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National Institute of Nutrition

(भारतीय आयुर्विज्ञान अनुसंधान परिषद)

(Indian Council of Medical Research)

ANNUAL REPORT

2010 - 2011

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
महानिदेशक, आई सी एम आर

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(स्वास्थ्य अनुसंधान विभाग)
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MESSAGE

The importance of optimal nutrition for health and human development is well recognized. Although most national development plans have included nutrition considerations for decades, the improvement in the nutritional status of the population is far from satisfactory. Further, there is growing recognition of the emergence of a "double burden" of malnutrition with under - and over nutrition occurring simultaneously among different population groups in our country.

National Institute of Nutrition (NIN) in its nearly 93 years of existence has always been keeping itself relevant in the ever-changing scenario of public health nutrition. During this centenary year, it is indeed delightful to note that the Institute is rising to the nutrition challenges confronting our country through its concerted research efforts in three broad settings -laboratory, clinical and community.

The country has been witnessing a surge in the prevalence of diabetes mellitus, hypertension, coronary heart disease and other diet related non-communicable diseases. This, in fact is a cause for concern, and the country is looking for cost - effective preventive and intervention measures on a war footing. For all these, we need to know of the magnitude and severity of these problems in diverse population groups. While large scale surveys provide data with utmost scientific rigour, well designed health and nutrition education campaigns could help tackle some of these public health problems effectively. The community studies carried out by the Institute in this direction provide newer insights into these realms.

It is also heartening to note that the institute has accorded priority to study the problems of some nutritionally vulnerable sections like lactating women, adolescent girls and HIV infected children. The food supplements using traditional foods like gingelly(til) to augment bone mineral density of nursing mothers, nutrition education for adolescents and assessing the health and nutritional status of children affected with HIV are some of the works which merit our attention here.

Institute's firm emphasis on basic science research this year is quite clearly visible. Studies on crop bio-fortification; relationship between vitamin B12, homocysteine and diabetic retinopathy and development of a suitable rodent model to study Type 2 diabetes are examples of good science in the area of basic studies.

Placing equal emphasis on both basic and applied aspects of research with public accountability in view is the need of the hour. I compliment the scientists and technical staff of the institute for providing fresh insights into various realms of nutritional sciences and I hope that their efforts will be further strengthened in future. It is expected that over a period of time, most of the research outcomes would get translated into a public health benefit. I am sure that scientists of NIN would continue to strive towards that goal.

(Vishwa Mohan Katoch)

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RESEARCH HIGHLIGHTS

1. COMMUNITY STUDIES

1.1 Prevalence and determinants of Hypertension and Type 2 Diabetes among 20-60 years old urban dwellers in Hyderabad, Andhra Pradesh

In the hypertension and diabetes study, 3102 urban adults (women: 50.6%) of 20-60 years were covered. The majority of urban men and women were literate (85%) and half of the men and one third of the women were educated (college and above). The mean BMI (kg/m^2) of men and women of 20-35 year adults was 23.1 (±4.36) and 23.8 (±4.84) respectively, while it was 25.4 (±4.44) and 27.4 (±4.91) among 36-60 years.

As per the BMI values for Asian, the prevalence of overweight and obesity was 46.2% and 51.9% among men and women of 20-35 years respectively, while it was 70.2% and 83.5% among 30-60 years. The prevalence of abdominal obesity (waist circumference; men: ≥ 90cm; women: ≥ 80cm) was 28.8% and 37.7% among men and women of 20-35 years, respectively, while it was 58.8% and 69.6% among 36-60 years.

The prevalence of hypertension (SBP ≥ 140 mm of Hg and /or DBP ≥ 90 mm of Hg) was 40.6% and 30.4% among men and women of 20-60 years respectively, while it was 55.8% and 49.3% among 36-60 years. The prevalence of T2 diabetes (≥ 26mg/dl) was 3.6% each in men and women of 20-60 years, while it was 21.9% and 19.4% among 36-60 years. It was significantly higher among the adult men and women who had obesity/abdominal obesity compared to normals.

Only 5% men and 4% women rated their health as excellent. About 80% men and 60% women were aware of their body weight and one third of men and half of the women were also aware that they had excess body weight and more than half of them were trying to lose their weight. Only one fourth of men and 10% women were doing mild to moderate exercise daily. Almost all men and women were watching TV at least two hours daily.

1.2 Survey conducted on physical and mental disabilities reported in the "Payakarao Peta" Legislative Assembly constituency of Visakhapatnam district in Andhra Pradesh

The objectives of the survey was to investigate high prevalence of physical deformities reported in "Payakarao Peta" assembly constituency of Visakhapatnam district and to recommend necessary interventions.

The preliminary survey conducted by NIN scientists revealed that 41 out of 11,350 people from four villages were affected with unusual disabilities. The overall prevalence was around 0.35% as against the national figure of 2.1%. In general, the disabilities were, mental retardation, physical deformities such as talipes equinovarus, genuvarum, kyphosis, deaf-mutism and blindness, with most of the cases among children of 10 years and above. About 50% of them reported history of birth asphyxia due to inadequate availability/utilization of health care facilities and history of consanguinity.

The expert committee survey in 7 villages covering 2.2 lakhs population revealed the presence of 58 out of 108 cases with mental retardation with or without cerebral palsy. Other deformities observed were microcephaly, deaf-mutism, blindness due to retinitis pigmentosa and

micropthalmia, dwarfism, cleft palate, talipes equinovarus, brachydactily, floppy child syndrome, choreo-athetosis, post polio residual paralysis, down's syndrome, trauma and hemiplegia. The independent survey conducted by the State government also revealed a prevalence of about 0.7%, which is also in line with the expert committee findings and much lower than the national average.

It was observed the reported problem was not unique/ specific to the area, reuiring in depth research evaluation. It was recommended that problem needs to be attended through medical, psycho-social, financial support and occupational rehabilitation through a multidisciplinary plan, involving local NGOs and philanthropic organizations. There is also need to generate data on community disability through a well designed epidemiological fact finder to guide future planning.

2. MICROBIOLOGY AND IMMUNOLOGY

2.1 To evaluate the efficacy of a *lactobacilli* preparation on bacterial vaginosis and vaginal immunity in healthy subjects and in patients with BV

- The three strains of *lactobacilli* (*L.brevis, L.salivarius Salicinius, L.plantarum*) induced resolution of BV in 76.5% of the women and restored normal vaginal flora (NVF) in 80 % of the women after 8 days local treatment.
- Cervical erosion and leucorrhea (vaginal WBCs) resolved substantially with local *lactobacilli* treatment.
- However, the effect on vaginal flora was very transient. The efficacy reduced to 60% by 15 days suggesting a longer and repeat treatment.
- The pH measurement for diagnosing BV is a good tool in resource-poor settings, because although it is not the most sensitive or specific test, it offers a middle ground on sensitivity and specificity compared with the more technologically demanding techniques. Moreover, this pH test performs better than the syndromic diagnostic algorithm.
- In symptomatic women, a high vaginal pH result would require further evaluation by a health care provider.

2.2 Effect of HIV on growth, morbidity and disease progression in HIV Infected children

- CED was prevalent in 19.5% at baseline and 23.9% at 1 yr.
- Undernourishment (38%), Stunting (45.5%) were quite prevalent.
- Nearly 50% were anemic, and they suffered from Iron, Folic acid and Vitamin D deficiency.
- In children receiving ART: Heights and weights increased significantly, Fat % and LBM were better and HIV viral load was lesser. Furthermore CD4 and CD8 counts were better.
- In children with morbidity – CD4 and Cd8 counts were lower and viral loads were higher.
- Serum albumin, Copper, Vit B_{12} correlated positively with CD4,CD8.
- Albumin correlated negatively with Viral load.
- Vitamin B_{12} correlated negatively with Ratio.

3. CLINICAL STUDIES

3.1 Calcium-rich food supplementation to lactating women from low socio-conomic group- Effect on bone density

Gingelly (til) a calcium-rich food when supplementated to under nourished women from the low income group resulted in significantly reduced loss of bone mineral density at the femoral neck and hip regions when compared to the control group. The study advances the knowledge in the area of bone metabolism during lactation and provides important information for the calcium requirements of lactating women.

3.2 Case control study of osteoporotic hip fractures

- Vitamin D levels were significantly (P< 0.001) lower in the fractures cases than the controls.
- There was high urinary fluoride excretion in the fractured cases and significantly (< P 0.001) more than the controls.
- At the femoral neck, 51% males and 76% females had osteoporosis, while, in the controls 19% males and 41% females had osteoporosis.

4. BASIC STUDIES

4.1 Establishment of screening facility for iron and zinc bioavailability using Caco2 cell- line

Biofortification of staple food crops such as rice, wheat and maize for iron, zinc and beta carotene through conventional plant breeding is considered as a sustainable strategy for improving their nutritional status in the population. During the process of development of a bio fortified product, screening for bioavailability of micronutrients assumes importance to ensure the intended beneficial outcome of these strategies. As a coordinating center for the nutritional studies of the Crop biofortification network project, a human intestinal cell line model, Caco-2 cell line, based bioavailability screening facility was established at this institute. Bioavailability screening methods for iron, zinc and carotenoids were developed and validated in the facility. The screening method for assessing the zinc bioavailability was used in selecting genotypes from 4 quality protein maize (QPM) variety and 6 hybrid maize genotypes with varying zinc content from 0.76- 2.34 mg/100g. Among the maize genotypes tested, 2 hybrid maize varieties showed the highest zinc content and bioavailability. Thus, the bioavailability screening method using Caco2 cell line can form an essential step in selecting genotypes for crop biofortification.

4.2 Development of a valid and reliable questionnaire for testing knowledge on micronutrients among adolescent students

Micronutrient deficiencies are rampant in India among all age groups. Absence of culture-appropriate nutrition education has been identified as one of the weakest links of nutrition intervention programs in India for targeting micronutrient malnutrition. One of the obstacles could be the lack of information on current nutrition knowledge of different communities, which in turn is attributable to the lack of validated questionnaire for testing knowledge among target groups. Since adolescence is an age group where maximum behaviour change is possible, the aim of the present study was to develop a psychometrically valid and reliable questionnaire for testing knowledge on micronutrients and to apply the test among a group of adolescents (16-18y) where micronutrient status was analyzed and to assess the relationship between knowledge and the biomarkers of micronutrient status. An 18 item questionnaire for adolescents was constructed with acceptable

validity and reliability. Knowledge on micronutrients measured using this questionnaire was found to be a significant predictor of plasma retinol status. This emphasizes the need for using validated questionnaires in nutrition research involving knowledge and if it is assessed correctly will relate to the nutritional status of the individual.

4.3 Maternal vitamin B12 and/or folate restriction induced changes in body adiposity, hyperglycemia and insulin resistance in Wistar rat offspring: molecular basis of the changes

In the earlier report, a Wistar rat model with chronic dietary restriction of folate and / or vitamin B_{12} increased body fat % (visceral adiposity) and fasting plasma glucose but not their insulin resistance status. While chronic maternal folate and / or vitamin B_{12} deficiencies increased the body weight of the offspring at/from weaning, altered their body composition (visceral adiposity) and induced insulin resistance (fasting and postprandial), most changes were mitigated at least partly by rehabilitation indicating their probable reversibility. Overall, chronic maternal folate and/ or B_{12} deficiency appeared to predispose the offspring to insulin resistance. Considering that abundant literature suggests trans-generational transfer of the effects of maternal under-nutrition in the offspring, we assessed whether or not the maternal folate and / or vitamin B_{12} restriction induced changes are transmitted to F2 generation. It was interesting to note that maternal folate and / or vitamin B_{12} restriction induced body composition changes were also seen in the F2 offspring. However, it was perplexing to see that changes were observed only in the male but not female offspring, both in F1 and F2 generations. The reasons for this gender bias are to be investigated.

4.4 Insulin, Insulin receptor and its signaling mechanisms in the brain and insulin sensitive target organs in NIN obese mutant rats (WNIN/Ob and WNIN/Gr-Ob) and Central regulatory mechanisms underlying obesity in WNIN Obese mutant rats

WNIN – Obese (sumo) rat developed at NCLAS, NIN, Hyderabad, India resemble Neuron specific insulin receptor knockout mice in that they are both hyperphagic, hyperinsulinemic, obese and infertile. These studies were conducted in six months old female WNIN/Ob rats to validate / negate the hypothesis, “impaired brain / hypothalamic insulin function / signaling could underlie the hyperphagia, obesity, in WNIN/Ob rats”. The findings suggest that impairment in brain / hypothalamic insulin signaling along with the impaired hypothalamic glucose uptake/energy homeostasis and the resultant failure to attain satiety in addition to decreased neuropeptide receptors and serotonin metabolism could either singly or together be responsible for the hyperphagia and obesity observed in the six months old female in WNIN/Ob rats.

4.5 Effect of different methods of cooking on natural antioxidant activity and phenolic content of green leafy vegetables commonly consumed in India.

Continuing the efforts to generate a database on phenolic content and antioxidant activity of plant foods commonly consumed in India including the effects of common domestic processing, this year the effect of conventional, pressure and microwave cooking on these parameters in green leafy vegetables was studied. In general, nine out of the eleven GLVs studies showed significant increase in their phenolic content and antioxidant activity on different types of domestic processing while only two them showed decrease. The fact that there was significant rank correlation among the PC and AOA among all GLVs studied both raw and processed suggests the importance of phenolics to the AOA of the GLVs studied in both these forms.

4.6 Vitamin B_{12} deficiency and hyperhomocysteinaemia in diabetic retinopathy

Although, many studies indicated an association between homocysteine and diabetic retinopathy (DR), the results so far have been equivocal. Amongst the many determinants of

homocysteine, B-vitamin status was shown to be a major confounding factor, yet very little is known about their relationship in DR. In the present study we found that higher homocysteine levels in DR were associated with lower vitamin-B_{12} but not with other B-vitamins. Additionally, hyperhomocysteineamia and vitamin-B_{12} deficiency do not seem to be related to the age, BMI and duration of diabetes. These results thus suggest a possible association between deficiency of plasma vitamin-B_{12}, hyperhomocysteinaemia and DR, for the first time. Further, these studies also indicate that vitamin B_{12} deficiency could be an independent risk factor for DR.

4.7 Animal model for type-2 diabetic complications

Chronic diabetes leads to various secondary complications. Although, there are many studies on complications of diabetes in experimental animals, most of the studies are conducted on type-1 diabetic animal models. However, barring some genetic models, there are no studies on type-2 diabetes (T2D)-induced complications in experimental conditions. A series of animal experiments were conducted with various T2D rodent models to evaluate a suitable animal model of T2D not only to understand the possible mechanisms involved in the development but also to prevent or delay diabetic complications. These studies indicate that neonatal-streptozotocin (nSTZ) WNIN-GR/Ob model could serve as a suitable model for studies on T2D-induced complications, particularly diabetic cataract and also for dietary intervention studies.

4.8 Novel ALR2 inhibitors

Aldose reductase (ALR2) catalyzed accumulation of osmotically active sorbitol has been implicated in the development of diabetic complications. A new natural active principle (piplartine) has been isolated from black pepper with ALR2 inhibitory potential, and using this natural molecule as a lead molecule, a novel hybrid compounds as ALR2 inhibitors by chemical transformation through Michael addition were synthesized. These novel compounds (particularly 3c, 3d, 2j and 3e) are more potent than the well know inhibitors sorbinil and fidarestat. Thus, these novel ALR2 inhibitors might be useful for the treatment and/ or prevention of diabetic complications.

4.9 Importance of α-crystallin heteropolymer

Eye lens α-crystallin exists as a heteropolymer composed of two homologous subunits, αA and αB. Despite the critical role of α-crystallin in many tissues, little is known regarding structural and functional significance of the two subunits. Herein, we describe a unique feature of αB-crystallin. At high temperatures not only αB-crystallin aggregates but also enhances the aggregation of other lens proteins. Intriguingly, αB-crystallin-mediated coaggregation involves β - but not γ-crystallin. Further, α*A*-crystallin, but not a mutant (F71L) α*A*-crystallin, prevented aggregation of αB-crystallin and also reduced coaggregation of αB- and β-crystallin. These studies explain the rationale for the existence of α-crystallin heteropolymer with αA subunit as a major partner that is vital for lens transparency. Hence, heteropolymer with 3:1 αA to αB ratio might be vital for eye lens transparency under diverse conditions to prevent cataract.

5. FOOD AND DRUG TOXICOLOGY

5.1 Flagship Project WNIN/Ob mutant rat model to studyDNA damage and mutagenicity testing

NCLAS at NIN has established two obese mutant rat models- WNIN/Ob and GR/Ob, former with euglycemia and the later with hyperglycemia. These animals show distinct physical, physiological and biochemical indices of obesity and age faster than the normal wistar rats. Apart from obesity these rats shows incidence of tumors (60%), cataract (10%), opportunistic infections (100%) and kidney abnormalities (80%). Carcinogenic process is known to be preceded by

damage to DNA. This is known to induce alterations in cellular genome and altered gene expression. Accumulations of such mutations are associated with ageing and other mutation based degenerative diseases like cancer, diabetes, cataract etc. Since these mutant rats shows obesity and obesity related degenerative chronic disorders, it is possible that they harbour large proportion of damaged DNA & accumulation of age related end products.

The result of the study showed that there was no significance in the antioxidant status of DNA damage and antioxidant enzymes in obese and lean rats compared to the WNIN rats as they did not exhibit any induction in the strand breaks in the blood tested as evidenced by the alkaline comet assay.

5.2 Assessment of Environmental Lead Exposure on Infection and Immunity

It is known that Lead, the ubiquitous environmental pollutant causes sub-clinical organ system damage specially to haemopoietic, renal and nervous system. Undernutrition *per se* may aggravate lead toxicity. Current evidences suggest that elevated lead levels alter immune functions by enhancing lymphocyte proliferation and possibly increase severity of infectious diseases. Micronutrient deficiency specially Fe may hamper immune function.

Elevated levels of lead and micronutrient deficiency may alters immunity and enhance lead induced cytotoxicity. Against this background a study was taken up with the following objectives,

1. To assess the immune function in Pb exposed iron deficient animal model.
2. To determine the effect of oral Pb exposure on intestinal microflora in iron deficient rats.
3. To evaluate the protective effects of thiamine on Pb induced inhibition of *Lactobacill* and *E. coli*.

The results revealed that chronic Pb exposure even at low levels can reduce the immune functions in iron deficiency. The *in vitro* bacterial culture studies have shown protective effect of thiamine against Pb induced bacterial inhibition.

6. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

6.1 Effect of Mucuna pruriens on WNIN obese rats

In Indian system of medicine, several medicinal plant extractions are used to prevent or to treat human diseases. The ancient Unani medicine, mentions the use of *Mucuna pruriens* mainly as an aphrodisiac. We tried to ascertain the ascribed medicinal properties of *M.pruriens* ethanolic seed (velvet beans) extract in an unique inbred obese mutant rat model – WNIN/GR-Ob, an obese rat with prediabetes established at our institute. The obese male and its lean counterparts were fed with 0.5 gm hand made pellet containing 6 mg of the seed extract (normal dose group), and 1.0gm hand made pellet containing 12 mg of the seed extract (high dose group) for 45 days. The rats were analyzed for lean body mass (LBM), body fat and extra cellular fluids by a Total Body Electrical Conductivity (TOBEC) instrument. The circulatory levels of plasma glucose, cholesterol, triglycerides, reproductive hormones like testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin were also measured. Levator ani (LA) muscle weight and semen analysis parameters like sperm motility, sperm count, gonadial index and histology of testis were investigated additionally, along with histology of liver and testis.

The experimental rats showed a significant decrease in body fat, blood glucose and lipids, with an increase in LBM, total body sodium and potassium and fat free mass as compared to controls. Serum testosterone, prolactin, LH and FSH hormone levels showed a significant increase in treated rats as compared to controls. Among the treated groups the high dose treated animals

showed significantly higher values compared to normal dose treated rats. The LA muscle weight was increased 2-3 times and also a significant increase in terms of sperm count and motility was found in extraction treated rats compared to control rats. Histologically, steatosis of fat was significantly reduced in the livers and significant improvement was seen in the architecture of the testes with densely packed spermatids in the seminiferous tubules of treated obese rats. Thus, the seed extract of *M.pruriens* was found to have significant benefits in terms of reduction in total fat content, lipids and increase in lean body mass as expected and additionally in hypoglycemic effect as well. It also showed its potential as an aphrodisiac in terms of improvement of reproductive hormone profile and semen quality in infertile WNIN obese rats. The hypolipidemic effect shown in this study, is a new finding which adds to the list of other benefits of this traditionally used aphrodisiac.

I. COMMUNITY STUDIES

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

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

The study was conducted in six states viz. Andhra Pradesh, Maharashtra, Tamil Nadu, Karnataka, Nagaland & Manipur, and four National highway segments, adopting a uniform protocol. The study was conducted at two points of time during the project period, the baseline survey was undertaken during 2005-07 while the second round was conducted during 2009 -10.

The National Institute of Nutrition carried out the baseline survey in Andhra Pradesh in the eight high prevalence districts of Visakhapatnam, East-Godavari, Guntur, Prakasam, Chittoor, Warangal, Karimnagar and Hyderabad. The results of the second round in comparison to the earlier round are presented in the current report.

OBJECTIVES

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

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

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

METHODOLOGY

It was a cross-sectional study, using two- stage sampling procedure. The districts were selected based on criteria of socio-cultural regions and the high proportion of female sex worker population. The study included female sex workers (FSWs), men having sex with men (MSM) and clients of female sex workers.

Two alternative sampling approaches viz; "conventional cluster sampling" for the selection of Brothel based FSWs and Clients of FSWs and Time location cluster sampling (TLCS) for the selection of street based FSWs and MSM, were used in the study. Within each of the selected cluster, simple random sampling procedure was used for the selection of study subjects. The sample size was estimated at 400 respondents for each group of subjects for each district.

The Behavioral indicators such as socio-demographic characteristics, sexual history and behavior and Knowledge, attitude and practices about STI and its prevention, and the biological

indicators such as prevalence of HIV and STIs such as, syphilis, neisseria gonorrhea, chlamydia trachomatis and herpes simplex virus type 2 (HSV-2) were studied.

The analysis of biological samples was carried out during the first quarter of the reporting year. The data on behavioral, biological, clinical aspects was checked and subjected to double data entry (one entry by the research agency and other by the NIN), as a check for entry errors. The errors were rectified and statistical analysis was carried out and report writing of MSM group was completed.

A workshop was conducted at NIN to disseminate the results to all the stake holders and the implementing partners and another workshop was conducted by National AIDS Research Institute to share the lessons learnt during round II of the study by the participating centers and document the same for future guidance in research. Two manuscripts were prepared and were communicated to Indian Journal of Medical Research and journal of Bio-med central.

RESULTS

A total of 400 respondents of each category of subjects viz; female sex workers, men having sex with men and clients of female sex workers were recruited for the study in each of the selected district.

Salient observations with regard to socio-economic, demographic particulars, risk behavior, prevalence of STI and knowledge, attitude about STI and HIV and practices towards control & prevention of STI and HIV across the select districts in Andhra Pradesh are presented by category of study subjects are given below.

