCTASE BY CURCUMIN

Prolonged exposure to chronic hyperglycemia in diabetes can lead to various complications, affecting the cardiovascular, renal, neurological and visual systems. Although mechanisms leading to diabetic complications are not completely understood, many biochemical pathways associated with hyperglycemia have been implicated. Among these, polyol pathway and advanced glycation end-products (AGE) formation have been extensively studied. Aldose reductase (ALR2 or AKR1B1; EC: 1.1.1.21) belongs to aldo-keto reductase (AKR) super family.

It is the first and rate-limiting enzyme in the polyol pathway and reduces glucose to sorbitol utilizing NADPH as a cofactor. Sorbitol is then metabolized to fructose by sorbitol dehydrogenase. Accumulation of sorbitol leads to osmotic swelling, changes in membrane permeability, and also oxidative stress culminating in tissue injury.

A number of studies with experimental animals suggest that ALR2 inhibitors (ARI) could be effective in the prevention of some diabetic complications including cataract, retinopathy, nephropathy and neuropathy. However, to date, most ARI have met with limited success, and some of the synthetic ARI were associated with deleterious side effects. In addition to ALR2, many ARI also known to inhibit closely related members of ALR2 (such as AKR1A1 and AK1B10) contributing to the poor outcome of ARI clinical trials. Previously, it was reported that ARI activity contained in a few spice/dietary sources including turmeric (and its active principle curcumin) prevented diabetic complications using *in vitro*, *ex vivo* and animal models. However, mechanism of inhibition, specificity with other AKRs and its functional significance has not been reported. In the present study was characterized and the inhibition of human recombinant ALR2 by curcumin insights into the nature of inhibition was provided. Further, the specificity of curcumin towards two closely related AKRs and its effects on intracellular sorbitol accumulation in red blood cells (RBC) under *ex vivo* high glucose conditions was investigated.

METHODOLOGY

1. **Expression and purification of recombinant human ALR2:** Recombinant human ALR2 was over-expressed in *E. coli* and purified essentially as described previously.
2. **Purification of ALR1 from bovine kidney:** ALR1 was partially purified from bovine kidney following the previously described methods.
3. **Enzyme assays:** The activity of ALR1 and ALR2 was measured as described previously. The change in the absorbance at 340 nm due to NADPH oxidation was followed in a spectrophotometer.

4. **Inhibition studies:** For inhibition studies, concentrated stocks of curcumin prepared in DMSO were used. Various concentrations of curcumin were added to assay mixtures of ALR2 or ALR1, and incubated for 5 min before initiating the reaction by NADPH. The percentage inhibition was calculated considering the activity in the absence of curcumin as 100%. The IC_{50} values were determined by nonlinear regression analysis of the plot of percent inhibition versus log inhibitor concentration.
5. **Enzyme kinetics:** K_m and V_{max} of recombinant ALR2 were determined with varying concentrations of substrate in absence and presence of different concentrations of curcumin by Lineweaver-Burk double reciprocal plots. Inhibitory constant (K_i) was derived by plotting slopes obtained from Lineweaver-Burk plots versus curcumin concentration.
6. **In vitro incubation and estimation of sorbitol in RBC:** Five mL blood was collected into heparinized tubes from healthy male volunteers after an overnight fast. Red blood cells were separated and incubated in Krebs-ringer bicarbonate buffer, pH 7.4 at 37°C in presence of 5% CO_2 for 3 hrs under normal (5.5 mM) and high glucose (55 mM) conditions. The effect of curcumin (25-100 nM) on sorbitol accumulation was evaluated by incubating the RBC with different concentrations of curcumin. At the end of the incubation period, sorbitol content of the RBC was measured by a fluorometric method as described previously using a spectrofluorometer.
7. **Molecular Docking:** Molecular docking studies were done by SYBYL FlexX software (Tripos). The FlexX module in SYBYL 7.0 was used to dock the ligand structures into the active site of the crystallographic structures, which was defined as all residues within 6.5Å° away from the inhibitor in original complex by using an incremental construction algorithm. For docking studies, coordinates of crystal structure of proteins (ALR1: PDB # 2A° and ALR2: PDB # 1PWM,) were taken from Brookhaven Protein Data Bank (PDB). The predicted protein ligand complexes were optimized and ranked according to the empirical scoring function ScreenScore, which estimates the binding free energy of the ligand receptor complex.

RESULTS

1. Curcumin inhibited human recombinant ALR2 with an IC_{50} of 10.0 ± 4.0 nM (Fig 36).
2. It was interesting to note that curcumin did not inhibit bovine kidney ALR1 upto 200nM concentration under the conditions employed in the study, signifying its marked specificity towards ALR2 over ALR1.
3. In the presence of different concentrations of curcumin, V_{max} was decreased but there was no change in K_m with glyceraldehyde as substrate (Fig. 37 & Table 27). These results suggested a non-competitive inhibition of ALR2 by curcumin.
4. Further, the inhibitory constant (K_i) was determined from the secondary plots of Lineweaver-Burk plots and K_i of curcumin for ALR2 was found to be 40×10^{-6} M (Fig. 38).
5. Molecular docking studies were conducted to substantiate the binding pattern and selective inhibition of ALR2 by curcumin. It was observed that curcumin is likely to interact with ALR2 at active site residues Tyr-48, Lys-21, Thr-19 and Gln-183. Further, there was hydrogen bonding with Leu-300 and Trp-111 (distance 2.70 and 2.22 Å°, respectively). Hence, it appears that curcumin might bind to ALR2 in a closed type of conformation (Fig. 39A).

Fig 36. Representative inhibition curve of human recombinant ALR2 by curcumin. ALR2 activity in the absence of curcumin was considered as 100%.

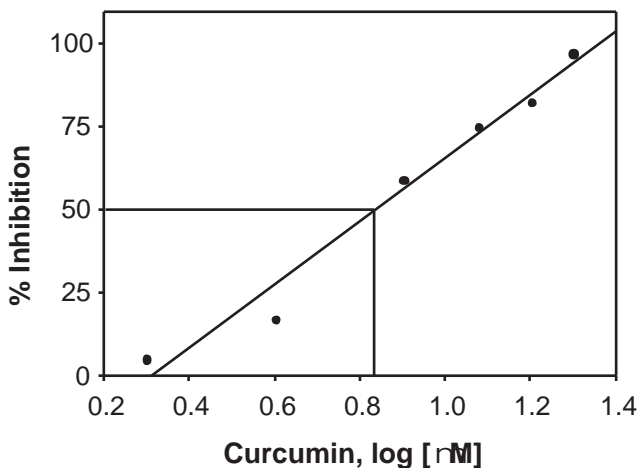


Table 27. Kinetics of human recombinant ALR2 in the absence and presence of curcumin. Data are the mean \pm SE (n=6). V_{max} is reported as μ moles NADPH oxidized/min/mg protein.

Curcumin	Km (mM)	Vmax
'0' μ M	0.232 \pm 0.08	0.074 \pm 0.019
10 μ M	0.243 \pm 0.07	0.045 \pm 0.011
30 μ M	0.240 \pm 0.05	0.034 \pm 0.014
45 μ M	0.244 \pm 0.11	0.022 \pm 0.013

Fig 37. Lineweaver-Burk plot of recombinant ALR2 in the presence of '0' (circles), 10 (triangles), 30 (diamond) and 45 μ M (hexagonal) curcumin.

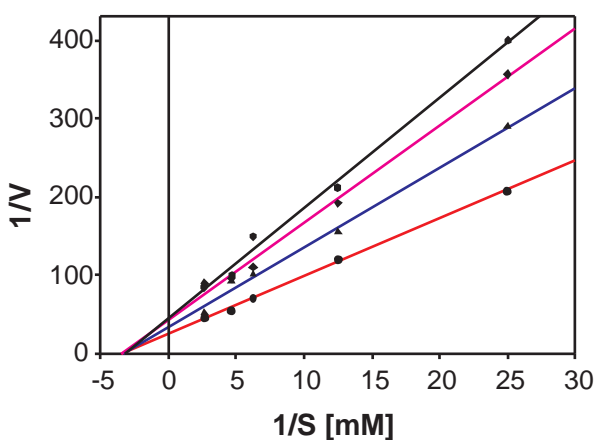
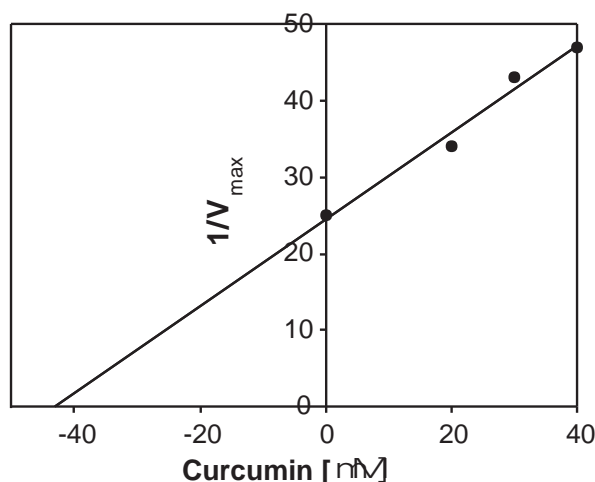
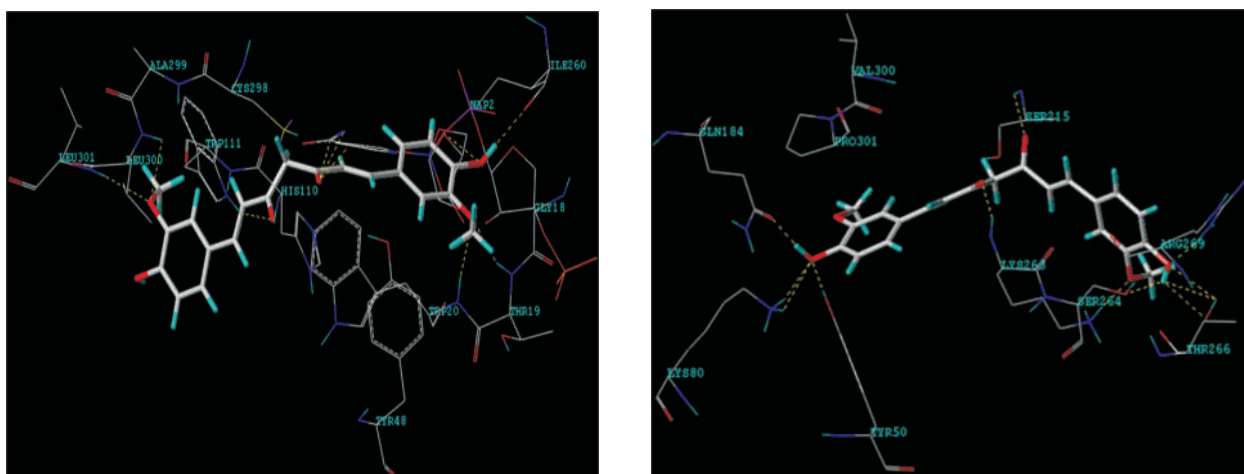


Fig 38. Determination of inhibitory constant (K_i) of curcumin with ALR2



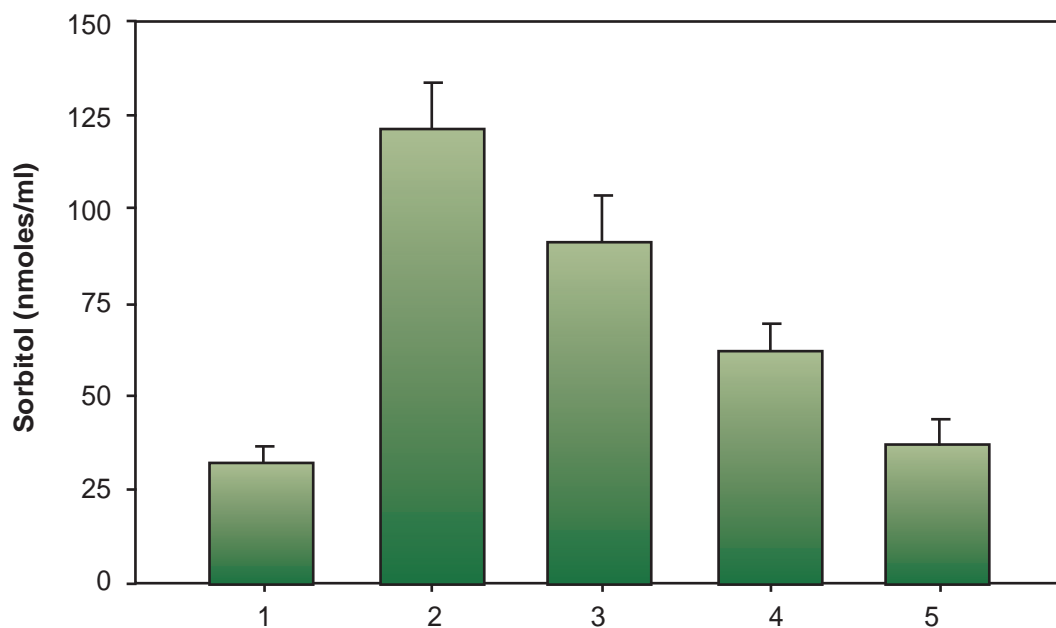
- In case of ALR1, hydrogen bonding was observed between curcumin and amino acid residues Tyr-50, Gln-184 and Lys-80 (Fig. 39B). Since Leu-300 and Leu-301 are replaced by Pro-300 and Val-301 in ALR1, curcumin did not interact with Pro-300 and Val-301. It is interesting to note that unlike with ALR1, curcumin interacted with Leu-300 and Leu-301 in ALR2 that are involved in imparting plasticity to ALR2. These observations indicate that curcumin might be a specific inhibitor of ALR2.
- Accumulation of sorbitol in RBC under high glucose conditions (*ex vivo*) to understand the significance of *in vitro* inhibition of ALR2 by curcumin, particularly the effect of curcumin on osmotic stress was assessed.

Fig 39. Stereoviews of ALR2 (Left panel) and ALR1 (Right panel) with curcumin (keto form) docked into active site. Hydrogen bonds shown in dashed yellow lines



8. *In vitro* incubation of RBC with 55 mM glucose resulted in the accumulation of sorbitol three- to four-fold higher than the control (Fig 40). Incubation of RBC in the presence of curcumin under high glucose conditions led to reduction in the accumulation of intracellular sorbitol in a dose dependent manner (Fig 40).
9. These results not only substantiate the inhibition of ALR2 by curcumin but also indicate the significance of curcumin in terms of preventing the accumulation of intracellular sorbitol.

Fig 40. Effect of curcumin on sorbitol accumulation in RBC. Sorbitol levels in RBC under normal glucose concentration (5.5 mM) (bar 1) under high glucose (55mM) conditions in the absence (bar 2) and presence of 50 μ M, 100 μ M and 200 μ M curcumin (bars 3-5, respectively).



Data are mean \pm SE (n=6).

CONCLUSIONS

Results of the present study indicate that curcumin inhibits human recombinant ALR2 in a non-competitive manner and this inhibition appears to be relatively specific towards to ALR2 over ALR1. Suppression of sorbitol accumulation in human erythrocytes under high glucose conditions by curcumin is suggestive of translating its impact to *in vivo* conditions which are supported by previous studies that curcumin delayed the progression of cataract and inhibited retinal VEGF expression in STZ-induced diabetic rats. Finally, these observations suggest that curcumin or its synthetic analogues could be explored for alleviating complications of diabetes.

11. MOLECULAR STUDIES ON CATARACTOGENESIS IN WNIN/OBESE RAT MODEL

Several serious medical conditions have been linked to obesity, including type-2 diabetes, heart disease, high blood pressure, and stroke. Cataract is a recent addition to the list of complications of obesity. Diabetes and insulin resistance are the part and parcel of obesity and diabetes is one of the major risk factors of visual impairment. Cataracts are 1.6 times more common in people with diabetes than in those without diabetes. Further many epidemiological studies suggest that there is a clear-cut association between obesity and cataract development. Hence there is a need for mechanistic studies to understand and prevent or delay cataract due to obesity. So use of a suitable animal model is of considerable importance in understanding the etiology and pathogenesis of cataract of this category.

The National Center of Laboratory Animal Sciences at the National Institute of Nutrition has developed two mutant obese strains, WNIN/Ob and WNIN/GR-Ob, the former with euglycemia and the later with glucose intolerance. However, the molecular basis of obesity and associated clinical features are yet to be unraveled. It was found that 15-20% of WNIN/Ob and WNIN/GR-Ob rats develop cataracts spontaneously by the time they reach 12-15 months of age. Since diabetes is an added risk factor of cataract, insulin resistance of WNIN/GR-Ob animals may be important regulator for the development of cataract. Keeping in view that these obese models are insulin resistant and there is a tendency in these animals to develop cataract, deciphering the molecular basis of cataractogenesis in these mutant strains may provide insights into the earlier biochemical events. The main aim of the project was to understand the possible molecular and biochemical basis underlying the accelerated cataractogenesis in these mutant animals.

METHODOLOGY

1. **Tissue collection:** Eye balls of 3-12 month old strains were collected (tissues were shared as a part of Flagship Project on WNIN/Obese rat). Lenses were dissected by posterior approach and their morphology was assessed for lens opacity by Slit-lamp Biomicroscope. Lens opacities, if any, were noted.

2. **Protein profile, crystallin distribution and protein aggregation:** A 10% homogenate of the lenses was prepared in aqueous buffers. Total, soluble and insoluble protein content was estimated in respective fractions by Lowry method and percentage soluble protein was calculated. Crystallin profile in soluble fraction was analyzed by size exclusion chromatography. Polypeptide profile of soluble and insoluble proteins was analyzed by SDS-PAGE. The tendency of the total soluble protein and individual crystallins, mainly β - and α -crystallins, towards aggregation due to heat and UV-irradiation was measured by light scattering methods.
3. **Oxidative stress and antioxidant defense system:** Lipid peroxidation and protein carbonyl content of soluble protein were analyzed by spectrophotometric methods. Activities of antioxidant enzymes; superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, glucose-6-phosphate dehydrogenase were measured in soluble fraction by spectrophotometric methods.
4. **Polyol pathway:** The status of osmotic stress in the eye lens of WNIN/Ob and WNIN/GR-Ob rats was assessed by analyzing the levels of sorbitol and activity of aldose reductase and sorbitol dehydrogenase.
5. **Non-enzymatic glycation:** Extent of glycation was measured by monitoring advanced glycation end-product (AGE) related non-tryptophan fluorescence by excitation at 370 nm and emission between 400 to 500 nm in a spectrofluorometer and also by estimating the protein carbonyl content.
6. **Molecular chaperone activity of α -crystallin:** α -Crystallin was isolated from the soluble fraction of lens homogenate by gel filtration on Sephacryl S-300 column and dialyzed for the chaperone studies. Chaperone-like activity of isolated α -crystallin was studied by measuring its ability to prevent the aggregation of other proteins induced by heat or chemical. Heat-induced aggregation of citrate synthase/ β _L-crystallin in the absence or presence of α -crystallin was measured by light scattering methods monitored spectrophotometrically.
7. **Susceptibility to galactose- and streptozotocin-induced cataract:** Susceptibility of WNIN/Ob and WNIN/GR-Ob rat to galactose-induced and streptozotocin-induced cataract was investigated in comparison to their respective lean control rats as described previously. While a group of 3-months old WNIN/Ob and their littermate lean rats was fed a normal stock diet based on the AIN-93 formula, another group was fed 30% galactose in the AIN-93 diet. Similarly, a group of 3-months old WNIN/GR-Ob and their littermate lean rats was fed on an AIN-93 and another group on 30% galactose in the AIN-93 diet. In case of streptozotocin treatment, a group of 3-months old rats (WNIN/Ob, WNIN/GR-Ob and their respective lean rats) received 0.1 M citrate buffer, pH 4.5 as a vehicle, whereas another group of animals (WNIN/Ob, WNIN/GR-Ob and their respective lean rats) received a single intraperitoneal injection of streptozotocin (35 mg/kg) in citrate buffer. Onset and progression of cataract in these animals was monitored by examining the eyes alternate days using a slit lamp biomicroscope and lenticular opacity was graded into four stages as reported previously.

RESULTS

- ♦ In general, insolubilization of otherwise soluble proteins is the major factor in the cataractogenesis. However, there was no change in total and soluble protein content in the lens of WNIN/Ob and WNIN/GR-Ob rat at different ages as compared to their lean controls of respective age.

- ◆ Distribution of major crystallins in soluble protein fraction was similar in WNIN/Ob and WNIN/GR-Ob lens as compared to their respective lean animals from 3-months age onwards as analyzed by size exclusion chromatography (Fig 41). Similarly, the sub unit profile of lens proteins is also not different in WNIN/Ob and WNIN/GR-Ob rats compared to their respective lean animals.
- ◆ Loss of chaperone-like activity of α -crystallin was observed in many types of cataract. However, there is no significant change in chaperone-like activity of α -crystallin in 3-12 months old WNIN/Ob and WNIN/GR-Ob eye lens as compared to their respective age-matched lean controls.
- ◆ Though, there were no significant changes in oxidative markers (lipid peroxidation and protein carbonyls), activities of antioxidant enzymes in WNIN/Ob and WNIN/GR-Ob eye lens were altered as compared to their respective age-matched lean controls. For example, activities of superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase but not glutathione-S-transferase were significantly higher in WNIN/Ob and WNIN/GR-Ob eye lens from 6-month old onwards as compared to their respective age-matched lean controls (Fig 42).
- ◆ Though, the activities of polyol pathway enzymes, aldose reductase and sorbitol dehydrogenase, were not significantly different between the eye lens of WNIN/Ob or WNIN/GR-Ob and their respective lean rats from 3-12 months, sorbitol levels were significantly high in WNIN/Ob and WNIN/GR-Ob lens compared to their respective lean control from 3-months age onwards (Fig 43).
- ◆ There is an increased susceptibility of total soluble protein to UV-induced aggregation in WNIN/Ob and WNIN/GR-Ob as compared to their lean controls (Fig 44). Like wise, there is an

Fig 41. Representative crystallin distribution profile of total soluble lens protein on a Sephacryl S-300 HR gel filtration column of lean and WNIN/Ob rats

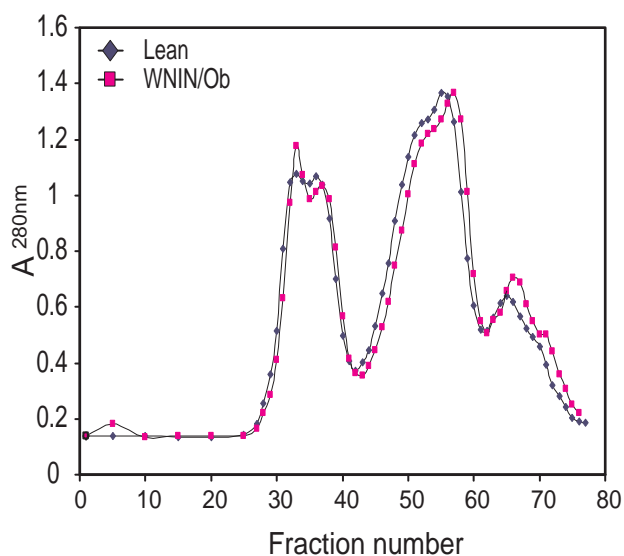


Fig 42. Activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PD) in the eye lens of 6-months old lean and WNIN/Ob rats

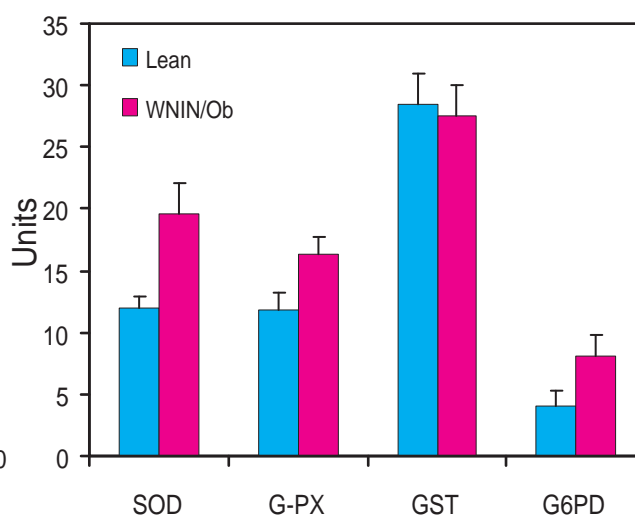
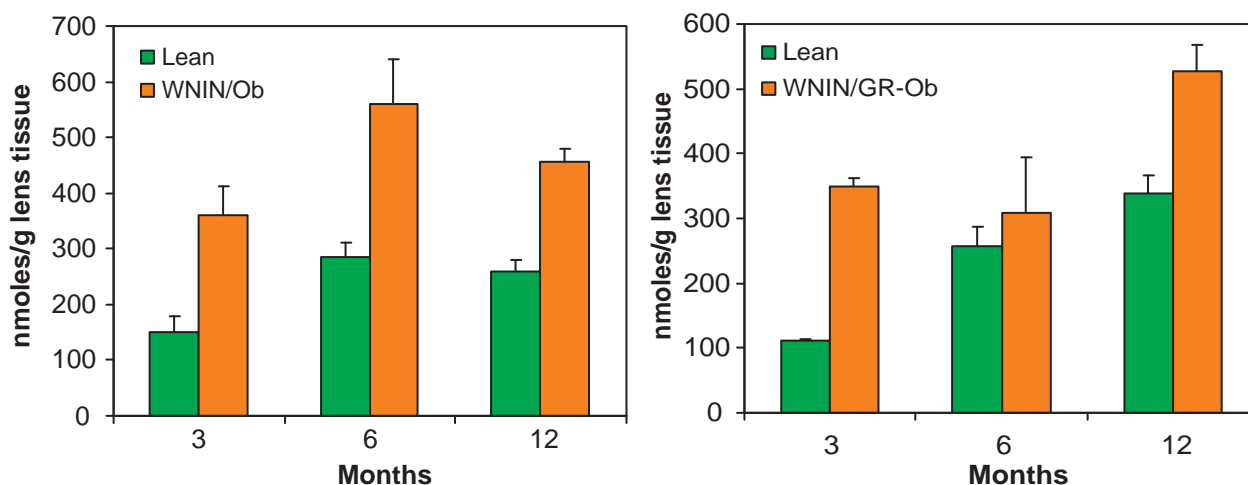
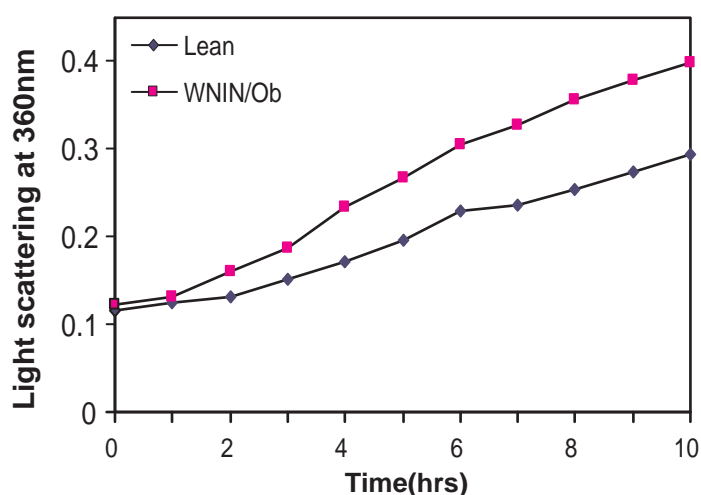


Fig 43. Levels of sorbitol in the eye lens of WNIN/Ob (Left panel) and WNIN/GR-Ob (Right panel) rats along with their lean controls from 3-12 months



increased susceptibility of total soluble protein to non-enzymatic glycation in vitro from WNIN/Ob and WNIN/GR-Ob eye lens as compared to their lean controls.