Female Sex Workers

- The mean age of the FSWs surveyed was about 30 years. A higher proportion (53%) of FSWs from Chittoor district were literate as compared to other districts.
- A large proportion of FSWs still reportedly depended on sex work as the main source of income in all the districts, ranging from a high of 67% in Prakasam district to a low of 35% in Visakhapatnam district.
- The mean age of first sexual contact (15 -16 years) and the mean age at initiation of commercial sex (22-26 years) across the districts remained similar during both the rounds.
- The average number of clients entertained on the last day worked by a FSW remained similar to that of first round, ranging 1.8 in Hyderabad to 2.8 in E.Godvari districts. While, the volume of clients entertained during the past week was low at 8.7 in Visakhapatnam and maximum at 13.2 in Prakasam district, an increase of client volume was observed in seven districts during the second round.
- The mean duration of sex work among the FSWs surveyed ranged from 3.9 years in the district of Chittoor to 8.7 in E.Godavari district.
- About 54-66% of the FSWs across the districts reported that their clients requested for having anal sex with them. While, the proportion of FSWs who actually ever had anal sex was low at 12% in E.Godavari district and high at 37% in Karimnagar district.
- Most of the FSWs (86 -100%) had occasional clients, while 57 – 99% had regular clients. The proportion of FSWs having regular non-commercial partners ranged from 63 – 83%.
- Barring Guntur, Chittoor and Karimnagar (32-34%) a majority of the FSWs (50 – 69%) in the other districts carried condom at the time of interview.

- The extent of consistent condom use with different types of clients ranged from 70-97% with occasional clients, through 61-96% with regular clients to a low <1-29% with regular non-commercial partners, in various districts. In general, there was an increase in consistent condom use with all the types of sexual partners.
- Majority of FSWs surveyed reported that they were aware of signs and symptoms of STIs among women as well as men and also were aware of HIV/AIDS. The proportion of FSWs who were aware of correct methods of prevention of HIV/AIDS ranged from 50-71% across the districts. There was an increase in the awareness about HIV & its correct preventive methods.
- The prevalence of HIV among FSWs surveyed was maximum in East Godavari district (23.3%) compared to only 6.5% in Karimnagar district. The prevalence of syphilis ranged from a high 17.9% in E.Godavari district to 1.5.% in Warangal district. Similarly, the prevalence of Chlamydia Trachomitis was high (10.3%) in E.Godavari district while Gonorrhea was highest (11.5%) in Hyderabad district. The prevalence of HSV-2 ranged from about 43% in Warangal district, to 90% in Hyderabad district.

Men Having Sex With Men

- A majority (27 to 51%) of MSM surveyed were *Kothis* in nature, followed by Bisexuals (15 to 30%). The mean age of respondents ranged from 26-29 years and a majority of them were literate.
- The proportion of currently married MSM ranged from 26% in Visakhapatnam to 52% in Guntur district.
- A small proportion (3 to 13 %) of MSMs reported that sex work was the main source of income.
- All of the respondents had multiple types of partners, which included, paying male, paid male/Hijra, paid female, other non-commercial male/hijra and regular female partners. Majority of respondents (>90%) had other non-commercial male/hijra partners, followed by paying male partners (28 – 64%), regular female partners (30-58%), paid male/hijra partners (6-45%) and paid female partners (<1 – 23%), across the districts.
- The proportion of respondents carrying condom at the time of interview ranged from a low 36% in Visakhapatnam to a high 54% in Hyderabad district. About 32 to 95% of respondents of Visakhapatnam district used condom consistently with all types of partners as compared to remaining districts. Within Visakhapatnam district the consistent condom use was maximum with paid female partners ((95%). A small proportion of respondents (8-32%) used condom consistently with their regular female partners.
- Majority of MSM (52 to 92%) reported that they were aware of atleast two signs and symptoms of STIs. Almost all the respondents were aware of HIV/AIDS. However, only 51-72% of respondents were aware of correct methods of prevention of HIV/AIDS.
- The prevalence of HIV was high among MSM of Hyderabad district (28.9%) compared to 4.9% in Visakhapatnam district. Similarly, the prevalence of syphilis was also high in Hyderabad district (12.6%) compared to 1.9% in Visakhapatnam district.
- The prevalence of HSV-2 was high (74%) among the MSM of Guntur district, as compared to a low of 38% in Visakhapatnam district.

Clients of Female Sex Workers

- The mean age of clients of FSWs across the districts surveyed ranged from 29 years to about 31 years. A majority of them (69% in E.Godavari to 96% in Warangal) were literate.

- About 46 to 64% of clients of FSWs were married.
- The mean age at first sexual act was about 18 to 20 years and the initiation of commercial sex was at about 20 -23 years, across the districts. On an average, each client had sex with 2.1 FSWs per month in Hyderabad district to a maximum of 2.9 in E.Godavari district. Similarly, the mean number of commercial sex acts during the past six months ranged from a low 6.3 in Visakhapatnam to a high 9.5 in E.Godavari district. In general, there was a reduction of mean commercial sexual acts during the current round as compared to the earlier round.
- The proportion of clients having regular FSWs was high at 84% in E.Godavari district and low at 45% in Visakhapatnam district. Almost all of the clients had occasional FSWs as their sexual partners.
- A small proportion of clients of FSWs (4 to 26%) carried condom at the time of interview across the districts.
- Consistent use of condom with occasional FSWs ranged from a low 70% in Visakhapatnam to 80% in Warangal district. While, the consistent condom use with regular FSWs ranged from a low 55% in Viskhapatnam to a high 71% in Hyderbad district. Similarly, consistent condom use with male/Hijra partners ranged from 58% in Hyderabad to 100% in Warangal district. In general, there an increase of consistent condom use with all the types of sexual partners during the current round as compared to the earlier round.
- Almost all of the clients of FSWs were aware of HIV/AIDS. A very small proportion of the respondents ranging from 2% in Guntur to 13% in Visakhapatnam district believed that they were at risk of being infected with HIV due to commercial sexual behaviour.
- The prevalence of HIV among clients of FSWs is relatively low (2.8 in Warangal, 9.6% in East Godavari) across districts as compared to the other risk groups studied. The prevalence of syphilis ranged from a low of 0.1% in Warangal district to a high of 2.1 in E.Godavari district. The prevalence of HSV-2 is high (43%) among the clients of FSWs who belonged to Warangal district compared to a low 23% in Hyderabad district.

2. PREVALENCE AND DETERMINANTS OF HYPERTENSION AND TYPE2 DIABETES AMONG 20 - 60 YEARS URBAN POPULATION, HYDERABAD, ANDHRA PRADESH

Demographic, epidemiological and nutritional transition in the world including India has made changes in population structure, eating behaviour and disease pattern. Proportion of middle aged and elderly population is rapidly increasing, leading to increase in the burden of chronic morbidity and mortality. Deaths due to cardiovascular diseases rank first, followed by diabetes both in the developed and developing countries in the world. Among the cardiovascular diseases, coronary artery disease (CAD) is the commonest one. Effective primary prevention of CAD requires an assessment of associated factors to categorize patients for selection of appropriate interventions. It also provides critical background information that can be used in the development of guidelines and for the treatment.

The major independent risk factors for CAD include tobacco smoking of any amount, elevated blood pressure, elevated serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), low serum high-density lipoprotein cholesterol (HDL-C), diabetes mellitus and advancing age. The Framingham Heart Study and other studies have elucidated the quantitative relationship between these risk factors and CAD.

The American Heart Association (AHA) also identifies obesity and physical inactivity as major risk factors for CAD. The adverse effects of obesity worsened when it is expressed as abdominal obesity, which is an indicator of insulin resistance. Among these, environmental factors and lifestyle patterns are modifiable. Thus, keeping in view, the paucity of data on the prevalence of these conditions among the urban population in India, it is proposed to assess the prevalence of hypertension and type-2 diabetes and it's associated factors in the urban community to help in the formulations of appropriate preventive strategies. Since, the risk and fatality is relatively high in the middle age group, the present survey will be carried out in these age groups.

OBJECTIVES

General Objective

To assess the prevalence of hypertension and type-2 diabetes among 20-60 year urban population living in the limits of Municipal Corporation of Hyderabad (MCH), Andhra Pradesh.

Specific Objectives

1. To assess the prevalence of obesity in terms of body mass index (BMI), Waist & Hip circumference and waist-hip-ratio.
2. To assess the prevalence of hypertension as per the JNC criteria VII.
3. To assess the prevalence Type-2 diabetes and impaired glucose tolerance by using auto-analyzer.
4. To assess the prevalence of hyperlipidemia in a sub-sample of subjects.
5. To assess the B_{12} and homocysteine levels in sub-sample of target population.
6. To assess the life style patterns of target population such as physical activity levels, dietary patterns, and leisure time spent, habits and addictions etc; and
7. To assess their knowledge and practices (K&P) in relation to hypertension and Type-2 diabetes mellitus.

METHODOLOGY

Study Design

It was a cross sectional study and cluster-sampling procedure was adopted.

Setting and the sampling frame

Urban community, living in the Municipal wards of Greater Hyderabad Municipal Corporation (GHMC), Andhra Pradesh, were used as sampling frame.

Subjects and selection procedure

Thirty municipal wards were selected from the list of municipal wards obtained from Commissioner of Municipal Corporation of Hyderabad, using 30 cluster sampling technique. From each cluster, about 120 adults (both men and women) of 20-60 years were recruited consecutively to carry out the proposed investigations by starting from northeast corner of the cluster, irrespective of their socio-economic status.

Outcome variables

The outcome variables were prevalence of overweight, obesity, hypertension (JNC VI/WHO Criteria), type2 diabetes mellitus (ICMR/WHO criteria), insulin resistance, micronutrient deficiencies and its associated factors and knowledge and practices of selected adults on diet related chronic non-communicable diseases.

Investigations

I) Socio-economic and demographic particulars

Socio-economic and demographic particulars such as age, sex, occupation, literacy level, family income, community and type of dwelling were collected with the help of structured and pre-tested schedules on all the selected adults covered for nutritional anthropometric measurements.

ii) Nutritional anthropometry

Anthropometric measurements such as height (cm), weight (kg) and mid upper arm, waist and hip circumference (cm), fat fold thickness at multiple sites (triceps, biceps, sub-scapular and supra-iliac areas) among adult men and women of 20-60 years were measured using standard equipment and procedures.

iii) Measurement of blood pressure

Systolic and diastolic blood pressure was measured in recumbent posture using mercury sphygmomanometer on all the adults selected for nutritional anthropometry. The measurements were carried out for three times, with a gap of 5 minutes between the measurements in order to avoid '*white coat effect*'. The average of the three measurements was considered as the final measurement.

iv) Estimation of blood glucose levels

Measurement of fasting blood glucose levels were assessed using one touch glucometer (Johnson & Johnson make) among most of the available adults and those were with compliance of fasting and glycosulated hemoglobin and *homa*-insulin was also estimated on a sub-sample of subjects.

v) Estimation of lipid profile and other biochemical parameters

Lipid profile (Total cholesterol, Triglycerides, high density and low density lipoprotein), micronutrients such as riboflavin, folic acid, B_{12}, homocysteine, serum creatinine and blood urea was estimated on a sub-sample of subjects.

vi) Food frequency questionnaire survey

For the purpose, specially prepared and pre-tested food frequency questionnaire, was used to assess the dietary patterns from all the subjects covered for the study.

vii) Life style patterns

Information about the physical activities such as games & sports, physical exercise, leisure time spending, duration of sleeping, TV viewing etc. and risk behaviours such as tobacco consumption, use of beverages and alcohol was collected with the help of pre-tested schedule from all the subjects covered for the anthropometry.

viii) Knowledge and practices

Knowledge and practices of the subjects on overweight and obesity, hypertension and diabetes was also collected with the help of structured schedule from all the adults covered for the anthropometry.

Sample size

The sample size required for various investigations among adult men and women of middle-aged individuals (20-60 years) is provided in the Table1.

Ethical issues

The project was approved by the Scientific Advisory Committee (SAC-NIN) and Institutional Ethical Review Board (IRB) of National Institute of Nutrition, Hyderabad. Individual written informed consent was obtained from all the subjects covered for the survey.

Training and standardization

The Medical Officers, Nutritionists, Social Workers and Technicians, who were involved in the survey, were trained and standardized at NIN for a period of three weeks, in various methodologies used in the survey. During the training, emphasis was given to achieve the maximum intra and inter-individual agreement in respect of all the measurements.

Quality control

Quality control was carried out by repeating some of the measurements already carried out by the investigators by the field supervisors of the division with periodical intervals (Table 1).

Table 1

Investigations	Estimated Prevalence	Design Effect	C.I	Relative Precision	Required Sample size	Sample required
Blood glucose estimation	10%	2	95%	20%	1730	1800
BP measurement	20%	2	95%	20%	770	1800
Anthropometry	-	-	-	-	-	1800
Socioeconomic & demographic particulars	All the subjects covered for Anthropometry					1800
Food frequency questionnaire survey	All the subjects covered for Anthropometry					1800
Knowledge & practices	All the subjects covered for Anthropometry					1800
Estimation of lipid profile, homa-insulin, glycosulated Hb, homocystein, etc.	Sub-sample of adults covered for Anthropometry					300
Micronutrients estimation (riboflavin, folic acid, B_{12})	Sub-sample of adults covered for Anthropometry					300
Renal function tests (blood urea, sr. creatine)	Sub-sample of adults covered for Anthropometry					300

The salient findings of the study are as follows:

Coverage

The urban adults covered in this survey was 3,102 (women: 50.6%) in the age group of 20-60 years in the Greater Hyderabad Municipal Corporation area.

Socio-economic profile

About 83% of men and women were living in *pucca houses,* while 16% were in semi-*kutcha*. Majority of the men were engaged either in service or business, while women in household chores. Majority of urban men and women were literate (85%) and half of the men and one third of the women were educated (college and above). The average monthly family per capita income was about Rs. 21,500/-

Anthropometry

The mean weight of men and women of 20-35 years was 65.6kg (±13.82) and 56.8kg (±11.94) respectively, while the mean height was 168.2cm (±6.62) and 154.4cm (±6.15) respectively. The mean BMI (kg/m^2) of men and women of 20-35 year adults was 23.1 (±4.36) and 23.8 (±4.84) respectively.

Similarly, the mean weight of men and women of 36-60 years was 70.3kg (±13.83) and 64.1kg (±12.40) respectively, while the mean height was 166.1cm (±6.92) and 153.0cm (±5.97) respectively. The mean BMI (kg/m^2) of men and women of 36-60 year adults was 25.4 (±4.44) and 27.4 (±4.91) respectively.

Prevalence of general obesity

Prevalence of overweight (BMI 25 - <30) was 23.9% and 25.1% among men and women respectively in the young adults (20-35 years), while obesity (BMI 30) was 6.7% and 10.6% among men and women respectively. As per Asian cut-offs, the prevalence of overweight and obesity was 46.2% and 51.9% among men and women respectively. Similarly, the prevalence of overweight was 35.5% and 40.4% among men and women in the older adults (36-60 years), while obesity was 14.7% and 26.4% among men and women respectively. As per Asian cut-offs, the prevalence of overweight and obesity was 70.2% and 83.5% among men and women respectively.

Prevalence of abdominal obesity

Prevalence of abdominal obesity (waist circumference; men: 90cm; women: 80cm) was 28.8% and 37.7% among men and women respectively in the age group of 20-35 years, while it was 58.8% and 69.6% among men and women respectively. The prevalence of central obesity (WHR ; men: 0.90; women: 0.80) was 52.1% and 56.5% among men and women respectively in the age group of 20-35 years, while it was 88.5% and 80.8% respectively.

Percent body fat

The mean sum of skin-folds was 64.7mm (±27.12) and 83.8mm (±29.91) among men and women respectively in the young urban adults, while in the older adults (36-60years), the sum of skin-folds was 73.4mm (±26.52) and 99.8mm (±26.27) among men and women respectively.

The total body fat per cent (BF %) was 21.6 and 33.6 among men and women in the age group of 20-35 years, while it was 28.8% and 39.7% among men and women respectively.

Prevalence of hypertension

In general, the mean systolic blood pressure levels were 120.5 mm of Hg (±12.37) and 112.9 mm of Hg (±13.12) among men and women respectively in the age group of 20-35 years, while it was 130.1 mm of Hg (±16.55) and 124.4 (±16.96) among adult men and women respectively in the age group of 36-60 years. In case of diastolic blood pressure, it was 81.8 of Hg (± 10.27) and 76.4 mm of Hg (±9.17) men and women respectively in the age group of 20-35 years, while it was 87.5 mm of Hg (±11.40) and 83.13 mm of Hg (±10.75) among men and women of 36-60 years. The prevalence of hypertension (140 mm of Hg SBP and /or 90 mm of Hg DBP) was 40.6% and 30.4% among men and women respectively in the age group of 20-60 years. However, it was significantly lower (P <0.001) in the age group of 20-35 years (men: 26.1%; women: 11.7%) compared to 36-60 years (men: 55.8%; women: 49.3%).

Prevalence of IGT and type-2 diabetes

The mean fasting blood glucose levels were 98.6mg/dL (±32.22) and 96.9 mg/dL (±34.67) among men and women respectively in the age group of 20-60 years.

The prevalence of T 2 diabetes (126mg/dl) was 3.6% each in men and women in the age group of 20-60 years, while it was significantly higher (P <0.001) in the age group of 36-60 years (men: 21.9%; women: 19.4%). The prevalence of impaired glucose tolerance (fbg 110 to <126mg/dL) was 3.4% and 2.2% in men and women respectively in the age group of 20-35 years, while it was significantly higher in the age group of 30-60 years (men: 7.2% and women: 5.7%).

The prevalence of hypertension was significantly higher among the adult men with abdominal obesity as compared to normal men, similarly among women also the prevalence of hypertension was significantly higher with abdominal obesity as compared to normal women.

Similarly, the proportion of hypertension was significantly higher among the adult men who were overweight and obese as compared to normal men and CED and similar trend was observed among women also.

Knowledge and practices of adults about obesity, hypertension and diabetes mellitus

The information on knowledge and practices of urban adults about obesity, hypertension and diabetes and their risk behaviours are as follows:

More than half of men and women stated that their health was good; while only 5% men and 4% women rated their health was excellent. Only one fourth of men and women were covered by health insurance and rest of them depended on out of pocket expenditure towards unexpected medical expenditures. About 80% men and 60% women knew their body weight and of them one third of men and half of the women knew that they had excess body weight and more than half of them were trying weight loss measures.

About 16% men and 0.3% women were smokers. About half of men and three fourth of women had monitored their blood pressure measurement at least once in a year. About one fourth of men and one fifth of women were diagnosed as hypertensives and majority of them were receiving anti-hypertensive drugs. Surprisingly, more than half men and women had been using extra-salt at dining table. Only less than 10% men and 5% women were using fruits daily, while 95% men and women were using vegetables once or twice in a day. About 10% men and women were eating chicken/meat once or twice daily. About 75% men and women were consuming chicken/meat at least once in a week. About one fourth men and 5% of women were going out at least once in a week either for lunch or dinner. Only one fourth of men and 10% women were doing vigorous to moderate exercise daily. Almost all men and women were watching TV daily.

3. ASSESSMENT OF PHYSICAL AND MENTAL DISABILITIES REPORTED IN THE "PAYAKARAO PETA" LEGISLATIVE ASSEMBLY CONSTITUENCY OF VISAKHAPATNAM DISTRICT IN ANDHRA PRADESH – ICMR EXPERT COMMITTEE STUDY

In response to the representation received from local MLA and on the instructions of Union Minister for Health and Family Welfare, a survey was carried out in the *'Payakarao Peta'* Legislative Assembly segment by an expert committee constituted by the Director General, ICMR, The survey was carried out at two different times, a rapid survey in the month of April followed by a more

comprehensice study during October of 2011. The state health staff also participated in the exercise.

OBJECTIVES

The aim of the survey was to investigate the prevalence of physical and mental disabilities if any and suggest remedial measures to the Union Government.

The members of the team made house to house visit in the some of the villages along with the local primary health staff, anganwadi workers and the 'ASHA' volunteers, visited the outpatient departments of PHCs, gathered records from the PHCs and also examined results of the independent survey on physical and mental deformities carried out by DM & OH, Visakhapatnam, through state health staff, on door to door basis, for arriving at reasonable conclusions about the situation reported.

SALIENT OBSERVATIONS

- The overall prevalence of disabilities in the areas surveyed was 0.35% as against the national prevalence of 2.1% (as per the census 2001 of India), which is lower than the overall prevalence of congenital deformities ranging from 3.2 to 3.7 per hundred live births reported for the country according to a study conducted during 1989-92, and reported in Indian Journal of Pediatrics.
- The survey carried out by DM & OH, Visakhapatnam through state health staff, on door to door basis, indicated an overall prevalence of 0.7% of both physical and mental health problems in the 4 mandals in the constituency.
- Out of 105 cases observed during the visit, 58 (55.2%) of subjects had mental retardation either with or without cerebral palsy. Two subjects were also having microcephaly. Idiopathic deaf mutism without MR was present in 5 individuals. Of the total cases of blindness (n=8, 7.6%), blindness due to retinitis pigmentosa was seen in 5 cases and micropthalmia was present in 3 cases. Dwarfism was present in 8 (7.6%) subjects. The blindness and dwarfism observed in these cases was found to be familial, except for one family. Other deformities either acquired or congenital include cleft palate, Talipes equinovarus, brachydactyly, floppy child syndrome, choreo-athetosis, post- polio residual paralysis etc in 17 (16.2%) cases.
- Home deliveries and birth asphyxia and other bad obstetric history was mostly reported among the children with physical deformities.
- Consanguineous marriages which are widely prevalent in the areas surveyed, could be one of the major reasons for the prevailing congenital deformities
- There was no evidence suggestive of environmental iodine deficiency in the form of prevalence of Iodine deficiency disorders or evidence of consumption of diets containing goitrogens.
- Identification of genetic mutations for prevention of selected genetic conditions can be carried out in collaboration with established centers in the country. The expertise of CCMB and CDFD can be used for the purpose.
- In general, there is a significant burden of physical deformities and mental retardation in the community, that needs a comprehensive multidisciplinary plan, involving local NGOs and philanthropic organizations besides medical care. It may require special financial allocation by the State/Central government to provide a life of dignity for the disabled children.

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 that the 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 caregivers 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'.*

OBJECTIVES

- To develop culture-appropriate home-based nutrition education intervention to improve feeding skills and caring behaviours of caregivers 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 caregivers 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 caregivers, 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.

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 and behaviors Interview schedule on child health and development.
5. Previous week morbidity schedule – every month
6. CES-D maternal depression scale
7. Maternal autonomy interview schedule
8. Maternal self esteem scale and locus of control scale
9. Demographic, household and socio-economic status schedule
10. HOME Inventory
11. Anthropometry: Infant supine length, weight - monthly from 4 to 15 mo
12. Maternal height & 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 that 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 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 R^2 was significant with a higher coefficient (B=0.17*) among children in complementary food group compared against the control group. None of the other nutritional indices were significantly different between the groups.

Development

The mean mental development scores were significantly different between the groups [F (2, 519) =4.08, p= 0.018 -Table 10] 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.

CONCLUSIONS

The 12 month educational intervention through pictorial flip-charts based 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 complementary food group 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.

II. MICROBIOLOGY AND IMMUNOLOGY

1. EFFECT OF HIV ON GROWTH, MORBIDITY AND DISEASE PROGRESSION IN HIV INFECTED CHILDREN

Children account for around 4% of total HIV cases, but 20% of deaths due to HIV infection occur in children. 25% of perinatally infected children progress rapidly to AIDS within one year and 50% of them die within 2 years. Malnutrition is a common complication of HIV infection and AIDS. State of immune system, viral load and the nutritional status critically determine the outcome.

HIV infected neonates are smaller than HIV exposed but uninfected neonates. In older children stunting is the most prevalent abnormality. 42% of prepubertal HIV infected children have growth velocity less than 5th percentile. Poor growth has been associated with poor survival in HIV infected children. In U.S. poor weight gain is an independent risk factor for death in HIV infected children. In Uganda, infants with low weight had a 5-fold increase in risk of death. Body fat is relatively preserved compared to body cell mass, which is distinct from nutritionally malnourished children. HIV replication is significantly associated with low fat free mass (FFM). The need to understand growth retardation associated with HIV infection and to find appropriate strategies to prevent it are very high priorities in the pediatric AIDS research agenda.

Limited studies have demonstrated that macronutrient supplementation positively impacted in people living with HIV. However, there is little information regarding the prevalence of nutrition deficiency, nutritional supplementation among HIV-seropositive individuals in India particularly in children. Though multivitamin supplements have shown beneficial effects in HIV-infected adults in Africa, there are no studies carried out in India either on adults or on children. Therefore, in the study, macro and micro nutrient intake, HIV viral load and disease progression in children were evaluated. The perceived outcome of the study was development of strategies to improve the quality of lives of HIV infected children.

AIMS AND OBJECTIVES

To determine nutritional status, body composition, macro and micronutrient intake of HIV infected children.

To assess the association of nutritional status with HIV viral load and disease progression (CD4 counts).

MATERIALS AND METHODS

This was a cross sectional study to assess the nutritional status of children with HIV. Base line anthropometric status, biochemical indicators of nutritional status (CBP, Vitamin A) were determined by HPLC; Vitamin D, B12, folic acid were done by RIA; serum zinc, iron, and calcium were done by AAS). Serum albumin was done by autoanalyser. Viral load was done by nucleic acid detection method by Realtime PCR. CD4/CD8 counts were done by Flow cytometry. 24-h dietary recalls questionnaire concerning dietary intakes during the 24 hr was collected at base line. Energy and nutrient intakes were derived by using a food-composition database. Anthropometry such as height and weight were collected every month. Skin fold measurements were done once every three months. Serum nutrient levels, CD4/ CD8 count and plasma viral load were assessed at baseline, 6 months and 1 year.

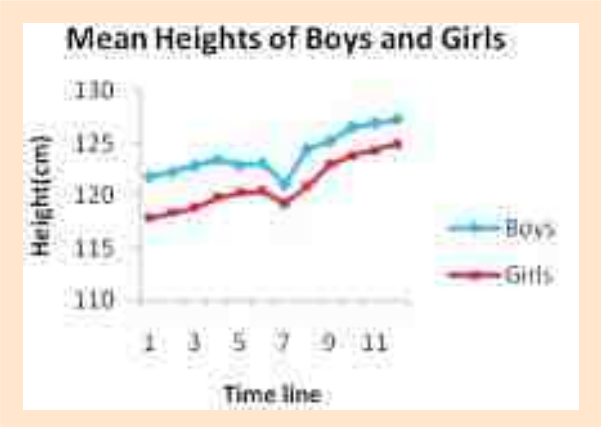
Work done during the year

A total of 77 children including 37 males and 40 females were recruited. These children were from two orphanages.

Baseline data was collected on 10th July 2009 and the children were followed for 1 year. The age ranged from 1.7 to 15.5 years and the mean age (standard deviation) was 9.1 years (38.2).The mean heights were 121.8cm and 117.9cm and weights were 22kg and 21.5 kg in boys and girls respectively at baseline. The mean heights and weights of the children increased consistently over a 12 month period, except for a dip in heights and weights at the 7th month, which could probably due to the increased incidence of several morbidities such as chicken pox, pneumonia, tuberculosis etc around that time (Fig 1). Proportion of children with chronic energy deficiency (CED) seemed to increase with time, being 19.5% at baseline, 23.7% at 6th month and 23.9% by the end of 1 year, with boys having more CED than girls(29.7% vs 10%) (Table 2). Fat (kg) and Lean body mass (LBM) had significantly increased with time (Table 3).

A total of 45.5% children were anemic (had Hb <11gm/dl) which reduced to 23.8% by 6 months. Vitamin D deficiency was prevalent in 51.9% and folic acid deficiency in 46.8% children. There was an increase in folic acid deficiency by 6th month (66.2%) which again tapered to 40.3% by 1 year. Serum zinc was deficient in 16.9% children at baseline which increased to 62.3% by 6 months. Low levels of serum albumin (<3.5g/dl) were detected in 36.4% children at baseline and gradually reduced to 7.8% children having low serum albumin by the end of 1st year (Table 4). The means of serum nutrients are depicted in Table 5.

Fig 1. Heights and Weights of HIV infected children over a 12 month period



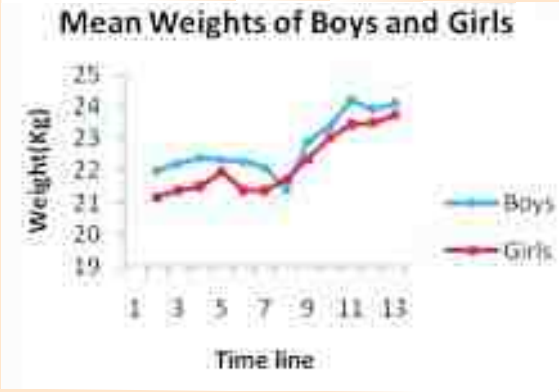


Table 2. Chronic energy deficiency in HIV infected children

	Baseline	6th month	12th month
Boys(n=37)	29.7(11)	24.3(9)	33.3(12)
Girls(n=40)	10(4)	23.1(9)	14.3(5)
Whole group (n=77)	19.5(15)	23.7(18)	23.9(17)

Values are Proportions.
Number of children in parenthesis

Table 3. Body composition parameters of HIV infected children

Parameter	Baseline (n=77)	6th month (n=74)	12th month (n=68)
Fat %	16.70 ± 5.05	16.84 ± 4.99	16.67 ± 5.17
LBM	17.84 ± 4.6*	18.3 ± 5.4	19.8 ± 5.03

Values are Mean ± SD.
** P<0.01(Significant difference between baseline and 6th month followup)*

Table 4. Proportions of HIV infected children with nutrient deficiencies

Parameter	Baseline (n=77)	6th month (n=77)	12th month (n=77)
Hb(gm%) (<11gm%)	45.5%(35)	23.4 %(18)	2.6%(2)
S.Vitamin A (<20 µg/dl)	14.3%(11)	9.1%(7)	ND
25 OH Vit D (<20 ng/ml)	51.9%(40)	42.9%(33)	24.7%(19)
S.Zinc (<70 µg/dl)	16.9%(13)	62.3%(48)	19.5%(15)
S.Iron (<70 µg/dl)	49.4%(38)	24.7%(19)	20.8%(16)
S.Copper (<60 µg/dl)	1.3%(1)	ND	ND
S.Folic acid (<10ng/ml)	46.8%(36)	66.2%(51)	40.3%(31)
Serum albumin (<3.5g/dl)	36.4%(28)	22.1%(17)	7.8%(6)

Values are proportions. Number of children with deficiency in parenthesis.
ND=Not done

Table 5. Comparison of nutrients at 3 time points

Parameter	Baseline	6th month	12th month
Hb(gm%)	11.87 ± 3.37	12.57 ± 2.06	13.57 ± 1.48
Vitamin A (µg/dl)	34.04 ± 15.32	36.15 ± 12.95	40.02 ± 12.19
Vitamin D (ng/ml)	19.83 ± 7.46	23.76 ± 15.85	17.86 ± 6.92
Zinc (µg/dl)	93.05 ± 40.83	66.14 ± 12.79*	84.81 ± 43.73
Iron (µg/dl)	87.42 ± 48.06*	112.26 ± 59.13	85.71 ± 41.09
Copper (µg/dl)	121.29 ± 32.58	116.88 ± 32.68	107.57 ± 26.85
Vitamin B12 (pg/ml)	717.19 ± 344.23	1231.21 ± 1289.73	1893.12 ± 1813.98
Folic acid(ng/ml)	13.3 ± 10.26	9.74 ± 11.68	11.41 ± 9.94
S.albumin(gm/dl)	3.63 ± 0.52*	3.99 ± 0.82	4.25 ± 0.55

Values are Mean ± SD.
** P<0.01(significance difference between baseline and 6th month followup)*

Clinically, the most common morbidities reported were Respiratory followed by Dermatological. Tuberculosis was the most common opportunistic infection. Absolute CD4, CD8 counts increased over time and HIV viral load reduced over time (Table 6). When data was analyzed based on whether children were receiving antiretroviral therapy or not, CD4:CD8 ratio was higher and HIV viral load were significantly lower in the children who were on ART (Table 7). Body composition and anthropometry parameters such as Mean Fat (kg), LBM, heights and weights were all

Table 6. CD4, CD8 counts and HIV viral load in children with HIV

Parameter	Baseline (n=76)	6th month (n=33)	12th month (n=29)
Abs CD 4 (cells/µl)	1058.97 ± 866.16	1173.96 ± 659.55**	990.41 ± 455.56
Abs CD 8 (cells/µl)	1488.64 ± 1423.2*	1511.37 ± 632.97	1498 ± 897.94
HIV viral load(IU/ml)	89818.11 ± 266769.38	86395.44 ± 337401.08*	51277.45 ± 123956.04

*Values are Mean ± SD., * P<0.05, **<0.01 (Significant difference between baseline and 6th month followup)*

Table 7. Comparison of Serum Nutrients in children with and without ART

Parameter	**ART** (n=41)	**No ART** (n=34)
Hb	11.58 ± 0.52	12.21 ± 0.57
Vitamin A	37.54 ± 2.66	30.24 ± 2.32*
25 OH Vit D	21.09 ± 1.08	18.27 ± 1.37
Zinc	99.65 ± 6.7	84.65 ± 6.29
Iron	94.04 ± 7.34	78.15 ± 8.75
Copper	128.5 ± 5.47	112.11 ± 4.57*
S.Albumin	3.69 ± 0.08	3.55 ± 0.09
Folic Acid	11.96 ± 1.28	14.89 ± 2.15
Vit B 12	816.89 ± 57.64	602.39 ± 51.58**
HIV viral load	7882.2 ± 3264.14	18500.17 ± 62994.44**

*Values are Mean ± SE. * P<0.05, **P<0.01 significant difference between groups*

Table 8. Comparison of CD4,CD8,Ratio and Viral load in children with & without morbidity

Parameter	Baseline (n=77)		6th month (n=33)	
	Children with morbidity (n=8)	**children without morbidity** (n=69)	**Children with morbidity** (n=5)	**children without morbidity** (n=28)
Abs CD4 (cells/µl)	755.82 ± 327.74	1094.63 ± 903.515*	1241.67 ± 705.04	869.27 ± 251.10
Abs CD8 (cells/µl)	1333.19 ± 552.06	1506.93 ± 1494.08	1533.07 ± 661.05	1413.71 ± 527.11
CD4:CD8 Ratio	0.56 ± 0.19	0.77 ± 0.42*	0.78 ± 0.27	0.65 ± 0.22 *
HIV viral load(IU/ml)	265659 ± 504624.57	69130.94 ± 221206.32*	102300.4 ± 372144.22	14823 ± 30077.93

*Values are Mean±SD * P<0.01(significant difference between children with and without morbidity)*

significantly more in children receiving ART. All serum nutrients were higher in children on ART compared to those not on ART.

On comparing the CD4, CD8 counts and viral load in children with and without morbidity, the absolute CD4, CD8 counts and ratio was significantly higher in children who suffered with morbidity while the HIV viral load was lower (Table 8).

2. TO EVALUATE THE EFFICACY OF A LACTOBACILLI PREPARATION ON BACTERIAL VAGINOSIS AND VAGINAL IMMUNITY IN HEALTHY SUBJECTS AND IN PATIENTS WITH BV- PHASE 1

Bacterial vaginosis (BV) represents the most common vaginal syndrome afflicting premenopausal and pregnant women, with an incidence rate ranging from 10% to 50%. BV is complex, polymicrobial disorder characterized by an overgrowth of strict or facultative anaerobic bacteria (*Gardnerellavaginalis, Prevotella, Mobiluncus, Mycoplasma horminis*) and a reduction of

lactobacilli particularly those producing hydrogen perioxide. BV is frequently underestimated since the symptoms are often insignificant, however, the clinical consequences could be important. BV facilitates the acquisition of sexually transmitted diseases such as *Neisseria gonorrhoeae, Chalmydia trachomatis,* HIV and Herpes simplex virus type-2 infection (HSV-2). Cure rate of BV with oral or local administration of metronidazole or intravaginal clindamycin, ranges from 48-85%, however, 40% of women relapse within 3 months after initiation of antibiotic therapy and up to 50% of women after 6 months. The high recurrence rates with repeated exposure to antibiotics, results in the emergence of drug resistant strains. Since eduction of lactobacilli and increase in pH are the main pathogenesis of BV, Probiotics have been suggested as a tool to treat and prevent BV.

The use of lactobacilli to re-establish a physiological microbial flora of the female uro-genital tract dates back to early 1990s. From the beginning of the nineties, there has been a renewed interest in the use of probiotic products in the treatment and prevention of BV. In the past years, some studies have evaluated the effect of probiotic or intravaginal pH lowering compounds (pH pessary) in the treatment of vaginal infections in an attempt to recreate an environment unfavourable to the growth of pathogens. Although treatment of BV with pH-lowering compounds has been reported to be successful in some studies, other trials have shown vaginal acidification to be ineffective treatment for BV. In the present double blind study, the efficacy of probiotics (3 different strains of lactobacilli in the form of vaginal pessary) and pH-lowering compounds in women with BV and without BV was evaluated.

MATERIALS AND METHODS

Subjects

Healthy non-pregnant, married women, living with husband, aged 20 to 40 years; were recruited from urban slum after taking written consent. The criteria for including subjects in the study were 8^{th} to 10^{th} day of menstrual cycle, absence of bleeding during examination, non utilization of oral antibiotics or contraceptives or vaginal medication in the last 10 days and no sexual intercourse for last 2 days before sample collection. All enrolled women denied using douches or tampon and were non-smokers. General and gynecological examinations were done to evaluate their general health. The study was conducted in accordance with Declaration of Helsinki and current Good Clinical Practice and was approved by the Institute's Ethical Committee, NIN. Written informed consent was obtained from the patients.

Study medication

The test preparation consisted of probiotic vaginal pessary containing at least 10^9 viable lactobacilli (*L.brevis, L.salivarius subsp. Salicinius, L.plantarum)* (Lactobacilli) and the pH lowering compound (pH pessary). Treatment consisted of one Lactobacilli pessary inserted into the vagina daily at bedtime for 8 days.

STUDY DESIGN

This was a randomized, double-blind, placebo-controlled study. Subjects were assigned to therapy with active or placebo preparation according to a computer generated randomization scheme. None of the staff or patients had access to the randomization codes during the study. The medications were dispensed by the investigator at the initial visit; compliance was assessed by counting returned tablets and questioning the patients.

Evaluation and scheduling

Demographics and medical history concerning previous history of reproductive tract infections, any chronic disease were assessed at baseline. Pelvic examination and assessment of clinical

signs and symptoms of BV was performed at baseline and after 9 days from the beginning of therapy. After Pelvic and speculum examination, swabs were collected for gram stain and cervico-vaginal wash for cytokine estimation. Gram stained smears were scored for gram negative and gram positive bacteria, clue cells, yeast and pus cells to diagnose bacterial vaginosis or vaginitis. BV was evaluated by Gram stain score of vaginal smears.

Statistical analysis

Since, the data were skewed, log transformation was performed to analyze continuous variables. Two way Repeat Measure ANOVA-F test was performed to assess interactions over time. ANOVA were used for descriptive data. Multiple linear regression models were used to evaluate the associations of different variables on the cytokine concentration of lavage samples. P-Values <0.05 were considered as statistically significant. Statistical analysis was performed using SPSS statistical software (SPSS Inc, Chicago, IL, USA).

RESULTS

Study population

Hundred and eighty nine subjects were screened, of whom 30 were excluded as they did not fulfill inclusion criteria. Of the 159 women, 67 had bacterial vaginosis (BV) (Nugents' score>7), 50 had intermediate flora (IF) (Nugents' score 4-7) and 42 had normal vaginal flora (NVF) whose Nugents' score was between 1 to 3; participated in the trial. Of the 67 patients with BV, 37 were randomly assigned to receive the lactobacilli preparation and 30 to receive ph pesary. There were 6 dropouts from the BV group (did not return for the follow-up visits) leaving 61 evaluable patients (active treatment, n=34, placebo, n=27). Baseline characteristics of women randomly assigned to test preparation or placebo were demographically and clinically similar (Table 9). Of the 42 women with NVF, 22 were randomly assigned to receive the test preparation and 20 to receive pH pessary. There were 3 dropouts from this group leaving 39 evaluable subjects (active treatment, n=20, placebo, n=19).Of the 50 IF cases, 23 were randomized to receive lactobacilli and 27 were randomized to receive ph pessary. With 2 dropouts, and had 48 evaluable cases of IF (lactobacilli, n=21, ph pessary, n=27). Baseline characteristics of women randomly assigned to lactobacilli preparation or pH pessary were demographically and clinically similar.

At the first follow-up visit on the 9th day, the proportion of women with >4.5 pH reduced in the lactobacilli treated group, while there was an increase in the pH pessary group, but significant change was not observed. On the other hand, mean nugent's score and proportion of women with Amsel's criteria decreased significantly on 9th day follow-up both in the lactobacilli or pH adjustment tablets group; however, more significant change was observed in the lactobacilli treated group (Table 9). Normal vaginal flora (NVF) increased significantly in the lactobacilli treated group. Similarly, proportion of women with clue cells reduced significantly ($p<0.001$) from the initial number in those treated with lactobacilli, while in the pH adjustment tablets although there was a change, it did not reach statistical significance. Leucorrhea (vaginal WBC) decreased by 36% in the lactobacilli group, while it increased in the pH adjustment tablets group (Tables 9 & 10). Thin homogenous discharge and vaginal candida infection did not show any change.

When percent change in various parameters was assessed, there was a 76 % reduction in the BV and 80% increase in the NVF with lactobacilli treatment compared to 55% and 52% respectively with pH adjustment tablets. Intermediate flora increased in both the groups. Cervical erosion decreased by 15% with lactobacilli, while it increased by 25% with pH adjustment tablets. Clue cells and vaginal WBCs decreased in lactobacilli treatment (Table 10 & 11). When subjects were categorized into BV and NVF, there was a significant improvement in vaginal flora as measured by

nugent's score with both pH adjustment tablets alone and with lactobacilli (Table 11) and a significantly higher proportion of women retained normal vaginal flora (NVF) with lactobacilli treatment (Table 12). However, as indicated in fig 2 the effect of lactobacilli (florisia) or pH tabs (placebo) on vaginal flora was very transient, with increase in proportion of women with BV and decrease in proportion of women with NVF by the second follow up, that is on 16th day from baseline.

Effect of lactobacilli on vaginal proinflammatory cytokines showed a significant reduction in all the three cytokines with lactobacilli treatment for 8 days, whereas, the pH tablets increased IL6 and had no effect on TNFα or IL1β (Fig 3).

Table 9

No	Parameter	Lactobacilli			Ph pessary		
		0 day	9th day	P value	0 day	9th day	P value
1	>4.5 pH	75.0% 62/82	66.6% 50/75	NS	66.6% 50/75	72.6% 53/73	NS
2	Mean Nugent's score	5.5±0.76	3.6±1.62	0.001	5.5±0.76	4.13±1.87	0.01
3	Amsel criteria + ve	70.7% (58/82)	50.6% (38/75)	0.02	63.3% (50/77)	42.4% (31/73)	0.05
4	Normal Vaginal flora	26.8% 22/82	48% 36/75	0.007	25.9% 20/77	39.7% 29/73	NS
5	BV +	45.1% 37/82	10.6% 8/75	0.0001	38.9% 30/77	16.4% 12/73	0.004
6	Women with clue cells	71.6% (58/81)	50.6% (38/75)	0.01	71.4% (55/77)	57.5% (42/73)	NS
7	Women with vaginal WBC cells >5 (leucorrhea)	23.2% (19/82)	16% (12/75)	NS	15.5% (12/77)	20.5% (15/73)	NS
8	Vaginal Candida infection	40.7% 33/81	44% 33/75	NS	35.1% 27/77	38.3% 28/73	NS

Table 10. Percent change in vaginal flora & other parameter after 8 days treatment with lactobacilli or Ph treatment

	lactobacilli	Ph tabs
Resolution of BV	76.5%	55. 6%
Increase in IM	47.6%	18.5%
Restoration of NVF	80.0%	52.6%
Clue cells decreased	34.5%	23.6%
Amsel criteria normalisation	34.5%	38.0%
Cx. erosion	-15.2%	+21.4%
Vaginal WBC	-36.8%	+25%
Candida infection	No change	3% increase

Table 11. Effect of florisia or pH adjustment tablets in patients with BV

Parameters (Mc nemar test or chi square test)	Lactobacilli (day 0 vs day 9) (p-value)	Ph tabs (day 0 vs day 9) (p-value)
Reduction in thin homogenous discharge	0.999	0.057
Reduction in Positive whiff	0.66	0.054
Reduction in Clue cells	0.039	0.999
Candida present	0.361	0.288
Trichomonas present	--	0.999
Improvement in vaginal flora by Nugent score (Binomial test)	0.009	0.006

Table 12. Effect of florisia or pH adjustment tablets in subjects with NVF

	Subjects with normal vaginal flora (NVF)	
	lactobacilli	Ph tabs
Reduction in thin homogenous discharge	0.195	0.999
Reduction in Positive whiff test	0.14	0.523
Candida present	0.17	0.999
Trichomonas present	--	--
Retaining normal vaginal flora (NVF) (Binomial test)	<0.001	0.359