Fig 44. Susceptibility to UV-induced aggregation of total soluble protein of the eye lens from 6-months old WNIN/Ob and its lean control rats



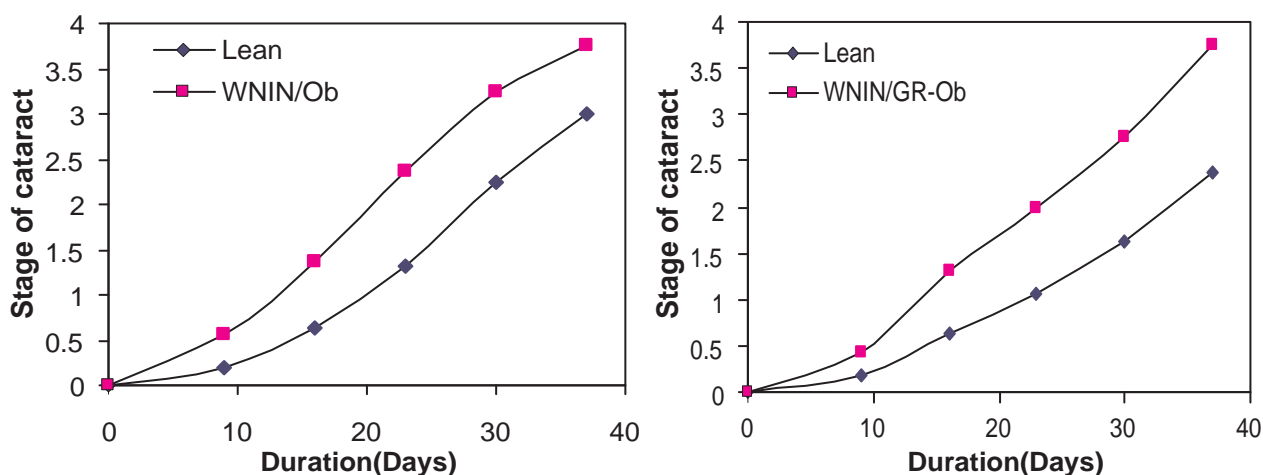
- Interestingly, onset and progression of galactose-induced cataract in WNIN/Ob and WNIN/GR-Ob was much faster as compared to their lean counter parts (Fig 45).

- Noteworthy observation is that WNIN/Ob and WNIN/GR-Ob rats could not tolerate the dose of streptozotocin similar to lean rats as there was 100% mortality with 35 mg/kg bw dose in WNIN/Ob and WNIN/GR-Ob rats but not in lean rats. Thus, the streptozotocin dose has to be optimized for WNIN/Ob

and WNIN/GR-Ob to follow the onset and progression of diabetic cataract. Though, there is no significant difference in the onset and progression of cataract in WNIN/Ob and WNIN/GR-Ob rat compared to lean rats, the required dose is about 6-8 mg lesser than the dose required for lean controls. These results indicated that the sensitivity of WNIN/Ob and WNIN/GR-Ob to streptozotocin is very high indicating an altered susceptibility of pancreatic β -cells to streptozotocin.

- Further, the data obtained with in vivo studies on galactose- and streptozotocin-induced cataract corroborate the importance of higher sorbitol levels in WNIN/Ob and WNIN/GR-Ob rat model.

Fig 45. Onset and progression of galactose-induced cataract in WNIN/Ob (Left panel) and WNIN/GR-Ob (Right panel) in comparison to their lean controls



CONCLUSIONS

The base-line data indicate that sorbitol levels were high in the eye lens of WNIN/Ob and WNIN/GR-Ob as compared to their lean controls from 3-months old age onwards. Activities of antioxidant enzymes were also altered in the eye lens of WNIN/Ob and WNIN/GR-Ob. Also, the susceptibility of total soluble protein of the eye lens of WNIN/Ob and WNIN/GR-Ob to UV-induced aggregation and *in vitro* glycation was higher as compared to their lean controls from 3-months old age onwards. These results may explain the increased incidence of cataract in WNIN/Ob and WNIN/GR-Ob rats. Increased susceptibility of WNIN/Ob and WNIN/GR-Ob rats to galactose- and streptozotocin-induced cataract further supports the early onset and higher incidence of cataracts in WNIN/Ob and WNIN/GR-Ob rats. These results also indicate that there might be a connection between increased sorbitol levels and obesity which needs to be investigated further.

12. IMPORTANCE OF α -CRYSTALLIN HETEROPOLYMER IN THE EYE LENS: SUSCEPTIBILITY TO PROTEOLYSIS AND MODIFICATIONS

The small heat shock protein, α -crystallin is abundant in the eye lens of almost all vertebrates, reaching levels up to 50% of lens soluble proteins. Chaperone-like activity (CLA) of α -crystallin was shown to be instrumental in maintaining eye lens transparency and prevention of cataract. α -Crystallin has been shown to prevent precipitation of aggregation-prone proteins by forming a stable, soluble high-molecular-mass complex with the 'substrate' or 'client' proteins.

The eye lens α -crystallin is a large heteropolymer composed of two subunits, αA and αB , each of 20 kDa and encoded by *CRYAA* and *CRYAB* genes, respectively. In most vertebrate lens, the

molar ratio of α A to α B is 3:1, though the ratio varies amongst species, from 1:3 in dogfish, 9:1 in kangaroo and 19:1 in catfish. Homopolymers of α A- and α B-crystallin are known to possess similar structural and functional integrity as that of native α -crystallin, although their relative CLA is not be identical. Studies from NIN and elsewhere, have demonstrated that α A and α B subunits, either recombinantly expressed or reconstituted from native source, form heteropolymers in the proportion they are mixed, due to subunit or intermolecular exchange. However, the significance of the existence of α -crystallin as a heteropolymer with a lens-specific subunit ratio is not known.

The eye lens accumulates modified proteins with aging due to failure of protective mechanisms with aging. This leads to gradual loss in the optical quality of the lens, and ultimately leading to opacity or cataract. Studies have shown that lens crystallins undergo extensive modifications with age, among those; non-enzymatic glycation (Maillard reaction) is a predominant post-translational modification involved in age related and diabetic cataracts. Apart from modifications, proteolysis is also known to influence the cataract formation. It is quite likely that the susceptibility of α -crystallin heteropolymer with 3:1 ratio to proteolysis and posttranslational modifications might have determining role for the selection of heteropolymer in the eye lens.

Various factors such as hydrophobicity, secondary and tertiary structure, oligomeric size and temperature are known to regulate the CLA of α -crystallin. Earlier, it was demonstrated that although, under physiological conditions α B-homopolymer has a relatively higher CLA, at elevated temperatures and upon structural perturbation, the CLA of α A-homopolymer or the heteropolymer with a higher α A proportion (3:1 ratio) was higher. In this study, the susceptibility of the α -crystallin heteropolymer towards proteolysis (trypsin digestion) and modifications by glyating agents such as fructose, glucose-6-phosphate (G6P) and methyl glyoxal (MGO) was investigated in comparison to homopolymers of α A- and α B-crystallin and heteropolymer with varying sub unit ratio.

METHODOLOGY

- 1. α -Crystallin variants used in the study:** Recombinant α -crystallin heteropolymers (with α A to α B subunit ratios of - 3:1, 1:1 and 1:3 were obtained by mixing recombinant α A- and α B-crystallins in appropriate proportions) and α A- and α B-crystallin homopolymers were used in this study. Native α L-crystallin isolated from goat lens served as the native control for the heteropolymer having 3:1 α A to α B ratio and henceforth is referred to as α L. Formation of heteropolymers of α -crystallin upon mixing in the desired ratios of subunits was confirmed by immunoprecipitation followed immunoblotting as described previously.
- 2. Proteolytic digestion:** Susceptibility of α -crystallin variants to trypsin digestion was studied by incubating 0.5 mg protein with trypsin (0.15 mg/ml) in a final volume of 500 μ l in 0.1 M Tris-Cl buffer, pH 7.4, containing 0.004 M $MgCl_2$, 0.01 M KCl and 0.01% Tween-20 at 37°C. The reaction was stopped at different time points by the addition of 2 μ l of 0.1 M PMSF (a serine protease inhibitor) and placed on ice. Samples were resolved on a 12% SDS-PAGE.
- 3. Modification of crystallins with glyating agents:** Stock solution of the glyating agents 0.1 M methyl glyoxal (MGO), 1.0 M glucose 6-phosphate (G6P), 1.0 M fructose were prepared in 0.1 M sodium phosphate buffer, pH 7.5, containing 0.1 M NaCl. α -Crystallin variants (3 mg/ml) were incubated either with MGO (0.005 M for 72 h) or G6P/fructose (0.1 M for 21 days) in dark after filtering through 0.22 μ syringe filter under sterile conditions. α -Crystallin variants incubated in the absence of glyating agents under similar conditions served as respective controls.

3.1 Non-tryptophan fluorescence: Advanced glycation end-product (AGE) related non-tryptophan fluorescence of unmodified and modified α -crystallin variants (0.1 mg/ml) in 0.02 M sodium phosphate buffer, pH 7.2 was monitored by excitation at 370 nm and emission between 400 to 500 nm in a spectrofluorometer.

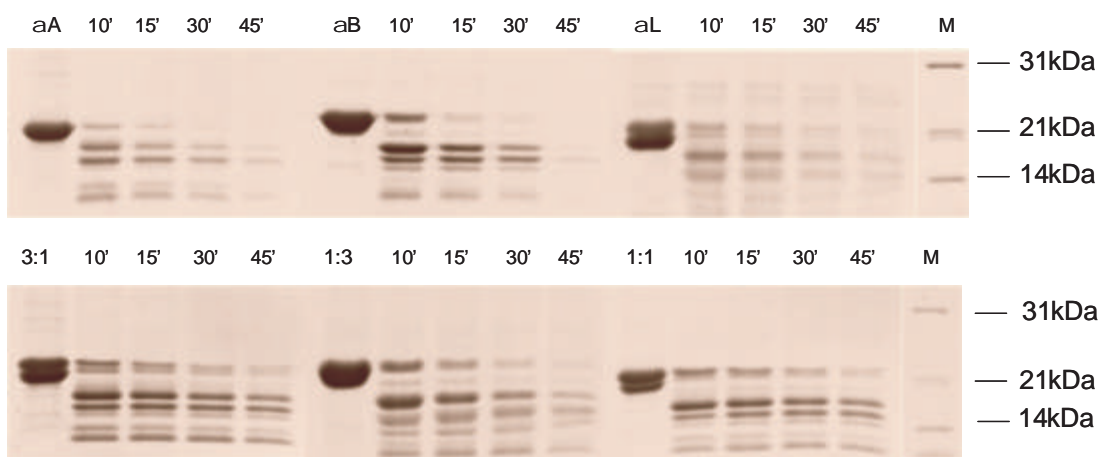
3.2 Tryptophan fluorescence: Tryptophan fluorescence of control and modified α -crystallin variants (0.1 mg/ml) in 0.02 M sodium phosphate buffer, pH 7.2 was monitored in a spectrofluorometer by exciting the samples at 280nm following the emission fluorescence from 300-400 nm.

3.3 Circular dichroism (CD) studies: Far-UV CD spectra of unmodified and modified crystallin preparations (0.1 mg/ml in 0.2 cm cuvette) in 0.02 M sodium phosphate buffer, pH 7.2 was recorded using a spectropolarimeter from 200-250 nm.

RESULTS

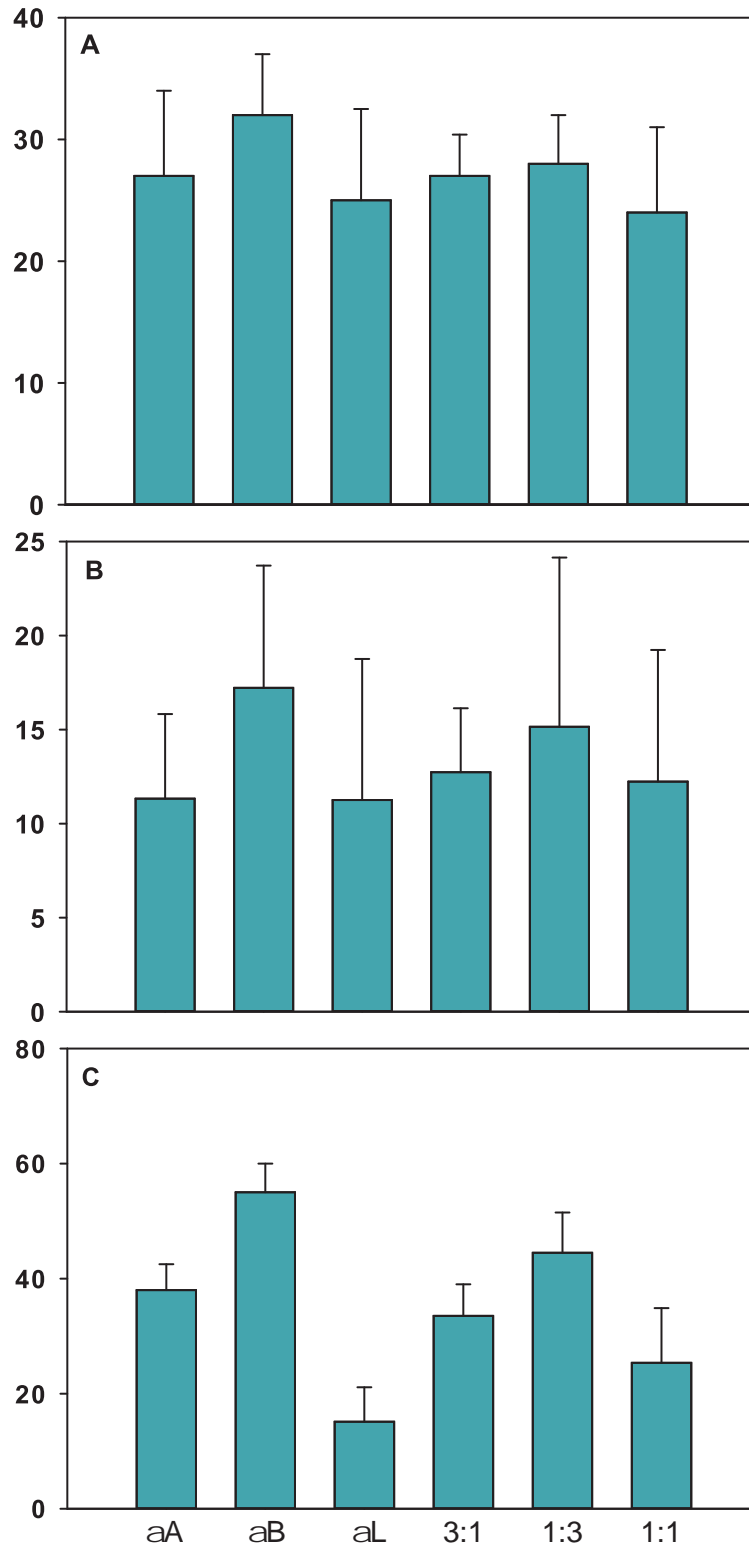
1. Trypsinolysis of the α -crystallin variants resulted in the formation of proteolytic fragments which was accompanied by the gradual disappearance of bands corresponding to the intact (undigested) protein.
2. It is interesting to note that while the disappearance of intact protein was more apparent and was completed by 30 min for the α A- and α B-homopolymers indicating total proteolysis, complete proteolysis was not observed until 45 min for α -crystallin heteropolymers indicating that heteropolymers are more resistant to the proteolysis (Fig 46). However, there is no significant difference among various heteropolymers.

Fig 46. Susceptibility of α -crystallin variants to proteolysis (trypsin digestion). Time of proteolysis (0-45 min) and molecular weight marker (M) are indicated



3. The relative resistance to proteolysis of heteropolymers could be due to a masking of the proteolytic cleavage sites (C-terminal Arg or Lys) due to sub unit contacts in heteropolymers as against that of the homopolymers.
4. Susceptibility to non-enzymatic glycation by MGO and G6P as monitored by non-tryptophan AGE fluorescence was not significantly different for α -crystallin variants (Fig 47A & B). However, α B-crystallin homopolymer and 1:3 polymer appear to be more susceptible to glycation by fructose as indicated by higher AGE fluorescence for α B-homopolymer and 1:3-heteropolymer when compared to other crystallin variants (Fig 47C).

Fig 47. Non-tryptophan AGE fluorescence intensity of α -crystallin variants modified with 0.005 M MGO (Panel A), 0.1 M G6P (Panel B) and 0.1 M fructose (Panel C) at 430 nm (emission maxima). Results are mean \pm SD (n=3)



- To understand the variability in glycation-mediated structural changes of α -crystallin variants, CD and fluorescence spectroscopy was performed.
- In the far-UV CD, α B-homopolymer and 1:3 displayed greater structural changes when incubated with MGO (Fig 48). α L and 3:1 were less susceptible to MGO-induced changes in secondary structure when compared to other variants (Fig 49A). However, G6P and fructose induced greater changes in secondary structure of α A-homopolymer compared to other variants (Fig 49B & C).

At the tertiary structural level as shown by percent change in the intensity of tryptophan fluorescence, α L and 3:1 heteropolymers were relatively less susceptible to modifications with all the three glyating agents (Fig 50).

Fig 48. Representative far-UV CD profiles of α B-homopolymer (panel A) and 3:1-heteropolymer (panel B) modified by glyating agents

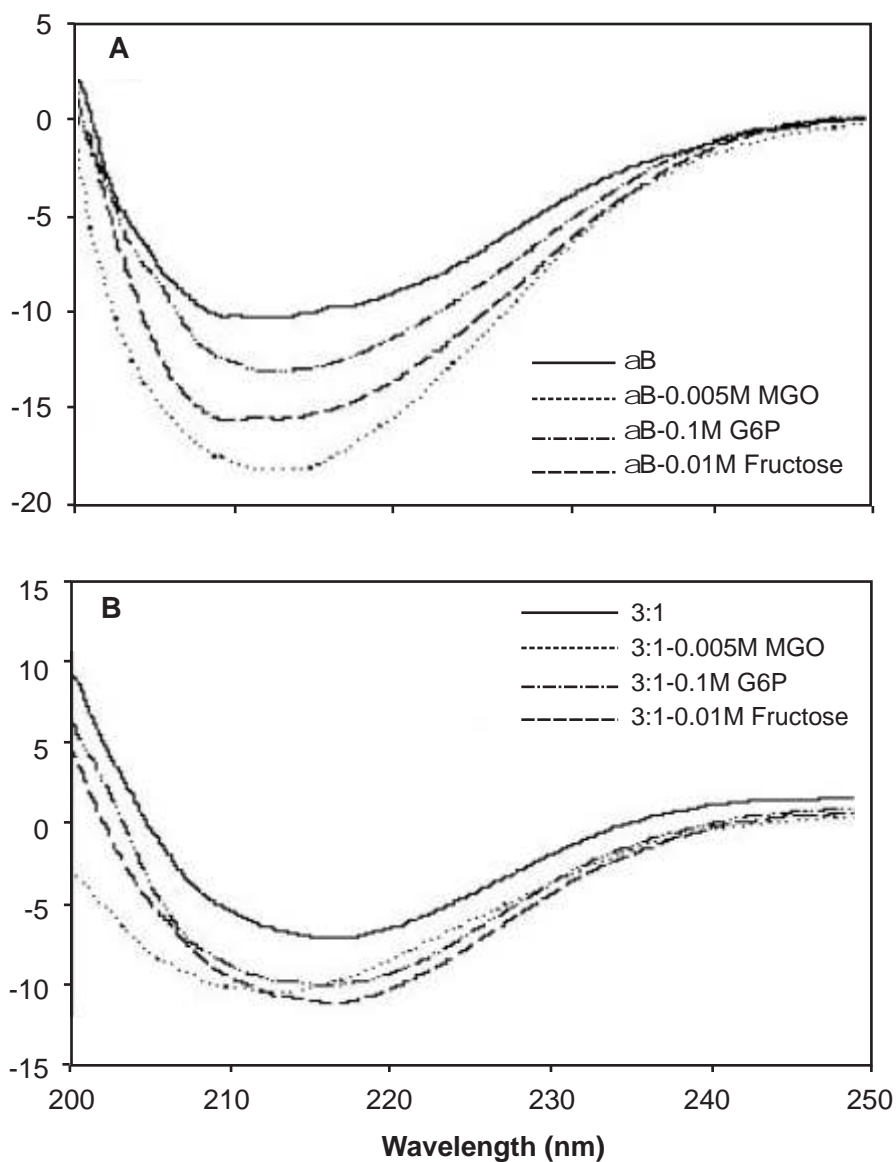


Fig 49. Percent change in the ellipticity (at 222 nm) of α -crystallin variants up on modification with 0.005 M MGO (Panel A), 0.1 M G6P (Panel B) and 0.1 M fructose (Panel C). Results are mean \pm SD (n=3).

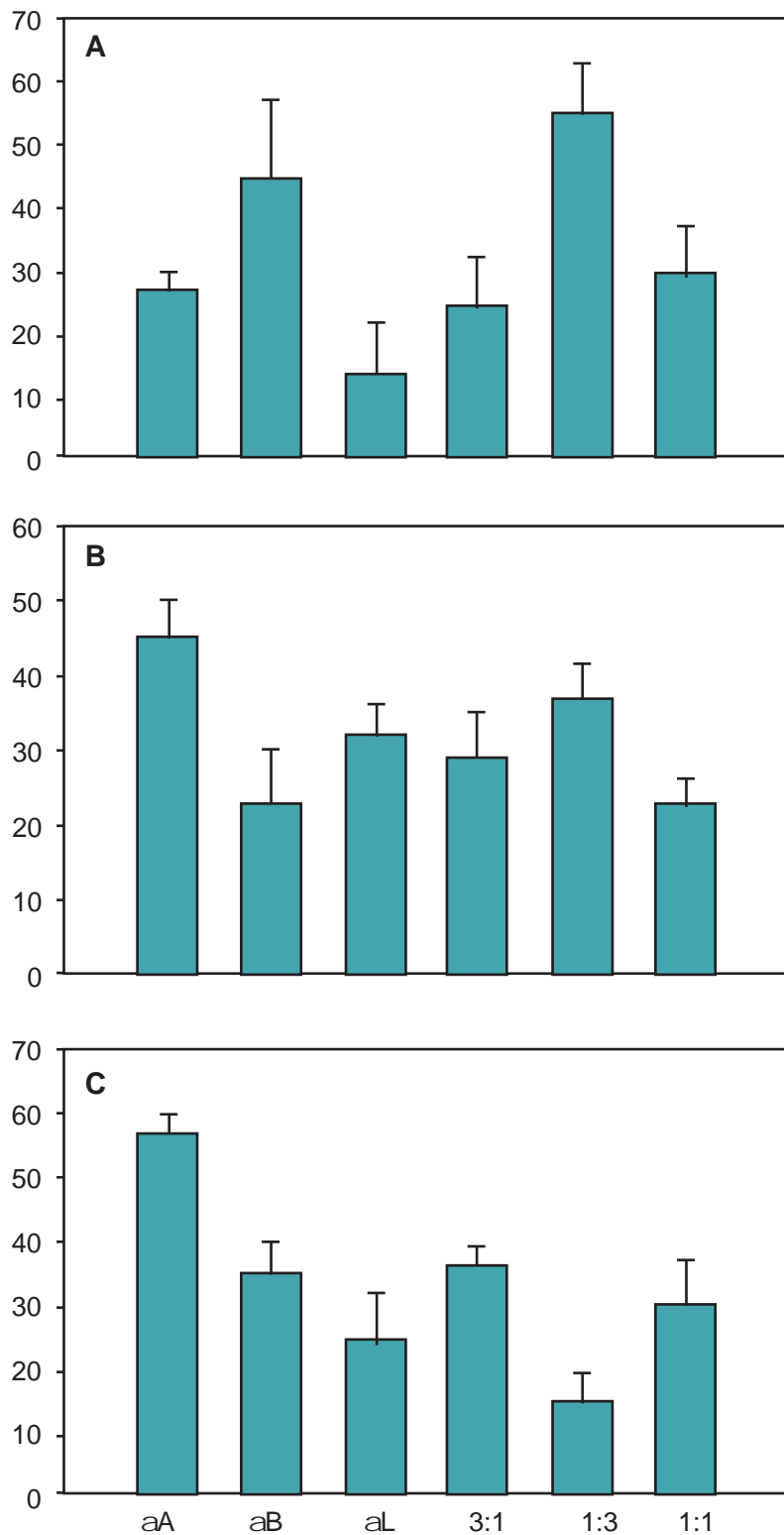
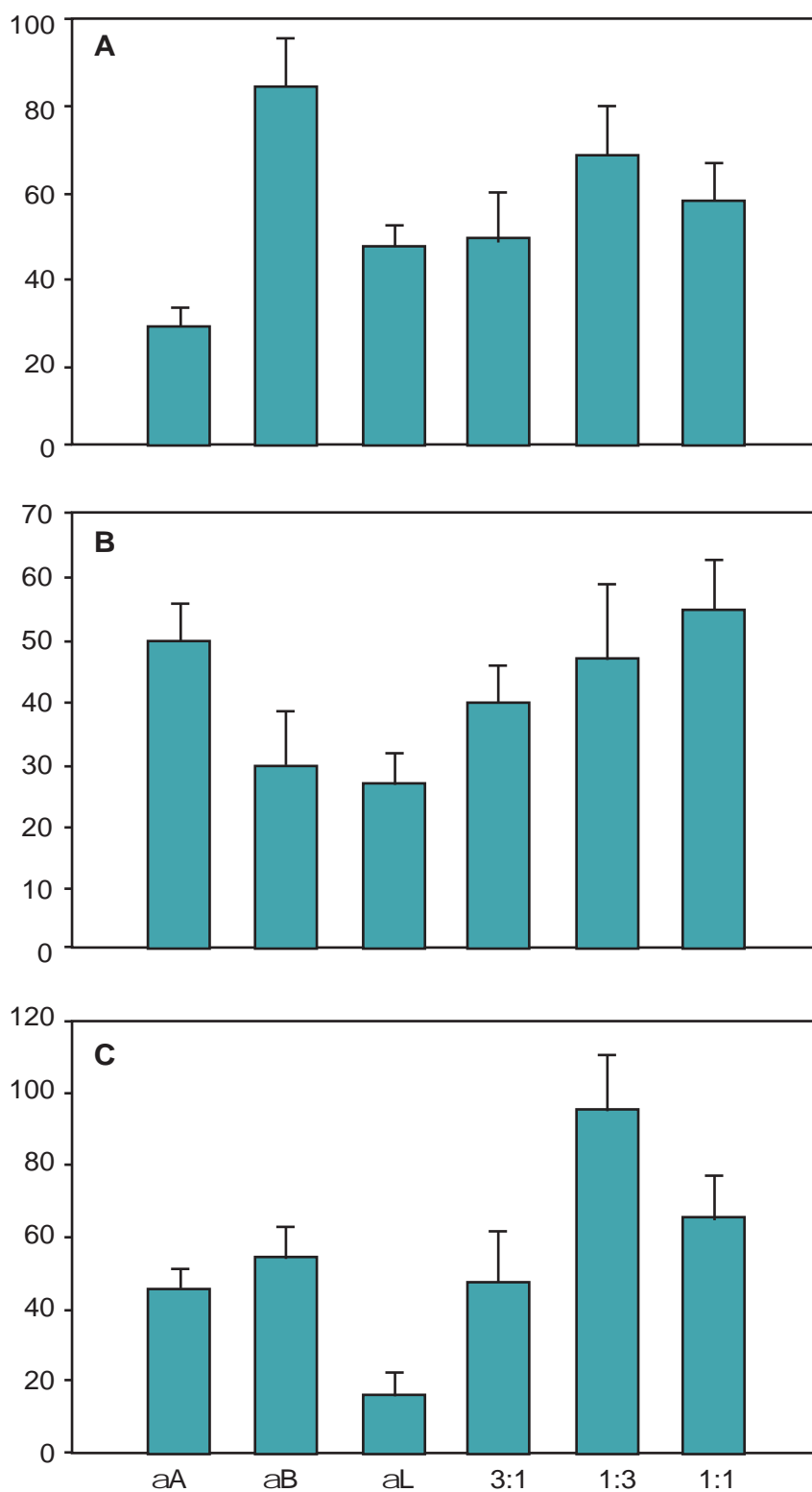


Fig 50. Percent change in tryptophan fluorescence intensity of α -crystallin variants upon modification with 0.005 M MGO (Panel A), 0.1 M G6P (Panel B) and 0.1 M fructose (Panel C) Results are mean \pm SD (n=3).