Fig. 2 Effect of lactobacilli and ph tabs on vaginal flora

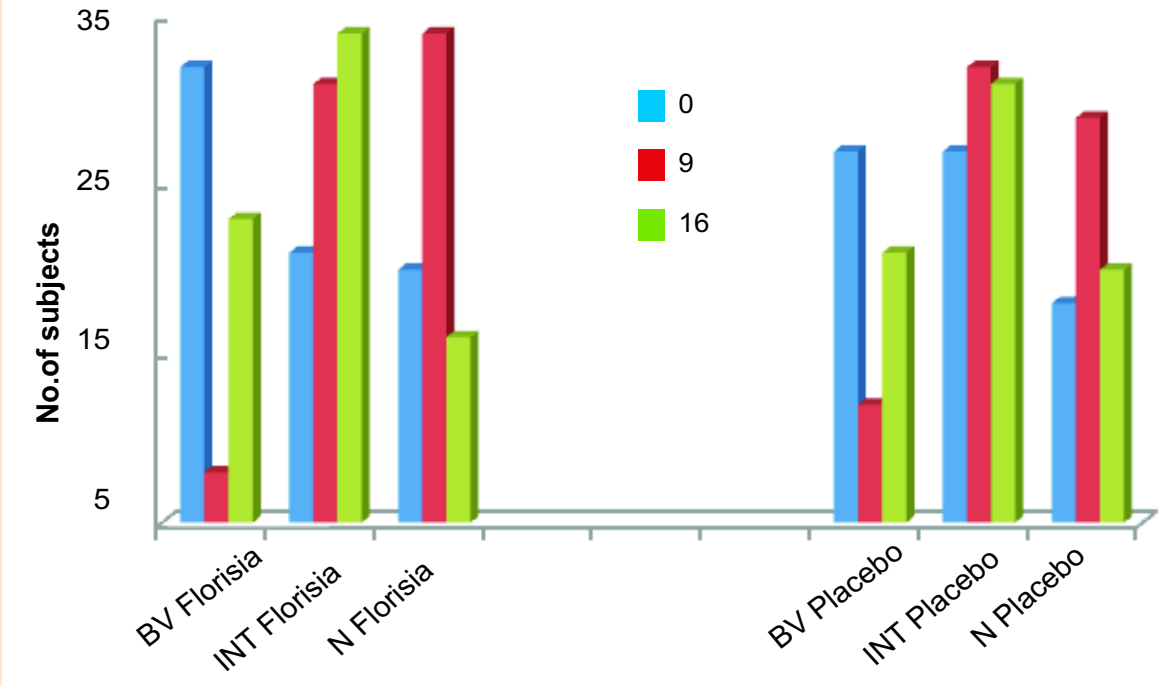
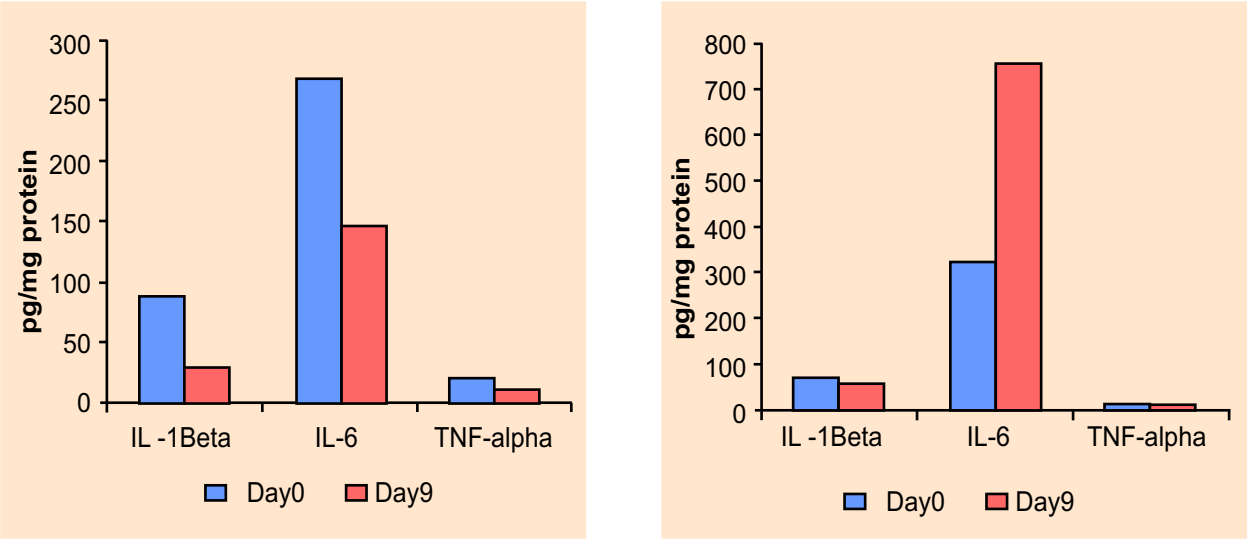


Fig 3. Effect of cytokine levels after administration of pH adjustment tablets with lactic acid bacteria



CONCLUSIONS

1. The three strains of lactobacilli (*L.brevis, L.salivarius Salicinius, L.plantarum)* induced resolution of BV in 76.5% of the women and restored normal vaginal flora (NVF) in 80 % of the women after 8 days local treatment.
2. Ph adjustment tablets induced resolution of BV and restoration of NVF in 55% and 53% respectively.
3. Cervical erosion and leucorrhea (vaginal WBCs) resolved substantially with local lactobacilli treatment.
4. However, the effect on vaginal flora was very transient. The efficacy reduced to 60% by 15 days suggesting a longer and repeat treatment.

Influence of hormones can be ruled out as all the study subjects were recruited on 9th & 10th day of the menstrual cycle and none were on any contraceptive method. Nevertheless, effect of attrition bias should not be excluded as 7 subjects dropped out of the study. Pharmacological therapies, such as clindamycin or Metronidazole generally show resolution of BV in 75% & 60% of patients with 2 weeks treatment. The study demonstrated a similar effect, but the effect was very transient, suggesting a longer course of treatment. The strengths of the current study are higher sample size and results are not influenced by contraceptives or douches; hormonal effect should be minimal as sample collection was restricted to 9^{th}& 10^{th} day of the cycle. But it must be noted that probiotic efficacy in resolving BV in the current study did not show 100% efficacy as reported earlier and the effect was transient, that is, the efficacy reduced to 60% by 15 days suggesting a longer and repeat treatment.

3. TO EVALUATE THE EFFICACY OF A LACTOBACILLI PREPARATION ON BACTERIAL VAGINOSIS AND VAGINAL IMMUNITY IN HEALTHY SUBJECTS AND IN PATIENTS WITH BV – PHASE 2: VALIDATION OF FLORISIA pH GLOVE

Reproductive and sexual health is fundamental to individuals, couples and families, and the social and economic development of communities and nations. Vagina is a unique environment for bacterial colonization. Among premenopausal women, ovarian hormones facilitate vaginal colonization with lactobacilli, which maintains a pH between 3.8 and 4.2. Abnormal vaginal discharge is one of the most frequent complaints in women attending gynecological clinics. Bacterial Vaginosis [BV] is the most common cause of vaginal discharge among women in reproductive age. It is a condition of vaginal flora imbalance, influenced by environmental, hygienic, hormonal and other factors. Assessment of intravaginal pH is a helpful, but frequently neglected, diagnostic procedure used to evaluate vaginal health. Awareness of the extent and consequences of reproductive ill health has increased over the past decades. This can be considered to improve women self examination techniques for good reproductive health. An easy, rapid and inexpensive self-diagnostic test for vaginitis may help to minimize the tendency to self-treat vaginitis inappropriately.

Florisia glove is a new research method forwarded to evaluate vaginal health and for improving the quality of life of women, especially in resource poor settings. Therefore, the aim of this study was to validate the Florisia glove as a screening tool to detect an abnormal pH and as an additional tool for the health care personnel or by extension, even for the self-diagnosis by the patient herself of abnormal vaginal flora in the presence of bacterial vaginosis and other RTI's.

METHODOLOGY

Study population

This cross-sectional study enrolled 464 volunteer women by convenience from gynecological outpatient clinics at Tertiary Care Government Hospitals and a Community health clinic located at Hyderabad beginning around October 2007. Informed consent was obtained from women offering explanations about the study. Some of the women were also recruited for a prospective study. The criteria for including subjects in the present study were as follows: 18-45 years of age, married, having menstrual cycles, non-pregnant women (only subjects with a negative urine human gonadotropin test result were allowed to continue), and absence of bleeding during the examination, no recent history of abortion/MTP in the preceding 3 weeks, no recent history of vaginal and cervical manipulations, no known primary immuno-deficiency and no history of gynecological cancer. This study was approved by Ethical Committee. A detailed interview gathered data on demographic characteristics and reasons for the clinic visit. Physical examination included general examination and anthropometric measurements. Gynecologic examination and laboratory monitoring were performed.

Criteria for diagnosing BV

The Gram stain is believed to be the gold standard for diagnosing BV. For Gram stain a standardized 0-10 point score was assigned by Nugent. A Nugent Score (NS) of 7-10 is classified as BV; 4-6 as intermediate BV and 0-3 as normal. Other clinical criteria for diagnosing bacterial vaginosis requires the presence of 3 of the following 4 Amsel criteria: 1) Thin homogeneous discharge; 2) Vaginal pH greater than 4.5; 3) Positive “whiff” test or release of amine odor with the addition of 10%KOH and 4) Clue cells on microscopic evaluation.

Sample collection

Vaginal swabs for vaginal pH, gram stain, wet mount, and whiff test; vaginal pH measurement with Florisia Glove; Cervicovaginal lavage collection. After inserting a non lubricated speculum vaginal secretions were collected by rubbing a cotton-tip applicator over the lateral vaginal wall, transferred on the Indikrom pH indicator strips (Qualigens fine chemicals, Glaxo India Ltd, Mumbai) and the color compared with the standard pH reference chart ranging from 3.5 to 6. Florisia Glove is a latex glove manufactured by CD Pharma with an absorbing pad on the fore finger and a pH strip on the thumb. The forefinger is inserted into the vagina and the secretions from the lateral vaginal wall which were absorbed to the pad were pressed against the pH strip and pH measured using a colored indicator ranging from 3.5-5.2. pH value >4.5 is used in the clinical diagnosis of BV. Cervicovaginal secretions were collected with a cotton tip applicator rolled in the posterior fornix of vagina and the tip dipped into an eppendorf tube containing 0.3 cc of distilled water. It was not used the secretions for measuring pH with pH meter for the present study. As the pH meter was not used and aimed at determining the concordance of vaginal pH measurements by Indikrom strips and Florisia glove in presence of BV. Secretions collected with other cotton tip applicators from the lateral wall were transferred on to a glass slide for Gram staining. The presence of Candida and Trichomonas was also checked under the microscope.

Statistical analysis

Data was entered and stored in Microsoft Excel software and analyzed by using SPSS software (version 15). The data of women with intermediate BV were excluded for final analysis. The sample for analysis included 270 women, in which 154 women were BV positive. Descriptive statistics were calculated for all demographic and clinical variables. Patient characteristics were compared between women with and those without bacterial vaginosis by using the Student test and Wilcoxon rank-sum test for continuous data and chi-square test for nonparametric categorical data. Specificity, sensitivity, area under curve and 95% confidence intervals (CIs) for each of the individual criterion, combinations of the criteria were calculated considering NS 7-10 as BV. Florisia glove was validated with Nugent score and Amsel criteria. Receiver operating characteristic curves (ROC curves) were generated for overall measurement of test performance in diagnosing bacterial vaginosis. Bland Altman plot was used to compare pH paper with pH Glove. All statistical significance was assessed using an α level of 0.05.

RESULTS

After excluding intermediate BV subjects a total of 270 women were recruited for the study. The mean age and BMI of the subjects were 28.8 and 22.2 respectively. The median parity of the subjects was two. Illiterate women and women with primary education have parity more than two compared to women with more than primary education (p=0.02).Majority (79.6%) of the women adopted permanent sterilization methods.

The women were grouped as BV and Normal and analysed for all variables. Descriptive statistics for all demographic and clinical variables are given in Table 13. Between the groups the factors such as age, BMI, parity had no significance. In the present study, the subjects with BV were 57% and 49.6% based on Nugent score and Amsel's criteria respectively. Vaginal discharge and foul smell were the symptoms in 84.8% and 37.8% of the subjects respectively. Women with BV were more likely to have foul smell as symptom (p =0.01). Vaginal discharge and thin homogenous discharge were similar in women with BV or normal. 22.5% of the women had cervical erosion on gynecological examination. There was no significant difference between the groups with regard to cervical erosion. Women with cervical erosion had vaginal discharge and thin homogenous discharge (p<0.0001). Women with cervical erosion had vaginal WBC >5 (p=0.03).Women with BV had higher vaginal pH and the difference was significant (p < 0.001).Women with vaginal WBC >5

had vaginal pH >4.5 and the difference was significant (p=0.002). There was a significant difference between the groups BV and normal in the presence of the Amsel's criteria (p< 0.001). Presence of Clue cells and positive Whiff test were significant for BV (P=<0.001).In the present study, 28.5% and 1.1% of the Women were infected with Candida and Trichomonas respectively, but there was no significant difference between the groups.

Table 13. Demographic and clinical characteristics of patients

	Bacterial Vaginosis (n=154)	No Bacterial Vaginosis (n=116)	P value	Total
Age(y)(Mean±SD)	29.1±4.8	28.5±5.2	NS	
Median Parity	2	2	NS	
BMI)(Mean±SD)	22.2±5.1	22.2±5	NS	
Vaginal discharge	130(56.8%)	99(43.2%)	NS	229(84.8%)
Foul smelling odor	67(65.7%)	35(34.3%)	0.01	102(37.8%)
pHGlove(Mean±SD)	4.9±0.4	4.6±0.4	<0.001	
pHPaper(Mean±SD)	5.0±0.6	4.6±0.5	<0.001	
Thin homogenous Discharge	121(58.7%)	85(41.3%)	NS	206(76.3%)
Clue cells present				
Positive Whiff test	147(92.5%)	12(7.5%)	<0.001	159(58.9%)
Amsel’s criteria ≥ 3	71(74%)	25(26%)	<0.001	96(35.6%)
Yeast infection	118(88.1%)	16(11.9%)	<0.001	134(49.6%)
Trichomonas	47(61%)	30(39%)	NS	77(28.5%)
Vaginal WBC >5	2(66.7%)	1(33.3%)	NS	3(1.1%)
Cervix erosion	32(60.4)	21(39.6%)	NS	53(19.6%)
Present	28(54.9%)	23(45.1%)	NS	51(22.5%)

NS- Not significant

The sensitivity, specificity, area under the curve, 95% CIs of the individual criterion and combination of criteria for diagnosing BV are displayed in table 2. Clue cells were the criteria with highest sensitivity (0.95) and specificity (0.90) in diagnosing BV. The sensitivity of pH paper and pH Glove were 72% and 79% respectively. The specificity of pH paper and pH Glove were 60% and 53% respectively. Amsel's criteria had 77% and 86% sensitivity and specificity respectively (Table 14). Thin homogenous discharge had the lowest specificity (27%). The area under the curve for pH paper and pH glove was 0.79 when compared with Amsel criteria, stating the concordance of two methods. Among the combination criteria clue cells and pH had highest sensitivity and specificity. The validity test results for Florisia glove when compared with various diagnostic methods are displayed in Table 15. The sensitivity and specificity of pH glove compared with Amsel criteria were 88% and 57% respectively.

Bland Altman Plot was used to portray variability of pH between the pH paper and pH Glove (Fig 4). There was good agreement between the two methods. The concordance correlation coefficient for pH paper and pH Glove was 0.8. ROC Curves for Amsel, pH paper and pH Glove were compared considering Nugent as the standard (Fig 5). Both the methods had good agreement.

Table 14. Sensitivity, specificity and 95% confidence intervals of the clinical criteria for diagnosing bacterial vaginosis

	Sensitivity	95% CI	Specificity	95% CI	AUC	95% CI
Thin homogenous Discharge	0.79	0.71-0.85	0.27	0.19-0.36	0.53	0.47-0.59
Paper pH >4.5	0.72	0.64-0.79	0.60	0.51-0.69	0.71	0.65-0.76
Glove pH>4.5	0.79	0.71-0.85	0.53	0.43-0.62	0.72	0.66-0.77
Positive amine odor	0.46	0.38-0.54	0.78	0.70-0.86	0.62	0.56-0.68
Clue cells present	0.95	0.91-0.98	0.90	0.83-0.94	0.93	0.89-0.95
P pH > 4.5 *and* thin homogeneous discharge	0.91	0.83-0.94	0.36	0.23-0.50	0.64	0.57-0.70
G pH > 4.5 *and* thin homogeneous discharge	0.92	0.85-0.97	0.29	0.18-0.43	0.64	0.57-0.70
P pH > 4.5 *and* amine odor	0.65	0.54-0.74	0.85	0.74-0.92	0.66	0.59-0.72
G pH > 4.5 *and* amine odor	0.72	0.61-0.81	0.81	0.69-0.90	0.66	0.59-0.72
P pH >4.5 *and* clue cells	0.98	0.93-1	0.90	0.81-0.96	0.81	0.76-0.87
G pH >4.5 *and* clue cells	0.98	0.94-1	0.89	0.78-0.95	0.85	0.8-0.9
Amine odor *and* thin homogeneous discharge	0.75	0.64-0.84	0.57	0.41-0.71	0.60	0.53-0.67
Amsel's criteria (≥ 3 of 4 criteria)	0.77	0.69-0.83	0.86	0.79-0.92	0.81	0.76-0.86

CI-confidence interval; AUC-area under the curve; PpH-Paper pH; G pH-Glove pH

Table 15. Sensitivity, specificity and predictive values of forisia glove when compared with the standard criteria

	PAPER pH	AMSEL	NUGENT
Sensitivity (95% CI)	0.95 (0.90-0.98)	0.88 (0.81-0.93)	0.79 (0.71-0.85)
Specificity (95% CI)	0.76 (0.67-0.84)	0.57 (0.49-0.66)	0.53 (0.43-0.62)
PPV (95% CI)	0.85 (0.78-0.90)	0.67 (0.60-0.74)	0.69 (0.61-0.76)
NPV (95% CI)	0.91 (0.84-0.96)	0.83 (0.74-0.90)	0.65 (0.54-0.74)

Fig. 4 Bland-Altman plot

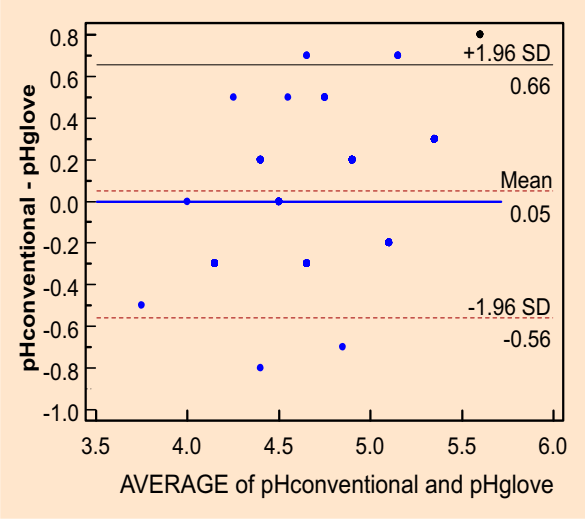
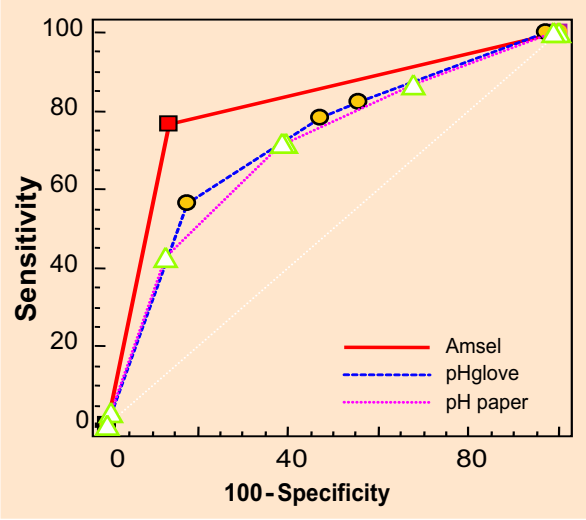


Fig. 5 ROC curves



CONCLUSIONS

1. In the present study, the sensitivity and specificity of Amsel criteria was 77% and 86% respectively.
2. As had been reported previously, clue cells were the most reliable single indicator for BV (sensitivity 95% and specificity 90%); however, identification of clue cells require on-site microscopy capabilities.
3. The sensitivity and specificity of pH paper was 72% and 60% and pH glove (Florisia*) was 79% and 53% respectively.

In case of mixed infections a diagnostic strategy that uses the pH is most likely a better solution in resource-poor settings because, although it is not the most sensitive or specific test, it offers a middle ground on sensitivity and specificity compared with the more technologically demanding techniques. Moreover, this pH test performs better than the syndromic diagnostic algorithm. In symptomatic women, a high vaginal pH result would require further evaluation by a health care provider. The Florisia glove had good agreement with pH paper (compared by Bland Altman plot and ROC curves). This glove can be used for self sampling or to be used by nursing personnel for evaluating vaginal health.

Self-sampling the vagina seems to be very acceptable to women of multiple ethnic groups. A better informed self-diagnosis would ultimately reduce individual financial expenditures, delayed treatment, and possible secondary complications. It would also lower health care cost for the medical industry.

III. CLINICAL STUDIES

1. CALCIUM RICH FOOD SUPPLEMENTATION TO LACTATING WOMEN FROM LOW SOCIO-ECONOMIC GROUP- EFFECT ON BONE DENSITY

Lactation is a nutritionally demanding process. It imposes a particular burden on the calcium economy of the mother because breastfeeding mothers secrete around 200mg of calcium into breast milk every day. Western studies have shown that a substantial part of this increased calcium demand is mobilized from maternal skeleton and bone mineral density (BMD) decreases by 4-7% in lumbar spine (LS) and hip regions during 3-6 months of lactation. Subjects in the above mentioned studies were well-nourished with high dietary intake of calcium and shorter duration of lactation (6 months).

Studies investigating the effect of calcium supplementation on bone density changes have detected no effect. But the subjects in these studies already had moderate to high calcium intake. Results of the above studies led to the conclusions that lactation related changes in bone mass may be independent of dietary calcium intake and unresponsive to increase in calcium intake. Studies from this Institute in undernourished lactating women from low income group indicated that the bone changes during lactation were determined by the nutritional status of the mother. Women having better weights and higher lean body mass could partially compensate for the extra demand of lactation and did not have any bone loss. The study also highlighted the fact that these lactating women had not attained their peak bone mass. Effect of calcium supplementation on lactation related bone changes in women who have not attained peak bone mass has not been sufficiently studied.

Based on the studies carried out at NIN, it was observed that diets of lactating women from the low socio-economic group were inadequate in all the nutrients including energy, proteins, calcium, vitamin A, iron and other B vitamins with calcium deficiency being more marked (Intake of calcium Vs RDA 400mg/day Vs 1000mg/day). It is important to explore whether the bone loss of the lactating women can be prevented by providing a food-based supplement which will increase the intake of calcium to meet the RDA and also provide additional energy and proteins as well. Sesame seeds (*Til*) is a rich sources of calcium (100g of sesame seeds 1450mg of calcium). They are traditionally eaten in this part of the country and a recipe using this foodstuff would add substantially to the calcium, energy and protein intakes of these women. This study was therefore planned to investigate whether a calcium rich food supplement in lactating undernourished women can prevent the bone loss at LS at 6 months postpartum and enhance the peak bone mass.

Hypothesis

Calcium rich food supplementation to young lactating women will prevent lactation related bone loss at 6 months postpartum and increase the bone mineral density at one year in the supplemented group as compared to the control group.

AIMS AND OBJECTIVES

Primary objective

To study the effect of calcium rich food supplementation on the lactation related bone changes at 6 months, 1 year and 1.5 years postpartum in women from the low income group and to compare these changes with those in control women from a similar socio-economic class.

Secondary objective

To study the effect of energy dense food supplementation on the fatty acid profile of the breast milk.

METHODOLOGY

Sample size: With 95% confidence interval and 80% power for the increment of bone density of S.D.4.7% and the difference 3.5%, the required sample size for each group was 56. Assuming 20% loss to follow-up, it was decided to enroll 70 women in each group.

All the postpartum women from an urban slum were contacted by house-to-house survey. After collecting their background information, their bone density measurements were carried out using DXA (Hologic Discovery) at lumbar spine, hip, forearm and whole body within one month after delivery. Their baby's bone mineral content was also measured by DXA. Their fasting blood sample was collected for biochemical investigations like hemoglobin, serum calcium, serum phosphorus, serum proteins, serum total and bone specific alkaline phosphatase and serum total and bone specific acid phosphatase. Estimation of serum vitamin D and Parathyroid hormone levels was also carried out.

Breast milk samples were collected after complete emptying of breast at 3 months postpartum for the analysis of fatty acid profile. Dietary intake was estimated by 24 hr recall method. Long term calcium intake was estimated by Food Frequency Questionnaire.

The participants were supplemented daily with a preparation containing *Til*, groundnuts and *jaggery* (which provided energy-500 Kcal, proteins-11g and calcium 750mg) daily for a period of one year. Approximate cost per person per day of the food supplement was ₹.10/-

Postpartum women from the similar socio-economic background and residing in the same community were enrolled as controls. These control group women were given *Rawa laddus* which provided similar amount of energy but had poor calcium content. All the women were followed up and BMD measurements were repeated at 6 months, 1 year and 1.5 years to estimate the change in BMD. Biochemical parameters and dietary intake estimations were repeated at the same time.

RESULTS

A total of 140 women (70 in each group) were enrolled in the study within one month of delivery and their baseline bone density measurements were carried out using DXA. A total of 62 women in the intervention group and 58 women in the control group completed supplementation and all the three follow ups. Remaining women discontinued the study due to reasons such as migration out of the study area, inability to come for follow up measurements due to job or other responsibilities etc.

Table 16. Background characteristics of the study participants at baseline

	Intervention group (n=70)		Control group (n=70)	
	Mean	SD	Mean	SD
Age (yrs)	24.0	3.3	23.5	3.4
Parity	2.6	0.7	2.3	0.6
Height (cm)	150.0	5.9	152.4	5.1
Weight (kg)	47.4	14.7	47.0	9.2
BMI	20.3	3.3	20.2	3.8
Birth Weight (kg)	2.86	0.42	2.72	0.45
Infant's weight at baseline (kg)	3.67	0.51	3.81	0.89
Dietary calcium intake (mg/d)	458	109	500	158

BMI- Body mass index

Background information

The women resided in an urban slum (Addagutta) and consumed diets that were cereal based. Intake of all the major nutrients was below the RDA. Their background characteristics and the bone parameters are presented in table 16 and 17, respectively. The characteristics of women and their baseline bone parameters

were not significantly different in the 2 groups except a higher bone mineral content (BMC) at the hip region in case of women in the control group.

Table 17. Baseline bone parameters in study participants

Bone parameters		Intervention group (n=70)		Control group (n=71)	
		Mean	SD	Mean	SD
F. Neck	BMC	3.17	0.49	3.28	0.50
	BMD	0.713	0.095	0.730	0.102
Hip	BMC	21.00	3.30	22.26	3.41*
	BMD	0.768	0.092	0.796	0.093
Spine	BMC	36.62	7.75	38.92	6.15
	BMD	0.788	0.106	0.814	0.097
Forearm	BMC	9.32	1.32	9.41	1.23
	BMD	0.501	0.040	0.501	0.040
WB	BMC	1492	213	1543	198
Total fat mass (kg)		14.0	4.2	15.1	4.4
Total lean mass (kg)		30.4	4.4	31.3	3.7
Total mass (kg)		45.9	8.2	47.9	7.6
Fat %		29.9	4.5	31.0	4.7

Notes : BMC –Bone mineral content in g; BMD- Bone mineral density in g/ cm^2
*F. Neck – femoral neck; WB- whole body * - p <0.05*

Bone density changes in the two groups of women

The bone density changes (per cent change from the baseline BMD) at 6 and 12 months of follow up at different skeletal sites in the two groups of women are presented in table 18. It can be observed that women in the intervention group had a significantly lower loss of BMD at the femoral neck and hip regions when compared to the control group women. The changes at the other skeletal sites were not significantly different in the two groups.

Table 18. Percent change in BMD at different skeletal sites in the two groups of women

	Intervention group (N= 58)		Control group (N= 57)		P value
	Mean	SD	Mean	SD	
F. Neck BMD - 6 mo	-5.8	5.2	-8.1	4.6	0.017
- 12 mo	-6.1	5.3	-6.3	5.9	0.854
Hip BMD - 6 mo	-1.6	3.7	-2.9	3.4	0.063
- 12 mo	0.9	4.4	-0.5	3.8	0.134
Spine BMD - 6 mo	-0.3	4.0	-0.7	4.2	0.653
- 12 mo	3.0	4.6	2.6	5.2	0.711
Arm BMD – 6 mo	-1.5	2.3	-1.4	1.8	0.742
- 12 mo	-1.9	2.4	-1.4	2.6	0.380

BMD- Bone mineral density; F. Neck – Femoral neck

Multiple regression analyses to assess the impact of the intervention on the bone density changes – Multiple linear regression models were constructed to assess the impact of the calcium rich food supplementation on the bone density changes at different skeletal sites after adjustment for the potential confounders including baseline body weight, body composition and bone mineral density at baseline as well as the changes in body composition during lactation. These analyses confirmed the earlier findings indicating that women in the intervention group had a significantly lower BMD loss at femoral neck and hip regions at 6 months when compared to the control group women (Table 19).

Table 19. Differences in per cent change in the BMDs at different skeletal sites in the supplemented vs control group after adjustment for baseline weight, body composition, BMD and change in body composition during lactation

Parameter	Difference in control vs intervention group	P value	R^2 (%)
Neck BMD – 6 mo	-1.9 ± 0.9	0.049	20.6
- 12 mo	-0.2 ± 1.2	0.880	18.9
Hip BMD -6 mo	-1.4 ± 0.7	0.037	22
-12 mo	-1.9 ± 0.8	0.024	32.5
Spine BMD – 6 mo	-0.1 ± 0.8	0.939	16.8
-12 mo	-0.7 ± 1.0	0.485	24.8
Total BMC – 6 mo	-8.3 ± 6.8	0.222	16.5
-12 mo	-11.3 ± 9.6	0.245	8.3

CONCLUSION

Calcium rich food supplementation to under nourished women from the low income group resulted in significantly reduced loss of bone mineral density at the femoral neck and hip regions when compared to the control group. The study advances the knowledge in the area of bone metabolism during lactation and provides important information for the calcium requirements of lactating women.

2. STUDY OF BONE PARAMETERS OF MEN AND WOMEN WITH OSTEOPOROTIC HIP FRACTURES (CASE CONTROL STUDY OF OSTEOPOROTIC HIP FRACTURES)

The fragility fractures due to osteoporosis are responsible for much morbidity and disability in the populations. Among all fractures, hip fractures causing more disability, morbidity and high health costs. In India, there is very limited data available on osteoporosis hip fractures and its epidemiological patterns. The study planned to generate bone parameters for the osteoporosis-fractured patients to prepare a treatment schedules with the bone mineral densities and to formulate preventive strategies for the management of osteoporosis.

OBJECTIVES

To study the bone parameters of men and women with osteoporotic hip fractures to explore the associated factors contributing to fractures.

Hypothesis

It is hypothesized that Bone mineral density of patients with confirmed fresh osteoporotic fractures is lower than the bone mineral density of normal age and sex matched controls.

Type of study: Case control study

Sample size

Assuming the 95% significance level, 80% of power, standard deviation of hip bone mineral density 0.15 g/cm2 and expected differences 0.07g/cm2 and the required sample size can be calculated as both cases and control groups and for males and females.

MATERIALS AND METHODS

Cases

Adult men and women of 30-70 age groups who had a trivial injury admitted in the Orthopedics ward of the Osmania General Hospital, Secunderabad were recruited for the study. The subjects were recruited for the period of April 2005 to March 2008. A total number of 73 male and 73 female cases were recruited. Subjects enrolled who had fresh fractures of hip with in the last three months history of trivial injury and clinical evidence of osteoporosis. Background information about family history of fracture, personal history, smoking, alcohol consumption, physical activity, social status, parity and menopause were recorded.

Controls

Age, sex and low socio-economic background matched controls were selected from low-income group of Hyderabad, India. A total number of 68 male and 46 female subjects of low-income group (LIG), Hyderabad were recruited to act as controls.

Exclusion criteria for enrolling the subjects

Subjects were selected on the basis of inclusion and exclusion criteria. Clinical examination, bio chemical parameters, anthropometric and Bone Mineral Density (BMD) measurements were recorded.

STATISTICAL METHODS

For demographic profile, bone parameters and prevalence of osteoporosis by T scores the chi square test was used and for the analysis of anthropometry and biochemical tests Data ANOVA test was used.

RESULTS

There was no difference in males and females between the cases and control subjects in the distribution. The cases and control are equally distributed between males and females. There is no difference between the mean age of the fractured cases and the healthy normal controls. The fractures occurred at the mean age of 59.1 for males and 63.6 for the females. This confirms that fractures were occurring at the early age than the western populations (Table 20).

The mean height of the cases in males and females were 164cms and 152cms respectively. The mean height of both males and females from cases group was significantly ($P< 0.001$, $P< 00.1$) more than controls subjects. The mean weight of cases were 48 (SD 11) and 45 (SD 9.7). The BMI was significantly ($P< 0.002$) lower in the fracture cases of females than the controls. The study confirms low BMI is strongly associated with osteoporosis either as a risk factor or as marker. However, the study shows that chronic energy deficiency (CED) was prevalent in the population and the cases (Table 21).

Table 23. Overview of Bio chemical parameters from the cases and controls

Variable	Cases Mean(SD)	Controls Mean(SD)	P value
Serum calcium (mg/dl)	8.7 (1.3)	9.2 (0.7)	< 0.20
Vitamin D (ng/ml)	14.4 (11)	33.8 (9.1)	*< 0.001
PTH (pg/ml)	56.7 (54.4)	37.6 (26.2)	< 0.081
Urinary fluoride ((µg/g)	2.7 (5.5)	0.84 (0.4)	*< 0.001

Test used: ANOVA Serum calcium normal range: 8.4 -10.2 mg/dl (AAS)
Vitamin D normal range --- : >15ng/ml (HPLC), Parathormone normal range: 9-55 pg/ml (DSL)
Urinary fluoride normal range : < 0.80 µg/g

There were lower levels of serum calcium and Vitamin D observed in cases with fractures. The vitamin D levels were significantly (P< 0.001) lower in cases than the controls. There were high parathorormone activity in fractures cases than the controls but there was a weaker non-significant association between cases and controls. The study results also showed that high excretion of urinary fluorides in the cases was significantly (< P 0.001) more than the controls.

Table 24. Prevalence of osteoporosis by "T" scores

	"T" score	Cases	Controls	P value
Hip	<-2.5	26 (36%)	4 (6%)	
Males	-2.5- 1	35 (49%)	46 (68%)	
	>1	11 (15%)	18 (26%)	*P <0.001
Females	<-2.5	55 (74%)	24 (52%)	
	-2.5-1	18 (24%)	17 (37%)	
	>1	1 (1%)	5 (11%)	*P<0.001
Wards Triangle				
Males	<-2.5	41 (57%)	16 (24%)	
	-2.5-1	24 (33%)	44 (65%)	
	1	7 (10%)	8 (12%)	*P<0.01
Females	<-2.5	63 (85.1%)	28 (61%)	
	-2.5 to 1	11 (15%)	15 (33%)	
	>1	0 (0%)	3 (7%)	*P<0.001
Spine				
Males	<-2.5	44 (59%)	34 (50%)	
	-2.5-1	18 (24%)	25 (37%)	
	>1	12 (16%)	9 (13%)	P= 0.316
Females	<-2.5	63 (85%)	40 (87%)	
	-2.5-1	7 (9%)	4 (9%)	
	>1	4 (5%)	2 (4%)	P= 0.998
Neck				
Males	<-2.5	37 (51%)	13 (19%)	
	-2.5-1	27 (38%)	45 (66%)	
	>1	8 (11%)	10 (15%)	*P<0.001
Females	<-2.5	56 (76%)	19 (41%)	
	-2.5-1	17 (23%)	22 (48%)	
	>1	1 (1%)	5 (11%)	*P<0.001

Test used: Chi square test

Table 20. Overview of demographic data from the cases and controls

Variables	Cases n (%)	Controls n (%)	P value
Sex			
Males	74 (50%)	68 (60%)	
females	74 (50%)	46 (40%)	P= 0.12
Religion			
Hindus	95 (64%)	95 (84%)	
Muslims & Christians	53 (36%)	18 (16%)	* P<0.001
Martial status			
Married	143 ((97%)	113 (100%)	
Un married	5 (3%)	0	*P<0.05
Communities			
SC+ST	26 (18%)	41 (36%)	
BC	59 (40%)	63 (56%)	
Others	63 (42%)	9 (8%)	*P<0.001
Age(Mean)			
Males	59.1	56.1	P= 0.121
Females	63.6	60.9	P= 0. 941

Statistical test used: Chi square test used. SC (scheduled caste), ST (Scheduled tribes) (Lower caste population) BC (Backward caste populations) Others (Upper caste communities)

Table 21. Overview of Anthropometric parameters from the cases and controls

Variable	**Cases** Mean (SD)	**Controls** Mean (SD)	P value*
Height (cms)			
Males	164 (6.1)	160 (6.2)	*< 0.001
Females	152 (6)	147 (5.4)	*< 0.001
Weight (kgs)			
Males	48 (11)	49 (8.8)	P= O.8
Females	45 (9.7)	47 (11)	P= 0.14
BMI (Kg/m2)			
Males	18 (3.7)	19 (3.3)	*< 0.27
Females	19 (3.9)	22 (4.3)	*< 0.002

The ANOVA test is used

Table 22. Over view of risk factors of osteoporosis

Variable	Cases(total)	Controls(total)	OR	95%CI
Regular Alcohol consumers	17 (148)	19 (113)	0.64	0.3-1.37
Smoking any amount	33 (148)	22 (113)	1.19	0.62-2.3
Menopause	34 (74)	26 (46)	0.65	0.29-1.5

This study observed that chronic smoking, regular alcohol and the post menopause had no significant impact on the occurrence of fractures. This may be due to the fact that controls may not be true representatives. Normally, most of the studies showed heavy smoking, chronic alcoholism and post menopausal period as the risk factors for osteoporosis (Table 23 & 24).

The bone mineral density "T" scores at hip region of the males in cases showed that 36% were osteoporotic and 49% were osteopenic. Bone mineral density of the cases was significantly lower than the controls (P< 0.001). In the female subjects of cases groups 74% were osteoporotic and 24% were osteopenic. Bone mineral density cases were significantly (P<0.001) lower than the controls at the hip region. However, in control population of males and females, 6% and 52% had osteoporosis respectively.

At the Wards triangle, among the male cases, 57% were osteoporotic and 33% were osteopenic. Cases bone mineral density was significantly (P< 0.01) lower than the controls. In the females 85.1% were osteoporotic and 15% were osteopenic. Cases bone mineral density was significantly lower than the (P<0.001) controls. At the Wards triangle, 24% males and 61% females had osteoporosis among the controls.

At spine region, 59% males among the cases had osteoporosis and 24% were osteopenic and there was a weak association with controls. In the female cases, 85% had osteoporosis and 9% were osteopenic and weak association with controls was observed. However, in normal control subjects 50% males and 87% females had osteoporosis.

At the femoral neck 51% of male cases had osteoporosis and 38% were osteopenic. The cases bone mineral density was significantly lower (P< 0.001) than the controls. In the female cases, 76% had osteoporosis, 23% were osteopenic. Cases bone mineral density was significantly (P<0.001), lower than the controls. However, in the controls, 19% males and 41% females had osteoporosis.

CONCLUSIONS

The study of bone parameters and risk factors among patients with fractures is a well-designed study and the first of its kind in the country. This study gave inputs about mean age of the fracture occurrence and the range of the bone mineral density fractures in which fractures were happening. The study showed high prevalence of osteoporosis in both cases and controls. The study also showed that the lower levels of calcium and Vitamin D levels, high excretion of urinary fluorides and increased trend of prathormone were correlated with fractures. The study also confirms that fractures were occurring 10 years earlier than the Western populations. To know the population risk, controls may be recruited from the middle and high socio-economic groups, with matched subjects from different castes and regions and in required numbers.

3. NUTRITION PROFILE OF MIGRANT TRIBAL IN HYDERABAD

Migrant population living in urban slums are normally more vulnerable to malnutrition related problems as well as to coronary artery disease, diabetes and other non-communicable diseases. Further poor socio-economic status and desadvantaged existence their vulnerability to several health problems.

Aim

The present study was carried out to assess the nutritional status and cardio vascular risk factors among migrant tribal population.

MATERIALS AND METHODS

A community based cross-sectional study was carried out among the adult tribal migrants in slum areas of Kondapur, Hyderabad, India.

RESULTS

A total of 275 subjects (138 men and 137 women) were covered in the study. The mean age of men and women were 42.85±10.7 (30-78) and 39.7±9.95 (30-73) respectively. Mean household size was 4.6, and majority (85%) of them were nuclear families. About 65.7% were migrated from the states of Karnataka, Maharashtra, Chattisgarh, Orissa and Bihar. Most of the subjects were construction workers (35%), followed by vegetable vendors (20%), domestic helpers (20%) and house keepers (25%). On clinical examination, nutritional deficiency signs like bitots spots, angular stomatitis were not observed. The mean (±SD) values of physical, anthropometric measurements and blood pressure by period of stay are presented in Table 25. No significant difference between various anthropometric variable by period of stay was observed. Similar association was observed with respect to blood pressure. The mean intake of different foods and distribution (%) of food groups according to recommended dietary intake (RDI) by gender is given in the Table 26. Majority of migrants subsisting on inadequate diets (<70% of RDI) and the proportion of migrants not meeting even 50% of RDI was highest for leafy vegetables (84-91%) followed by other vegetables, milk and milk products, pulses, sugar and jaggery.

Table 25. Physical and anthropometric variables of migrant population by duration of stay (Mean± SD)

	Males (Years)				Females (Years)			
	1-5	5-10	10-15	P value	1-5	5-10	10-15	P value
Age (years)	39.4±8.3 (27)	40.36±7.7 (44)	45.85±12.74 (67)	<0.005	36.9±8.4 (31)	38.3±7.9 (34)	41.5±11.1 (72)	0.62
Height (cms)	163.75± 5.2 (24)	161.97±6.8 (42)	163.1±7.44 (67)	0.570	152.96±5.2 (31)	150.4±7.0 (33)	148.5±4.9 (72)	0.001
Weight (kgs)	60.5±10.56 (24)	61.5±12.8 (42)	61.0±13.7 (67)	0.956	57.9± 10.9 (31)	54.38±12.9 (33)	52.87±10.7 (72)	0.123
BMI(kg/m²)	22.59±3.8 (24)	23.37±4.22 (42)	22.8±4.2 (67)	0.701	24.8±4.7 (31)	24.1±5.92 (33)	23.35±4.6 (72)	0.733
Mid Arm circumference (cms)	27.49±3.48 (25)	27.57±3.1 (43)	27.42±4.22 (67)	0.981	27.99±3.43 (31)	26.04±4.68 (33)	26.5±3.48 (72)	0.101
Waist circumference (cms)	85.1±10.67 (25)	85.8±11.58 (43)	85.2±11.9 (66)	0.955	79.9±12.5 (31)	78.6±14.1 (33)	77.22±11.1 (72)	0.576
Hip circumference (cms)	89.7±6.2 (25)	92.2±8.4 (43)	90.0±8.0 (65)	0.289	94.3±9.0 (30)	93.1±11.98 (33)	91.34±10.3 (72)	0.931
Triceps circumference (mm)	10.4± .87 (25)	10.48±4.21 (42)	11.21±5.3 (67)	0.612	15.68±4.31 (26)	14.67± 4.6 (29)	13.7±4.4 (74)	0.114
Biceps (mm)	5.413± 2.55 (23)	5.4±2.16 (39)	5.681±2.74 (69)	0.826	7.03±3.19 (26)	6.55±2.24 (28)	6.418±2.92 (74)	0.636
Sub scapular (mm)	14.88±6.39 (25)	16.3±7.5 (43)	15.97±7.4 (67)	0.739	15.3± 4.6 (31)	15.0± 6.0 (33)	13.9±4.7 (72)	0.362
Supra Iliac (mm)	9.0 ± (3.5) (25)	9.96 ± 4.5 (43)	10.2 ± 5.0 (67)	0.573	11.3±5.03 (31)	12.1± 5.63 (32)	11.03±4.6 (72)	0.54
Fat (%)	25.7±6.5 (25)	23.9±7.7 (42)	23.6±6.7 (67)	0.442	23.6±7.2 (31)	24.9±6.5 (32)	25.3±6.3 (67)	0.48
Fat free mass (kgs)	42.4±6.7 (25)	43.2±10.8 (42)	42.4± 9.7 (67)	0.917	44.6±7.59 (31)	44.6±7.6 (32)	42.6±7.9 (72)	0.233
SBP(mm hg)	122.57± 9.2 (25)	123.6±11.66 (44)	125.4±14.45 (67)	0.526	115.7±18.8 (31)	118.4±19.1 (34)	119.3±15.53 (72)	0.619
BP(mmhg)	81.5±5.0 (25)	81.2±8.5 (44)	81.3±7.8 (67)	0.986	77.9±8.5 (31)	80.1±7.8 (34)	79.7±8.8 (72)	0.555

( ) No of subjects studied

Consumption of milk and milk products, sugar and jaggery was significantly (p<0.000) higher in females than males. Mean (±SD) of nutrients and distribution (%) of nutrients according to recommended dietary allowances (RDA) by gender is presented in Table 27. More than 70% of people were consuming adequate (=70% of RDA) amounts of total fat (96-100%), energy (64-89%), protein (61-82) and folic acid (69%), while a majority were not meeting even 50% of RDI for micro-nutrients such as iron (80-84%), vitamin A (81-83%) and riboflavin (67-84%).The intakes were significantly (p<0.05) different among men and women with respect to protein, energy, thiamine, riblflavin and niacin. The distribution (%) in individuals by biochemical and nutrition parameters profile is given in Table 28. Men (p<0.010) had higher levels of glycosylated haemoglobin than the females. The prevalence of anaemia was significantly (p<0.000) higher in women (69%) as compared to men (10.9%). About 39% of men had higher (=15) concentrations of homocysteine (p<0.001), while none of the women had the same. The prevalence of obesity in terms of BMI was higher in women (24.3%) compared to males (14.3%), while the prevalence of abdominal obesity and hypertension was comparable between both sexes.