CONCLUSIONS

Extensive modification of α -crystallins with glycosylating agents were shown to cause aggregation and decreased CLA leading to cataract. It is also known that a compromised CLA of α -crystallin isolated from diabetic rat and human lens was due to glycation. Further, cross-linking of proteolytically cleaved products are known to cause cataract. Results of the present study indicate that heteropolymers particularly α_L and 3:1 are relatively less susceptible to glycation-induced modifications and proteolysis compared to other variants, gain merit with regard to the physiological significance of the existence of α -crystallin in the eye lens as a heteropolymer with a specific subunit ratio. Together with previous studies a rationale was provided for the existence of α -crystallin heteropolymer with 3:1 α_A to α_B ratio in terms of chaperone function, structural stability and susceptibility to posttranslational modifications that could explain the importance of the heteropolymer of α -crystallin in the eye lens. Thus, the existence of 3:1 heteropolymer might be vital for eye lens transparency under diverse conditions to prevent cataract.

13. DEVELOPMENT OF PCR METHOD FOR DETECTION OF GENETICALLY MODIFIED FOODS

Due to globalization of trade, a wide range of genetically modified (GM) foods are appearing in the market of several countries and are likely to enter the Indian market also. A testing facility with appropriate detection methods that are validated, sensitive as well as cost effective for screening, identification and quantification of GM foods is required so as to monitor the movement of GM foods. Genetically modified cotton popularly known as Bt-cotton, is the only GM crop that has been approved for commercialization in India and many GM food crops are in the pipeline for release and are likely to be commercialised in future. The first edible GM food, Bt-brinjal is likely to be released soon in India. With the increasing availability of genetically modified products, it is a necessity to develop sensitive techniques for the detection and identification of such products which will help in adhering to labelling requirements for the consumers, post marketing surveillance and other safety regulations. For such purposes, methods need to be developed and validated before the GM products are released in the market.

AIMS AND OBJECTIVES

The objectives of the project is to develop and validate PCR based assay for the detection of biomarkers associated with genetically modified (GM) crops/foods like cotton, maize, brinjal and soybean.

METHODOLOGY

A set of oligoprimers specific for the markers of these GMOs were designed *in silico*. The test samples were procured from the developers. In absence of appropriate test samples, certified

Table 28. List of oligoprimers designed for the present study

Primer Name	Target gene	Specificity	Amplicon (bp)	Reference
35S 195	35S	CaMV35S promoter	195	Lipp 1999
Nos 138	<i>Nos</i>	Nos terminator	138	Xu 2006
Sad1 309	<i>Sad1</i>	Cotton genome	309	This work
Invertase 242	<i>Invertase</i>	Maize genome	242	This work
Lectin 161	<i>Lectin</i>	Soybean genome	161	This work
UGPase 245	<i>UGPase</i>	Solanaceae genome	245	This work
Cry1Ac 355	<i>Cry1Ac</i>	Cry1Ac	355	This work
EPSPS 240	<i>EPSPS</i>	CP4-EPSPS	240	This work
Cry1Ab 282	<i>Cry1Ab</i>	Cry1Ab	282	This work
C-MON-531	<i>531event</i>	Cotton genome-insert	72	JRC 2007
B-EE1	<i>EE1 event</i>	Brinjal genome-insert	680	Monsanto

reference materials were used. The genomic DNA was purified from these samples. The PCR conditions were optimised for individual markers for singleplex and multiplex PCR. Fluorescent based capillary gel electrophoresis technique was optimised for the analysis of GMO markers. Quantitative estimation of GM material present in a sample was done with the help of real time PCR.

RESULTS

The present report includes the PCR analysis of genetically modified cotton, brinjal, maize and soybean. The most important factor for the successful development of PCR methods is the availability of the oligoprimers which can amplify sets of specific markers repeatedly. In the present study, majority of the primers for their respective annealing temperature (T_m) for singleplex and multiplex PCR were designed and optimized (Table 28).

Bt-Cotton

Genetically modified cotton expressing Bt-toxin, commonly known as Bt-cotton, is the only GM crop that has been approved for commercialization in India. Present study analysed the markers associated with the Bt-cotton derived from the event MON 531. The salient findings are described below:

- ❖ The qualitative PCR based assay was developed to detect cotton sample for the presence of GM material based on common GMO marker (*Nos*), endogenous reference gene (*Sad1*) and transgene (*Cry1Ac*) specific markers. The method may be useful to detect any unknown cotton sample for the determination of GMO status.
- ❖ Qualitative PCR assay was validated in spiked seed samples and was found to be capable of detecting Bt-seeds up to 0.1% mixture with non-Bt seeds. This assay with spiked seeds may be useful to check purity and identity of cotton seed.
- ❖ The limit of detection (LOD) based on the transgene (*Cry1Ac*) and the transformation event marker (*MON 531*) was achieved at the level of 0.01%.
- ❖ PCR detection kit for Bt-cotton was validated successfully by several independent groups within Institute. The kit is ready for external third party validation.
- ❖ The fluorescent-tagged primer based multiplex PCR assay was also developed in Bt-cotton for simultaneous detection of four markers. This technique can be used for higher throughput qualitative detection by multiplexing different combinations of dyes and fragment sizes together.

- ❖ Real time PCR was also developed to quantify the percentage of GM material contamination in cotton. The limit of quantification (LOQ) was found at 1% seed level.

Bt-Brinjal

Bt-brinjal is undergoing the final stage of approval process in India. If approved, this would be the first food of GM origin in India. Bt-brinjal samples (EE-1 event) were procured from the Mahyco, India. This Bt-brinjal event is being reviewed intensively for its final approval to release in the market. In this context, the development of an optimized assay to distinguish between GM and non-GM would be useful not only to protect purity of the seeds but also to support species identity. The salient findings on its detection are described below:

- ✦ PCR method was developed to detect markers associated with Bt-brinjal. The qualitative assay is capable to detect common GM marker (*Nos*), endogenous reference gene (*UGPase*), transgene (*Cry1Ac*) and genome-insert junction regions of the event EE-1.
- ✦ The LOD based on EE-1 event specific marker was achieved at the level of 0.01%.

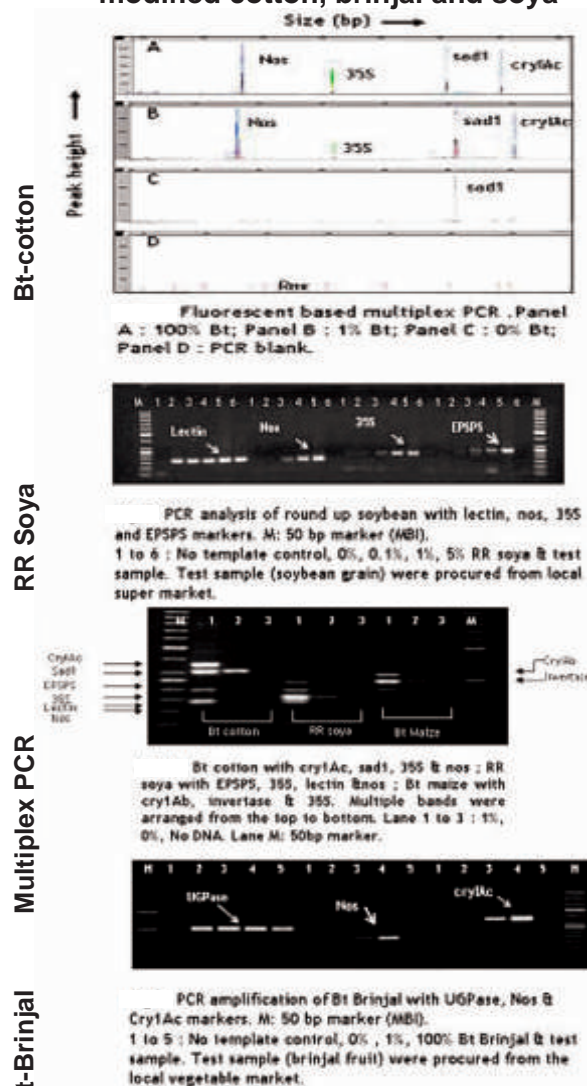
Bt-Maize

The genetically modified corn is being cultivated worldwide and the global acreage has been increased substantially over the past few years. In the present study, the certified reference material of the maize event Bt-176 was used to develop the detection assay. This assay was aimed to detect the GMO markers and transgene sequence resides in the gene construct of the Bt-corn with the help of PCR. Bt-176 corn was detected for the presence of 35S and *Cry1Ab* sequences along with *invertase* gene. The insert specific PCR assay can be used to detect the presence or absence of transgene and common GMO marker in Bt- maize.

RR-Soya

Genetically modified soybean, roundup ready (RR) is the widely cultivated GM soybean worldwide. In absence of any GM soya sample in India, certified reference material (CRM) of RR-soya grain powder from IRRM, Belgium was procured. Soybean grains purchased from the local market were also included in the present assay. The marker sequences of the RR-soya was detected for the presence of *lectin* (endogenous), 35S & *Nos* (common GMO element) and *EPSPS* (transgene) markers by qualitative singleplex and multiplex PCR assay. The assay was sensitive to detect as low as 0.1% GM-soya contamination at grain level. The limit of detection (LOD) of 0.1% may be important for the purpose of RR-soya detection (Fig 51).

Fig 51 PCR analysis of genetically modified cotton, brinjal and soya



CONCLUSION

These DNA based methods can detect marker elements associated with genetically modified cotton, brinjal, corn and soybean with help of PCR techniques. These methods may be useful to know the GMO status of food samples.

14. POTENTIAL ROLE OF DIETARY NUTRIENTS VITAMIN A AND POLYUNSATURATED FATTY ACIDS (PUFA) ON REGULATION OF DEVELOPMENT AND/OR CONTROL OF OBESITY USING A GENETIC OBESE MUTANT RAT MODEL (WNIN/GR-Ob) NUTRIENT-GENE INTERACTION

Obesity, a global epidemic is threatening millions of population around the world. It is estimated that 154 million people worldwide are obese. Notably, prevalence of childhood obesity has increased dramatically over the past 20 years. This sudden rise can be attributed to high calorie-dense foods and reduced physical activity. Obesity is an outcome of unfavorable interactions between the genetic pool and environmental factors. Dietary fats are known to play a role in the onset and progression of various diseases such as atherosclerosis, obesity, hypertension, type 2 diabetes and certain types of cancers. Recent understanding of the role of PUFAs and their metabolites on gene regulation made a significant impetus in the field of lipid research. Therefore, the study focused on the impact PUFA (high n-6 PUFA) and with lowered ratio of n-6 to n-3 PUFA diets on obesity and glucose clearance by using a genetically obese rat model with impaired glucose tolerance.

AIM OF THE STUDY

To understand the role of PUFA-rich oil diets (n-6 and a blend of n-6 & n-3) supplementation on obesity and associated disorders using glucose-intolerant obese rats of WNIN/GR-Ob strain.

OBJECTIVES

1. To study the impact of PUFA feeding on physical indices of obesity [body weight gain, adiposity index (AI), body mass index (BMI)]
2. To study the effect of PUFA supplementation on biochemical and molecular markers of dyslipidemia.
3. To study the effect of PUFA supplementation on liver and adipose tissue lipid metabolism.
4. To study the effect of PUFA supplementation on biochemical and molecular markers of glucose homeostasis.

METHODOLOGY

Standard methodologies/protocols were followed

1. Western blotting for hepatic mtGPAT and PPAR α
2. Histological analysis of liver
3. Diaphragm glucose up-take
4. Soleus muscle triglycerides (TGs) and membrane phospholipids (PLs)
5. Immunoblotting of glucose transporter-4 (GLUT4) of soleus muscle

Table 29. Experimental Design

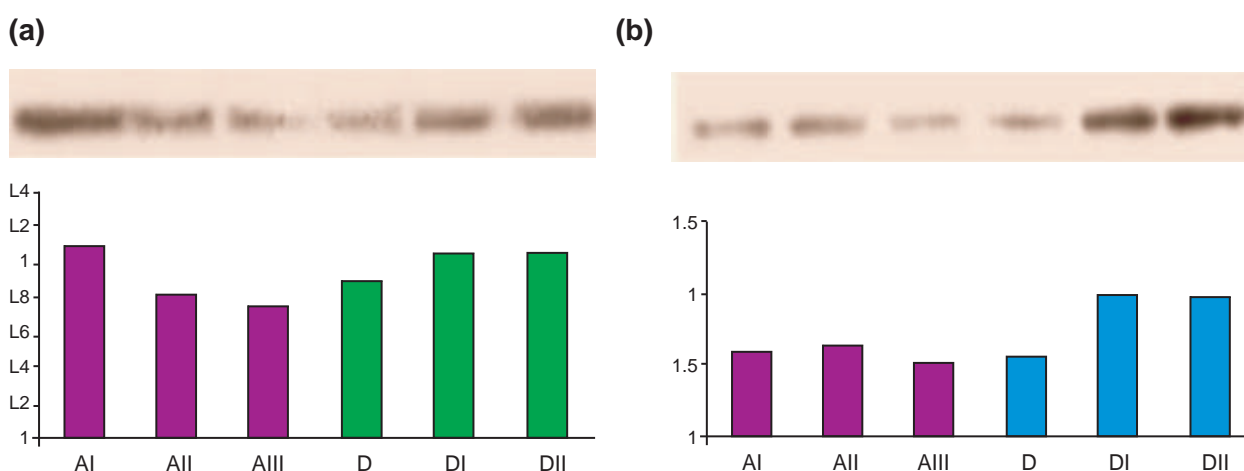
4% Groundnut oil-containing diet		4% Safflower oil-containing diet		4% blend of safflower and soybean oil containing diet	
AI	BI	AII	BII	AIII	BIII
Lean (n=8)	Obese (n=8)	Lean (n=8)	Obese (n=8)	Lean (n=8)	Obese (n=8)
Animals were fed on their respective diet for a period of 3 months					

RESULTS

1. Regulation on hepatic levels of mtGPAT and PPAR α

An increased trend in the levels of PPAR α was observed in PUFA-fed obese rats, compared to control obese rats (Fig 52a). However, no such changes were seen in PUFA-fed lean rats. Hepatic levels of mtGPAT remain unaltered in PUFA-fed lean and obese rats, as compared to their respective controls (Fig 52b).

Fig 52. Effect of PUFA feeding on hepatic proteins

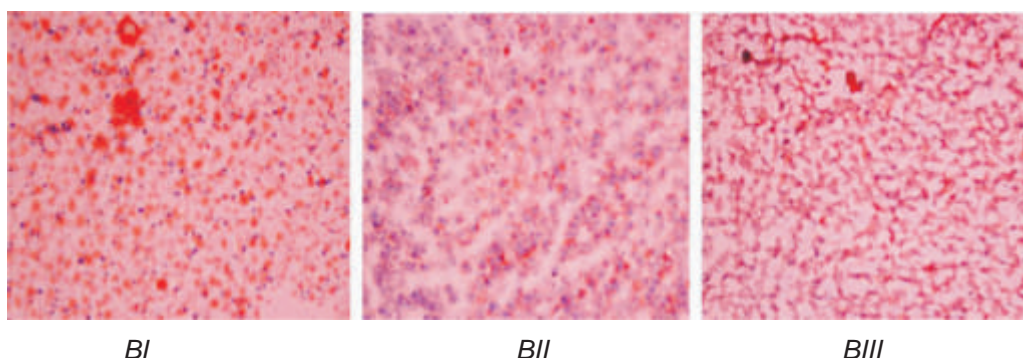


Top Panel shows the representative Western blots of (a) PPAR α and (b) mtGPAT in lean and obese rats fed on control and PUFA diets. Bar graph shows the densitometric analysis of 3 rats, representing each of the dietary group. Bars are means \pm S.D. *Significant at P 0.05 level. (Abbreviations: PPAR α ; peroxisome proliferator activated receptor- α , mtGPAT; mitochondrial glycerol 3-phosphate acyl transferase, PUFA; polyunsaturated fatty acid).

2. Effect of PUFA feeding on hepatic lipid accumulation in obese rats.

Representative Oil red-O images of liver sections at a magnification of 200X. Frozen tissue sections were stained with lipid specific Oil red-O dye (orange red). Hepatic lipid accumulation was reduced in PUFA-fed obese rats compared to control obese in accordance with biochemical measurements of liver triglycerides (Fig 53).

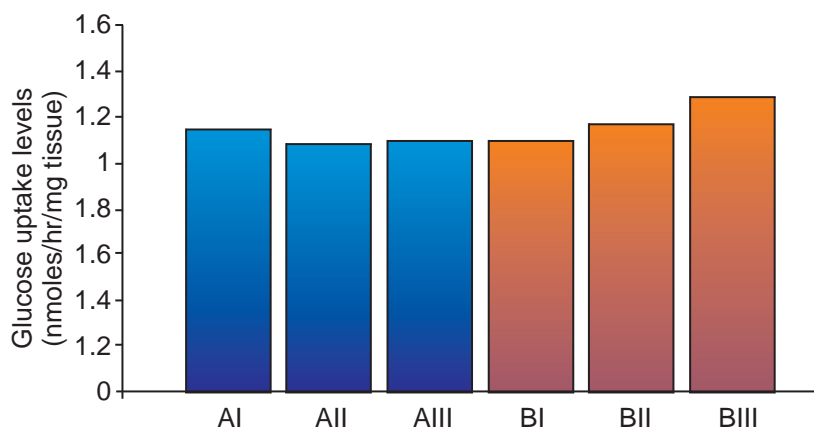
Fig 53. Effect of PUFA feeding on hepatic lipid accumulation in obese rats



3. Effect of PUFA feeding on insulin-stimulated glucose uptake by diaphragm

Glucose uptake study, using diaphragm muscle showed that tissue glucose uptake under basal conditions i.e. in the absence of insulin were similar in lean and obese rats. Further, upon stimulation with insulin, glucose levels increased in the diaphragm of both phenotypes. However, the insulin-stimulated glucose uptake levels were significantly higher in obese rats fed on blends of *n*-6 & *n*-3 PUFA (BIII), as compared to obese rats fed on groundnut oil diet (BI) (Fig 54).

Fig 54. Effect of PUFA feeding on insulin-stimulated glucose uptake by diaphragm muscle



Results are means \pm SD of 3-4 rats. Comparisons were made between control Vs experimental groups of each phenotype (i.e. A-I Vs A-II, A-III & B-I Vs B-II, B-III).

4. Effect of PUFA feeding on soleus muscle lipids

Triglyceride concentration of soleus muscle in either of the phenotypes was not influenced by any of the dietary regimens (Table 30a). Fatty acid composition of membrane phospholipids (PLs) showed that the levels of palmitic acid (C16:0) were significantly lower in obese rats fed with a diet

containing both n-6 & n-3 PUFAs. Identical dietary treatment reduced the levels of stearic acid (C18:0) in the PLs of lean rat. There was a significant reduction in the total saturated fatty acid (SFA) content of the soleus muscle of both lean and obese rats fed the diet containing n-6 & n-3 PUFAs. Feeding PUFA-enriched diets had no effect on long chain PUFA content of the PLs of obese as well as lean rats (BII, BIII) (Table 30b).

Table 30a. Effect of PUFA feeding on triglyceride levels in the soleus muscle

Soleus muscle	AI	AII	AIII	BI	BII	BIII
Triglycerides (mg/gm tissue)	2.6±1.61	1.4±0.66	2.1±0.02	3.1±1.65	3.1±1.56	3.7±1.34

Comparisons were made between control and experimental groups of each phenotype (i.e. A-I Vs A-II, A-III & B-I Vs B-II, B-III). Values are means ±SD (n=3-4).

Table 30b. Effect of PUFA feeding on fatty acid composition of membrane PLs in soleus muscle

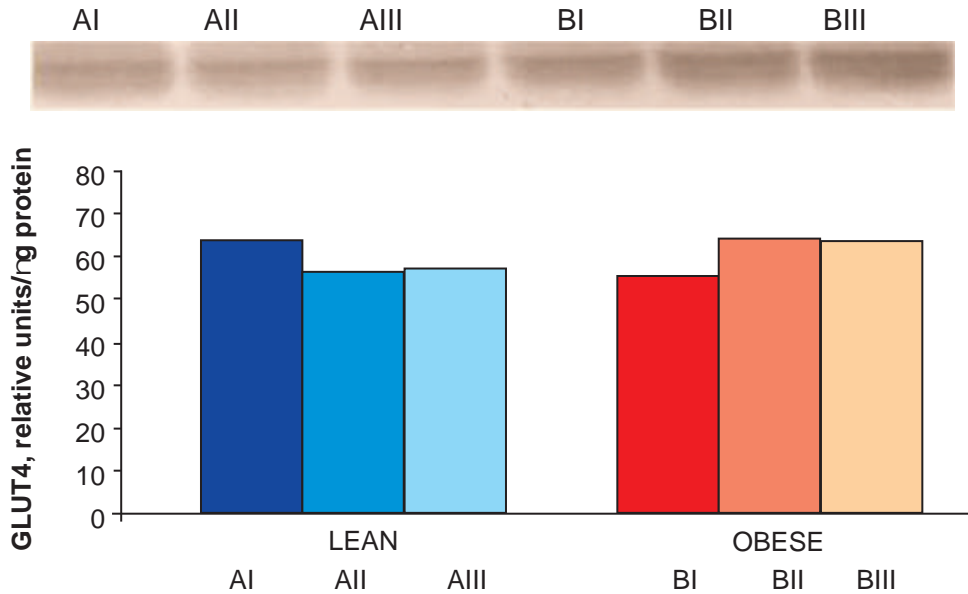
Fatty acids(%)	AI	AII	AIII	BI	BII	BIII
16:0	21.4±1.2	18.8±1.3	18.9±1.8	20.9±2.1 ^a	19.6±0.6 ^{a,b}	18.4±1.5 ^b
16:1	0.8±0.4	1.2±0.1	1.2±0.4	1.4±0.4	1.5±0.4	1.±0.2
18:0	14.9±0.6 ^a	14.2±0.3 ^{a,b}	13.0±0.8 ^b	12.8±1.4	12.4±0.9	13.1±1.2
18:1	3.7±1.5	4.1±0.3	3.9±0.8	6.1±1.6	8.0±2.4	6.3±1.0
18:2	21.7±1.2	22.3±1.9	21.0±2.3	22.8±1.0	21.3±1.5	21.4±1.2
20:2	3.4±0.6	3.9±0.6	4.4±1.1	4.6±2.4	4.9±0.8	3.8±0.2
20:3n6	1.0±0.2	1.0±0.07	2.3±1.4	2.2±0.5	2.2±0.2	1.7±0.2
20:4	18.6±1.1	19.7±2.0	18.2±0.8	15.3±2.0	16.0±1.8	17.5±0.4
22:0	2.8±0.7	3.0±0.6	3.4±1.5	3.5±1.9	3.6±1.1	2.7±1.5
22:5n3	2.0 ±0.9	1.9±0.5	3.0±0.9	2.8±0.5	2.3±0.3	3.5±1.1
22:6n3	7.3±1.0	7.7±1.0	6.8±1.2	5.6±0.8	5.7±0.4	5.2±1.6
24:1	2.2±0.7	2.0±0.4	2.3±0.6	1.8±0.3	2.6±0.4	3.6±1.7
SFA	39.1 ^a	36.0 ^{a,b}	34.3 ^b	37.3 ^a	35.5 ^{a,b}	33.4 ^b
MUFA	6.7	7.4	9.9	9.4	12.0	10.1
PUFA	54.2	56.6	56.0	53.3	52.4	56.2

Comparisons were made between control and experimental groups of each phenotype (i.e. A-I Vs A-II, A-III & B-I Vs B-II, B-III). Means with different superscripts are significantly different at $P < 0.05$. Bars are means±SD (n=3-4)

5. Effect of PUFA feeding on microsomal GLUT4 levels

Levels of microsomal membrane-associated GLUT4 in the skeletal muscle (soleus) of obese rats were low compared to the lean phenotype as shown by immunoblot analysis. Further, chronic consumption of PUFA-rich diets did not alter the levels of this glucose transporter in either of the phenotypes compared to their respective controls (Fig 55).

Fig 55. Effect of PUFA feeding on microsomal GLUT4 protein levels in soleus muscle of lean and obese rats



Bars are means \pm SD of 3 rats, representing each of the dietary group. No significant changes in GLUT4 protein levels were observed. Equal loading was ensured by PonceauS staining.

CONCLUSIONS

The results of the study suggest that compared to the diet with safflower oil (*n*-6 PUFA) alone, a blend of safflower and soybean oils (*n*-6/*n*-3 PUFA at 13/1) was more effective in reducing hepatic steatosis, enhancing skeletal muscle glucose uptake and thereby improving insulin resistant condition of genetically obese glucose-intolerant rats of WNIN/GR-Ob strain. Thus, the results underscore the importance of the balance between *n*-6 and *n*-3 PUFA in the diet to combat various components of metabolic syndrome such as dyslipidemia, hepatic steatosis, glucose intolerance and insulin resistance.

15. BASAL CHARACTERIZATION OF WNIN/Ob & WNIN/GR-Ob RATS WITH RESPECT TO HDL-C, LIPOPROTEIN PROFILE, CERTAIN IMMUNOLOGICAL PARAMETERS, RETICULOCYTE DIFFERENTIATION, FEMALE INFERTILITY & CARDIOVASCULAR PHYSIOLOGY

Obese rats of WNIN/Ob strain are hyperlipidemic, hyper-cholesterolemic, hyperleptinemic and hyperinsulinemic. The increase in plasma total cholesterol was mainly due to an increase in HDL-C fraction. In addition, these obese animals also displayed higher reticulocyte counts. Based on these results and some of the recent reports on SR-B1 knock out mice, it was assumed that the expression of SR-B1 receptors in obese rats of WNIN/Ob might be low, thereby resulting in the elevated plasma HDL-C levels.

In view of the well established role of SR-B1 in reverse cholesterol transport (RCT), a mechanism by which excess cholesterol from peripheral tissues is transported to liver, steroidogenesis, reticulocyte differentiation, intestinal absorption by extrinsic factors (nutrients and chronic infection) and intrinsic factors (IL-4, IL-6 and TNF α), it would be of great interest to study the expression of these receptors in the obese rats of WNIN/Ob and WNIN/GR-Ob strains. In addition, these studies may help in understanding the mechanism of female infertility in obese rats of WNIN/Ob and WNIN/GR-Ob strains. Whether, these receptors also mediate the estrogen-dependant activation of endothelial nitric oxide synthase (eNOS) and thereby offer cardio-protection can be established by determining eNOS activity and SR-B1 binding in the femoral artery of female obese rats of both strains.