Correlation coefficients and risk factors

The correlation coefficients are given in the Table 29 and 30. BMI was significantly positively correlated (R= 0.577) with triglycerides in men whereas it was significantly correlated with total cholesterol (R= 0.283), LDL (R= 0.223), triglycerides (R= 0.258) and with glycosylated haemoglobin ®= 0.350) in women .

Table 26. Mean intake of different foods and distribution (%) of food groups according to RDI by gender

		Mean ±SD	P value	<50 (%)	50-70(%)	>70(%)	P value
Cereals	Males (100)	315.3 ±113.6	0.753	24.0	28.0	48.0	0.012
	Females(96)	301.1±88.8		9.4	40.6	50.0	
Pulses	males(100)	22.3±26.8	0.763	55	13.0	32.0	0.796
	Females(96)	20.87±25.8		54.2	10.4	35.4	
Leafy - vegetables	Males(100)	9.85±22.8	0.365	84.0	2.0	14.0	0.28
	Females(96)	10.49±20.9		90.6	5.2	4.2	
Vegetables	Males(100)	28.3±58.2	0.727	70.0	3.0	27.0	0.092
	Females(96)	30.1±55.1		66.7	0	33.3	
Roots and tubers	Males (100)	36.8±50.1	0.712	52.0	16.0	32.0	0.449
	Females(96)	34.0±35.6		45.8	22.9	31.3	
Milk and milk products	Males (100)	42.2±67.5	0.602	70.0	20.0	10.0	0.000
	Females(96)	44.9±64.6		64.6	4.2	31.3	
Fats and oils	Males (100)	22.2±18.9	0.761	17.0	19.0	64.0	0.882
	Females(96)	22.79±16.3		16.7	21.9	61.5	
Sugar and jaggery	Males (100)	11.7±8.8	0.065	73.0	11.0	16.0	0.000
	Females(96)	14.3±12.2		37.5	17.7	44.8	

( ) No of subjects studied

Table 27. Median intake of nutrients and Distribution according percent of RDA by gender

Nutrients		Median, range	"P" value	<50 (%)	50-70 (%)	>70(%)	P value
Protein	Males (100)	45.30 [34.88,63.92]	0.931	18.0	21.0	61.0	<0.004
	Females (96)	45.20 [37.65,56.30]		8.3	9.4	82.3	
Energy	Males (100)	1861.30 [1514.85,2224.53]	0.905	13.0	23	64.0	0.000
	Females (96)	1871.15 [1562.30,2146.10]		2.1	9.4	88.5	
Calcium	Males (100)	270.55 [163.75,403.10]	0.521	34.0	19.0	47.0	0.252
	Females (96)	273.35 [193.73,409.45]		25.0	27.10	47.9	
Iron	Males (100)	9.85 [718,13.48]	0.769	80.0	13.0	7.0	0.631
	Females (96)	9.70 [7.23,13.08]		84.4	11.5	4.2	
Vitamin A	Males (100)	110.40 [68.73,182.20]	0.801	83.0	4.0	13.0	0.587
	Females (96)	112.95 [77.53,177.65]		81.3	2.1	16.7	
Thiamine	Males (100)	0.60 [0.40,0.88]	0.697	54.0	21.0	25.0	0.002
	Females (96)	0.60 [0.40,0.80]		29.2	28.1	42.7	
Riboflavin	males(100)	0.50 [0.30,0.68]	0.474	84.0	11.0	5.0	0.017
	Females (96)	0.50 [0.30,0.60]		66.7	21.9	11.5	
Niacin	Males (100)	9.45 [7.53,12.73]	0.606	32.0	35.0	33.0	0.000
	Females (96)	9.30 [7.00,12.68]		13.5	26.0	60.4	
Vitamin C	Males(100)	27.65 [17.00,44.55]	0.849	32.0	18.0	50.0	0.876
	Females (96)	27.15 [18.88,45.55]		30.2	20.8	49.0	
Folic acid	Males (100)	93.05 [60.08,132.93]	0.986	19.0	12.0	69.0	0.673
	Females (96)	93.50 [63.28,132.90]		15.6	15.6	68.8	
Total fat	Males(100)	35.15 [24.80,46.98]	0.362	1.0	3.0	96.0	0.065
	Female(96)	39.40 [26.78,50.70]		0	0	100	

( ) No of subjects studied, [ ] Inter quartile range

Table 28. Distribution (%) of bio-chemical and nutrition profile of migrant population

Variables	**Males** No=46	**Females** No=83	P value
Total Cholesterol (≥220mgs/dl)	32.6	27.7	0.559
LDL mgs/dl (≥175)	23.9	14.5	0.179
HDLmgs/dl (≥30)	89.1	96.8	0.102
Triglycerides mgs/dl (>165 M)(≥134F)	23.9	22.9	0.895
Glycosylated hemoglobin (≥6.7%)	39.1	18.3	0.010
Hb (g/dl) (≥12g/dl)	89.1	30.9	0.000
Protein (gms/dl) (≥6gms/dl)	97.8	97.6	0.932
Albumin (gms/dl) (≥3.5)	6.5	93.5	0.036
Vitamin D (≥15ngs/ml)	82.6	86.4	0.563
Homocysteine (≥15 umol)	39.1(N-23)	0.09 (N-23)	0.001
BMI (Kg/m²) (≥27.5)	14.3	24.3	0.160
Fat percentage (≥25, M) (≥30F)	52.2	23.0	0.000
Abdominal Waist circumference (cms) (≥90cm, M) (≥80 cm, F)	35.1	47.1	0.045
Waist hip ratio (≥0.9,M, ≥0.8,F)	72.2	75.6	0.0529
Hypertension (HTN mm) ≥140/90	18.4	16.8	0.729

( ) Cut off values, M = Males. F = Females

Table 29. Correlation coefficients of obesity indicators with cardiac risk factors

	Total cholesterol	LDL	HDL	TG	Glycosylated Hb	Homo cysteine
Males						
BMI	0.108	0.068	0.111	0.577**	0.242	- 0.062
Waist circumference	0.174	0.067	0.040	0.551**	0.240	0.004
Waist hip ratio	0.242	0.061	-0.040	0.450**	0.334*	0.047
Fat %	0.142	0.147	-0.005	0.177	0.146	-0.230
Females						
BMI	0.283**	0.223*	0.087	0.258*	0.350**	-0.245
Waist circumference	0.341**	0.201	0.013	0.295**	0.395**	-0.55
Waist hip ratio	0.360**	0.177	-0.048	0.328**	0.318**	0.109
Fat %	0.203	0.178	0.116	0.198	0.139	0.038

** P< 0.05, ** p< 0.01, T – Trend (p<0.010)*

Table 30. Correlation coefficient of obesity with bio chemical nutritional factors

	Hb	Protein	Albumin	Vitamin D
Males				
BMI	0.198	0.218	0.135	0.280(T)
WC	0.222	0.176	0.205	0.177
WHR	0.147	0.162	0.150	0.109
Fat%	0.033	0.270 (T)	-0.073	0.033
Females				
BMI	0.252*	0.030	-0.039	-0.144
WC	0.289**	0.025	-0.046	-0.079
WHR	0.146	0.011	-0.105	-0.018
Fat%	0.048	0.110	-0.014	0.00

** P< 0.05, ** p<0.01*

Waist circumference was significantly correlated with triglycerides ®=i0.551) in men whereas in women it was significantly correlated with total cholesterol (R= 0.3411), triglycerides (R= 0.295), and glycosylated haemoglobin (R=0.395). In men, waist-hip ratio was significantly correlated with triglycerides (R=0.450), glycosylated haemoglobin(R=0.334) whereas in women it was significantly correlated with total cholesterol (R=0.341), triglyceride (R= 0.258) and glycosylated haemoglobin (R= 0.395). Hypertension in both men and women was significantly correlated with total cholesterol (OR= 4.0 95% CI 1.8-10.8), Waist circumference (OR =1.9, CI: 1.02-3.06), WHR (OR= 3.5, CI: 1.3-9.3) and BMI (OR=2.5, CI: 1.2- 4.4).

CONCLUSIONS

The tribal population migrating to cities were more prone to obesity and metabolic syndrome than their brethren. The tribal folks who continue to live in natural habitat back home. This may be attributed to rural to urban migration and subsequent changes in their lifestyles including dietary behaviour. These migrant population need screening for non-communicable diseases, health education on balanced diet (RDA) and physical activity. The results indicate that the National and State campaigns need to be created to bring about awareness on lifestyle modifications in relation to diet and physical activity.

IV. BASIC STUDIES

1. ESTABLISHMENT OF SCREENING FACILITY FOR IRON AND ZINC BIOAVAILABILITY USING Caco2 CELL- LINE

The aim of the Caco-2 cell culture facility was to establish and develop methods for screening bioavailability of iron and zinc from biofortified wheat, rice and maize crops developed under the India Crop Biofortification program. A facility was established which is now geared up for taking up screening of biofortified wheat, rice and maize for iron and zinc bioavailability. The project was extended for two more years in order to develop similar methodologies for assessing provitamin A carotenoid bioavailability. During the current year, zinc bioavailability has assessed from 10 biofortified maize genotype obtained from Crop Improvement Program Division, Vivekananda Parvatiya Krishi Anusandhan, Almora, Uttarakhand.

AIMS AND OBJECTIVES

To test the bioavailability of zinc in bio-fortified maize developed under the crop-bio-fortification network program.

Work done during the year

Assessment of zinc bioavailability from Biofortified maize samples:

Ten varieties of maize genotypes (~ 20g) namely CM122, VQL1, CM145, VQL2, VIVEK341, VQL17, VIVEKTHYB-9, VIVEK.QPM-9, VIVEK334, VIVEK336 were obtained for screening for zinc bioavailability.

METHODOLOGY

The zinc bioavailability was assessed in maize samples using the coupled simulated in vitro digestion/Caco-2 cell method developed previously in the laboratory.

Sample processing

The maize genotype samples (20g) were made into fine powder in cclone sample mini flour mill (UDY Corporation, M.S/No 2482) and stored at RT until further analysis.

Estimation of zinc content

Digested about 0.5 g of maize powder in 2 mL of 65% ultra pure nitric acid (Merck) and 1 mL of 33 % H_2O_2 in a microwave digester (Mars Xpress). The zinc content in the digest was estimated by atomic absorption spectrometer (Varian 220 -version 2.1).

In vitro digestion

Hydrated 1 g of seed powder for 30 min with 5 mL of milli E water in 50 mL falcon tube and microwaved for 5 min. Samples were then homogenized and subjected to simulated in vitro digestion in triplicate.

Caco-2 cell zinc uptake

Digest (1.5 mL) containing ^{65}Znwas introduced into the upper chamber of the transwell insert ring and incubated in a humidified CO_2 incubator at 5% CO_2 at 37°C for 2 h with continuous shaking at 150 rpm. At the end of incubation, the insert was removed and incubated for 1 h and the medium aspirated. The monolayers were washed, the cells were scraped in 400 µL of PBS and ^{65}Zn

radioactivity counted in an auto-gamma counter (Perkin Elmer auto gamma counter, 1480 Wizard3).

Statistics

The radio activity of Zn obtained as CPM was converted to DPM (44% counting efficiency) and the exact amount of zinc taken up from each sample was calculated using specific activity. The descriptive statistics such as mean and SD were calculated using Microsoft excel and one way ANOVA with post-hoc 't' test using SPSS version seven package. The results were considered significant if $p<0.05$.

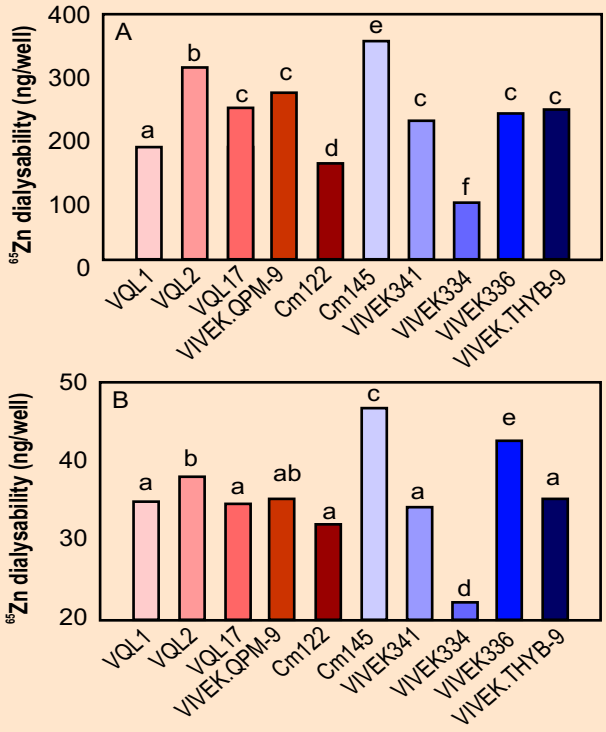
RESULTS

Zinc content of maize samples

The zinc content of maize genotypes is given in Table 31, which varied from a low of 0.76 mg/100g in VIVEK 334 to a high of 2.34 mg/100g in VIVEK 336.

Dialyzability and Caco-2 cell uptake of zinc from maize samples: The ^{65}Zn dialyzability and % uptake is given in Table 31

Fig 6. Bioavailability of zinc from biofortified maize genotypes



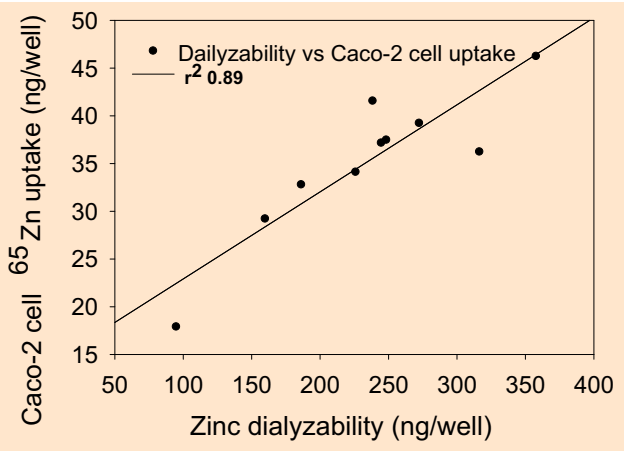
The maize seed samples were cooked and traced with ^{65}Zn and subjected to simulated in vitro digestion and the dialysability and uptake of zinc was measured in differentiated Caco-2 cells. Bars that do not share common superscript differ significantly ($p<0.05$)

Table 31. Zinc content, dialysability and Caco-2 cell uptake from biofortified maize genotypes

S. No	Maize sample ID	Zinc (mg/100g)	Dialyzable Zinc (ng/well)	% Dialyzable	Zn uptake in Caco-2 cells (ng/well)	% Uptake
QPM versions						
1	VQL1	1.43	186^{a} ±4.3	11.3	32.78^{a} ±4.13	1.98
2	VQL2	1.46	316^{b} ±2.1	18.8	36.22^{b} ±2.03	2.15
3	VQL17	1.30	248^{c} ±16.4	16.5	32.46^{a} ±1.04	2.16
4	VIVEK QPM-9	1.71	272^{c} ±19.6	13.8	33.22^{ab} ±3.37	1.68
NORMAL INBREAD/HYBRID						
5	CM122	1.42	159^{d} ±6.5	9.7	29.20^{a} ±1.32	1.78
6	CM145	1.87	357^{e} ±17.2	16.5	46.23^{c} ±1.27	2.14
7	VIVEK341	1.45	226^{c} ±27.5	13.5	32.10^{a} ±0.76	1.92
8	VIVEK334	0.76	95^{f} ±9.1	10.8	17.89^{d} ±1.44	2.04
9	VIVEK336	2.34	238^{c} ±18.0	8.8	41.55^{e} ±2.75	1.54
10	VIVEK THYB-9	1.69	244^{c} ±10.7	12.5	33.15^{a} ±2.17	1.70

All are inbread except Vivek THYB- 9 and Vivek QPM 9. The values with different superscript differ significantly ($p<0.05$).

Fig 7. Relationship amongst dialyzable zinc content and uptake in Caco-2 cells



Dialyzable zinc content from maize genotypes showed a significant correlation (r^2=0.89) with zinc uptake in Caco-2 cells.

and Fig 6. Among the maize genotypes tested the dialyzability of zinc from CM145 and VQL2 was significantly higher compared to all the other genotypes. The variety VIVEK 334 showed the lowest dialyzability. The zinc uptake in Caco-2 cells was the highest in CM145 followed by VIVEK 336 and VQL2 compared to all the other genotypes. There was a significant correlation between dialyzability and the uptake of zinc in Caco-2 cells (Fig 7. $p<0.001$).

CONCLUSIONS

The zinc bioavailability from the maize variety CM 145 appears to be higher compared to other maize genotypes tested.

2 DEVELOPMENT OF A VALID AND RELIABLE QUESTIONNAIRE FOR TESTING KNOWLEDGE ON MICRONUTRIENTS AMONG ADOLESCENT STUDENTS

Despite the intuitive appeal of education as a means of improving diet, many studies in this area have failed to find significant associations between nutritional knowledge and dietary intake. This could be due to the application of knowledge questionnaires that are not psychometrically validated. Therefore, the aim of the present study was to develop a statistically validated knowledge questionnaire suitable for use among adolescent students on micronutrients and to apply the test and assess the relationship between knowledge and micronutrient status among a group of adolescents.

METHODOLOGY

The study design for development of questions and its application among adolescent students is presented in Fig 8.

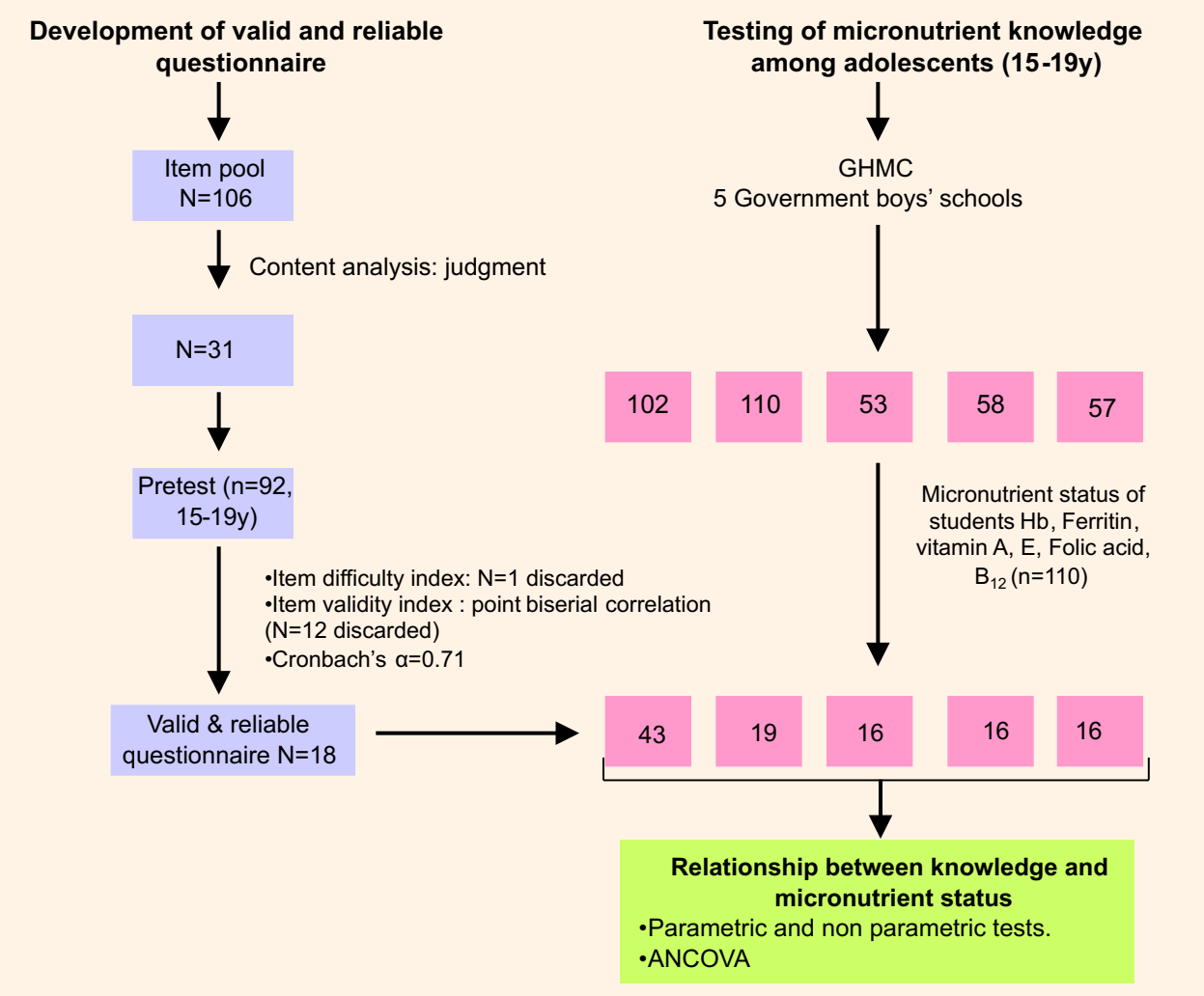
Development of questionnaire

The questionnaire was developed according to the method suggested by Kline (The Handbook of Psychological Testing: 1993) and Garret and Woodsworth (The reliability and validity of test scores, in statistics: in psychology and education, 1969). Briefly, the method consists of the following steps:

Item pool

Considering the major micronutrients iron, vitamins A & E, vitamin B_{12}, vitamin C, folic acid and other B vitamins, an item pool of 106 questions were designed. The three major domains included

Fig 8. Development, validation and testing of knowledge questionnaire on micronutrients 'N' indicates number of questions and 'n' and the numbers in the box indicates number of students



were functions, signs of deficiencies and sources of micronutrients. A few questions on general health and balanced diet were also included. The item pool was subjected to content analysis.

Content analysis

Content analysis was done by the judgment method by two reviews done by an expert panel from the field of nutrition, psychology medicine and biochemistry to select the best in terms of clarity of the questions, accuracy of the dietary knowledge being tapped, and interpretability. Some questions were reworded for ease of understanding. The content analysis resulted in a selection of 31 items retained within the 3 domains.

Questionnaire pre-test

The questionnaire was pre-tested in a group of higher secondary students aged 15-19 years from a school catering to students of middle income group (n=92). The group consisted of students from science and commerce streams (n=46 per stream respectively) of both genders (37 girls and 55 boys).

Item difficulty (DI)

The item difficulty was determined by the number of students in the group who performed correctly on the item. The percentage of students scoring correctly on each item has been calculated and averaged to get the DI. The students who scored in the top and bottom 27% on the knowledge scale were only included for analysis. DI of >0.1 and <0.9 were considered acceptable.

Item validity

The validity index of an item is determined by the extent to which the given item discriminates among examinees who differ sharply in the function measured by the test as a whole. Point bi-serial correlation was used for item validity test using SPSS version 16.0. Bi-serial 'r' gives the correlation of an item with the total score on the test. Items having zero validity and those with negative validity were discarded and a minimum 'r' value of ≥0.1 was considered valid.

Determining the reliability of the test

Rational equivalence method was used to assess the reliability of the questionnaire using Cronbach's alpha in SPSS version 16.0. Cronbach's alpha estimates the reliability of test scores with respect to (a) how well the individual items of the scores fit together, and (b) whether they assess the same construct. This is also referred to as internal consistency. The inter-correlations of the items in the test and the correlations of the items with the test as a whole were assessed. A Cronbach's alpha of above 0.7 was considered as having good internal consistency.

Application of the test

The knowledge questionnaire was applied to a group of students participating in a 2 year study on 'Stress, allostatic load and micronutrient status among students; impact of dietary advice'. For this purpose 5 boys' schools from three different geographical locations of Greater Hyderabad Municipal Corporation were selected. From a total of 380 students, a sub-sample of 110 where micronutrient status was available formed the sample frame for testing the validated knowledge questionnaire. The micronutrient status of the students was analysed (Fig. 8)

Micronutrient status

The baseline blood samples were collected after an overnight fast and analyzed for hemoglobin by cyanmethemoglobin method and plasma for ferritin by sandwich ELISA, folic acid and vitamin B12 (RIA kit by Siemens Inc). Simultaneous determination of plasma retinol and α –tocopherol was carried out by HPLC. Ascorbic acid assay was done on the same day using α- α dipyridyl micromethod. The ferritin ELISA and HPLC methods for retinol and alpha tocopherol were done in-house, using methods externally validated with VITAL-EQA program (CDC), Atlanta. The percentage inadequacy of micronutrient status was computed from cut-off values derived from literature.

Statistical analysis

All statistical analysis was done using SPSS version 16.0 (SPSS, Inc., Chicago IL, USA). Descriptive statistics were used to summarize the results. The relationship between knowledge questionnaire and micronutrient status was tested by independent samples 't' test between groups above and below 50th percentile of knowledge score. For micronutrients which were not normally distributed, nonparametric Mann–Whitney U test was done. Analysis of co-variance was performed taking standard of living index as a covariate, knowledge as a predictor and micronutrient status as the dependent variable. Linear regression was also done with micronutrient status as the dependent variable and knowledge scores as a predictor.

RESULTS

The process of validating the questionnaire included item difficulty, item validity and reliability.

Item difficulty

The DI of the 31 item questionnaire ranged from 0.12 to 0.90. One question showed a DI of 0.9 and was therefore discarded. A DI of 0.2-0.8 was considered as questions of ideal DI which included 23 questions. Three questions were difficult i.e., DI of 0.10-0.20 and 4 questions were easy (0.80-0.90) (Table 32).

Table 32. Difficulty index (DI) for the domains of knowledge questionnaire (n=50)

Domain	No. of questions	% of students scored correct in the top 27%	% of students scored correct in the bottom 27%	Difficulty Index
Micronutrient functions and deficiencies	15	28-100	4-80	0.22-0.88
Dietary sources	16	16-96	0-84	0.12-0.9

The percentage of students scoring correctly on each item has been calculated and averaged to get the DI. The students who scored in the top and bottom 27% on the knowledge scale were only included for analysis. DI of >0.1 and <0.9 were considered acceptable.

The relatively easy questions (0.8-0.9) were considered based on literature citing that if the study group under consideration is supposed to have a lower knowledge, a higher cut-off can be used. Three of the four difficult questions were retained, considering its content validity of the attribute not being assessed by any other question.

Item validity

A total of 12 items were removed at different stages based on validity test showing point bi-serial correlation below 0.1. The resulting 18-item questionnaire had a correlation ranging from 0.1-0.6. 14 questions had item validity above 0.2. Apart from item validity, content validity was also considered while discarding the items (Table 33). Based on this, four items in the range of an r value of 0.1-0.2 were retained.

Table 33. Item analysis and reliability: Point bi-serial correlation (r) and corresponding Cronbach's alpha reliability

Stages of analysis	No. of items (N)	Range of point bi-serial 'r'	Cronbach's alpha
1	30	-0.3451 to +0.4808	0.4969
2	23	+0.0289 to + 0.5751	0.6697
3	20	+0.0675 to +0.6040	0.6971
4	18	+0.1000 to +0.6227	0.7100

Point bi-serial correlation was calculated using SPSS version 16.0.

Item validity index, measures the ability of the item to discriminate between those who do well on the test and those who do not. A large number of items belonging to the domain of dietary sources did not show an item to total correlation. The variability of the components related to nutrition knowledge and its wide applications might be a reason for this. The items included 'groundnuts are an important source of vitamin E, and some items like cooking leads to a loss of B vitamins', 'food is the major source of nutrients for the body'.

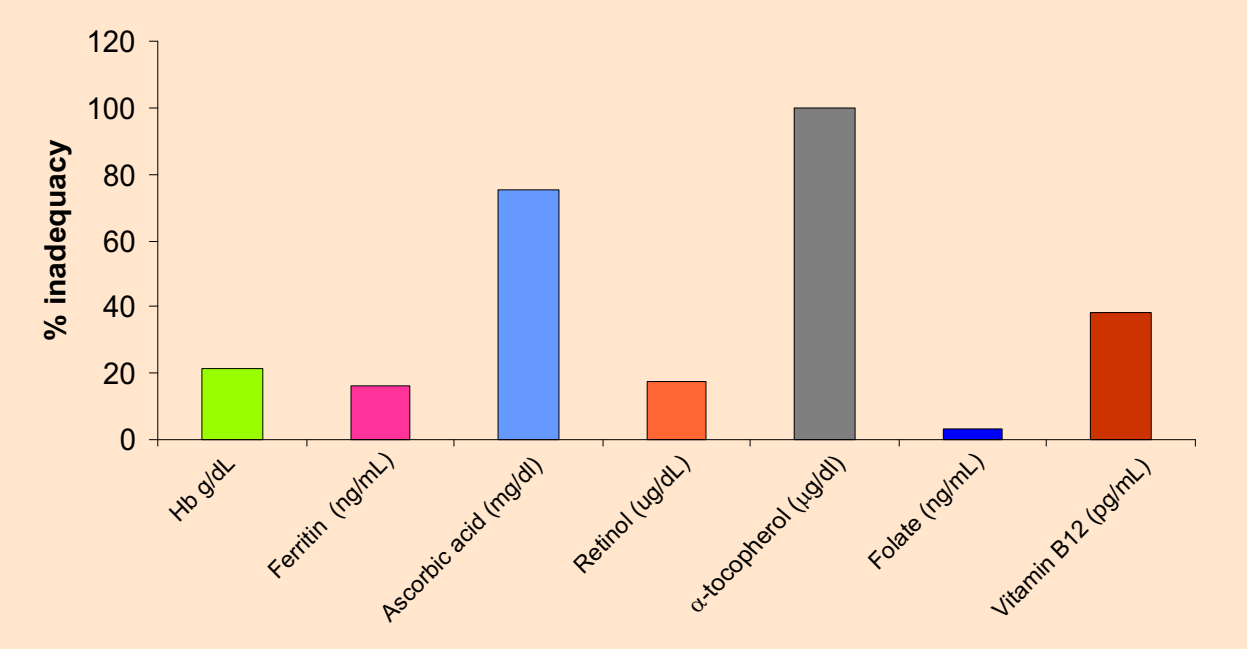
Reliability

A Cronbach's alpha value of 0.71 was obtained for the 18 item scale. The domains did not show an adequate reliability but the internal consistency improved on combining the domains. The alpha value was considered adequate for a reliable test. The developed questionnaire had shown an adequate reliability and therefore can be used among adolescents of 15-19years.

Micronutrient status

The mean±SD values were 14.1 ± 1.63 g/dL for hemoglobin, 44.6 ± 80.1 ng/mL for ferritin, 0.42± 0.29 mg/dL for ascorbic acid, 26.3 ±7.4µg/dL for serum retinol, 328.9 ±164.42 µg/dL for alpha tocopherol, 5.5 ± 1.9 ng/mL for folic acid and 245.0 ± 110.00 pg/mL for vitamin B_{12}. The percent inadequacy of these micronutrients is presented in Fig 9. The deficiency of vitamin E (100%), vitamin C (75%) was very high among students. The percent inadequacy of vitamin B_{12} was 38%, the status of iron (21% anemia and 41% iron deficiency) and folic acid (3.5%) and retinol (21%).

Fig 9. Percent inadequacy of micronutrients among adolescent boys of Government schools (n=110)



Knowledge scores

The knowledge scores of the students ranged from 0-14 with a mean of 5.2± 2.68. About 59% of the students scored below 50th percentile of knowledge scores (score of 5.0). The relationship between micronutrient status and knowledge was tested among students below and above 50th percentile scores (Table 34).

Knowledge and micronutrient status among students

The relationship between micronutrient status and categorical scores of knowledge is presented in Table 34. Serum retinol status showed a statistically significant change between the groups above and below 50th percentile knowledge scores (P=0.022). Other micronutrients did not show any change between groups. Linear regression and ANCOVA showed knowledge to be a significant predictor of serum retinol status ((P=0.022, P=0.018 respectively).

Table 34. Indicators of micronutrient status based on below and above 50th percentile of knowledge scores

Indicators	Knowledge category	N	Micronutrient status	
			Mean	SD
Hb g/dL	Below	65	13.7	2.36
	Above	45	14.1	2.64
Ascorbic acid (mg/dL)	Below	64	0.42	0..282
	Above	45	0.42	0.308
Retinol µg/dL	Below	65	24.8*	6.69
	Above	45	28.0	7.67
α-tocopherol µg/dL	Below	65	326	200.0
	Above	45	331	99.5
Folate ng/mL	Below	65	5.4	1.84
	Above	45	5.6	2.09
B_{12} pg/mL	Below	65	244	108.1
	Above	45	248	114.9
Ferritin ng/mL	Below	65	46.9	101.47
	Above	45	40.4	35.06

The knowledge scores below indicates knowledge scores less than 50th percentile.
** indicates P<0.05.*

CONCLUSIONS

- A valid and reliable 18-item questionnaire was constructed for assessing knowledge on micronutrients among students which could discriminate in serum retinol between students above and below 50th percentile of knowledge scores. The relationship established between serum retinol and micronutrient knowledge among adolescents is first of its kind and emphasizes the need for using validated questionnaires.

3. MATERNAL VITAMIN B_{12} AND/OR FOLATE RESTRICTION INDUCED CHANGES IN BODY ADIPOSITY, HYPERGLYCEMIA AND INSULIN RESISTANCE IN WNIN RAT OFFSPRING: MOLECULAR BASIS OF THE CHANGES

In an attempt to validate/negate the hypothesis that maternal vit B12 and/or folate deficiency increases the body adiposity and insulin resistance in the offspring perhaps through changes in epigenetic mechanism(s), has demonstrated the following in the Wistar rat model earlier.

i) Developed animal (rat) models of folate and / or vitamin B12 deficiency that simulate real life human situations.

ii) Demonstrated changes in body composition, central adiposity (Adiposity Index), plasma lipid profile, glycemic status, oral glucose tolerance, oral glucose tolerance / glucose stimulated insulin levels in the offspring of different groups at quarterly intervals between 3 and 12 months of age.

iii) In view of the changes observed in the development and function of adipose and muscle tissues, glucose and lipid metabolism. The underlying / associated biochemical changes like: glucose uptake (basal and insulin stimulated) by muscle (diaphragm), activity of rate limiting enzymes involved in carbohydrate metabolism (Glycolysis: glucokinase, pyruvate kinase; Gluconeogenesis: glucose-6-phosphotase, Fructose-1,6-bisphosphatase, phosphoenol-pyruvatecarboxykinase), circulating and adipose tissue levels of various adipocytokines, activities of fattyacid synthase, acetyl-CoA – carboxylase was studied.

iv) Although changes were observed in all the vitamin deficient offspring , considering that they were significant in only B12 restricted offspring, the reversibility/preventability of the changes was assessed by rehabilitating the B12 restricted mothers from conception, parturition and their offspring from weaning and showed that most of the changes were preventable/ reversible by B12 rehabilitation from conception and parturition .

1) Increased oxidative stress and/or changes in antioxidant enzyme activity (in liver) and glucocorticoid stress (plasma cortisol levels) could be the probable common mechanism(s) associated with these effects of maternal folate and/or vitamin B12 deficiency in the offspring have been determined.

Work done during the year

Considering that literature is replete with reports of trans-generational transfer of the effects of maternal under-nutrition in the offspring, whether or not the maternal vitamin B12 and/or folate restriction induced changes observed in F1 offspring are transmitted across to the next generation (F2) was assessed using the Wistar rat model.

Experimental

Weaning (21 day old), Wistar female rats (n=24) were divided into four groups: control, folate restricted, vitamin B12 restricted (B12R) and folate and vitamin B12 restricted (Dual deficient, DD) (n=6 each).They were fed *ad libitum* for three months, a casein based (20 % protein) (AIN 76 A) control diet or the same diet restricted in folate and/or vitamin B12 (M/S Research Diets Inc, USA). B12R and DD diets also contained 50g pectin / kg diet (in addition to cellulose).

After ensuring vitamin B12 and /or folate deficiency in them (by plasma vitamin B12and / or folate levels), the animals were mated with control males and the dams continued on their respective diets through out pregnancy, lactation and weaning. Body weight gain of pregnant dams, birth and weaning weights of the pups were recorded. A uniform litter size of six pups (equal numbers of males and females wherever possible) was maintained with each mother from day 3 of lactation.

To study the trans-generational effects, female F1 offspring (n= 24 for each group) of different groups were maintained on their respective mothers' diet from weaning and at 3 months of age, their body composition was monitored by TOBEC and their plasma levels of vitamin B12, folic acid and lipid profile were determined. GTT was conducted to assess insulin response to glucose challenge. Fasting levels of plasma glucose and insulin (and HOMA IR) were determined as also the AUC of glucose and insulin during an oral glucose tolerance test.

F1 females were then mated with control males and they continued on their respective diets through pregnancy, lactation and their offspring (F2) were weaned on to their respective diets. Trans generational effects (body composition and biochemical parameters) were assessed both in male and female offspring at three months of their age The data has been analyzed statistically using SPSS statistics package (version 10.0).

Salient observations in F1 offspring

1. All the effects seen in Wistar colony females after feeding folate and / or vitamin B12 restriction diets for three months were also observed in F1 female offspring at three months of their age .
2. Although no effects were seen in the reproductive performance the F1 females, as observed earlier in F1 male offspring, birth weight of F2 female B12R offspring was significantly lower than that of F2 controls. However, weaning weights were comparable among F2 offspring of the different groups. As expected, their plasma folate and/or B12 status continued to be lower than their F2 controls although food intake was comparable among groups. Unlike our earlier observations in F1 male offspring, body weights, BMI and body composition of F1 female offspring were comparable among groups but for the decreased fat % and increased LBM % & FFM% in DD rats. Also, neither visceral adiposity nor tissue associated fat was altered among F1 female offspring of different groups. Plasma lipid profile was comparable among the groups but for the increased circulating FFA levels in B12R offspring. Fasting plasma glucose, insulin, HOMA-IR, AUCs of glucose & insulin during OGTT and the AUC ratio were comparable among the groups. Interestingly, reproductive performance was comparable among F1 female offspring of different groups.

Salient observations in F2 offspring

- The F2 B12R offspring weighed significantly less compared to F2 offspring of control and other groups. However, weaning weights were comparable among the groups. Plasma folate and/or B12 levels were lower than their respective controls.
- Food intake, body weights and BMI were comparable among F2 male offspring of different groups but a significant increase in body fat% (and visceral adiposity) and decrease in LBM% & FFM% were observed in F2 offspring of all vitamin restriction groups while tissue associated fat was significantly decreased in DD rats only. Plasma cortisol and lipid profile was comparable among the groups. Fasting plasma glucose, insulin, HOMA-IR and AUCs of glucose and insulin during OGTT and the AUC ratio were also comparable among groups.
- Except for the decreased vitamin B12 and or/ folate levels in the respective vitamin restricted groups, all parameters were comparable among F2 female offspring of different groups.

Summary of the findings

- That altered body composition seen by us earlier in F1 male offspring born to vitamin restricted mothers was observed in F2 male offspring is in line with literature reports of similar nature. Further, the results stress the importance of maternal folate and/ or vitamin B12 deficiency, both widely prevalent among Indian women/ mothers in trans generational transfer of the effects on body composition of the offspring, which are known to underlie insulin resistance and associated diseases in later life.

However, no alterations were seen in body composition or any other biochemical parameters in the female offspring of either F1 or F2 generations is perplexing and probably suggests variable effects of maternal folate and/or vitamin B12 deficiency in the offspring of different sexes and need further confirmation.

4. INSULIN, INSULIN RECEPTOR AND ITS SIGNALING MECHANISMS IN THE BRAIN AND INSULIN SENSITIVE TARGET ORGANS IN NIN OBESE MUTANT RATS (WNIN/Ob AND WNIN/Gr-Ob)

&

CENTRAL REGULATORY MECHANISMS UNDERLYING OBESITY IN WNIN OBESE MUTANT RATS

Obesity is caused due to genetic factors and /or altered energy homeostasis. Brain receives/ responds to a number of factors that influence energy homeostasis. Insulin, one such factor binds to its receptors in the brain/ hypothalamus and inhibits food intake/ increases energy expenditure. A rat model for obesity: WNIN- Obese (sumo) rat has been developed at NIN and they resemble Neuron specific insulin receptor knockout mice in their physiological and biochemical parameters (hyperphagic, hyperinsulinemic, obese and infertile). Impaired brain/ hypothalamic insulin function/ signaling could underlie the hyperphagia, obesity, hyperlipidemia, hyperinsulinemia and infertility in WNIN obese rats were hypothesized. The salient observations of the studies conducted in six months old, female, WNIN/Ob rats and their lean, carrier phenotypes and WNIN control females of comparable age are presented below.

Salient observations

Levels of plasma glucose and circulating endocrine hormones (especially insulin and glucagon) involved in glucose homeostasis and pituitary hormone levels (eg. TSH) were altered (increased) in obese rats compared to lean controls, especially during fed state. Indicating altered energy utilization / homeostasis in WNIN / Ob rats under fed conditions. That, despite significant peripheral hyperinsulinemia WNIN-Obese rats were hyperphagic, suggests probable impairment in insulin action in the brain /hypothalamus.

Considering the earlier reports that Zucker rats had low levels of insulin in hypothalamus, it was considered important to decipher whether the hyperphagia in WNIN/Ob rats was due to low levels of hypothalamic insulin and/ or impaired hypothalamic insulin signaling. For this purpose, insulin intra-cerebroventricularly (@ 240 mU/day) continuously infused for a week using an indwelling Aztec osmotic pump and observed that ICV infusion of insulin decreased food/ energy intake in control but not in WNIN – Ob rats. These findings appear to suggest that hyperphagia in WNIN/Ob rats may be due to impaired hypothalamic insulin signaling but not low levels of insulin.

Since, insulin acts through its cognate receptor and signaling molecules, the expression of important membrane bound and cytosolic insulin signaling molecules (and their phosphorylated/ active forms) were quantified by Western Blotting in the hypothalamus of overnight fasted and fed rats. Insulin signaling molecules especially the insulin receptor and its phosphorylated form, IRS1, p-IRS1, Glut-4 and Glut-3, and Akt-1 were significantly decreased in the hypothalamic membrane of obese rats compared to lean indicating impaired insulin signaling as well as a probable decrease in glucose uptake and/ or altered glucose homeostatsis in the hypothalamus. The resultant failure of hypothalamic neurons to attain satiety probably sends signals (eg. increased plasma glucagon level) that could result in hyperphagia and the consequent hyperinsulinemia.

Since, insulin modulates the expression of orexigenic and anorexigenic neuropeptides which are important in regulating food intake, expression (mRNA) of neuropeptides were checked by semi-quantitative PCR. Although their expression (mRNA) was comparable between WNIN-Ob and lean control rats, the expression of the receptors for these neuropeptides (for eg, MC4R and

NPY5R) was altered in obese rats compared to their lean counterparts. These findings appear to suggest altered (impaired) binding of neuropeptides to their cognate receptors and the resultant altered food intake (hyperphagia) in WNIN/Ob rats.

The neurotransmitters Dopamine (orexigenic) and Serotonin (anorexigenic) levels were comparable between WNIN/Ob and lean controls. However, when compared between fasting and fed states within a given phenotype, WNIN/ob rats had a decrease (albeit not significant) in serotonin and a significant decrease in HIAA, a metabolite of serotonin in fed the state. These findings probably indicate a defect in serotonin (the anorectic neuro transmitter) metabolism and hence the resultant hyperphagia.

The impairment in brain/hypothalamic insulin signaling, impaired hypothalamic glucose uptake/ energy homeostasis, failure to attain satiety, decreased neuropeptide receptors and serotonin metabolism in obese rats could either singly or together be responsible for the hyperphagia and obesity observed in the six months old female WNIN obese mutant rats.

5. EFFECT OF DIFFERENT METHODS OF COOKING ON NATURAL ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF GREEN LEAFY VEGETABLES COMMONLY CONSUMED IN INDIA

Epidemiological evidences underline the crucial role of diets in prevention of chronic degenerative diseases. Plant derived phenolic compounds are reported to have multiple biological effects including antioxidant activity. Plant foods are rich sources of phenolic antioxidants. It is hypothesized that phytochemicals in plant foods exert health beneficial effects beyond nutrition and combat oxidative stress and maintain balance between oxidants and antioxidants. Data is scanty on phenolic content (PC) and antioxidant content (AOC) of Green Leafy Vegetables (GLVs) commonly consumed in India. Since, GLVs are often consumed in some cooked form or the other, changes/ losses if any in the phenolic content and AOA of a few commonly consumed GLVs during different types of domestic cooking have been assessed.

MATERIALS AND METHODS

Commonly consumed GLVs (based on the NNMB survey) were collected from three markets of Hyderabad. Samples were analyzed in duplicates and mean values are presented on fresh weight basis. Total quantity of each sample collected was between 50 -100g. Edible portions of the sample were started out and washed in tap water and blotted on blotting paper. Each sample (10 grams each) was made into four parts. First portion was processed as such to know its natural (raw) antioxidant activity, while the 2^{nd}, 3^{rd} and 4^{th} portions of the sample were subjected to conventional, pressure and microwave methods of cooking respectively. Briefly, 10 grams of GLV sample was cooked in 50 ml of water for 3 - 10 minutes (in case of conventional cooking it took about 10 minutes, pressure cooking was done for about 5 minutes and micro wave cooking for 3 minutes). Cooking was in general done with the cooking vessel covered with a lid except in conventional cooking. Whereas to estimate natural (raw) antioxidant content 1^{st} portion of 10g GLVs sample was extracted as such in 80% methanol containing 0.1% HCl and final volumes of all the four extracts were made equal with 80% methanol. Standard extraction and estimation protocols described earlier were

adopted (Annual Reports 2006-2010). While the phenolic content (PC) was determined by the Folin-Ciocalteu method and anti-oxidant content (AOC) was determined by two different methods. 1. FRAP (Ferric Reducing Scavenging) 2. DPPH Radical Scavenging activity.

RESULTS

PC and AOA of the raw food samples

1. A total of eleven GLVs were chosen for the study, among which PC content of raw curry leaves was the highest (1077 mg/100g) followed by mint (440 mg/100g) and the lowest was in spinach (77mg/100g; Table 35).
2. DPPH content of mint was the highest (1368 mg/100g) followed by curry leaves (1020mg/100g) and the lowest was in spinach (21mg/100g) among the raw GLVs (Table 36).
3. FRAP activity showed similar trends like DPPH in their natural AOC, the highest being in mint followed by curry leaves and the lowest was in Spinach. The FRAP values were 27827, 2027 and 1380 mg/100g respectively (Table 37).