OBJECTIVES

- ❖ To establish the authenticity of HDL-C by more sophisticated method like FPLC method for lipoprotein analysis from plasma which gives information on the cholesterol content and the determination of Apo A1 in cholesterol-enriched fractions helps in knowing whether there is an increase in Apo A1 or not? and is it a just increase in HDL-particle size.
- ❖ To assess the reticulocyte status of female rats. Given the fact, the male obese rats of WNIN/Ob strain had higher reticulocyte count compared to their male carrier and lean counterparts, it is necessary to establish whether the same is true for female rats or the situation is much worse.
- ❖ To assess the basal expression of SR-B1 in liver, adrenals and gonads of male and female rats from three phenotypes of WNIN/Ob & WNIN/GR-Ob strains.
- ❖ The basal levels of TNF α , IL4 & IL 6 will be determined in male and female obese, lean, and carrier rats of WNIN/Ob & WNIN/GR-Ob strain.
- ❖ Basal endothelial nitric oxide synthase activity will be assayed in the femoral artery of the male and female rats of lean, carrier and obese phenotypes of WNIN/Ob & WNIN/GR-Ob strain and relate it to the SR-B1 expression of the same tissues.
- ❖ Morphological examination of adrenals and gonads.

METHODOLOGY

- FPLC- lipoprotein fractionation was done and cholesterol was analyzed in plasma of 330 days old male lean and obese rats of WNIN/Ob rats strain.
- Analysis of plasma MCP-1 cytokine level in 35, 90 and 330 days old male lean and obese rats of WNIN/Ob rat strain using commercially available kit (Milleplex).
- Adrenal SR-BI gene expression was analyzed in 365 days old male lean and obese rats of WNIN/GR-Ob strain.
- Adrenal SR-BI gene expression was analyzed in 180 days old female lean and obese rats of WNIN/Ob strain.

RESULTS

Plasma-lipoprotein cholesterol profile revealed that obese rats of WNIN/Ob strain had 3 fold higher levels of HDL-C, compared to their age and sex-matched lean counterparts.

Fig 56. Plasma-lipoprotein cholesterol profile of 330 days old WNIN/Ob strain rats by FPLC

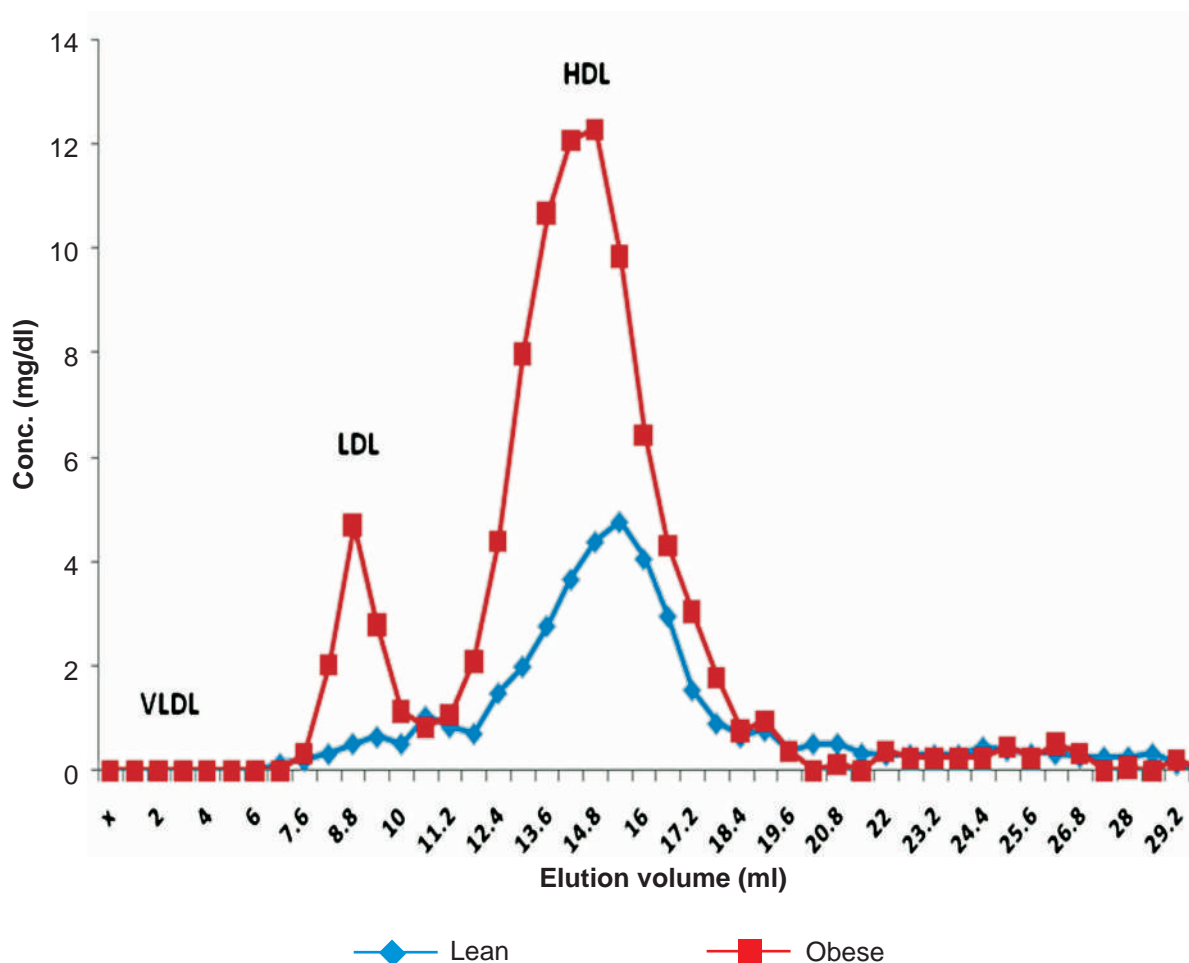


Table 31. Plasma MCP-1 cytokine in lean and obese rats of WNIN/Ob strain at various age points

	WNIN/Ob					
	35 days		90 days		330 days	
	Lean	Obese	Lean	Obese	Lean	Obese
MCP-1	751 – 654.0	392 – 290.2	299 – 204.0	2532 – 4738.4	92 – 184.1	291 – 430.0

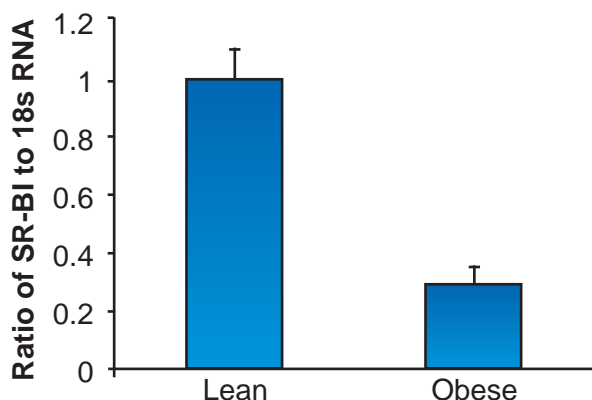
Values are mean±SD of 4 animals from different age groups.

There were no significant differences in the levels of Plasma MCP-1 of lean and obese rats of WNIN/Ob rats at various age points.

Adrenal SR-BI gene expression in male WNIN/GR-Ob rats

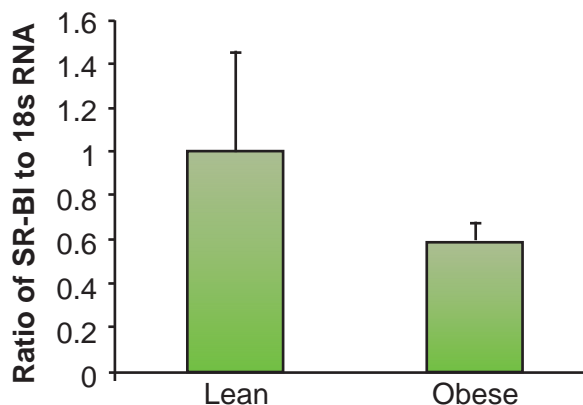
Total RNA was isolated from adrenals and relative SR-BI gene expression was analysed by semi-quantitative RT-PCR. The 365 days old lean and obese rats were used for adrenal SR-BI mRNA expression analysis (Fig 57 A & B).

57 A. Adrenal SRB1 expression in 365 days old male WNIN/GR-Ob rats



Adrenal SR-BI expression was higher in 365 days old male lean rats as compared to their obese counterparts of WNIN/GR-Ob strain.

57 B. Adrenal SRB1 expression in 180 days old female WNIN/Ob rats



Adrenal SR-BI expression was higher in 180 days old female lean rats as compared to their obese counterparts of WNIN/GR-Ob strain.

Adrenal SR-BI gene expression in female WNIN/GR-Ob rats

Total RNA was isolated from adrenals and relative SR-BI gene expression was analysed by semi-quantitative RT-PCR. 180 days old lean and obese rats were used for adrenal SR-BI mRNA expression analysis

16. EFFECT OF DIETARY NUTRIENTS ON SUB-CHRONIC AND CHRONIC DRUG TOXICITY IN SPRAGUE DAWLEY (SD) RATS

Under the United States National Toxicology Program, the National Institute of Health (NIH)-07 open formula non-purified diets are the recommended diets for rodents, in toxicology and carcinogenesis studies. It is reported that the protein and mineral concentrations of the NIH-07 diet may have increased some diet and age associated lesions such as nephropathy.

It is also reported that non-purified diets with lower protein and higher fat and fiber concentrations than the NIH-07 diet, decreased or delayed diet and age associated lesions and increased survival in 2-year studies.

The toxicological study of various drugs/products is being done in the Indian context throughout the country using the presently available dietary formulations from NIN. Based on the recommendations of the National Toxicology Programme of USA, it is proposed to look into the effect of the involved nutrients in NIN diet on the outcome of subchronic and chronic studies in comparison to commercially available diets as well as NTP diet.

OBJECTIVES

To study the effect of NTP diet with low protein (14.5%), high fat (8.2%) and high fiber (9.3%) on the toxic effects (subchronic & chronic studies) of selected pharmaceutical drugs with nephrotoxic properties (gentamycin) in comparison to NIN stock diet (20% protein, 8% fat and 10% fibre), special diet (pr. & fat - 8 %, fibre 10%) and commercial diet (CD) (pr. 22%, fat 5% and fiber 4%).

METHODOLOGY

A total of 240 SD rats, belonging to both sexes in equal numbers, were randomized and divided into two major groups, Group A and Group B. Animals in each of the groups A and B were further subdivided into four groups of 30 animals each. The four groups received four different diets (NIN, NTP, LP and Commercial Diet). The animals of each subgroup were fed their respective diets for 40 days followed by intra-peritoneal administration of Gentamycin at two dose levels (viz. 1X (20mg) and 2X (40mg) of the therapeutic dose). Feed intake and body weights were recorded twice weekly.

The experiment was conducted in two phases. In phase one, the animals were maintained on the respective diets for 40 days followed by treatment with Gentamycin (40 mg/kg b.wt.) and observed until day 90 (referred as Gentamycin 1 experiment). In the second phase, the animals were fed respective diets for 70 days followed by Gentamycin treatment and observation upto day 90 (referred as Gentamycin 2). The changes in the hematological, biochemical and histopathological values were studied in NTP diet in comparison to the other diets.

RESULTS

No significant changes were observed in any of the parameters such as live phase, cage side observations, neurological examination and allergenicity profile of all the animals from all the groups.

A) BIOCHEMISTRY

GENTAMYCIN 1

No significant changes were observed at Baseline, 1 X groups of NTP Vs

✎ 1 X & 2 X groups of NTP Vs. 2 X of NIN, LPD and CD.

✎ In C groups of NTP Vs NIN, LPD and CD

Baseline --- Urea – was significantly increased in NTP control group as compared to LPD group.

Post Exposure --- ALT was significant increased in NTP control as compared to LPD group.

✎ In 'C' NTP Vs '1 X' NTP & 'C' NTP Vs '2 X' NTP

Post Exposure --- Creatinine was significantly higher in 2x group as compared to control group.

Recovery period --- Triglyceride was significantly decreased in 2x as compared to control.

GENTAMYCIN 2

✎ 'C' groups of NTP Vs NIN, LPD, CD, "1 X" groups of NTP Vs 1 X of NIN, LPD, CD and "2 X" groups of NTP Vs. 2 X of NIN, LPD and CD

No significant changes were seen.

✎ 'C' NTP Vs '1 X' NTP & 'C' NTP Vs '2 X' NTP

Post Exposure: AST was significantly increased in 2x group as compared to control.

B) HEMATOLOGY

GENTAMYCIN 1

✎ 'C' groups of NTP Vs NIN, LPD, CD

Baseline and Recovery period: No significant changes were observed.

Post exposure: WBC- NTP group had significantly lower counts as compared to CD group.'

✎ '1 X' groups of NTP Vs 1 X NIN, LPD, CD

Baseline, Post exposure and Recovery period: No significant changes were observed.

Euthanization day: HGB, HCT -Significantly lower in NTP group as compared to NIN group. WBC-Significantly higher in NTP group as compared to LPD group.

✎ '2 X' groups of NTP Vs 2X NIN, LPD and CD

Baseline, Pre-exposure, Post-exposure and Recovery period: No significant changes were observed.

Euthanization day: HGB, HCT, MCV-significantly lower in NTP as compared to NIN group. WBC-significantly higher in NTP as compared to LPD group.

GENTAMYCIN 2

'C' groups of NTP Vs NIN, LPD and CD :

Baseline: No significant changes were observed.

Post Exposure: WBC-Significantly higher as compared to LPD group.

Euthanization Day : HGB-Significantly lower in NTP as compared to NIN and CD group. HCT,MCH--Significantly lower in NTP as compared to NIN group. MCV,WBC -Significantly higher in NTP as compared to LPD.

'1 X' groups of NTP Vs 1 X NIN, LPD and CD :

Baseline: WBC- Significant decrease in NTP group as compared to NIN group.

Post Exposure: MCH- Significant increase in NTP group as compared to NIN group. WBC-Significantly lower in NTP as compared to CD group and significantly higher in NTP as compared to LPD group.

Euthanization day : HGB, HCT, WBC: Significantly lower in NTP as compared to NIN group. MCV: Significantly higher in NTP as compared to LPD and CD group.

'2 X' groups of NTP Vs 2X NIN, LPD and CD

Baseline: No significant changes were observed.

Post Exposure: HGB-Significantly higher in NTP group as compared to NIN, CD and LPD group.

Euthanization day: HGB, HCT,MCV-Significantly lower in NTP group as compared to NIN group. WBC-Significantly higher in NTP as compared to LPD group.

C) EUTHANISATION

Organ Weights: Kidney and spleen weights were observed to be significantly higher in NTP group as compared to LPD group. Within the group no differences in organ weights were observed between control, 1 X and 2 X dose groups.

HISTOPATHOLOGY EVALUATION

GENTAMYCIN 1 (N=5)

1. 'C' NTP Vs NIN, LPD and CD

One animal each from NIN, CD and NTP groups males showed proximal tubular swelling and necrosis in the kidneys. In the females, only one animal from NIN group showed the changes in the tubules.

2. '2 X' NTP Vs. 2 X NIN, CD and LPD

In males only one animal from NIN group showed the tubular changes in the kidneys. In females 2 animals (n=5) from NIN, 3 animals (n=5) from NTP and one animal each from CD and LPD diet groups showed tubular changes in the kidneys.

3. "C" NTP Vs 2 X NTP

In control males 1 animal (n=5) showed tubular changes. In 2 X group (n=1) no changes were observed in the remaining one animal. In female control group no changes in the kidney were observed. In the 2 X NTP diet group 3 animals showed Tubular changes in the kidneys.

GENTAMYCIN 2 (N=5)

1. "C" NTP Vs NIN, LPD and CD

No changes were observed in the control groups between NTP and other diets.

2. "2 X" NTP Vs. 2 X NIN, CD and LPD

In males at 2 X dose 2 animals from NIN (n=5), one animal from NTP group (n=4), one animal from CD group (n=5) and no animal from LPD group (n=5) showed tubular changes in the kidneys. In females 1 animal each from NIN, NTP and LPD groups showed tubular changes in the kidneys.

3. "C" NTP Vs 2 X NTP

In the NTP control group males (n=5) no animal showed changes whereas in the 2 X group one animal (n=4) showed tubular changes in the kidneys. In female control group all animals were normal whereas in the 2 X group one animal (n=5) showed tubular changes in the kidneys.

Based on the experiments conducted, it was seen that the various biochemical (urea, creatinine, AST etc.), haematological (Hb, HCT, WBC etc.), and organ weights were significantly different in NTP diet fed animals as compared to other groups. This was observed in both short term and long term feeding of respective diets to the animals. Preliminary data analysis shows significant increase in creatinine levels in 2 X group animals from the Gentamycin 1 study post exposure and a significant decrease in the triglycerides. In the Gentamycin 2 study post exposure, in 2 X group an increase in AST levels was observed. In hematology, significantly higher WBC counts were observed in the NTP group as compared to other groups in the Gentamycin 2 study. At necropsy, higher kidney and spleen weights were observed in the NTP group. Histopathologically, a higher number of animals from the 2X group showed tubular necrosis in the kidneys as compared to the other groups.



VI. FOOD CHEMISTRY

CAROTENES AND XANTHOPHYLL CONTENT OF INDIAN FOODS

Vitamin A is made up of a family of compounds called the retinoids. There are essentially 3 forms of vitamin A: retinols, beta carotenes, and carotenoids. Retinol, also known as preformed vitamin A, is the most active form and is mostly found in foods of animal sources. Beta carotene, also known as provitamin A, is the plant source of retinol from which mammals make two-thirds of their vitamin A. Carotenoids, the largest group, contain multiple conjugated double bonds and exist in a free alcohol or in a fatty acylester form.

Vitamin A deficiency (VAD) begins as a silent, unseen threat which, if untreated, can eventually rob children of their eye sight and their lives. The macula at the back of the eye is a tiny area about 5 mm in diameter, with the fovea at its centre. There are no blood vessels, but lots of cells full of photosensitive pigments that allow us to see details in the centre of vision. With the process of aging, the cells with retinal pigment become less efficient, the membrane degenerates, some cells atrophy and waste products build up and central vision is gradually lost which is age-related macular degeneration (AMD). Lutein and zeaxanthin has been shown to have beneficial effects in combating AMD.

While there is ample data on the carotenoids content in plant foods (Table 32) data on carotenoids, bioavailability is still limited. Intestinal absorption is a major determinant of bioavailability. Carotenoids are hydrophobic molecules, whose absorption pathway closely follows that of lipids. Numerous factors can interfere at different levels of this pathway, including competition between various lipid-soluble compounds or matrix effects. Interactions between carotenoids during intestinal absorption have been investigated in several studies. The available evidence in humans concerns only the interactions between purified or synthetic carotenoids: β -carotene with lutein, lycopene, or canthaxanthin. Although the picture is not always consistent, it appears that providing various purified or synthetic carotenoids in the same meal results in modifications in the postprandial response of the carotenoids. Mechanisms potentially involved include competition for incorporation into lipid droplets and then into mixed micelles, resulting in competition for intestinal absorption. Macronutrient deficiencies and micronutrient deficiencies are contributing serious health problems in under developing and developing countries. Recent report of NNMB Technical Report 23 revealed that about 62% of Indian children have less than 20 $\mu\text{g}/\text{dL}$ of Vitamin A in blood indicating sub clinical VAD.

AIMS AND OBJECTIVES

1. To provide database for carotenes and xanthophylls content of commonly consumed Indian foods including tribal foods.
2. To evaluate processing losses due to various storage and cooking methods.

3. To evaluate bioavailability of carotenes, xanthophylls in commonly consumed green leafy vegetables.
4. To develop carotenoids rich recipes for disease prevention.

Stability of β -carotene in leaf nutrient concentrate

Green leafy vegetables are excellent sources of micronutrients and to some extent macro nutrients. Recommended dietary allowances for various micronutrients are unable to meet the requirements of our children and geriatric population. To meet these nutrients, one has to consume large quantities of these green leafy vegetables. Gastric capacity is a limiting factor for children, there must be a viable, sustainable alternative to overcome this problem. A nutrient dense product i.e. leaf nutrient concentrate to augment the prevalent problems related to micronutrient deficiencies were proposed in India. One kg green leafy vegetable was taken and made into leaf juice using a fruit grinder (Fig. 58). Leaf juice was coagulated by heat treatment at boiling temperature and precipitate was filtered through muslin cloth. This dense leaf nutrient product can be used in many recipes for better acceptability and palatability. Study revealed that commonly used curry leaves in Indian culinary is one of the GLV which can be prepared for leaf nutrient concentrate and can be made into recipes. The other two GLVs, Drum stick leaves and fenugreek leaves can also be used both fresh and dried form in many recipes. The study indicated that solar cabinet drying was better than shade drying for retention of carotenoids in these leaf nutrient concentrates. Storage study revealed that these dried leafy vegetable nutrient concentrate can be stored at room temperature and can be used during off-season in various recipes for providing vitamin-A precursors.

This leaf nutrient concentration (LNC) can be further developed by using a biotechnology approach, like LNC yoghurt. LNC can also dried and can be developed as supplementary products for children to combat vitamin A deficiency and complementary product in aged population for age related macular degenerative diseases (Fig. 59).

Table 32 Carotenoids content ($\mu\text{g}/100\text{g}$) of some medicinal plants

No	Name of the plants	α -Carotene	β -Carotene	Lutein	Lycopene
1	Commiphora caudate(leaf)	4166.28	-----	81.63	21.45
2	Syzgium alterifoloym (leaf)	1476.24	-----	-----	-----
3	Strichros potatorum (fruit)	907.79	-----	-----	-----
4	Boswellia serrata (leaf)	11674.16	-----	-----	112.96
5	Boswellia serrata (stem bark)	-----	-----	-----	-----
6	Boswellia ovalifolalta(leaf)	12580.17	-----	47.59	-----
7	Boswellia ovalifolalta (stem bark)	6100.78	-----	-----	-----
8	Altanita monophylla (fruit)	-----	0.0598	-----	-----
9	Rhychosia beddo mil (leaf)	3159.23	-----	-----	-----
10	Curcuma neiligherrisis (leaf)	9900.01	-----	2520.83	-----
11	Boswellia serrata (gum)	1225.94	-----	-----	-----

Fig. 58 Quantitative separation of leaves and their processed products from 1 kg of fresh leafy vegetables

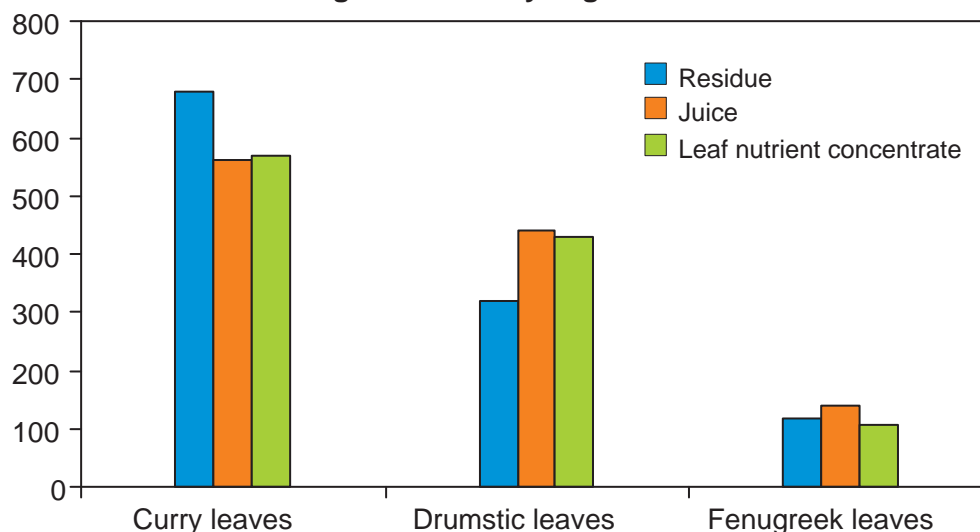
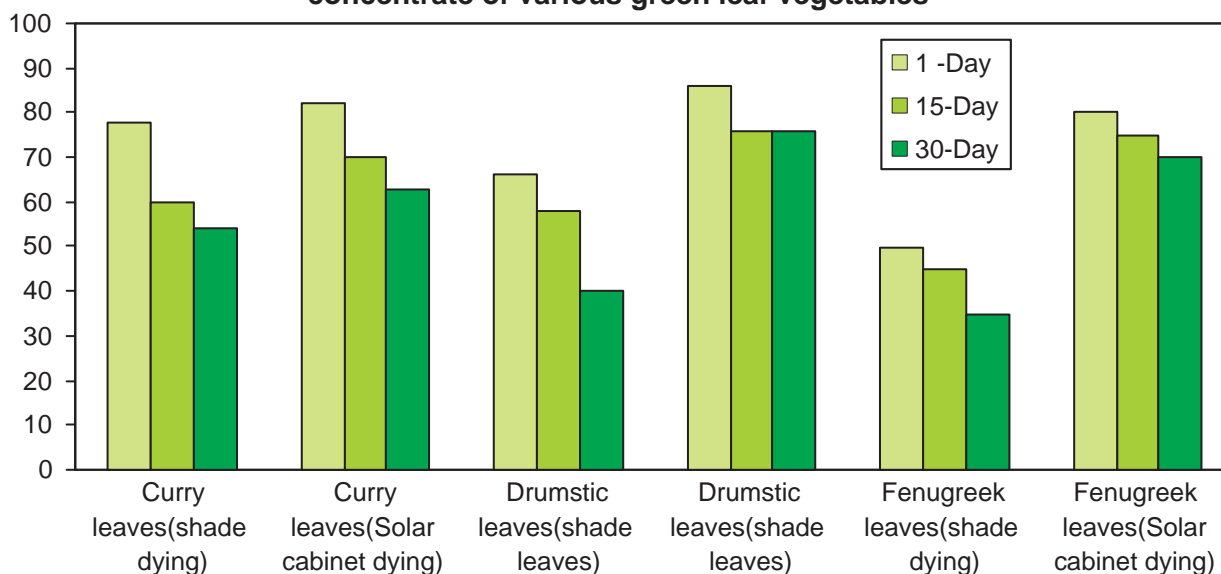


Fig.59 Effect of drying and storage on β -Carotene retention (%) in leaf nutrient concentrate of various green leaf vegetables



The *in vitro* method simulates digestion in the gastrointestinal tract and gives an estimation of maximum amount of carotenoids released from the food matrix, at optimal physiological and dietary conditions, it would be possible to absorb in to the human mucosa. Although transport and metabolic mechanisms cannot be effectively produced, studies can provide a simple predictive instrument to investigate the potential bioavailability of dietary carotenoids by assessing their stability under conditions mimicking the GI tract. In addition, these studies allow for the screening and comparison of multiple samples, thus providing information about the influence of different food matrices on the recovery of individual carotenoids. Thus, this *in vitro* method for bioaccessibility of β -carotene from leafy vegetables and vegetables can be standardized and can used for routine screening of various foods. There is a need to standardize the *in vitro* method for bioaccessibility for other important carotenes and xanthophylls, which are under progress.

VII. EXTENSION AND 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 Diet and Diabetes, Some Therapeutic Diets and Nutritive Value of Indian Foods.

2. TRAINING PROGRAMMES

Regular Training Programmes:

This year, a total of thirty two candidates have attended the regular training programmes of the Institute viz.

- (I) MSc (Applied Nutrition) - 16 participants
- (ii) Post-Graduate Certificate Course in Nutrition - 10 participants
- (iii) Annual Training Course in Endocrinological Techniques - 6 participants



3. EXTENSION ACTIVITIES

3.1 Exhibitions

- ❖ An exhibition was organized on Nutrition and health by displaying posters in Bharat Olympics organized for physically challenged sports persons at Ramachandra Puram, BHEL, Hyderabad. (24th -26th Sept. 2009).
- ❖ Organised a Health and Nutrition camp and exhibition in the National Camp on Roller Skating and Health Promotion for intellectually disabled, organized by Special Olympic Bharat, AP Chapter (17th -19th September 2009). Over 300 mentally challenged adolescent and young-adult sports persons, their coaches and care takers took part. Health camp included taking their anthropometric measures, measuring blood glucose, administering food frequency questionnaires and nutrition awareness included organizing lectures by NIN scientists, diet counseling and putting up an exhibition of posters.
- ❖ Participated in the Public Health Awareness Programme on Nutrition organized by Health Unit of South Central Railway and put up Nutrition poster exhibition for the benefit of railway employees and their families. Over 500 of them witnessed the exhibition (26th Nov. 2009).
- ❖ An exhibition on nutrition and health was organized in association with GMR Varalakshmi Centre for Empowerment and Livelihood, Shamshabad, Hyderabad for anganwadi workers (16th Dec. 2009).
- ❖ Participated in the 15th Agriculture-Industry-Tourism & Science Festival and Health Fair, organized by Contai Palpara Saradadevi Mahila Mondal, Kolkata, West Bengal and displayed different posters related to nutrition and health in the exhibition at Baruipur Village (23rd -30th Jan. 2010).

3.2 Popular Lectures/Awareness Camps

- ❖ A lecture was delivered on “Body Mass Index and Dietary Guidelines for Healthy Eating” at a Training Programme for NTPC employees on health conducted by NTPC, Hyderabad (15th May 2009).
- ❖ Lectures on “Balanced diet and improving dietary pattern among pregnant and lactating mothers” and “Significance of role of Nutrition among adolescent girls” were delivered in an induction training programme to the participants of Eye Sight Universal at NIN (20th May 2009).
- ❖ A lecture was given on “Balanced Diet and Dietary guidelines to Healthy eating” in a training programme for accountants in Southern Railways, Hyderabad (3rd July 2009).
- ❖ Delivered two lectures for two different batches on Nutrition and Health for security personnel working with Chief Minister's security wing, Hyderabad. (8th Sept. 2009).
- ❖ Participated in a training programme organized by Food & Nutrition Board, Hyderabad on nutrition and health education organized for CDPO's, ACDPO's at Nalgonda District. About 50 CDPO's/ ACDPO's and health workers participated in the programme (15th Sept. 09).
- ❖ About 7 extension lectures were given to different batches of police personnel on Nutrition and Health on different dates. In each batch about 50 police personnel belonging to different cadres including Constables, Head Constables, Sub-Inspectors, Inspectors, and DSPs participated in the programme. (28th & 29th August, 12th & 13th, 18th, 19th, 22nd Sept, October, 6th & 9th November, 26th December 2009).
- ❖ Delivered a popular lecture to the housewives of Air Force personnel in a Workshop on Nutrition organized by Air Force Wives Welfare Association (AFWWA), Hyderabad. About 150 house wives of the Air Force personnel participated in the programme. (12th Oct. 2009).
- ❖ Delivered a lecture on “Importance of nutrition” to the students of Railway Mixed High School, South Lallaguda, Hyderabad. About 100 school children of adolescent age participated in the programme. (15th Oct. 09).
- ❖ A talk was given on “Early childhood Nutrition” for teachers and parents at Global Indian International School, Hyderabad (14th Nov. 2009).
- ❖ A talk was given on “Women's Nutrition” in the International Day for the “Elimination of Violence against women”, organized by Railway women employees association and South Central Railway Employees Sangh at Secunderabad. (25th Nov. 2009).
- ❖ Delivered a lecture to anganwadi workers of the villages in and around Shamshabad village on nutrition and health awareness organized by GMR Varalakshmi centre for empowerment and livelihood, Shamshabad, Hyderabad. About 30 anganwadi workers participated in the programme (22nd Jan. 2010).
- ❖ Delivered a popular lecture on nutrition and health to the villagers during 15th Agriculture-Industry-Tourism & Science Festival and Health Fair, at Purba Mednipur district, West Bengal. (23rd-30th Jan. 2010)
- ❖ A Talk on “Dietary guidelines for Healthy Life” was given to the officers and employees of Punjab National Bank circle office Hyderabad (25th Feb. 2010).

- ❖ A Talk on “Tips for healthy eating for aged people” was given in a “Course on Retiring officers who attained 58 years of Age” organized by National Academy of Customs, Excise & Narcotics, Regional Training Institute, Hyderabad (3rd March, 2010).
- ❖ Two extension lectures were given to the villagers of Venkatapuram village, Turkapalli Mandal, Nalgonda district in a village welfare programme organized by Sri Satya Sai Seva Samithi, Hyderabad. On each occasion, about 100 villagers including children, adolescents, pregnant and lactating women and geriatric population participated in the programme (3rd March 2010).
- ❖ Delivered a lecture on the importance of nutrition and physical activity in geriatric population at a Senior Citizens Welfare Forum, Bagh Amberpet, Hyderabad. About 40 senior citizens including women participated in the programme. (7th March 2010)

3.3 Popular articles in print media

The following articles were published in the print media during the year:

- ❖ Alternative pulses in place of red gram dal titled “Vanda Pappula Kanna Chouka Pappule Minna” in EENADU, Telugu daily, (12th August, 2009)
- ❖ “Low cost nutritious pulses” in EENADU, Telugu daily (4th Oct. 2009)
- ❖ “NIN Seeks tips on nutrition” in Chennai Edition of Deccan Chronicle (January, 2010)
- ❖ ” 3 Vs 6 Meals” English daily news paper Times of India (2nd February, 2010)
- ❖ Diet during examinations titled “Parikshalu.. Parikshalu“ about nutrition was published in Health section of Andhra Jyothi, Telugu daily news paper (3rd February, 2010)
- ❖ “Good eating habits” in EENADU, Telugu daily, Hyderabad edition (March 2010)
- ❖ Fast food titled ”Fast food tho fat muppu“ in the health section of Telugu daily news paper Saakshi (1st March 2010)
- ❖ “Change cooking oils often, says NIN” in Chennai Edition of Deccan Chronicle (9th March 2010)

4. SPECIAL EVENTS

National Nutrition Week Celebrations (1-7 Sep.2009)

- ❖ In connection with the National Nutrition Week theme “Good Nutrition – Foundation for Healthy Life”, organized a nutrition awareness programme for the school children of Railway School, Hyderabad. About 100 students participated in the programme (Sept. 2, 2009).
- ❖ An elocution competition was conducted on this year's theme “Good Nutrition – Foundation for Healthy Life”, for school children. About 30 students participated and trophies and certificates were given away.

5. DIET COUNSELING

Diet Counselling Center was established at NIN during the Nutrition Week in September 2006. Since then, the counselling center has been functioning regularly. Counselling was given three days in a week i.e. Tuesday to Thursday from 3.30p.m. to 5.30p.m. As part of center's activity,

counselling was given regularly to the people on appointment and so far diet counselling was given to 200 members, of which majority of them came to seek counselling for diabetes, reduction of weight and various other health issues.

6. ACTIVITIES OF SECRETARIAT FOR WHO SOUTHEAST ASIA (WHOSEA) NUTRITION RESEARCH-CUM-ACTION NETWORK

The Institute continues to be the Secretariat for the WHOSEA Nutrition Research-cum-Action Network. This year, the status of the Institute as 'WHO collaborating centre in nutrition and primary health care' has been renewed for a period of four years i.e. 2010-2014. In addition, the Institute has signed the Agreement of Performance of Work (APW) with WHO SEA Regional Office, New Delhi for bringing out the Newsletter of the Network.

B. RESEARCH ACTIVITIES

ASSESSMENT OF KNOWLEDGE, FOOD PREFERENCES AND PRACTICES AMONG URBAN SLUM ADOLESCENT GIRLS

Adolescent phase is a transitional and critical phase of life between childhood and adulthood. During this period, rapid physical growth and development takes place and this is characterized by physiological and emotional changes. Food and nutrition play a very important role during this period. Adopting healthy lifestyles and following dietary guidelines are essential for the adolescent population to ensure optimal growth and development. During this phase, there is a demand for adequate nutrient requirement for meeting the requirement of body and bone growth. Adolescent girls are the “future mothers”, hence creation of awareness about good nutrition among these girls is the vital and important activity of the nutrition extension. Maternal nutrition plays an important role in the development of foetus and the outcome of pregnancy. Poor nutritional status and lack of awareness of the nutrition during pregnancy and lactation among the low body weight expectant mothers in urban slums result in delivering low birth weight babies and other complications during delivery. Looking at the background of the poor socio-economic status of people living in urban slums of Hyderabad, it is expected that the children too will lack proper knowledge about nutrition and family life unless educated properly. Lack of nutrition and family life education is one of the main reasons behind the high morbidity and mortality rate in pregnant and lactating women and children due to diseases which are preventable in these urban backward areas.

Therefore, a study was carried out among the school going adolescent girls to find out their nutrition knowledge related to pregnancy, health and nutrition, lactation and family life education. The study was carried out in two schools of Hyderabad (Uppal and Malkajgiri areas) with the following objectives:

- ▲ To assess the health and nutrition knowledge of adolescent girls (10-18 yrs.) during pregnancy and lactation.
- ▲ To find out the knowledge levels of the adolescent girls between the age of 10-18 yrs related to health and nutrition during pregnancy and lactation.
- ▲ To develop information education and communication (IEC) material on maternal health and nutrition.
- ▲ To carry out nutrition education intervention with the IEC materials developed.

- ▲ To study the impact of nutrition education on adolescent girls vis-a-vis knowledge pertaining to health and nutrition.

MATERIALS AND METHODS

The study was carried out in two Government Schools of Hyderabad. Selection of the schools for this study was done based on matching the students for demographic profile and also other socioeconomic factors. Demographic details of the students such as age, gender, household income, occupation of their parents were recorded. A quota sampling method was used for this study. A sample of 186 students/each from both the schools were recruited for the cohort study. Based on the preliminary discussion with the students, a structured interview schedule was developed and pre-tested among the small group of the students. The pre-tested interview schedule has 82 questions comprising open and closed ended questions and it was administered to the students to assess their nutrition knowledge levels. The 82 open and closed ended questions covered main themes viz., 1) Adolescent phase, 2) Breast feeding and complimentary feeding concepts, 3) Food groups and balanced diet, 4) Vitamin A, 5) Anaemia and folates, 6) Iodine deficiency disorders, 7) Family Life Education, 8) Health and Nutrition Education (IEC). After analysis of the base line data collected from the schools of intervention and control on above themes, a set of 6 folders covering above themes were developed and pre-tested. The pre-tested IEC materials and a vernacular folk artform audio visuals covering all the themes for intervention were used in the school for educating the adolescent girls. Post intervention data were obtained from both the schools and the data were statistically analyzed and compared to assess the efficacy of the IEC material. After completion of the study, nutrition education was also given to the adolescents girls of the control group. χ^2 test was used to study the association between control and intervention schools.

RESULTS

The base line data of the both Intervention and control group were similar and matched as there was no significant difference in the knowledge levels of the adolescent girls. Intervention with IEC material developed on 6 themes (Fig. 1) was carried out in intervention school and the results of the main themes tested are as follows. The results of these themes are given in Table 33.

Demographic details of the adolescent girls

The age of adolescent girls ranged from 11 to 16 years with the mean being 12.813 years and nearly 70% of the girls were in the age group between 12-13 years. Most of the girls had no knowledge about their height (98%) and weight (89%). About 64% of the mothers were illiterate and rest of them were educated. Around 35% of the girls' mothers were housewives and rest of the 65% were labourers, maids and were involved in other menial jobs to earn their livelihood. Around 58% of the girls' fathers were illiterate and rest of them had primary and higher secondary education. Similarly, 66% of the girls's fathers were involved in construction work, labour work or painting and rest of them were serving as watchmen and cab drivers. The average total family income was ₹2500-3000 per month. From the occupation and income it can be concluded that the study subjects belonged to lower socio-economic class.

1) Adolescent Phase

Analysis of the baseline data of control group and intervention group indicated that about 66% and 70% the children indicated the correct age group (10-18yrs) of adolescent phase respectively. Intervention with the folders significantly improved ($P < 0.01$) the knowledge levels of these girls increased their knowledge to 92% in interventionschool as against no significant improvement was observed between the baseline and endline. As regards the physical and physiological changes such as height, weight and other changes, about 54% of children at the base line indicated that they

were aware of these changes and the awareness was increased to 66% after the intervention (Table 33). With regard to the importance of physiological changes that occur during adolescent phase, intervention with the educational material significantly improved the knowledge levels among the children from 72% to 89% in the intervention school. In the control group where intervention was not carried out, there was no improvement as indicated by no significant difference in knowledge levels between the baseline and endline, indicating impact of intervention with the IEC material in the experimental group. Calcium requirement is very high during this period and at the base line 50% girls indicated its importance in the bone development as the peak bone mass takes place during this period. A significant increase ($P < 0.01$) in the knowledge levels adolescent girls (82%) on the calcium requirement was observed in post intervention data.

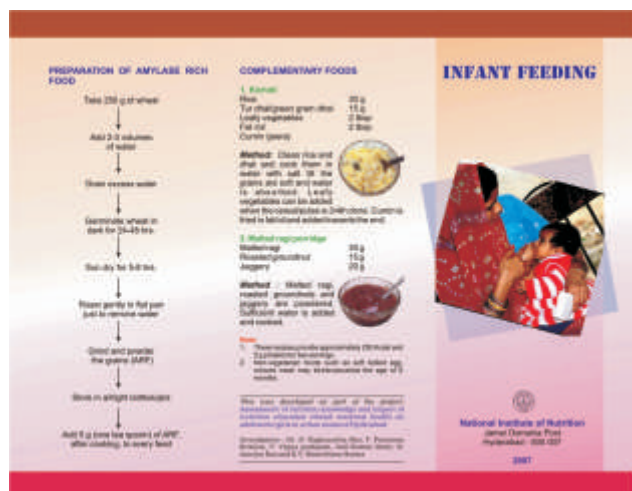


Most of the adolescent girls (84%) were aware of the importance of nutrition as it was indicated at the baseline and this was increased to 94% at the endline. As the peak bone mass formation occurs during the adolescent phase about 67% of the girls indicated the importance of calcium during this phase at the base line as against 90% in the post intervention group.

2) Breast Feeding and Complementary Feeding

Mother's milk contains all the essential nutrients including carbohydrates, proteins and micronutrients such as iron, vitamin A etc. Hence, it is often considered as wholesome food for the infants. Details about breast-feeding, complementary feeding, importance of green leafy vegetables (GLVs), fruits, amylase-rich foods (ARFs) and their preparation were more emphasized during the intervention. As regards the exclusive breast feeding practices for the first 6 months, about 77% expressed their awareness at the baseline and this was increased to 94% in the post intervention. All the improvements in the knowledge levels were statistically significant ($P < 0.01$). Although, 95% of the adolescent girls indicated that the breast feeding for infants is important to the growth of the infant, only 44% of these girls at the baseline indicated the presence of colostrum in the mothers' milk immediately after the delivery of the baby. About 16% of the adolescent girls indicated the presence of disease fighting factors i.e., maternal antibodies in the colostrum at the base line. After intervention, there was a significant improvement in the knowledge levels as 77% the adolescent girls indicated the importance of colostrum. Also, 30% of these girls indicated the presence of maternal antibodies in the post intervention as against 16% of girls before the intervention.

Regarding knowledge levels of complementary feeding, 36% children reported that complementary feeding should be initiated after 6 months at the baseline and there was significant increment (59.2%; $P < 0.01$) in the



knowledge levels of the adolescent girls after intervention in the Malkajgiri school.

3) Food Groups and Balanced Diet

Rapid physical growth demands more energy and nutrients. In order to follow dietary guidelines, it is important for the adolescent girls to know about the classification of foods and the functions they perform. Foods are classified in to three groups based on the functions they perform. They are energy yielding foods, body building foods, protective foods. A detailed emphasis was laid on the macronutrients, micronutrients and balanced diet during the intervention. About 38% adolescent girls indicated the important groups of foods and the classification of foods at the base line and there was a significant increment (P < 0.01) in the knowledge levels of food groups after the intervention as 61% of the girls indicated the right answer. Energy yielding foods include cereals, millets, oils and fats, Body building Foods include pulses, animal foods etc., and Protective foods include vegetables and fruits. With regard to energy yielding foods 5% of the adolescent girls were aware of energy yielding foods at the baseline and after intervention there was a significant improvement in the knowledge levels (P<0.01) as 23% of the adolescent girls gave correct answer. With regard to the sources of energy, at the base line 49% of the children indicated various food items like rice, wheat and millets viz. foxtail millets, finger millets etc. After intervention there is an increment of 86% in the knowledge level of these adolescent girls. Similarly when the adolescent girls were asked about the nutrients responsible for growth 46% answered that they were aware of the nutrients at the baseline and after intervention it raised to 71% of the adolescent girls. At the base line, with regard to the knowledge about protein rich foods about 36% of the adolescent girls indicated the pulses, egg, milk and meat as protein rich foods and after intervention about 52% of the girls indicated the same. About 63% of the adolescent girls indicated the importance of fat in the diet and in the endline after intervention 83% of the girls indicated the right answer. Oils and Fats which are included under energy yielding foods plays a very important role in the balanced diet. Of, these girls 21% of the girls at the baseline had mentioned that the fats are important for the growth and also they contain essential fatty acids (EFAs) and at the endline after the intervention there was a significant improvement in the knowledge levels of 54%.

Similarly when the adolescent girls were asked about the nutrient responsible for growth and development, 46% of the adolescent girls answered that proteins are responsible for the growth and development. Intervention improved the knowledge among the adolescent girls and about 71% answered correct answer in the post intervention. As regards the knowledge levels of the protective foods there was a significant improvement (P<0.01) observed between the baseline and endline.

4) Micronutrients

In developing countries, vitamin A

FUNCTIONS OF FOODS

Protective foods provide vitamins and minerals, which are essential for the upkeep of our health.

- Green leafy vegetables, (carrot, papaya and ripe mangoes) and nuts are rich in beta carotene (Vitamin A) and fibre.
- Fruits contain most of the essential micro-nutrients.

Balanced Diet

- Inclusion of all the foods belonging to the above mentioned three groups in proper proportions is referred to as balanced diet.
- About sixty percent of the energy should be derived from carbohydrates, 10-15% of energy should be drawn from proteins, and 20-25% energy should be derived from fats and oils.

PROTEIN RICH FOODS

Soya beans	43.2
Groundnut, Cashewnut	26.0
Fish	20.0
Mutton	21.0
Milk	4.3
Egg	13.3

This was developed as part of the project on nutrition education and health promotion for adolescent girls in urban slums of Hyderabad.

Developed by: Dr. D. Rajagopal Reddy, Dr. Prasad Kumar, Dr. T. Vijaya Prakash, All India Institute of Medical Sciences, Hyderabad.

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MICRONUTRIENTS

Calcium

- Adolescence leads to rapid bone growth.
- Calcium deficiency in adolescents leads to:
 - Fracture disorders
 - Impaired bone growth
 - Poor mental development
 - Delayed menstruation
 - Osteoporosis
- Calcium is the substance that we need to build strong bones. The body needs calcium to build strong bones.
- Look for the logo **calcium star** on calcium products.
- The skeleton accounts for at least 99% of the body's calcium.
- See a doctor if you have lost weight or feel tired and weak.
- About 45% of the adult skeleton mass is formed during adolescence.
- Requirement of calcium for the growth of the skeleton is highest during adolescence.
- More requirement and rapid deposition of Calcium occurs during early adolescence, between 10 and 14 years in girls and 12 and 18 years in boys.
- During peak adolescent growth, calcium intake is 1.5-2.0 g/day, about 300mg/day in girls and 300 mg/day in boys.

Iron

- Adolescence of peak bone mass during childhood and adolescence is critical to reduce the risk of osteoporosis in later years.
- Recommended calcium intake is possible by regular intake of smaller servings of dairy products such as milk, curd and cheese.
- Protein intake, fish and green leafy vegetables are good sources of calcium.
- Physical activity in the morning and exposure to morning sun which provides vitamin D is essential for building and repair bone strength in children.
- Dairy intake of calcium, other sources of calcium, like vitamin D and phosphorus are needed for building of bones.
- Strong respiratory muscles need extra amount of calcium for their healthy growth.
- Some calcium rich foods

This was developed as part of the project on nutrition education and health promotion for adolescent girls in urban slums of Hyderabad.

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deficiency, iron deficiency anaemia (IDA) and iodine deficiency disorders (IDD) are significant public health problems. It is particularly prevalent among pre-school children, adolescent girls and pregnant and lactating women. As per the recent National Family Health Survey-3 (NFHS-3), about 70% infants between 6 months and 3 years of age and 75% of women are anaemic. About 50% of pregnant women continue to be anaemic. As compared to the earlier survey, carried out 5 years ago, the proportion of anaemia in fact had marginally increased today. Hence, causes of anaemia, its consequences, symptoms and prevention are clearly explained. Also, folic acid, green leafy vegetables and cooking methods were also emphasized during the intervention. With regard to different aspects of vitamin A there was significant improvement in the knowledge levels in intervention school at the endline as compared to the control school. As regards the vitamin A rich foods at the baseline 38% of adolescent girls indicated that they were aware of these foods like GLVs, yellow coloured fruits. After intervention there was an increment of knowledge levels to 84% among these girls.

5) Anaemia and Folates

In developing countries, IDA is a significant public health problem. It is particularly prevalent among pre-school children, adolescent girls and pregnant and lactating women. Recent survey conducted by the Family and Health department indicated that about 75% infants between 6 months and 3 years of age are anaemic. In the intervention school, about 56% of the children indicated that they were aware of anaemia and after intervention there was knowledge increment among 91% of children. Similarly, there was knowledge increment in the intervention school on the causes and prevention of anaemia. As regards the folate rich foods about 28% of the children indicated the awareness and after intervention the knowledge increment had gone up 43%.

Iron and Folate Rich Foods and their nutrient values

Iron rich green leafy vegetables (mg/100g)

Amaranth (purslane)	27.0
Amaranth (spinach)	22.0
Amaranth (methun)	18.0
Amaranth (chilo)	18.0
Amaranth (chilo)	18.0
Palak (spinach)	16.0

Folate rich foods (µg/100g)

Chilly leaves	50.0
Wet leaves	37.0
Spinach	33.0
Amaranth (purslane)	14.0
Cherry leaves	14.0
Leafy (spinach)	100.0
Parasprout seeds	80.0
Egg (hard)	70.0
Leaf (chard)	170.0
Leaf (chard)	100.0
Wet (spinach)	8.0
Spinach (chard)	11.0

ANAEMIA AND FOLATES

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6) Family Life Education

It is necessary to impart 'Family Life Education' to adolescent girls as most of them get ready to enter into a married life. They comprise a major portion (about one-fifths) of country's population. Important concepts like puberty, STD and HIV education, avoiding early marriages, other hygienic issues were emphasized. Most of the adolescent girls were aware of the age at which girls attain menarche and there was no difference between the baseline and endline. When the girls were asked about AIDS, 63% of the children expressed that they were aware of AIDS and 50% of the children indicated the mode of transmission. After intervention awareness about AIDS and mode of transmission was increased to 95% and 92% respectively.

FAMILY LIFE EDUCATION FOR ADOLESCENT GIRLS

Use of sanitary toiletries.

Appropriate contraceptive methods including condoms use help plan the family and protect the health of women.

Mothers and other health educators should educate adolescent girls on sex and sexuality.

4. OTHER PREVENTIVE METHODS

- Use of sanitary napkins during menstruation.
- Consult medical doctor even for small problems like white discharge.
- Reproductive tract infection can disturb fetal growth during pregnancy.
- Maintenance of personal hygiene like defecation may be encouraged.

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Table 33. Assessment of knowledge and food preferences, practices among urban slum adolescent girls

SNo	Parameter	CONTROL		EXPERIMENTAL	
		Uppal		Malkajigiri	
		Base line	Final	Base line	Final
1.	Adolescent phase				
	Adolescent phase	75.2	70.6 (NS)	75.0	95.1**
	Adolescent age (10-18 yrs)	66.4	64.0 (NS)	69.5	91.5**
	Physiological changes during adolescent phase	68.1	58.8 (NS)	71.9	89.4**
	Type of physiological changes (Height, weight and facial changes)	9.4	8.1 (NS)	53.9	66.2**
2	Importance of nutrition				
	Requirement of nutrients	84.0	77.2 (NS)	84.4	93.7*
	Calcium requirement	55.0	51.5 (NS)	67.2	90.1**
	Importance of calcium for bone development	27.7	22.1 (NS)	50.0	82.4**
	Knowledge about calcium diet food Milk & milk products, egg, meat	12.7	11.8 (NS)	22.7	35.0**
3	Breastfeeding and complementary feeding				
	Exclusive breast feeding (6 months)	74.6	69.1(NS)	76.6	93.7**
	Importance of colostrum	26.1	14.7*	44.5	76.8**
	Complimentary feeding (after 6 months)	22.5	25.0	35.9	59.2**
4	Food groups and balanced diet				
	Food group classification	13.4	5.1*	46.9	85.2*
	Knowledge about foods (Energy yielding foods)	10.1	2.2	4.7	22.5**
	Sources of energy	63.5	63.2 (NS)	49.2	85.9**
	Protein rich food	21.8	12.5 (NS)	39.1	49.3**
	Knowledge about protein rich foods Pulses, egg, milk, meat	12.7	14.0	35.9	52.1*
	Vitamin and mineral rich food	33.9	31.6*	63.3	84.5**
	Balanced diet	15.0	5.9**	46.9	79.6**
	Define balanced diet (Meat, GLV, rice, vegetable, dal)	2.6	1.4	1.6	31.7**
5	Knowledge about Vitamin A				
	Knowledge about night blindness	71.7*	60.3*	77.3	90.8**
	Causes of night blindness Deficiency of vitamin A	36.8	22.1**	44.5	73.9**
	Symptoms of vitamin A deficiency Bitot spots	9.4	11.0	43.8	68.3**
	Prevention of vitamin A deficiency Consulting doctor	30.6	24.3	9.4	16.2**
	Dietary modification of vitamin A	40.1	25.0**	50.0	83.8**
	Vitamin A rich foods (GLV, Yellow coloured fruits)	54.1	47.8	37.5	83.8**

SNo	Parameter	CONTROL		EXPERIMENTAL	
		Uppal		Malkajigiri	
		Base line	Final	Base line	Final
6	Anaemia and folates				
	Symptoms of anaemia	39.7	21.5*	55.5	88.0**
	Causes for anaemia Deficiency of iron	4.6	5.1	36.7	64.1**
	Importance of folates	4.6	3.7 (NS)	17.2	33.8**
	Symptoms of anaemia	19.2	11.8*	46.9	70.4**
	Prevention of anaemia Take iron tablets	6.2	3.7	37.5	76.8**
	Knowledge about iron rich food items (GLV)	14.3	11.8	34.4	46.5**
	Importance of folates	19.9	14.0**	46.1	78.9**
	Folate rich foods (GLV)	5.5	0.7	28.1	43.0*
7	Family life education				
	Menarch age what	73.6	66.2**	80.5	82.4 (NS)
	Right age – marriage 18<	87.0	83.1 (NS)	85.2	85.9*
	AIDS – Do you know	53.4	35.3**	63.3	95.1**
	Mode of transmission of AIDS	36.2	19.9**	50.0	92.3*

NS-Not significant; *P<0.05; **P<0.01

CONCLUSION

The IEC material which was developed for adolescent girls on different focal themes significantly improved the knowledge levels among the adolescent girls. The material can serve as a resource material for the trainers to train the front line health functionaries who are involved in health education.



VIII. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

1. TOTAL DIET STUDY - ANDHRA PRADESH

Diet is a source of toxicants as well as nutrients. Human exposure to toxic elements and nutritional imbalances are known and may be a basis for promoting or causing diseases like cancer, kidney and liver dysfunction, hormonal imbalance, immune system suppression, birth defects, old age dementia and learning disabilities. Most of these conditions can be attributed to past and current exposure to chemicals in the foods. Ensuring the availability of safe food is one of the essential public health functions of any country. It is impossible to totally eliminate contaminants in food supply which passes through various stages in food chain. However, it is possible to compare their levels present in food in the manner they are consumed with their corresponding toxicological reference intakes, such as the acceptable daily intake (ADI) or provisional tolerable weekly intake (PTWI). This is the purpose of conducting total diet study.

OBJECTIVES

To analyze the most commonly consumed foods in Andhra Pradesh for the following selected contaminants after processing as for consumption.

Mycotoxins	: Aflatoxins B1 and M1, Fumonisin B1, T2 toxin
Pesticide Residues	: DDT, BHC, Aldrin, Dieldrin, Endosulfan, Chlorpyrifos, Cypermethrin
Toxic Metals	: Lead and Cadmium
Others	: Fluoride

RESULTS

Twenty two types of foods belonging to eleven food categories were selected for the study. The choice was made on the basis of most commonly consumed foods in Andhra Pradesh as per National Nutrition Monitoring Bureau 2004-06. The sampling design was stratified random sampling design to cover the entire state of Andhra Pradesh.

The objective of the survey was to collect four samples of each food from each district. However, depending on the availability, the number varied. Thus, a total of 503 samples were taken up for analysis of contaminants. Since water is a component of food, water samples were also collected. The contaminants namely fluoride, heavy metals (lead, cadmium), mycotoxins (aflatoxins B1, fumonisin B1, aflatoxin M1 and T2 toxin) and pesticides were analysed in food samples that were high risk for the presence of a particular contaminant. Prior to analysis, the food samples were processed as they are consumed in a "table ready" state.

The dietary intake of each food item for each age group was taken from NNMB dietary intake data (*NNMB 2006*). The estimated dietary intakes for contaminants were calculated for a range of age groups, sedentary man and pregnant women. These estimated values were compared to the ADI or PTWI/PTDI. Fluoride was estimated in water, sorghum, rice, red gram dal and spinach. The estimated levels in food composite were within the safe limits for any of the age groups or category of individuals. The highest contributor among the food items tested appeared to be water. For pesticide analysis QuEChERS method was followed. It is an emerging sample preparation

technique in the area of multi residue pesticide analysis in the food and agricultural produces. Twelve food items were taken up for analysis. A total of 19 residues were detected by GC equipped with electron capture detector (ECD). Representative samples were analysed by GC-MS for characterization and confirmation. The selection of these pesticides was made on their reported occurrence commonly in foods and from Food and Drug Administration - Total Diet Study (FDA - TDS). None of the samples were free from pesticides contamination. All the samples including water had one or the other of the 19 pesticides analyzed, aldrin and 4, 4, DDT were detected in almost all food samples. Among the eight physiological groups, children of 7-9 years and 10-12 years were more at risk to Aldrin due to high intake of milk and rice.

Mycotoxins, resulting from exposure to fungal toxins, present in certain foods continue to be a major food safety concern world-wide. The reasons are poor post-harvest technology management, like improper storage, transport, unhygienic conditions till retail consumption etc. The mycotoxins analysed were aflatoxin B1, fumonisin B1, aflatoxin M1 and T2 toxin. The samples that were analysed were jowar, groundnut oil, red chillies and milk. The results indicated that levels of mycotoxins analysed in selected food items were either below detectable level or were present at significantly low concentrations compared to permitted levels. Since all of them are carcinogens there are no safe limits. Toxic metals, namely lead and cadmium, were analyzed in all 22 selected food items and water. Among the food items, sorghum samples showed highest concentration of lead whereas, amaranth had highest levels of cadmium. Among all the food items, cereals were the maximum contributor for toxic metals consumption.

The above observations on fluoride, pesticides residues, mycotoxins and toxic metals were consistent for various sampling locations in Andhra Pradesh. The estimated dietary intake levels of contaminants reported in this study across in all age groups, sedentary workers and pregnant women were uniformly much lower than ADI or PTWI of a particular contaminant. At highest levels of food consumption, contaminants like aldrin, cadmium and lead were exceeding the safe limits. However, at higher levels of food consumption, the intake of nutrients, protective factors in diet as well as antioxidants levels would be greater which may confer higher level of protection against adverse effects arising from exposure to contaminants present in the diet.

2. DEVELOPMENT OF PCR AND RT-PCR BASED DIAGNOSTIC KITS FOR DETECTION AND SPECIES SPECIFIC IDENTIFICATION OF FOOD AND WATER BORNE PATHOGENS

Foodborne illnesses are due to presence of contaminants, which include biological, physical and chemical agents. Among the biological contaminants bacterial agents are most predominant. Conventional methods of culturing and identification are time consuming as well as sometimes less sensitive as is the case with resistant strains. Sensitive technique using generic DNA sequence analysis is accurate and quick. This technique will be applied to identify food and waterborne pathogens.

AIMS AND OBJECTIVES

To develop PCR and RT-PCR based diagnostic kit to detect food and water-borne bacteria involved in pathogenesis in humans by selecting several appropriate species indicating *E.coli*, *Vibrios*, *Shigella*, *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Campylobacter jejuni* and *Yersinia enterocolitica*.

RESULTS

The study was initiated by preparing the primers to *E.Coli*, *Vibrio cholera*, *Vibro parahaemolyticus*, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus* to develop PCR based uniplex detection method.

The primer sequence selected for the construct of primers was unique (Details not provided here as they need to be patented). The conditions of PCR have been standardized. The methods were then cross checked for their cross reactivity with the standard reference isolates obtained from NCIM, Pune and IMTECH, Chandigarh. After it was established that they were working to detect the specific pathogen, the market samples of food (4 different food matrices) were obtained to test newly developed methods for their specificity and sensitivity and compared with the conventional methods. The results of the validation are provided in table 34.

Table 34. Sensitivity and specificity of the uniplex PCR methods

	<i>S.aureus</i>	<i>Vibrio cholera</i>	<i>Salmonella spp</i>	<i>E.coli Spp.</i>
Sensitivity (Limit of detection)	4 cfu/g	2cfu/g	8cfu/g	5 Cfu/g
False positive	0	0	0	0
False negative	0	0.08%	0.1%	0

RT PCR method could not be developed due to non-availability of funding to collaborating center (Bio-Serve Biotechnologies).

3. BIOMARKERS FOR TRANSPLACENTAL GENOTOXIC EFFECTS AND THEIR CHEMOPREVENTION (INDO-BULGARIAN PROJECT)

a) Genotoxic effects of B(a)p exposure in tissues of turmeric fed rats (*In vivo*)

Diet is the most important source of chemopreventive agents acting upon human beings during their individual life. Turmeric, which is used in Indian culinary, is reported to have many medicinal properties. A lot of compounds most of them being from natural origin have already been assessed as inhibitors of chemical mutagenesis and carcinogenesis by employing different experimental systems.

Many of them including turmeric are thought to be safe enough to be applied to humans for long periods of time as shown by centuries-old traditional diet practices. This proposal is envisaged to evaluate *in vivo* genotoxic effects of BP exposure in tissues and the preventive role of turmeric feeding through diets.

Hypothesis

In vivo genotoxic effects of B(a)p exposure may be prevented by turmeric feeding through diet.

AIMS AND OBJECTIVES

To study the genotoxic effects of B(a)p exposure in tissues of turmeric fed rats (*In vivo*).

Work done during the year

Turmeric powder was fed through diet to WNIN rats at 1%, 3% and 5% for one month. At the end of the feeding period carcinogen B(a)p was injected (5 mg/animal) via i.p. route 0.5ml blood/rat was collected through orbital plexus. Rats were sacrificed by euthanasia and tissues such as liver, lung and kidney were collected. DNA damage in blood and tissues such as liver, lung and kidney was analysed by comet assay. Plasma MDA levels were estimated.

RESULTS

1. A significant difference of comet ratios was observed between the basal and B(a)P treated groups in blood. There was decrease in the extent of DNA damage in the turmeric fed groups compared to the B(a)P treated group (Table 35).

Table 35. Effect of turmeric on B(a)P induced DNA damage in rat blood

Treatment	Normal	B(a)P treated
Control	0.96±0.016	0.86±0.042
1%Turmeric	0.94±0.028	0.90±0.017
3%Turmeric	0.94±0.032	0.92±0.027
5%Turmeric	0.94±0.033	0.92±0.039

Values are mean±SD of 6rats/ group. Statistical significance between normal and B(a)P treated groups: p<0.05 by ANOVA

2. Reduction in the malondialdehyde (MDA) levels was observed in turmeric fed groups compared to control. Decrease in the MDA levels was also observed in turmeric fed groups treated with B(a)P compared to B(a)P control (Table 36).
3. Tail parameters were (Comet assay in tissues) calculated automatically using the Komet 5.5 image analysis software. Tail parameters used in this study were %DNA Tail(%DNA T), Olive Tail moment (OTM) and Tail length(TL) Statistical differences for %DNA T, OTM and TL between control and B(a)P treated groups were analysed by two-tailed test and with a significance level of p<0.05 was used to define the dose response relationship using SPSS 14.0 window version. In addition, for controls and individual treatment the correlation coefficients between %DNA T, OTM & TL were analysed statistically by Pearson's correlation coefficient.
4. B(a)p exhibited significantly higher mean levels of DNA damage in OTM & TL parameters in all the organs namely liver, kidney and intestine compared to the control group. A significant decrease in the comet length was observed

Table 36. MDA levels in turmeric (T) fed rats

Groups	MDA(rM)
Control	0.95* ± 0.065
Control +B(a)p	1.10* ± 0.101
1% T	0.87* ± 0.068
1% T+B(a)p	0.89* ± 0.108
3 % T	0.92* ± 0.095
3 % T + B(a)p	1.00* ± 0.089
5 % T.	0.87 ± 0.088
5 % T+B(a)p	0.93 ± 0.109

Values are mean±SD of 6 rats/ group. Mean differences are significant at p<0.05 analysed by one-way ANOVA compared to Control.

in B(a)P+ turmeric groups compared to their respective control. Dose response relationship in DNA repair was observed in all the organs (Table 37- 39). Decrease in the tail length was

Table 37. B(a)P induced DNA damage parameters determined by comet assay in Liver of rats fed with Turmeric(T)

Groups	%DNA Tail	Olive Tail Moment	Tail Length
Control	39.89 ^a ± 9.615	7.15 ^{a,d} ± 1.018	21.16 ^a ± 5.088
B(a)P	31.94 ^b ± 10.892	9.41 ^b ± 1.964	27.14 ^b ± 5.145
1% T	54.995 ^{c,f} ± 22.617	6.49 ^{c,d} ± 1.758	18.59 ^c ± 6.74
1%T+B(a)P	30.112 ^d ± 10.891	9.36 ^b ± 2.51	27.12 ^{d,b} ± 7.229
3%T	56.86 ^c ± 16.762	6.19 ^c ± 1.352	18.50 ^{e,c} ± 5.483
3%T+B(a)P	56.07 ^{c,f} ± 17.291	7.02 ^d ± 1.592	20.89 ^a ± 6.160
5%T	47.20 ^e ± 15.625	6.82 ^d ± 1.898	19.76 ^{a,c} ± 6.191
5%T+B(a)P	52.59 ^f ± 20.213	7.02 ^d ± 1.962	19.49 ^{a,c} ± 5.829

Table 38. B(a)P induced DNA damage parameters determined by comet assay in Kidney of rats fed with Turmeric(T)

Groups	%DNA Tail	Olive Tail Moment	Tail Length
Control	35.24 ^a ± 11.768	5.21 ^a ± 1.358	39.28 ^a ± 8.61
B(a)P	17.25 ^b ± 5.422	17.08 ^b ± 4.321	48.13 ^b ± 11.703
1% T	47.40 ^c ± 18.636	7.46 ^c ± 2.720	22.75 ^c ± 10.300
1%T+B(a)P	24.96 ^d ± 7.145	8.42 ^{d,c} ± 2.589	33.16 ^{d,g} ± 7.341
3%T	27.54 ^{e,d} ± 8.408	7.68 ^{e,c,d} ± 2.386	29.84 ^{e,d} ± 7.88
3%T+B(a)P	38.64 ^a ± 14.415	10.81 ^f ± 1.437	36.93 ^{a,g} ± 7.684
5%T	73.949 ^f ± 11.479	5.23 ^a ± 0.632	16.02 ^f ± 5.511
5%T+B(a)P	66.24 ^g ± 10.849	7.99 ^{g,c} ± 1.428	40.23 ^a ± 12.302

Table 39. B(a)P induced DNA damage parameters determined by comet assay in Lung of rats fed with Turmeric(T)

Groups	%DNA Tail	Olive Tail Moment	Tail Length
Control	35.24 ^a ± 11.768	5.21 ^a ± 1.358	39.28 ^a ± 8.61
B(a)P	17.25 ^b ± 5.422	17.08 ^b ± 4.321	48.13 ^b ± 11.703
1% T	47.40 ^c ± 18.636	7.46 ^c ± 2.720	22.75 ^c ± 10.300
1%T+B(a)P	24.96 ^d ± 7.145	8.42 ^{d,c} ± 2.589	33.16 ^{d,g} ± 7.341
3%T	27.54 ^{e,d} ± 8.408	7.68 ^{e,c,d} ± 2.386	29.84 ^{e,d} ± 7.88
3%T+B(a)P	38.64 ^a ± 14.415	10.81 ^f ± 1.437	36.93 ^{a,g} ± 7.684
5%T	73.949 ^f ± 11.479	5.23 ^a ± 0.632	16.02 ^f ± 5.511
5%T+B(a)P	66.24 ^g ± 10.849	7.99 ^{g,c} ± 1.428	40.23 ^a ± 12.302

Values are mean±SD of 6 rats/group.

Variations in superscripts indicate significance of mean differences among groups (p<0.05).

observed with the increase in the concentration of turmeric namely 1%, 3% & 5% both in the turmeric fed groups and B(a)P+turmeric fed groups compared to their respective control.

3. In Pearson's correlation analysis OTM was highly correlated with TL & %DNA T (Table 39).

CONCLUSION

The study demonstrated the protective role of turmeric in B(a)P induced DNA damage in rats.

4. ASSESSMENT OF ENVIRONMENTAL LEAD (Pb) EXPOSURE ON INFECTION AND IMMUNITY

Among the various heavy metal toxicities reported, lead toxicity is reported from all parts of the world especially in growing children and pregnant women. In the recent past the anemia caused due to various factors specially due to lead exposure is being considered as one of the most important factors. The literature also suggests that most of the times, sub-clinical toxicity caused due to low level of lead exposure is known to inhibit basal δ -Amino Levulenic Acid Dehydratase (ALAD) activity thus altering heme synthesis pathway. Earlier studies suggested a significant correlation between basal ALAD activity and elevated blood lead levels. Therefore, in the present investigation blood lead levels were monitored in anemic and non-anemic children to assess the correlations among clinical and sub-clinical indicators. In addition, the interactions with other nutrients like Zn, Cu, Mg were also investigated.

OBJECTIVES

1. To monitor blood Pb levels in anemic and non-anemic children, to evaluate serum Fe, Cu and Zn levels.
- 2) To determine subclinical hemopoietic toxicity, basal ALAD activity and Hb levels.

METHODOLOGY

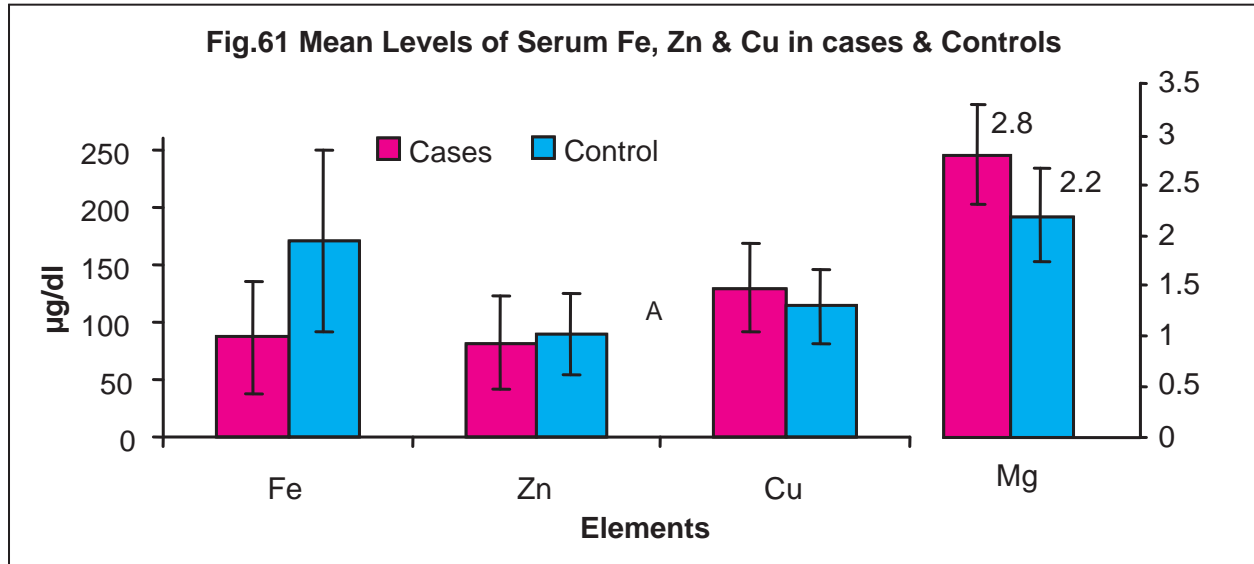
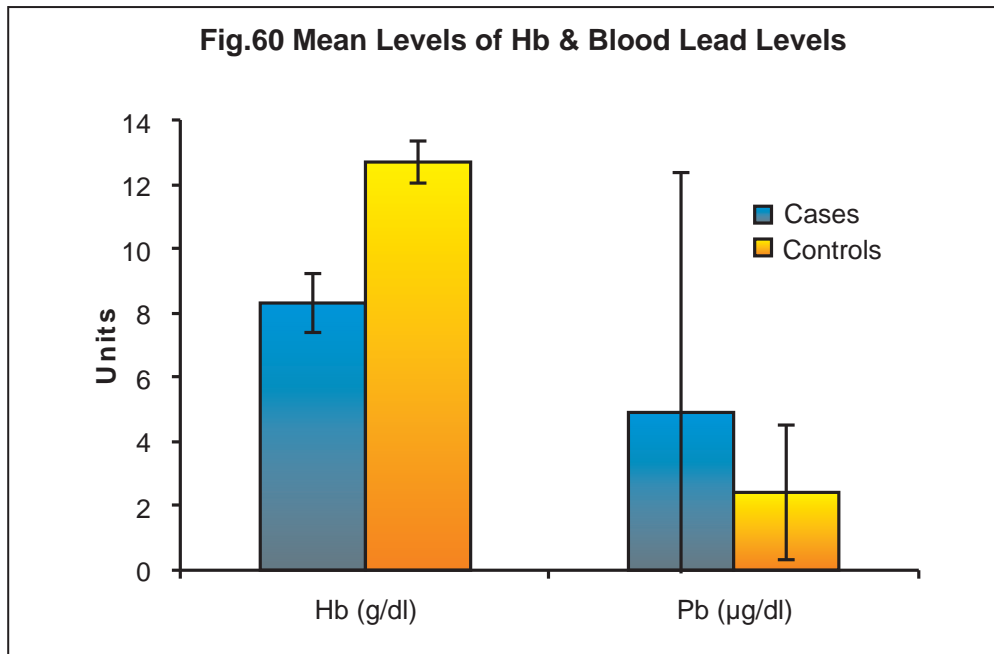
One hundred and twenty children aged 6 months to 12 years attending the Niloufer hospital consented to participate in the study. Socio-economic, and nutritional profile of the children were collected using standardized pre-tested questionnaire. Anthropometric measurements were done using standard procedures. The clinical condition of anemia was described based on hemoglobin levels less than 11.0g/dL.

Basal δ -amino levulenic acid dehydratase (ALAD) activity has been determined by using the method of Granick et al. Blood Lead, serum Fe, Zn, Cu, Mg levels were estimated using GF-AAS. The serum albumin and blood hemoglobin were estimated by standard procedures.

RESULTS

1. 63.3% of the children belonging to middle socio-economic group with rural background had less than 11.0g/dL Hb as compared to control groups.
2. In 10% of the children blood lead levels were above 10 μ g/dL (Fig 60).
3. Decreased ALAD activity was found in 20% of the subjects.

4. Serum Fe was deficient in 21.7%, whereas Zn was deficient in 48.3% children.
5. Significant difference was recorded with reference to serum Fe ($P < 0.01$), Cu ($P < 0.05$), Pb ($P < 0.05$) and ALAD activity ($P < 0.01$) between anemic and control groups (Fig. 61).



CONCLUSION

In majority of the cases, the presence of anemia, zinc, Iron deficiency was recorded irrespective of socioeconomic group. The study results suggested that the lead toxicity may be one of the causative factors for anemia.

5. DETOXIFICATION OF MYCOTOXINS BY LACTIC ACID BACTERIA ISOLATED FROM FERMENTED SORGHUM AND CASSIA TORA

Earlier studies at the Institute showed that natural fermentation of moldy sorghum along with *Cassia tora* seeds resulted in improvement in nutritive value and reduction in mold growth and mycotoxins that was attributed to high numbers of lactic acid bacteria. On the basis of various reports on the mould and mycotoxins degrading potential of lactic acid bacteria, the present study has been undertaken to assess the mold and mycotoxin degradation/reduction potential of LAB species isolated from fermented sorghum and compare with lactobacillus species known to reduce mycotoxin levels.

Earlier observations in the present study showed that lactobacillus isolated from fermented sorghum reduced aflatoxin B1 to 88% when incubated in PBS for a period ranging from 0 to 48hrs at 37°C. Incubation of lactobacillus isolate from sorghum with fumonisin producing *Fusarium moniliforme* culture showed decrease in mould growth at 48hrs of incubation at 28°C. The present study has been undertaken to assess removal of aflatoxin B1 by *Lactobacillus rhamnosus* strain GG (ATCC 53103) with the following objectives:

OBJECTIVES

- i) To assess removal of aflatoxin B1 by *Lactobacillus rhamnosus* strain GG (ATCC 53103) from liquid media at 0, 24 and 48 hrs.
- ii) To assess the extent of removal of aflatoxin by *Lactobacillus rhamnosus* strain GG from sorghum at 0 and 24hrs.

METHODOLOGY

- i) Assessing effect of *Lactobacillus rhamnosus* GG on aflatoxin B1 in liquid medium:** Lactobacillus working cultures were prepared from stock cultures in 10ml MRS broth. For assessing effect on aflatoxin B1, 24 hrs broth cultures with 6.6×10^8 cfu/ml were centrifuged at 5000rpm for 10mins and supernatant discarded. The bacterial pellet was washed twice with 4ml of 10mM PBS, pH7.3 and centrifuged as before. Aflatoxin B1 at concentrations of 1000ng were dissolved in 1.5ml 10mM PBS and added to PBS washed bacterial pellets and incubated at 37°C for 24 and 48hrs. Controls comprised PBS with aflatoxin alone at 0, 24 and 48 hrs incubation. The incubated cultures were centrifuged at 5000rpm for 10mins and supernatant removed for analysis of aflatoxin B1. The resulting pellet was homogenized in 2ml PBS five times and centrifuged as before. Aflatoxin was extracted from the supernatant of the pellet. Chloroform extracts containing aflatoxin were subsequently evaporated and derivatized with trifluoroacetic acid and analysed by HPLC as per AOAC procedures. HPLC was performed using reverse phase column (Waters Spherisorb ODS-2, 4.6 x 250mm, 10µm particle size), mobile phase consisting of water acetonitrile-methanol-tetrahydrofuran (55:30:15:1) at a flow rate of 1ml/min and fluorescence detection at excitation 365nm and emission 440nm wavelengths. Quantification of aflatoxin was carried out by comparing the peak areas of positive controls and aflatoxin in the supernatant.

ii) Assessing effect of *Lactobacillus rhamnosus* GG on aflatoxin B1 in sorghum: Sorghum was powdered and defatted with hexane and 5g were spiked with 1000ng aflatoxin B1 in chloroform. After chloroform vapours got evaporated, bacterial pellet processed as above and containing 6.6×10^8 cfu/ml broth was suspended in 10ml PBS at pH 7.3 and added to the spiked sorghum sample. The sample was incubated at 0 and 24hrs at 37°C and analysed for aflatoxin levels. Control samples comprised sorghum with 10ml PBS without bacteria. The growth of lactobacillus after incubation with AFB1 in sorghum was assessed by taking counts on MRS agar plates at 0 and 24 hrs of incubation. Aflatoxin from sorghum was analysed as per AOAC procedure and consisted of extraction with methanol-water (55:45), followed by chloroform. HPLC analysis was performed after evaporating chloroform extract and derivatization with trifluoroacetic acid as for bacterial cultures.

OBSERVATIONS

- i) Effect of *Lactobacillus rhamnosus* GG on aflatoxin B1 in liquid media: The percentage of AFB1 removed by lactobacillus that was observed in the supernatant was 78.9% (Table 40). Incubation for 24 and 48hrs resulted in 66 and 53.4% removal in AFB1 respectively.
- ii) Effect of *Lactobacillus rhamnosus* GG on aflatoxin B1 in sorghum: Addition of *Lactobacillus rhamnosus* GG to sorghum resulted in removal of 58% of the initial aflatoxin level at 0 hours and 77% at 24 hours of incubation (Table 41). Limited change was observed in bacterial growth during the incubation period.

Table 40. Effect of *Lactobacillus rhamnosus* GG on aflatoxin B1 in liquid media

Component	Aflatoxin levels (ng)*		
	Incubation (h)		
	0	24	48
Control: Aflatoxin B1+PBS	883.2±19.51	739.5±8.99	757.5±10.61
Test: Aflatoxin B1+LBGG**	697.42±55.274	484.7±129.117	404.49±2.355
% removal of Aflatoxin B1	78.9	66.1	53.4

*Mean±SD for 2 replicates ***Lactobacillus rhamnosus* GG

Table 41. Effect of *Lactobacillus rhamnosus* GG on aflatoxin B1 in sorghum

Component	Aflatoxin levels (ng)*	
	Incubation period (h)	
	0	24
Control: Sorghum+Aflatoxin B1	756.5 ± 6.36	611 ± 22.06
Test: Sorghum+AflatoxinB1+LBGG	319 ± 5.65	138.57 ± 18.99
% removal of AFB1	57.8	77.3
<i>Lactobacillus rhamnosus</i> Counts (cfu/ml)	1.4×10^8	1.1×10^8

*Mean±SD for 2 replicates ***Lactobacillus rhamnosus* GG

CONCLUSIONS

- i) The present study indicated that *Lactobacillus rhamnosus* strain GG has good potential to reduce aflatoxin levels in contaminated grains such as sorghum.
- ii) The extent of removal of aflatoxin by lactobacillus species isolated from fermented sorghum is observed to be comparable to that observed with *Lactobacillus rhamnosus* strain GG.
- iii) Detailed studies with different lactobacillus species on aflatoxin levels in grains are required to be carried out to fully explore the potential of such strains in removal of mycotoxins.

6. INHIBITION OF NITROSAMINES IN FOODS BY DIETARY SUBSTANCE

Nitrosamines are a group of chemical substances. Some of these compounds, if formed from secondary amines tend to be carcinogens. Ninety percent of secondary nitrosamines are proved in animals to be carcinogenic. The nitrosamines once formed cannot be deactivated easily. At least those that are to be formed by the action of nitrates/nitrites with secondary amines can be prevented. Indian diet contains several constituents which can prevent such formation like turmeric, ginger, onions etc. The inhibitory potential of several dietary constituents on the formation of nitrosamines is reported. Some spices form nitrosamine in the stomach and the inhibitory action of the other protective foods on them was studied.

OBJECTIVES

- 1) To estimate the inhibitory potential of dietary spices and condiments on commonly consumed foods.
- 2) To compare the inhibitory potential of such foods substances with a standard dose of vitamin C.

METHODOLOGY

Foods were obtained from four major markets four areas in Hyderabad and Secunderabad. The foods so brought were subjected for analysis. The procedure involved the use of the method developed at NIN. Initial formation of Nitrosamines was obtained from 10g of spice powder likely to form nitrosamine and subjecting it to *in vitro* artificial gastric juice for 1 hour. The contents were then vacuum distilled and the distillate was collected at $<-30^{\circ}\text{C}$. Finally, it was concentrated to 1 ml. Nitrosamines were estimated on the Gas Chromatography and Thermal Energy Analyser. To 10g of the same spice powder differing doses of protective foods were added and incubated under artificial gastric juice for 1hr in the presence of NO_2 solution. Nitrosamines were estimated on the GC-TEA. The difference was the inhibitory potential of the protective food.

RESULTS

The result of the amount of nitrosamines formed is given in Table 42. The nitrosamine dimethyl amine is very consistent and important in this study.

The inhibitory potential as percent of inhibition is shown in tables 43 - 47. The protective action of onions was found to be weak and hence, the studies on further nitrosating spices were terminated. Similarly, studies on garlic had to be stopped as it was also aiding the formation of nitrosamines.

The different doses used were arbitrary and is to understand the potential inhibition.

Table 42. NOC from select spices formed under simulated gastric juice (ppb)

S. No	Spice (10g)	Nitrosodimethyl amine	Nitrosopiperidine	Nitrosopyrrolidine
1	Black Pepper	46.4±1.51	1192.1±13.15	48.1±0.56
2	Dry Coriander	57.2±1.5	ND	20.6±1.08
3	Cumin	50.7±0.56	ND	80.7±0.3
4	Red Chilies	175.1±12.26	ND	122.4±2.96
5	Mustard Seeds	228.8±2.54	ND	69.9±1.94

Values are Mean ± SEM.

N=10 observations

Table 43.(A) Percentage inhibition by turmeric at 0.75 g dose under simulated gastric juice

S. No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	26.4	23.8	21.0
2	Dry Coriander	32.5	ND	21.1
3	Cumin	11.8	ND	13.8
4	Red chilies	23.3	ND	17.6
5	Mustard seeds	13.2	ND	15.8

Table 43.(B) Percentage inhibition by turmeric at 1.5 g dose under simulated gastric juice

S. No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	35.6	37.0	88.2
2	Dry Coriander	54.0	ND	39.9
3	Cumin	24.6	ND	36.2
4	Red chilies	49.0	ND	72.1
5	Mustard seeds	23.1	ND	39.4

Table 43.(C) Percentage inhibition by turmeric at 3.0 g dose under simulated gastric juice

S.No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	51.5	49.5	98.1
2	Dry Coriander	68.5	ND	52.7
3	Cumin	51.6	ND	75
4	Red chilies	64.8	ND	83.9
5	Mustard seeds	36	ND	70.6

Table 43.(D) Percentage inhibition by turmeric at 5 g dose under simulated gastric juice

S.No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	64.4	60.4	100
2	Dry Coriander	87.2	ND	63
3	Cumin	60.5	ND	95.9
4	Red chillies	82.0	ND	88.2
5	Mustard seeds	63.7	ND	88.2

Table 43.(E) Percentage inhibition by turmeric at 7 g dose under simulated gastric juice

S.No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	79.1	80.74	100
2	Dry Coriander	95.4	ND	75.1
3	Cumin	72.5	ND	100
4	Red chillies	95.7	ND	100
5	Mustard seeds	78.9	ND	95.4

Table 44.(A) Percentage inhibition by Ginger at 0.75 g dose under simulated gastric juice

S.No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	8.4	16.8	12
2	Dry Coriander	16.3	ND	19.1
3	Cumin	19.7	ND	22.0
4	Red chillies	14.9	ND	16.6
5	Mustard seeds	29	ND	11.1

Table 44.(B) Percentage inhibition by Ginger at 1.5 g dose under simulated gastric juice

S.No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	19.8	41.3	31.6
2	Dry Coriander	33	ND	34.1
3	Cumin	40.4	ND	36.1
4	Red chillies	32.9	ND	36.9
5	Mustard seeds	43.7	ND	42.2

Table 44.(C) Percentage inhibition by Ginger at 3 g dose under simulated gastric juice

S.No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	30.3	70.6	52.8
2	Dry Coriander	46.0	ND	51.5
3	Cumin	55.4	ND	53.2
4	Red chillies	46.5	ND	50.8
5	Mustard seeds	55.9	ND	56.9

Table 44.(D) Percentage inhibition by Ginger at 5 g dose under simulated gastric juice

S. No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	59.2	90.0	80.0
2	Dry Coriander	64.1	ND	70.5
3	Cumin	66.6	ND	73.7
4	Red chilies	60.1	ND	63.0
5	Mustard seeds	65.1	ND	70.3

Table 44.(E) Percentage inhibition by Ginger at 7 g dose under simulated gastric juice

S.No	Nitrosating agent (10g)	NDMA	NPIP	NPYR	NDEA*
1	Black Pepper	81.4	98.3	95.6	6.1
2	Dry Coriander	81.9	ND	79.3	5.9
3	Cumin	80	ND	76.5	6.1
4	Red chilies	76.9	ND	69.5	6.6
5	Mustard seeds	70.8	ND	85.5	7.1

*NDEA-Nitrosodiethylamine is additionally detected.

Table 45. Percentage inhibition by onions at different dose the NOC formed by black pepper under simulated gastric juice

S.No	Inhibitors of nitrosation	NDMA	NPIP	NPYR
1	Onions at 2.0 g	3.2	9.5	1.6
2	Onions at 5 g	6.2	12.9	7.9
3	Onions at 10 g	12.0	14.3	11.8
4	Onions at 15 g	17.0	15.5	14.1
5	Onions at 20 g	16.3	15.8	14.3

Table 46. Percentage inhibition by fresh garlic at different doses the NOC formed by Black Pepper under simulated gastric juice

S. No	Substance	NDMA	NPIP	NPYR	NDEA*
1	Garlic at 0.75g	-1.2	-0.16	-0.2	42.9
2	Garlic at 1.5.g	-1.0	-12.9	-7.9	44.0

*NDEA-Nitrosodiethylamine is additionally detected.

Table 47. Percentage inhibition by Ascorbic Acid at 5 g dose on NOC formed under simulated gastric juice

S.No	Nitrosating agent (10g)	NDMA	NPIP	NPYR
1	Black Pepper	62.4	92.4	100
2	Dry Coriander	67.6	ND	100
3	Cumin	60.6	ND	95.0
4	Red chilies	72.0	ND	98.2
5	Mustard seeds	69.0	ND	94.6

CONCLUSIONS

The nitrosamines NDMA, NPIP, NPYR are the frequently occurring carcinogens. NPIP is generally detected in certain substances like pepper. The red chilies had large variations in nitrosamine content. The formation occurred in the presence of artificial gastric juice.

Turmeric was inhibiting more at increasing doses. It completely inhibited the formation of NPYR at the highest dose adopted. Of the four substances studied, two were observed to be superior to onions and garlic. Garlic was showing the formation of another nitrosamine and had to be stopped. Onions was a weak inhibitor and large amount could not be used in the study and therefore had to be terminated. The reference substance vitamin C could inhibit all the above spices to a similar degree as turmeric powder.



IX. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

SERVICE ACTIVITIES

1. BREEDING AND SUPPLY OF ANIMALS

During the 12 months period, 36,026 animals were bred and out of which 28,086 animals were supplied for research including the parent institution. There was a slight dip in supply compared to last year, however, the income generated amounted to Rs.49.64 lakhs, almost same as last year. The details of individual animals species and strain names, bred and supplied are shown in tables 48 and 49.

2. SUPPLY OF ANIMAL FEED

a. Stock Animal feed

Apart from stock feed of 30,256 kg of animals under our care, an additional, 25,704 kgs of animal feed (23,893 kg of rat/mouse feed; 1711 kg of g.pig/ rabbit feed) was supplied during the period generating an amount of Rs.25.39 lakhs. There was a decrease of over 12% in the supply of feed compared to last year, but the income generated was same as last year.

b. Experimental Animal Feed

Need based supply of experimental animal feed of 404 kg, was continued during the period. The details of experimental diets are given in table 50.

C. Blood and Blood products

During this period, a total of 1774 ml of blood and blood products (Blood 220 ml, Plasma 1241 ml) were supplied to 12 different institutions on 42 occasions and a sum of Rs.2,15,880 was realized. Apart from the above, 313 ml of blood was supplied within the Institute.

In addition, during this period the tissues and organs from hamsters were supplied to two Institutions and a sum of Rs. 500 was realized.

3. TECHNICAL CONSULTANCY ON ANIMAL HOUSE DESIGN, ANIMAL EXPERIMENTATION AND ANIMAL WELFARE

1. Dr.P. Suresh attended the CDFD Animal House Construction Advisory Meeting on 30th March 2009, to evaluate the specifications and facility requirements in view of the proposed animal experiments for transgenic and knock out animals.
2. As CPCSEA appointed Site Inspector, Dr.P. Suresh reviewed and inspected M/s. Hetero Drugs Ltd., Animal facilities on 5th April 2009 and submitted report.
3. Dr.P. Suresh attended the review committee meeting of animal facilities of Sai Adv. in Pune from 8th-10th Oct. 2009, as part of their plan to acquire AAALAC accreditation programme.

Table 48. Details of breeding and supply of different species and strains of laboratory animals during the period 1.4.09 to 31.3.10

No.	Species	Strain or Breed	Stock As on 1.4.09	Total Number of animals							Balance as on 31.3.10	
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disp. Old/ Sick age		
1	Mouse	BALB/c An. N (inbred)	458	1546	2004	-	1564	1564	22		418	
		C57BL/6J (inbred)	935	4761	5696	0+2	3532	3534	985		1177	
		N:NIH(S) Nude (inbred)	190	569	759	-	-	140	140	433		186
		NCr.Nude	314	336	650	-	126	126	126	387		137
		FVB/N (in bred)	76	255	331	-	50	50	50	99		182
2	G. Pig	Swiss (in bred)	1140	9023	10163	-	7762	7762	372		2029	
		N:HART (Hartley)	313	1455	1768	8	1225	1233	103		432	
		N:NIH (Coloured)	255	877	1132	4	940	944	62		126	
3	Rabbit	New Zealand white	36	223	259	8+0	175	183	17		59	
4	Monkey	Macaca mulatta (Rhesus)	24	-	24	-	-	-	-		24	
	TOTAL		3741	19045	22786	22	15374	15536	2480		4770	

Percentage of animals supplied to other Institutions: 67.47%

Table 49. Details of breeding and supply of different species and strains of laboratory animals during the period 1.4.09 to 31.3.10

Sl. No.	Species	Strain or Breed	Stock as on 1.4.09	Total Number of animals							Balance as on 31.3.10
				Bred during the period	Available	Supplied to NIN	Supplied to other Insttts.	Supplied Total	Died	Disp. Old Age/ Sick	
1	Rat	CFY/NIN (inbred)	96	50	146	-	-	-	63	23	60
		Fischer 344 N (inbred)	108	118	226	20	30	50	35	30	111
		Holtzman (inbred)	63	163	226	-	-	-	85	21	120
		SD (Sprague Dawley) (Outbred)	837	4142	4979	-	3067	3067	175	576	1161
		Wkyoto (inbred)	65	106	171	-	-	-	43	20	108
		WNIN (inbred)	1613	10081	11694	199	8299	8498	80	551	2565
2	Hamster	WNIN/GR-Ob	747	474	1221	33	-	33	53	219	916
		WNIN/Ob-Ob (inbred)	664	569	1233	209	-	209	109	110	805
		Golden (inbred)	561	1278	1839	-	693	693	20	243	883
3	Sheep		1+0	1+0	-	-	-	-	-	1+0	
		Total	4755	16981	21736	461	12089	12550	663	1793	6730
		Total (1+2)	3741	19045	22786	22	15374	15536	2480	-	4770
		Grand Total	8496	36026	44522	483	27463	28086	3143	1793	11500

Percentage of animals supplied to other Institutions: 77%

4. Dr.P. Suresh CPCSEA nominee inspected M/s.Aptus Bio Sciences Pvt. Ltd. on 20th Nov. 2009 and submitted the report.
5. As AAALAC nominee Dr.P. Suresh visited M/s.Vimta Labs and did a mock audit & reviewed their facility on 1st Dec. 2009 to examine the sustainability of their facility for AAALAC accreditation.
6. Dr.P. Suresh attended a meeting in HLL, Chennai on 28th Jan. 2010 to hold discussion and review their animal facility construction and specifications.
7. Dr.N.V.Giridharan visited IISER, Trivandrum and gave technical advice on the construction of their animal facilities on 1st March 2010.
8. Dr.P. Suresh and Dr.N.Harishankar are CPCSEA nominees for six private and government Institutions.

Table 50. Experimental feed supplied from 1.4.2009 to 31.3. 2010

S.No.	To whom supplied	Type of diet	Quantity (Kgs)
1	CCMB, Hyderabad	Maltodextrine	47
2	CCMB, Hyderabad	High fat	5
3	MDRF, Chennai	Fructose	37
4	MDRF, Chennai	High fat	40
5	Hamdard University, Delhi	High fat	39
6	S.V.University, Tirupati	High fat	12
7	Natural Remedies, Bangalore	AIN Salt mixture AIN Vitamin mixture	70 25
8	CDRI, Lucknow	25% Protein 5% Protein 10% Protein 17% Protein	10 10 12 10
9	S.K.University, Ananthapur	Fructose	42
10	Osmania University, Hyderabad	Vit. Deficient Antherogenic	3 5
11	Vimta Labs, Hyderabad	60% Kcal fat	40

4. HEALTH MONITORING

During this period, a total of 402 samples from mice and rats (bacterial) were screened for micro biological (bacterial) monitoring.

Samples were taken from 190 Mice (BALB/C 46, Swiss 42, C57/6J – 28, FVB/N 36, Nude hetero 34, Nude 4) and 132 Rats (WNIN - 42, Holtzman – 8, SD - 62, Fischer - 8, WKY - 6, CFY – 6). Samples were also taken from other species like Hamsters 22, Rabbits 10, G.pigs 10 and other sources (water, feed, bedding, water bottles and canopies) 28.

The results of the bacterial screening is given in tables 51 and 52.

Among the samples tested for bacteria, higher incidence of Corynebacterium and Klebsiella were seen in more than 6 months old rats belonging to all strains. The same pattern was also

Table 51. Microbial colonies detected in different mice strains

Organisms Isolated	BALB/c (46)		C-57/6J (28)		Swiss (42)		FVB-N (36)		Nude-Hetero (34)		Nude NCI (04)	
	4m (06)	>6m (40)	4m (04)	6m (24)	4m (4)	>6m (38)	2m (04)	>6m (26)	4m (10)	>6m (24)	2m (04)	>6m
Coryn	N	N	N	N	N	N	N	N	N	N	N	-
Staphylococcus	03	16	01	02	04	26	02	12	02	20	02	-
Streptococcus	03	14	01	02	04	26	02	12	03	20	02	-
Klebsiella	N	N	N	01	N	N	N	04	N	04	N	-
Proteus	N	N	N	N	N	08	N	N	N	N	N	-
Tumors	N	N	N	N	N	N	N	N	N	N	01	-
Spleen Enlarged	N	N	N	N	N	N	N	N	N	N	N	-
Ecto Parasites	03	25	01	02	04	28	02	12	04	16	N	-
Liver Cyst	N	04	N	N	N	03	N	N	N	N	N	-

Values in brackets are the total number of samples tested: N- Not detected, m=months age

Table 52. Microbial colonies detected in different rat strains

Organisms isolated	WNIN (44)			S D (60)		Fisher (08)		Kyoto (06)		CFY (06)		Holtzman (08)		Hamster (22)
	2m (04)	4m (02)	>6m (38)	4m (06)	>6m (54)	4m (06)	>6m (02)	2m (04)	6m (02)	4m (04)	>6m (02)	2m (04)	4m (08)	>6m (22)
Coryn	N	04	26	02	12	01	02	01	03	01	03	N	01	N
Klebsi ella	N	1	16	06	08	02	04	01	01	01	01	N	01	04
Proteus	N	N	08	04	06	N	04	N	01	N	01	N	N	N
Staphylococ	02	02	12	02	20	02	04	02	03	02	03	03	01	N
Streptococc	02	02	12	02	14	02	06	02	02	02	02	02	01	N
Tumors	N	N	08	N	06	N	N	N	N	N	N	N	02	N
Spleen Enlarge	N	02	08	01	08	N	04	N	N	N	N	N	01	N
Giardia spp.	N	N	N	N	N	N	N	N	N	N	N	N	N	12
Liver Cyst	N	03	08	02	07	N	02	N	02	N	02	N	02	N

Values in brackets are the total number of samples tested: N- Not detected m=months age

observed in mice strains. In addition, *staphylococcus* and *streptococcus* continued to be the predominant pathogenic bacteria.

During the period, 92 sera samples were tested for Mycoplasma and the results showed that 17/46 rats and 10/46 mice were positive for mycoplasma (Table 53). All strains of rats showed high percentage of incidence of infection excepting WNIN and in mice BALB/c and C57 showed high incidence except Swiss strain. This indicates that infection was less in the animals that were under continuous breeding and supply.

As part of testing for PCT, 164 Rabbits were screened for their health during quarantine period.

During this period, a total of 610 animals (rats – 288, mice – 310, rabbits – 8, hamsters - 4) meant for culling due to old age were examined for organ abnormalities. 25% of the mice showed skin abnormalities, while 2% had tail abnormality and enlarged spleens. In rats also, about 17% of them showed skin problems in terms of dermatitis and loss of hair and about 4% rats showed lung lesions, liver cyst and enlarged spleen.

Table 53. Results of Mycoplasma testing in rats and mice during April 09 to March 2010

S.No	Virus	No. of samples Tested	Rat strains						Result Positive
			WNIN	SD	Fisher	Holtzman	CFY	Kyoto	
1	Mycoplasma	46	1/8	6/8	4/8	1/8	3/6	2/8	17

S.No	Virus	No. of samples tested	Mice strains							Result Positive
			BALB/c	C57	Swiss	NIH Hetero	NIH Nude	NCI Hetero	NCI Nude	
1	Mycoplasma	46	3/8	3/8	1/6	0/8	0/6	2/8	½	10

A Total of 46 samples tested for Mycoplasma

5. HUMAN RESOURCE DEVELOPMENT

- ❖ Under junior level training course - LATTC, 11 participants were trained and under senior level training course - LASTC - 4 participants were trained.
- ❖ *Ad hoc* training ranging from 1 week to 3 weeks was given to 30 candidates from January to April.
- ❖ The center conducted a 4-day Workshop on 'Non invasive techniques in laboratory animals' from Dec.14-19 and 23 participants selected from all over India participated in the workshop.

6. RESEARCH SUPPORT

- ♦ Main laboratory including Pre - Clinical Toxicology (PCT)
- ♦ During the period, 30 research projects were approved by IAEC for implementation. Out of these, 2 studies were completed, 20 are in progress and 8 are yet to be initiated.



X. PRE-CLINICAL TOXICOLOGICAL STUDIES

1. PRE-CLINICAL TOXICITY EVALUATION OF PROPHYLACTIC ANTI HUMAN PAPILOMA VACCINE (HPV)

Cervical cancer results from infection caused by Human Papilloma Virus (HPV) specially HPV type 16 & 18 (HPV16 & HPV18). Baud D *et.al* have developed salmonella based vaccine against HPV using single oral route that induces mucosal immunity in different stages. Indian Immunologicals Ltd. has developed recombinant vaccine with *salmonella* organisms that assemble HPV16 & 18 VLPs that can induce high titers of neutralizing antibodies in mice after oral or nasal route with live bacteria. It is reported that attenuated *salmonella Typhimurium* strains with deletion mutations have been indicated to be safe oral vaccine.

The above product is being manufactured for the first time by Indian Immunologicals Ltd. in India. As per DCGI Guidelines it has to undergo preclinical toxicity trial. In addition, the vaccine is a construct of new generation of *salmonella* organism that assemble HPV 16 & 18VLPs.

The present investigation has been completed as per the protocol approved by RCGM of DBT.

OBJECTIVE

To assess the safety profile of anti HPV vaccine in mice, rat and rabbit.

METHODOLOGY

Test compound, Anti-HPV vaccine (Recombinant salmonella HPV16 & 18VLPs) has been supplied by sponsor in the required dosage strength along with certificate of analysis. The following are the test details.

RESULTS

Acute Toxicity - Mice & Rat (14days): No mortality was observed at 10XTD in mice and 10-50 XTD in rats only, whereas 15% mortality was seen at 50XTD in Mice.

Sub-chronic Toxicity – Rat: Mortality in Rats - EC-IN (4%), TD-IN (8%) after dosing the anesthetized animals was recorded. Clinical chemistry, hematology parameters were in normal range. Spleen weight in TD-IN, AD-IN suggest immune response. No abnormal histopathology finding were observed.

Sub-chronic Toxicity – Rabbit: Five percent Mortality was observed in three groups (EC-IN, TD-IN, EC-OR) of Rabbits- Clinical chemistry, hematology parameters were in normal range. No significant changes in organ weights. No abnormal histopathology finding was observed.

Allergenicity Test in Balb/C Mice: Immunogenic response (IgG) was observed in 5XTD-Intranasal route (IN). There were No Allergenic Potential recorded.

Table 54

S.No	Test details	Test * compound exposure	Test species	Number of Animals	Study duration
1	Single dose toxicity study (Acute toxicity test)	10x TD & 50XTD- Single exposure 1	Mouse	40(20M+20F)	14 days
			Rat	40(20M+20F)	
2	Repeated dose toxicity study (Sub chronic toxicity test)	2 Dose levels- (1XTD & 5XTD) 2	Rat	144(72M+72F)	90 days + 30days
			Rabbit	100(50M+50F)	
3	Allergenicity test	TD & 5TD	Balb/c mice	48(24M+24F)	78 days+ 15 days
* Route of administration : Intranasal and Oral					

CONCLUSION

The study demonstrated that NOAEL for Prophylactic Anti Human Papilloma Vaccine (HPV) was at 5XTD given orally and intranasally. The studies also confirm the rationale of selecting intranasal route and species by immunogenic reactions.

2. PRE-CLINICAL TOXICITY EVALUATION OF PROPRIETARY PEPTIDE MOLECULE GENOPEP 1 (ISSAR 1)

The role of natural peptides as a substitute to anti-microbials is very well documented. In the recent past, the synthetic peptides viz. D2A21, MIMs (Membrane Interactive Molecules) etc have been investigated for anti-tumor activity in addition to anti-microbial activities in rats. The attempts are on to use such preparations in clinical practice.

The Issar Pharmaceuticals has developed a synthetic peptide, Genopep 1 (Issar 1). The peptide is chemically pure, stable; contains 23 amino acids and is manufactured by solid phase peptide synthesis to promote it as an anti-cancer agent.

The anti-cancer activity testing of Genopep 1 (Issar 1) *in vitro* at ACTREC, Tata Memorial Centre, as per their reports, indicated that it has potential anti-tumor activity.

The preclinical toxicity evaluation of this proprietary peptide molecule was carried out earlier using 0.143 mg/kg as clinical dose and was found to be safe. However, *in vivo* testing of Genopep 1

(Issar 1) with HCT-15 xenografts in nude mice at ACTREC, Tata Memorial Research Center, a concentration of 50.12 mg/kg in mice was shown to be very effective in tumor growth regression. Therefore, the present investigation (Pre-clinical toxicity evaluation) was carried out with higher dosage strengths and intravenous route for the evaluation of safety data as per schedule 'Y' to promote it for clinical use.

The present protocol used was designed based on the guidelines of FDA / schedule 'Y' of DCGI.

OBJECTIVE

To assess the safety profile of intravenously administered Genopep1 (Issar-1) in mice, rats and rabbit.

METHODOLOGY

The sub acute toxicity test by intravenous route was undertaken in rats and rabbits with three different dosages as shown in the table 55.

S.No.	No. of Animals/sex	Group	Dosage mg/kg	Duration of Exposure	Study Period
Rats					
1	12 (6M + 6F)	Vehicle Control (VC)	-	(5 days/ week) 2 (IV) & (5 days/ week) 2 (SC)	43 days
2	12 (6M + 6F)	Therapeutic Dose (TD)	0.9		
3	12 (6M + 6F)	Average Dose (TD X 2)	1.8		
4	12 (6M + 6F)	High Dose – (TD X 3)	2.7		
Rabbits					
5	8(4M + 4F)	Vehicle Control (VC)	-	(5 days / week) 4	43 days
6	8(4M + 4F)	Therapeutic Dose (TD)	0.465		
7	8(4M + 4F)	Average Dose (TD X2.5)	1.1625		
8	8(4M + 4F)	High Dose - (TD X5)	2.325		

The study parameters viz., cage side observation physical examination, body weight monitoring, neurological examination, urine analysis and blood chemistry, hematology profile were generated in the animals exposed immediately and 15 days post exposure. Similarly, necropsy and histopathology of vital organs were also evaluated.

RESULTS

Rat

In sub chronic toxicity test, there was no mortality in animals exposed to vehicle control, test compound at TD, AD (2XTD), and HD (3XTD) dose till end of the experiment. Test compound induced a reaction at the site of injection (tail) from 4th day of exposure in animals exposed to 2TD (30%) and 3TD (50%) which reduced during the recovery phase. There was no significant difference in body weight gains, live phase, physical activity and neurological activity between the control and test groups throughout the study period. There were no significant abnormalities in hematology, blood chemistry profile tested after 24h and 5th and 15th days of exposure. At necropsy,

no gross lesions were found in the organs in all groups. No significant difference was observed in the histopathology of various organs between control and test groups at different dose levels. In genotoxicity test, there was no significant difference between the groups.

Rabbit

In sub chronic toxicity tests, there was no mortality at therapeutic dose (TD), average dose (2XTD) and high dose (3XTD) level of exposure in rabbits. There were no differences in body weight gains, live phase, physical activity and neurological observation between the control and test group animals throughout the study period. However, there was a reaction at the site of injection from 4th day. The wound healing activity was recorded after 3 days of last exposure. No significant abnormality in biochemical profiles were recorded in serum investigated 24 hrs after fifth and 48hrs after 10th exposure and on 15th day of last exposure (29th day of study). At necropsy, no abnormal gross changes were recorded in animals euthanized immediately after 48hrs of exposure, and at the end of 15 days (recovery phase) in major organs.

There was no gross and histopathological changes in various organs viz., heart lungs, spleen, brain, skin, kidney etc., in rabbits exposed to the test compound as compared to unexposed test group which could be attributed to exposure to tests compound. Genotoxicity test as shown by the micro nucleated polychromatic erythrocytes were also normal in all groups exposed to test compounds as compared to the vehicle control.

CONCLUSIONS

The results of the study concluded that the NOAEL of geno pep 1 by intravenous (2XTD) is 1.8 mg/kg in rats & 0.93 mg/kg in rabbits.



INSTRUMENTATION SERVICES

Details of Instruments received at NIN,FDTRC and NCLAS with institutional funds:

2009

1. Real Time PCR- Roche
2. Genetic Analyzer-ABI Prism 3100
3. BOD POD Body Composition Analyzer- Life Measurement
4. High Throughput Genotyping Discovery System-Sequenom
5. Benchtop Flow Cytometer with Cell Sorter-BD Biosciences
6. HPLC-2 nos. – Shimadzu
7. Digital Power Quality Analyzer –Mohan Marketing
8. High Pressure Waterjet Cleaner – UT Pumps
9. Homogeniser- Kinematica
10. Indoor Air quality Analyzer –Swan Environmental
11. Microplate washer –sowar Pvt.Ltd.,
12. Modular Walk-in Cold Room- Vista Biocell
13. Nitrogen Evaporator Organomaiton/Indo Systems
14. Plasma Display Panel – 5 nos – Samsung
15. Sonicator- Sonics
16. Variable Frequency Drive System Amtech Electronics
17. Western Blot Developing Kit Chemfil Scientifics
18. X-Ray Film Processor – Medisun Medical Products
19. Stirred Cell Concentrator –Chemfil Scientifics
20. Stadiometers – 15 nos –SECA/Himbi
21. Water Purification Systems- 3 nos – Inexus Biotech
22. UV-VIS Spectrophotometer- Shimadzu
23. AC Plant for Conference Hall- Twenty Four Assured Aircon Pvt.
24. Spectrofluorimeter –Annapurna Scientifics
25. Wireless Portable Ergospirometry –Espee Dynamics
26. Elisa Plate Reader –Biotech Instruments/Medispec
27. 12-Channel ECG for Oxycon Pro –Esbee Dynamed
28. Tabletop Refrigerated Centrifuge -4nos-Sigma
29. Animal Containment Workstation –York Sales
30. Adult Weighing Scales -15 nos – Rustagi surgicals
31. Skin fold Calipers- Cranlea
32. Gradient PCR – Eppendorf
33. Environmental Particulate Air Sampler –Tulip Diagnostics

34. Refrigerated Shaker Incubator – New Brunswick
35. Molecular Imager- Synoptics
36. Portable Lactate Analyzer – EKF /Esbee Dynamed
37. Tabletop Anaesthesia System –Harvard Apparatus/Marsap
38. Stereotaxic Apparatus – Stoelting
39. CO2 Incubator – Thermofisher
40. Incubator – ThermoElectron

2010

The following equipment were received for 'Nutritive value of Indian foods' Project:

1. Binary Gradient Fast HPLC – 8 nos – Dionex
2. High Speed GC – 2 nos- LCGC
3. Atomic Absorption Spectrometer – Analytik Jena
4. Photochemical Analyzer – 2 nos-Analytik Jena
5. Single beam Diode Array UV-VIS Spectrophotometer – 3 nos- Analytik Jena
6. Fully Automated Kjeldahl Nitrogen Analyzer- 2 nos-Foss
7. Dietary Fibre Filter System- 2 nos- Foss
8. Digital Rotary Flash Evaporator – 3 nos- Buchi
9. Microwave Digestion System – CPM

The following equipment are (all codal formalities are completed) pending with ICMR for Financial Concurrence:

2010

1. Maldi ToF ToF – ABI 5800
2. Electro Retino Gram –
3. Atomic Absorption Spectrometer – 2 nos- Shimadzu
4. Automated Solvent extraction System - Dionex



LIBRARY AND DOCUMENTATION SERVICES

Library continued to cater to the documentation and information needs of the Institute and other Research Organizations, Home Science and Medical Colleges. The library has played a key role in reference activities by offering information dissemination services like MEDLINE Searches, Proquest Medical Library Full Text Database of journals and other online retrieval activities using the LAN Network of the Institute. Library continued to participate in exchange of data, journals and information using the URL <[http://Groups.yahoo.com/group/ICMR Librarians](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).

MODERNISATION OF LIBRARY AND INFORMATION NETWORK

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

- a) ICMR has renewed the subscription to Proquest Medical Library Full Text Database of the journals. During the period total of 4395 Proquest ML Full Text Database Searches were made.
- b) Subscription of JCCC@ICMR and J-Gate has been renewed by Indian Council of Medical Research through M/s. Informatics India Pvt. Ltd., Bangalore, JCCC@ICMR covers more than 1934 journals received collectively at 29 Institutions/Centres Consortia of ICMR Libraries. And J-Gate is an electronic gateway to global e-journals literature. It presently has massive database of journal literature indexed from more than 17,991 e-journals with links to full text at publisher sites and provides free access to full-text of 1700+ journals with e-author e-mail address and also one can find the availability of the journal in a local library.
- c) NIN Library is also a member of NML – ERMED Consortia for accessing more than 2672 Journals
- d) Online Subscription of 5 Core Journals such as BMJ, LANCET, NATURE, NEJM, SCIENCE has been renewed by ICMR is also accessible.
- e) The following equipments were procured for the library
 - ❖ 500 GB backup drive

NEW JOURNALS ADDED

Indian Journals

1. Heritage Amruta
2. Nutrition, Immunity & Health
3. Plant Horti Tech (INPHOM)
4. Yoga the Science

Foreign Journals

5. Analytical Biochemistry
6. Archives of Biochemistry & Biophysics

7. Archives of Environmental Contamination & Toxicology
8. Archives of Environmental & Occupational Health
9. Biochemical & Biophysical Research Communication
10. Biochemical Journal
11. Biochemical Pharmacology
12. Cellular and Molecular Life Sciences
13. Clinical Chemistry
14. Food Control
15. Food Quality & Preference
16. Food Research International
17. International Archives of Occupational & Environmental Health
18. International Journal of Occupational & Environmental Health
19. International Journal of Toxicology
20. Journal of Clinical Immunology
21. Journal of Diabetes and its Complications
22. Journal of Immunology
23. Journal of Microbiology
24. Journal of Plant Nutrition
25. Nutrition Metabolism & Cardiovascular Diseases
26. Stem Cells

The following library services were expanded as detailed below:

1. NEW ADDITIONS

Books	129
Reports	171
Journals (New Subs.)	26
Thesis / Dissertations	-
CDROMS	41
PC Quest CD's	19
General CD's	22

2. OTHER ACTIVITIES

Journals Bound	1,783
Visitors using the Library	3,209
Circulation of Books/Journals etc	1,201
MEDLINE Abstracts provided	200
No. of E-mails sent outside	2,000
No. of E-mails received	10,000
Photocopying (No.of pages)	2,78,120
Number of Annual Reports mailed	465
No. of duplicate journals sent out	100
No. of INTERNET searches provided	250
No. of reprints sent	600
Proquest Full Text Database searches provided		4,395

3. TOTAL LIBRARY COLLECTIONS

Books	17,194
Journals (Bound Volumes)	32,720
Journals subscribed for 2009	340
Journals received (Gratis/Exchange)	310
Microforms (Microfiche)	1,080
Slides	280
Reports	12,636
Theses & Dissertations	372
MEDLINE CD Rom Discs	383
Current Contents on Diskettes with abstracts. . . .		664
Proquest (Full Text E-Journals) on CD Rom		495
General CD's	83



Ph.D PROGRAMMES

Ph.D. Awardess

S. No.	Research Scholar	Title of thesis	University	Year
1.	Megha Saraswat (2003)	Screening of aldose reductase inhibitors and antiglycating agents from dietary sources and assessing their anticataractogenic potential	Osmania	2009
2.	Kiran Kumar B. (2002)	Genetic typing of WNIN/Ob and WNIN/GR-Ob strains using microsatellite markers	JNTU	2010

Research Scholars Registered for Ph.D.

S.No.	Research Scholar	Title of Thesis	Guide
1.	Mrudula. T (2003)	Characterisation and significance of a novel fatty acid elongase of the eye lens	Dr.Bhanuprakash Reddy G.
2.	Prashant A (2003)	Role of scavenger receptor class B1 (SR-B1) in reticulocyte differentiation, absorption of fat and fat soluble vitamins and female infertility using WNIN/Ob rat model	Dr.Vajreswari A.
3	Md.Naseeruddin (2004)	Understanding the role of micronutrients in pathogenesis of <i>Mycobacterium Tuberculosis</i>	Dr.Sudeep Ghosh
4.	Padmavathi I.J.N. (2004)	Role of maternal chromium status in the development of insulin resistance in the offspring.	Dr.Raghunath M.
5.	Satyanarayana B. (2004)	Biological significance of phytoferritins	Dr.Madhavan Nair K.
6.	Sreenivasulu K. (2004)	Caco-2 cell as a model to study bioavailability, mechanism of absorption and cytoprotective effects of zinc	Dr.Madhavan Nair K.
7	Vasuprada I. (2005)	Studies on the response and interactions of iron and zinc in Caco-2 cells	Dr.Madhavan Nair K.
8	Shashikiran G (2005)	<i>In vitro</i> regeneration of the insulin secreting cells from the adult pancreatic ductal epithelial cells (progenitors/stem cells)- The role of specific nutrients	Dr.Vijayalakshmi V.
9.	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.

S.No.	Research Scholar	Title of Thesis	Guide
10.	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.
11.	Manisha Ganeshan (2005)	Foetal origins of adiposity and insulin resistance: Role of peri/postnatal manganese status	Dr.Raghunath M.
12.	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.
13.	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.
14.	Pratibha B. (2005)	Immune status of WNIN mutant rats with reference to leptin and obesity	Dr.Giridharan N.V.
15.	Sreevani M. (2005)	Understanding & dissecting the role of resistin in etiology of insulin resistance using obese rat model	Dr. Nasreen Z. Ehtesham
16	Yadagiri Reddy P. (2006)	Biochemical studies on obesity induced cataractogenesis using WNIN obese rat model	Dr.Bhanuprakash Reddy G.
17	Naga Bala Shankara Srinivas P. (2006)	Studies on the significance of μ -crystallin heteropolymer in the eye lens	Dr.Bhanuprakash Reddy G.
18.	Anand Kumar K. (2006)	Maternal vitamin B12 restriction induced changes in body adiposity, hyperglycemia and insulin resistance in WNIN rat offspring: Molecular basis of the changes	Dr.Raghunath M.
19.	Priyanka Shankar (2006)	Study on high fluoride and low calcium on bone metabolism in rats: Biochemical mechanisms	Dr. Arjun L. Khandare
20	Y. Srinivasa Reddy (2006)	Effect of environmental lead exposure on infection and immunity in undernutrition	Dr.Kalpagam Polasa
21	Little Flower Augustine (2007)	Stress allostatic load and micronutrient status among higher secondary students: Impact of dietary advise	Dr. Madhavan Nair K.
22	P.Muthenna (2007)	Characterization of active principles from dietary sources, Mechanism of action of aldose reductase inhibitors and protein glycation inhibition	Dr.Bhanuprakash Reddy G.
23	A.Satyanarayana (2007)	Diabetic retinopathy - Role of micronutrients	Dr.Bhanuprakash Reddy G.

S.No.	Research Scholar	Title of Thesis	Guide
24	Agatha Betsy (2008)	Dietary assessments of exposure to select contaminants and intake of select nutrients among the various socio-economic groups of Hyderabad	Dr.Kalpagam Polasa
25	Sh.Prathipati Vijayakumar (2008)	Role of polyunsaturated fatty acids on regulation of obesity in an obese mutant rat model – WNIN/Ob	Dr.Vajreswari A.
26	Swarnim Gupta (2008)	Dietary diversification of Indian vegetarian diet to improve iron bioavailability: Studies using Caco-2 cell model	Dr. Madhavan Nair K.
27	Soundarya (2008)	Establishment of propoyable cell lines from adult adipose tissue of WNIN mutant rats (WNIN Ga/Ob and Ob/Ob)	Dr.Vijayalakshmi V.
28	Deethu Sara Varghese (2008)	Assessment of body composition in Indian females using different techniques	Dr.Venkataramana Y.
29	B. Sankar Anand (2009)	Role of T-cells and secreted cytokines in insulin resistance and obesity	Dr.Sudip Ghosh
30	Ramesh Athe (2009)	Meta analysis approaches on “Micronutrient food fortification and its effect on health, social and economic factors” – A statistical model building	Dr. Vishnuvardhan Rao M.
31	Nimgulkar Chetan Chandrakant (2009)	Evaluation of herbs/nutraceuticals products for anti-atherosclerotic agent	Dr. Dinesh Kumar B.
32	Mehraj-Ud-Din Bhat (2009)	Role of UPP in vitamin D deficiency induced muscle atrophy and hypo insulinemia	Dr.Ayesha Ismail
33	Azmeera Gandhi Nayak (2009)	Vitamin A metabolism in obesity	Dr.Vajreswari, A.
34	Anupama Tyagi (2009)	Anti-inflammatory potential of n3 PUFA in experimental ulcerative colitis: Biochemical and molecular study.	Dr. Ahmed Ibrahim S.
35	V. Sudhakar Reddy (2009)	To be registered	Dr.Bhanuprakash Reddy G.
36	Pallavi Namburi (2010)	Regulatory role of zinc in Hecpidin mediated iron metabolism	Dr.Madhavan Nair K.
37	N. Bindu (2010)	Effect of polyphenol action on cancer cell	Dr.Ayesha Ismail
38	Jitendra Kumar Sinha (2010)	IGF-1 and BDNF signaling in the brain of WNIN Obese mutant rats during ageing: Effect of calorie and micronutrient restrictions	Dr. Raghunath M.
39	Himadri Singh (2010)	Establishment of propagable cell lines from pancreas (Ductal Epithelial Cells) from WNIN-Ob and WNIN-Gr Ob rats	Dr.Vijayalakshmi V.
40	G. Kishore Kumar (2010)	To be registered	Dr.Bhanuprakash Reddy G.
41	Sara Sarin Joseph (2010)	To be registered	Dr.Bhanuprakash Reddy G.

AWARDS/ HONOURS RECEIVED BY THE SCIENTISTS DURING THE YEAR 2009-2010

Name of the Scientist	Award/ Honour received
Dr.A.Vajreswari <i>Scientist 'F'</i>	Selected as one of the IBC's Top 100 Scientist– 2009 in the field of Science by the International Biographical Centre, Great Britain.
Dr.R.Hemalatha <i>Scientist 'E'</i>	Awarded Best Paper Award for her paper entitled "Immunomodulatory activity of triticum aestivum (wheat grass) in Swiss Albino Mice" in the 6 th Indo-Australian Biotechnology Conference 2009.
Dr.D.Raghunatha Rao <i>Scientist 'E'</i>	Received Netherlands Fellowship to undergo training in Food and Nutrition Security. Awarded PG Dip. in Food & Security from Wageningen International, Wageningen University, Netherlands
Mr.GM.Subba Rao, <i>Scientist 'C'</i> Mr.SSV.Prasad, <i>SRF</i> Ms.Vasuprada Iyengar, <i>SRF</i> Mr.B.Satyanarayana, <i>SRF</i>	Received the <i>Young Scientists Travel Grant Award</i> to attend and present their work at the 19 th International Congress of Nutrition, held at Bangkok, Thailand during 4 th – 10 th October 2009.
Dr.N.Harishankar <i>Scientist 'B'</i>	Awarded K.R.Bharadwaj <i>Best Poster award</i> for his poster presentation entitled "WNIN/Ob, A novel obese rat model for the study on osteoporosis and osteoarthritis", at the International Conference on Laboratory Animal Sciences, Applications in Biomedical Research, held at Mumbai.
Dr.P.Suresh <i>Scientist 'E'</i>	Admitted as Fellow of Academy of Sciences for Animal Welfare F.A.Sc (AW).
Dr.G.Jagjeevan Babu <i>Scientist 'D'</i>	Awarded Masters in Public Health from Royal Tropical Institute, VU University, Amsterdam, Netherlands under Netherlands Fellowship Programme.
Dr.Sylvia Fernandez Rao <i>Scientist 'B'</i>	Awarded Young Scientist Award in the senior category for Community Nutrition in the 41 st Annual Conference of the Nutrition Society of India for her Paper entitled "Maternal well-being and locus of control as mediators in intervention for infant and young child feeding".
Dr.G.Krishna Swetha, <i>SRF</i>	Awarded the Best Oral Presentation in Clinical Nutrition category in the 41 st Annual Conference of the Nutrition Society of India for her Paper entitled "Micronutrient status and IL1 β , IL-6 in women with bacterial vaginosis".
Dr.A.Laxmaiah <i>Scientist 'E'</i>	Nominated to the International Health Professional of the Year for 2010 by the International Biographical Centre of Cambridge, England.
Ms.B.Prathibha, <i>SRF</i>	Awarded best poster presentation for her paper entitled "Immune response to hepatitis B vaccine in WNIN Obese mutant rats" in the International Conference on "Environment, Occupational and Life style concerns, Transdisciplinary Approach", held at Bangalore.

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

Date	Name of the Scientist	Conference/Meeting/Workshop/Seminar
2009		
March30 – June 12	Dr.D.Raghunatha Rao	Training in Food and Nutrition Security, at Wageningen International, Netherlands (NFP Fellowship).
May 3 - 7	Dr.G.Bhanuprakash Reddy	Annual Meeting of Association for Research in Vision and Ophthalmology (ARVO), Florida, USA. He delivered a talk on “A novel mutation in alpha-crystallin associated with age-related cataract results in defective chaperonelike function despite unaltered structure”.
July 6 - 8	Mr.T.Longvah	International Workshop on the “2010 Biodiversity Indicators and Post-2010 Indicator Development and Activities”, organized by UK Department of Environment, Food and Rural Affairs (Defra), Convention on Biological Diversity (CBD) and the United Nations Environment Programme World Conservation Monitoring Centre (UNEP - WCMC), United Kingdom.
Sept. 09 to Nov.24	Dr. K. Rajender Rao Visiting Scientist	Haward Hughes Medical Institute, The Rockefeller University, New York, USA. WNINOb/Ob Rat F ₂ population genotyping at Dr.Jeferry Friedman Laboratory.
Oct. 1 - 3	Mr.T.Longvah	8 th International Food Data Conference, organized by Institute of Nutrition, Mahidol University, Thailand. Presented a paper on “Cultivar differences and the effect of processing on the nutrient compositionof rice”.
Oct. 11 - 15		Workshop on “Culture, Environment and Agriculture for Food and Nutrition Security of Indigenous Peoples”, organized by International Union of Nutritional Sciences (IUNS)”, Kanchanaburi, Thailand. Presented a paper on “Food systems and nutritionof the indigenous people in North-east India”.
Oct. 4 - 8	Dr.S.Chennaiah	14th Workshop on Vitamin D, Brugge, Belgium. Presented a paper on “Effect of the supplementation of dietary rich phytoestrogens in altering the vitamin D levels in diet induced osteoporotic rat model”.
Oct.11 - 24	Dr.K.Bhaskarachary	Training programme at the 19 th International Graduation Course on the “Production and Use of Food Composition Data in Nutrition”, in Wageningen, Netherlands.

19 th International Congress of Nutrition, held in Bangkok, Thailand. The following members of the staff participated in the Congress and presented Papers (Oct.4-9)		
Symposium		
Dr.V.Sudershan Rao	Food safety awareness, practices and enabling assets in India –A nation-wide needs assessment study.	
Oral presentations		
Dr.A.Laxmaiah	The impact of 'positive deviance' programme for prevention and control of undernutrition among under 3 years in West Bengal, India	
Ms. Vasuprada Iyengar	Iron interactions with zinc during uptake in Caco -2 cells: evidence for the presence of an iron transporter other than DMT-1	
Mr.SSSV. Prasad	Effect of vitamin A feeding on 11beta-hydroxysteroid dehydrogenase 1 activity in liver and visceral fat of WNIN/Ob lean and obese rats	
Distinguished Posters		
Dr.P.Uday Kumar	Does maternal undernutrition affect human fetal pancreas morphology in second trimester of pregnancy? - An exploratory study	
Dr.M.Vishnuvardhan Rao	Millennium Development Goals: Where India stands in relation to nutrition and health	
Mr.GM.Subba Rao	Identifying critical issues and suitable approaches for food safety communication in India- Inferences from a nation-wide study	
Mr.B.Satyanarayana	Iron content and bioavailability of six rice genotypes using <i>in vitro</i> digestion/ coupled Caco- 2 cells	
Posters		
Mr.M.Maheshwar	Coverage of nutrition related topics by print media; A comparative analysis of leading newspapers in India	
Mr.GM.Subba Rao	Issues concerning implementation of inter -sectoral food and nutrition plans and policies in South - East Asian countries	
Dr.N.Harishankar	Determination of body composition in Syrian hamsters by non-invasive methods	
Nov. 12-13	Dr.A.Laxmaiah	Workshop on "Bridging Systems Sciences to Longitudinal and Cross -sectional data to set New Frontiers for the Multi-level Study of Childhood Obesity and other Diet and Lifestyle related Health Problems", at McGill University, Montreal, Canada.
Nov. 23-Dec.4	Dr.N.Balakrishna and Dr.KV.Radha Krishna	Meeting on growth modeling and determining growth trajectories of South Indian pre -school children, at Loughborough University, United Kingdom.

WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN

I. WORKSHOPS/CONFERENCES/SEMINARS

1. Workshop on "SAM Treatment in India: A Dialogue on Community based Interventions" in collaboration with Medicines Sans Frontieres (April 1-2, 2009).
2. Conference on "New Approaches to Infant and Young Child Feeding and Development", based on the results of the INDO-US Collaborative Study "The efficacy of an integrated feeding and care intervention among 3 to 15 months old rural children in Andhra Pradesh, India"(April 9, 2009).
3. NIN-WHO Joint Workshop on "Food Labelling Scenario" in India (June 24-25, 2009).
4. In connection with the World Food Day celebrations, a one day programme was organized on the theme "Achieving Food Security in Times of Crisis", in association with Association of Food Scientists & Technologists and Oil Technologists Association of India, Hyderabad (Oct. 16, 2009).
5. Pre-Conference Workshop on "Pre-Clinical Toxicology and Ethics in Animal Experimentation and GLP" as part of the XXIX Annual Conference of Society of Toxicology (STOX-2009) (Nov. 4, 2009).
6. XXIX Annual Conference of Society of Toxicology (STOX), India and International Symposium on "Current Trends in Toxicology and Pre-Clinical Toxicology" (Nov. 5-7, 2009).
7. Pre-conference Workshop on "Assessment of Nutritional Status and Dissemination on New RDA for Indians" for students delegates as part of the National Conference of Nutrition Society of India (Nov. 19, 2009).
8. The 41st National Conference of the Nutrition Society of India on the theme "Chronic Diseases: The New Pandemic" (Nov. 20-21, 2009).
9. Meeting of the NNMB Steering Committee (Dec.7, 2009).
10. Consultative Meeting on "Women's health in the context of hysterectomies-Medical and ethical issues", organized in association with *Life*-HRG and KICS (Jan. 9, 2010).
11. First National Conclave on "Defining Healthy Packaged Foods and Beverages", organised in association with Healthy You Foundation, New Delhi (Jan. 18, 2010).
12. National Seminar on "Traditional Indian Diets and Health Care", jointly organized by National Institute of Nutrition and Institute of Scientific Research on Vedas (I-SERVE), Hyderabad (Feb. 4-5, 2010).

II. TRAINING PROGRAMMES

1. 41st laboratory animal technicians training course was conducted by National Centre for Laboratory Animal Sciences (NCLAS) (15th June to 31st July, 2009).

2. Thirty eighth Annual Training Course on “Endocrinological Techniques and their Applications”. (August 17th - September 25th, 2009)
3. NIN-MoHFW Training of Trainers for the National Programme for Prevention and Control of Fluorosis. (Nov.10-11, 2009)
4. Training Workshop on “Non Invasive Techniques in Small Laboratory Animal Physiology”, was organized by National Centre for Laboratory Animal Sciences and Centre for Advanced Research in Pre-Clinical Toxicology. (Dec. 15-19, 2009)
5. 47th Post-Graduate Certificate Course in Nutrition. Eleven candidates from different States of the country participated in the Course. (Jan. 4-March 19, 2010)
6. Re-orientation Training in Nutrition organized for the officials of Food and Nutrition Board, Ministry of DWCD, Government of India, New Delhi. (March 8-12, 2010)



SERVICES RENDERED TOWARDS INCOME GENERATION

1. PATHOLOGY SERVICES

During the year, a total income of Rs. 3,38,770/- was generated from various projects of Institute's preclinical toxicology and surgical pathology and cytology samples.

2. TRAINING PROGRAMMES

An amount of Rs.3,98,000/- was generated from tuition fee collected from 16 participants of MSc (Applied Nutrition) course and 8 private candidates admitted to the regular training programmes viz., Post Graduate Certificate Course in Nutrition (7 participants) and Training Course on Endocrinological Techniques and their Applications (1 participant).



NIN SCIENTIFIC PUBLICATIONS – 2009

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

- 1 Amarendra Reddy G; Bharati Kulkarni; Veena Shatrugna; Thilak Ravindra Reddy P; Balakrishna N; Ajeya Kumar P; Usha Rani K : Bone mass of overweight affluent Indian youth and its sex – specific association with body composition. Arch Osteoporos. 4 : 31-39, 2009.
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- 12 Dinakar N; Nagajyothi PC; Suresh S; Damodharam T; Suresh C : Cadmium induced changes on proline, antioxidant enzymes, nitrate and nitrite reductases in arachis hypogaea L. J Environ Biol. 30 : 289-294, 2009.

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B. PAPERS PUBLISHED IN PROCEEDINGS/BOOKS

- 1 Anand Kumar K; Padmavathi IJN; Lalitha A; Manisha Ganeshan; Rajender Rao K; Mahesh Kumar J; Chandak G; Raghunath M : Chronic maternal Vitamin B12 restriction induced changes in the Wistar rat offspring are partly correctable by rehabilitation. *CMR@JOURNAL,* 2009, 95p.
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- 7 Bhanuprakash Reddy G : Nutritional and dietary factors in diabetic ocular complications, In “ 41st National Conference, Nutrition Society of India, Theme: Chronic diseases – The New Pandemic, 20-21 November, 2009, Hyderabad, National Institute of Nutrition, 2009, (SYM1-03) 25-26pp.
- 8 Bhanuprakash Reddy G : Structure and functional significance of heteropolymer of eye lens small heat shock protein, α -crystallin, Symposium speaker at the National Conference on Cellular and Molecular Biophysics conducted by Indian Biophysical Society, CCMB, Hyderabad, January 22-24, 2009.
- 9 Bhanuprakash Reddy G; Bhagyalaxmi SG; Srinivas PNB; Padma T; Barton KA; Petrash JA; Kumar KR; Vidyavathi M : A Novel mutation (f711) in α - Crystallin associated with age – related cataract results in defective chaperone – like function despite unaltered structure. Invest Ophthalmol Vis Sci. 50 : E.- Abstract 1637, 2009.
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- 12 Brahmam, GNV : Epidemiology of malnutrition. In : Symposium –cum – Training Programme, Developing capacity building of young scientists for carrying out basic, clinical and operational research in the field of nutrition – Agenda & Abstracts, March 21-23, 2009, National JALMA Institute for Leprosy and other Mycobacterial Diseases, Agra (U.P.), Organised by Indian Council of Medical Research, New Delhi, p.2.
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