Table 35. Effect of domestic processing on polyphenol content of commonly consumed Green Leafy Vegetables

Sl. No.	Common Name	Botanical Name	Phenolic Content (mg/100g Gallic acid Eq)			
			Raw	Conventional	Pressure	Microwave
1	Amaranth	*Amaranthus gangeticus*	253.0 [a] (100)	275 [b] (108)	355 [c] (140)	312 [d] (123)
2	Ambat chuka	*Rumex vesicarius*	100.3 (100)	90 (89)	93 (92)	91 (91)
3	Coriander leaves	*Coriandrum sativum*	239.6 [a] (100)	417 [b] (174)	451 [c] (188)	506 [d] (211)
4	Curry leaves	*Murraya koenigii*	1077.0 [a] (100)	1434 [b] (133)	1184 [c] (109)	1377 [d] (127)
5	Fennel leaves	*Foeniculum vulgare*	251.3 (100)	268 (106)	265 (105)	312 (124)
6	Fenugreek leaves	*Trigonella foenum graecum*	163.3 [a] (100)	180 [a] (110)	176 [a] (107)	220 [b] (134)
7	Purslane leaves	*Portulaca oleracea*	94.6 [a] (100)	128 [b] (135)	138 [b] (146)	128 [b] (135)
8	Gogu	*Hibiscus cannabinus*	191.3 (100)	194 (101)	211 (107)	213 (111)
9	Mint	*Mentha spicata*	440.3 [a] (100)	657 [b] (149)	796 [c] (180)	761 [c] (172)
10	Ponnanganni	*Alternathera sessilis*	136.3 (100)	122 (89)	110 (80)	123 (90)
11	Spinach	*Spinacia oleracea*	77.0 [a] (100)	96 [b] (125)	125 [c] (162)	117 [c] (152)

Mean values were compared (n=3) by Non-parametric kruskal walies one way ANOVA. Differences in alphabets are significantly different at p < 0.05.Percent gain or loss calculated when raw value taken as 100%. Percent recovery values are given in parentheses. Decimal points are not given due to higher numbers.

Table 36. Effect of domestic processing on DPPH activity of commonly consumed green leafy vegetables

Sl. No.	Common Name	Botanical Name	DPPH (mg/100g Trolox Eq)			
			Raw	Conventional	Pressure	Microwave
1	Amaranth	*Amaranthus gangeticus*	405.6 [a] (100)	520 [b] (128)	527 [b] (129)	476 [b] (117)
2	Ambat chuka	*Rumex vesicarius*	85 .3 (100)	87 (101)	83 (97)	94 (110)
3	Coriander leaves	*Coriandrum sativum*	471.0 [a] (100)	886 [b] (181)	948 [b] (201)	1100 [c] (233)
4	Curry leaves	*Murraya koenigii*	1020.6 [a] (100)	950 [b] (93)	1724 [c] (168)	1418 [d] (138)
5	Fennel leaves	*Foeniculum vulgare*	545.3 (100)	592 (108)	540 (99)	746 (136)
6	Fenugreek leaves	*Trigonella foenum graecum*	144.3 (100)	142 (98)	127 (87)	193 (134)
7	Purslane leaves	*Portulaca oleracea*	138.3 (100)	162 (117)	165 (119)	151 (109)
8	Gogu	*Hibiscus cannabinus*	346.0 (100)	365 (105)	334 (96)	456 (131)
9	Mint	*Mentha spicata*	1368.6 (100)	2055 (150)	1856 (135)	2020 (147)
10	Ponnanganni	*Alternathera sessilis*	173.0 (100)	172 (99)	203 (117)	198 (114)
11	Spinach	*Spinacia oleracea*	21.6 [a] (100)	69 [b] (321)	85 [c] (393)	104 [d] (481)

Mean values were compared (n=3) by Non-parametric kruskal walies one way ANOVA. Differences in alphabets are significantly different at p < 0.05.Percent gain or loss calculated when raw value taken as 100%. Percent recovery values are given in parentheses. Decimal points are not given due to higher numbers.

Effect of cooking on PC and AOA of GLVs

In general, there were significant differences among different cooking methods in the effects on PC and AOA of the GLVs studied (Table 35-37).

1. Effect of cooking on PC is presented in Table 35. Except *ambat chukka* and *ponnaganti* which showed a 10 -20% decrease in their PC on cooking and *gogu* showed very little or no effect on cooking, all other GLVs showed an increase in PC during different cooking methods. PC content of cooked GLVs ranged from 108 -146 % of their respective uncooked/ raw sample. Similar findings were reported earlier in spinach leaf extracts.
2. Coriander, mint and spinach showed a significant increase in PC in different cooking methods and the percent increase ranged from 125 -211 (Table 35).
3. As compared to natural GLVs, percent increase or decrease in their antioxidant properties, on different types of cooking showed a mixed trend. Most of the GLVs showed an increasing trend ranging from 128 -168%, whereas coriander and spinach showed an enormous increase ranging from 181-233%, 321-481% respectively (Table 36).

4. Effect of cooking on FRAP in GLVs (Table 37) also showed a mixed trend. Most of the GLVs (nine out of eleven) showed an increase, ranging from 119 -181%. Here again, coriander and spinach showed an enormous increase, ranging from 261-277% and 231-253% respectively. On the other hand two GLVs : ambat chukka and ponnaganti showed a decrease of 10%.

5. The findings are in line with literature which also shows a complex trend on cooking, which is unexplainable and requires further research. The possible mechanism for the increase or decreasing trends in various cooking methods could be that the phenolics were stored in pectin or cellulose networks of plant foods and are released during thermal processing.

That there were significant correlations (rank correlation) among the PC, FRAP and DPPH among the GLVs both in raw as well as cooked (by different methods) forms suggests that phenolics are important contributors to the AOA of the GLVs studied both in the raw and processed forms (Table 38).

Table 37. Effect of domestic processing on FRAP activity of commonly consumed Green Leafy Vegetables

Sl. No.	Common Name	Botanical Name	FRAP (mg/100g $FeSo_4$ Eq)			
			Raw	Conventional	Pressure	Microwave
1	Amaranth	*Amaranthus gangeticus*	8237.6 ^{a} (100)	11370 ^{b} (138)	12102 ^{b} (146)	11786 ^{b} (143)
2	Ambat chuka	*Rumex vesicarius*	3511.6 (100)	3270 (93)	2946 (83)	3243 (92)
3	Coriander leaves	*Coriandrum sativum*	7125.6 ^{a} (100)	18636 ^{b} (261)	16123 ^{c} (226)	19802 ^{d} (277)
4	Curry leaves	*Murraya koenigii*	20275.0 ^{a} (100)	18533 ^{b} (91)	24213 ^{c} (119)	27392 ^{d} (135)
5	Fennel leaves	*Foeniculum vulgare*	9238.6 ^{a} (100)	10128 ^{a} (109)	9970 ^{a} (107)	13362 ^{b} (144)
6	Fenugreek leaves	*Trigonella foenum graecum*	3409.6 ^{a} (100)	3919 ^{b} (114)	4799 ^{c} (140)	5429 ^{d} (159)
7	Purslane leaves	*Portulaca oleracea*	2863.3 ^{a} (100)	4327 ^{b} (151)	4800 ^{c} (167)	4030 ^{b} (140)
8	Gogu	*Hibiscus cannabinus*	5254.0 (100)	7274 (138)	6921 (131)	7107 (135)
9	Mint	*Mentha spicata*	27827.6 ^{a} (100)	42562 ^{b} (152)	48909 ^{b,c} (175)	50401 ^{c} (181)
10	Ponnanganni	*Alternathera sessilis*	5068.3 (100)	4280 (84)	4837 (95)	4327 (85)
11	Spinach	*Spinacia oleracea*	1380.6 ^{a} (100)	3196 ^{b} (231)	3471 ^{b} (251)	3502 ^{b} (253)

Mean values were compared (n=3) by Non-parametric kruskal walies one way ANOVA. Differences in alphabets are significantly different at $p < 0.05$.Percent gain or loss calculated when raw value taken as 100%. Percent recovery values are given in parentheses. Decimal points are not given due to higher numbers.

Table 38. Rank Correlation between phenolic content vs. DPPH and FRAP in different cooking methods of GLVs

TPC Vs AOA	Raw	Traditional	Pressure	Microwave	Homogenity
TPC Vs DPPH	0.945	0.936	0.918	0.945	χ^2 =0.23, p=0.97
TPC Vs FRAP	0.955	0.936	0.927	0.973	χ^2 =1.23, p=0.74
DPPH Vs FRAP	0.964	0.973	0.991	0.991	χ^2 =3.23, p=0.36

All correlations are significant at p<0.001(n=11)

6. DIABETIC RETINOPATHY-ROLE OF MICRONUTRIENTS: (I) EVALUATION OF MICRONUTRIENTS STATUS OF DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes and ranks as a common cause of blindness worldwide. DR is a major public health problem considering the global prevalence of diabetes. While, the prevalence of DR varied (20-60%) in different studies, a recent study indicated that the estimated prevalence of DR was 28.5% among US adults with diabetes. The prevalence of DR was 0.5% in the general rural populations of Southern India and 10.5% among diabetic patients. In DR there is appearance of vascular lesions of increasing severity, culminating in the growth of new vessels. The early or non-proliferative DR (NPDR) is marked by retinal vascular microaneurisms, blot hemorrhages, and cotton-wool spots and there is a loss of retinal pericytes, increased vascular retinal permeability, alterations in regional blood flow, and abnormal retinal microvasculature, all of which lead to retinal ischemia. The more severe state of proliferative DR (PDR) is marked by the formation of abnormal fragile new blood vessels that are prone to hemorrhage. Studies have shown that the prevalence of DR increases with diabetes duration and intensive glycemic control could delay the development of DR. In principle, all diabetic patients might be expected to develop diabetic microvascular complications if hyperglycemia alone were the triggering factor for diabetic complications. It is however noteworthy that some patients may still develop DR even with good glycaemic control. Conversely, some patients with poor glycaemic control are spared from this complication and notably, in long surviving patients with type 1 diabetes (T1D), the association between DR and glycaemic control is less well supported. Thus, multiple factors are likely to be involved in predisposing diabetic subjects to complications, as evidenced by the fact that many but not all diabetic patients develop one or more microvascular complications.

If the predisposing susceptible factors are known, it may be possible to delay the onset and progression of these complication(s) with appropriate strategies. Thus, first and foremost there is a need to understand susceptible factors that predispose the diabetic subjects to complications. While a number of studies focused on the genetic susceptibility, role of environmental factors such as nutritional and dietary factors in the development of DR has not been investigated. Supplementation of vitamins and minerals for the management of Type-2 Diabetes though has

been reported, their role in the prevention and development of T2D in general and diabetic complications in particular has not been established clearly. Therefore, the main aim of the study was to evaluate the association between micronutrients (vitamins and minerals) and diabetic retinopathy with the broad objective to understand the role of micronutrients in the prevention or delay of diabetic complications.

METHODOLOGY

A hospital based case control study involving a total of 600 subjects (250 with diabetic retinopathy, 250 diabetic subjects without any complications and 100 normal non-diabetic subjects) with a well-defined inclusion/ exclusion criterion was undertaken with the approval of the Institutional Ethics Committee. The fundus of each subject was evaluated by both direct and indirect ophthalmoscopy and DR was defined and classified as NPDR or PDR. Full history of the subject with respect to age, sex, clinical symptoms, duration and type of diabetes, medication and socioeconomic back ground with the help of a well-designed questionnaire were collected.

Blood was drawn into anticoagulant tubes and red blood cells (RBC) were separated from plasma by centrifugation for the following analyses.

Biochemical parameters: Glucose, HbA1C, insulin and lipid profiles such as total cholesterol, triglycerides, HDL, LDL were analyzed. Standard operating procedures (SOP) were followed for the sample collection and in the analysis process. For the analysis of minerals, standards and reference materials were obtained from National Institute of Standards and Technology certified authorities. Appropriate standards and controls were obtained and used for the vitamin analysis.

Estimation of minerals: Analysis of trace elements such as Mg, Ca, Fe, Cu, Mn, Co, Cr, Se, Zn was done in plasma using Atomic Absorption Spectroscopy (AAS). For the analysis of Ca, Mg, Zn, Fe and Cu flame ionization method was used. For the estimation of Se, Co and Cr microwave digested samples were analyzed by Graphite Furnace AAS. Appropriate Standard reference Materials (SRMs) were obtained from National Institute of Standards and Technology (NIST) and other sources.

Determination of vitamins: Analysis of blood levels of vitamins A, B1, B2, B6, D, E and homocysteine was carried out by HPLC method using UV and fluorescent detection methods. Vitamin C levels were measured by spectrophotometric method. Vitamin B12 and folic acid were analyzed by dual count radioimmuno assay kit method.

RESULTS

1. There was no significant difference ($p>0.05$) between male and female subjects in all the three groups with respect to demographic profile and the measured parameters. Therefore, the data for both men and women in respective groups were pooled analysed as a whole. Mean age, body mass index (BMI) and hemoglobin levels were comparable between the groups. As expected the levels of glucose, insulin and glycosylated hemoglobin (HbA1C) were high in diabetic patients (without and with DR) compared to normal control subjects. While plasma total cholesterol and LDL were comparable between the groups, the levels of triglycerides were higher and HDL were lower in diabetic groups compared to control group. However, there were no significant difference in clinical and demographic profile between diabetic patients without and with DR.
2. While vitamin C levels were not significantly different between control and diabetic patients (without and with DR), levels of vitamin A and E levels were slightly higher in DR patients compared to diabetic patients without DR and control subjects. Vitamin D levels were found to be not only significantly lower in diabetic subjects when compared to normal subjects but also below normal range. However, there were no significant difference in vitamin D values between diabetic patients without and with DR (Table 39).

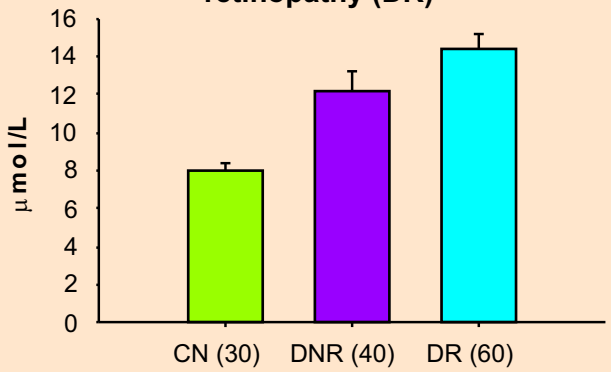
Table 39. Blood levels of vitamins A, C, D and E. CN-control subjects, DNR-diabetic patients without retinopathy and DR-diabetic retinopathy patients.

Vitamin	CN	DNR	DR
Vitamin A (µg/dL)	35.6±10.2 (n=30)	36.4±11.6 (n=30)	40.4±19.1 (n=45)
Vitamin C (µg/mL)	8.4±7.2 (n=60)	12.4±10.1 (n=60)	11.3±6.4 (n=60)
Vitamin D (ng/mL)	24.9±12.4 (n=40)	14.1±7.50 (n=40)	16.4±9.5 (n=80)
Vitamin E (mg/dL)	0.60±0.27 (n=30)	0.77±0.37 (n=28)	0.81±0.44 (n=45)

Values are mean ± SD

3. The mean plasma homocysteine levels were significantly higher in T2D patients compared to control subjects and a further increase ($p<0.05$) was found in DR group compared to DNR group (Fig 10).

Fig. 1 Plasma homocysteine levels in non-diabetic control and diabetic subjects without retinopathy (DNR) and with retinopathy (DR)



Data represent mean ± SE.

4. Blood levels of vitamin B1, B2 and B6 were determined by HPLC method. While, vitamin B1 levels were significantly different ($p<0.05$) between the groups, the levels were not different amongst DNR and DR (Table 40). Although, the plasma levels of vitamin B2 were comparable between the groups, plasma vitamin B6 levels were not only significantly ($p<0.05$) lower in diabetic groups (DNR and DR) compared to control group (Table 40) but also below the normal range. However, there was no significant difference in the mean levels of vitamin B6 between DNR and DR groups (Table 40). Similarly, plasma folic acid levels were lower in diabetic groups (DNR and DR) compared to control group but comparable between DNR and DR groups, although the values were in the normal range in all the groups (Table 40).

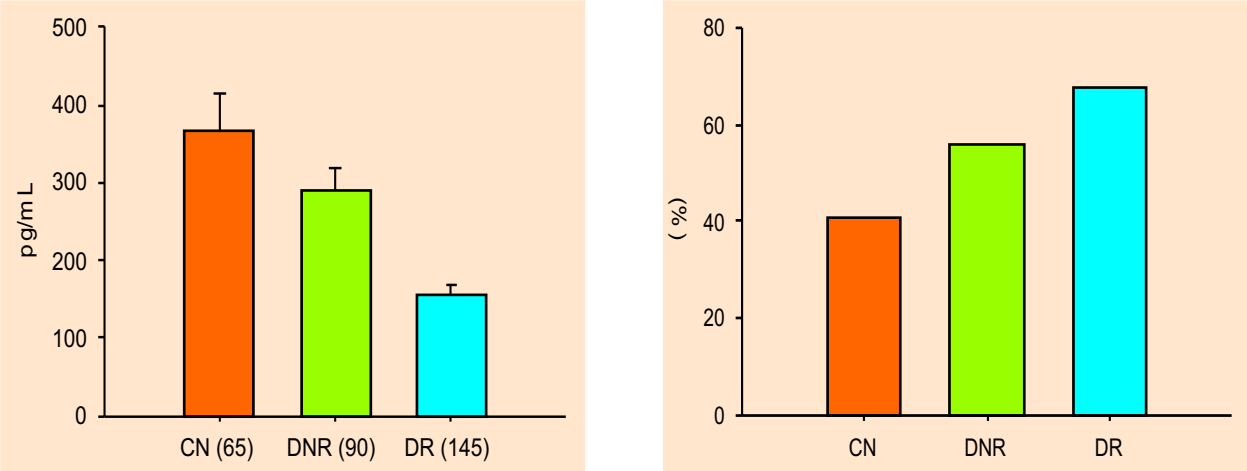
5. Plasma vitamin-B12 levels were significantly lower ($p<0.01$) in diabetic groups compared to control group (Fig 11). Interestingly, in this study a significantly lowered ($p<0.05$) plasma vitamin B12 levels was observed in DR patients compared to DNR patients (Fig 11A). Considering 200 pg/mL as the cut-off level, the prevalence of vitamin B12 deficiency was significant ($p<0.001$) between the groups; 67% in DR, 55% in DNR and 41% in control, respectively (Fig 11). Further,

Table 40. Blood levels of vitamin B1, B2 and B6 and folic acid in non-diabetic control (CN) and diabetic subjects without retinopathy (DNR) and with retinopathy (DR)

Vitamin	CN	DNR	DR
B1 (ng/mL)	57.6±3.1 (n = 30)	72.1±3.8 (n = 30)	67.2±3.0 (n = 45)
B2 (ng/mL)	231±9.3 (n = 30)	248±8.2 (n = 30)	238±7.0 (n = 45)
B6 (ng/mL)	20.6±1.3 (n = 40)	13.0±1.1 (n = 40)	14.6±1.0 (n= 60)
Folic acid (ng/mL)	10.0±0.9 (n = 65)	7.8±0.6 (n = 90)	7.2±0.4 (n = 145)

Values are mean ± SD

Fig 11. Plasma vitamin B12 levels (left panel) and percentage distribution of vitamin B12 deficiency (right panel) in non-diabetic control (CN) and diabetic subjects without retinopathy (DNR) and with retinopathy (DR)



Data represent mean ± SE. Sample number is indicated in the parenthesis.

the ratio of vitamin B12 and folic acid was also significantly different ($p<0.05$) between control and DR groups, but not between DNR and DR groups. These results indicate that there is a deficiency of vitmamin B12, amongst all the B-vitamins, in DR patients. However, there was no significant difference in homocysteine and vitamin B12 levels between NPDR and PDR patients.

6. Homocysteine levels were inversely related to vitamin B12 and folic acid but not with vitamins B1, B2 and B6 (Table 41). Interestingly, homocysteine levels were not related to the age, BMI and duration of diabetes (Table 3). Likewise, vitamin B12 levels were significantly associated with homocysteine but not with age, BMI and duration (Table 41). Therefore, vitamin B12 probably is a more important determinant for increased homocysteine particularly in older people. Vitamin B12 becomes the limiting nutrient for the maintenance of normal plasma concentrations, once the folate levels are optimised. It should be noted in the study that the mean age of subjects is 55 years and the folate levels are still in the normal range, although lowered mean levels were found in diabetic patients.

Table 41. Correlation of B12 and homocysteine with demographic and vitamin parameters

Parameter	B12		Homocysteine	
	r-value	p-value	r-value	p-value
Age	0.111	0.055	0.088	0.328
Duration	0.074	0.263	0.162	0.113
BMI	0.082	0.292	0.083	0.493
Vitamin B1	-0.062	0.571	0.294	0.066
Vitamin B2	-0.059	0.577	0.284	0.056
Vitamin B6	0.047	0.614	-0.137	0.383
Folic acid	0.443	0.000	-0.323	0.002
Vitamin B12	-	-	-0.465	0.000
Homocysteine	-0.465	0.000	-	-

7. Among trace elements, the Mn and Co levels were decreased in diabetic patients (without and with DR) compared to control subjects (Table 42). Whereas, Mn and Co levels were further decreased in DR patients compared to diabetic patients without DR. While, the levels of Zn showed no significant differences between normal and diabetic patients without retinopathy, there is a marginal decrease in diabetic retinopathy group (Table 42).

Table 42. Blood levels of trace elements in non-diabetic control (CN) and diabetic subjects without retinopathy (DNR) and with retinopathy (DR)

Trace elements	CN (N=50)	DNR (N=60)	DR(N=70)
Mg (µg/ml)	19.34±3.18	17.30±3.06	18.09±2.60
Ca (µg/ml)	105±16.75	105±12.73	107±20
Fe (µg/ml)	1.36±0.45	1.26±0.58	1.19±0.45
Cr (ng/ml)	0.86±0.26	0.82±0.25	0.90±0.40
Cu (µg/ml)	0.88±0.14	0.96±0.18	0.92±0.21
Mn (ng/ml)	3.05±1.01	2.85±0.91	2.30±1.13
Zn (µg/ml)	0.88±0.42	0.88±0.45	0.77±0.40
Se (ng/ml)	110±15.5	101±19.6	105.6±20.4
Co (ng/ml)	6.2 ±3.2	3.6 ±1.9	3.1 ±1.7

Interestingly, while in the study lower levels of three B vitamins were observed in diabetic patients irrespective of presence of retinopathy, lower levels of vitamin B12 was associated only with DR. Likewise, the data indicate that among all the minerals, there is an association between DR and lowered levels of Mn and Co. The results also signify an implication that a deficiency or inadequacy of vitamin B12 may predispose the diabetic patients to DR. It should be noted that low levels of vitamin B12 have been recognized in Indians for a long time and recent studies confirm low concentrations of vitamin B12 and its implications for diabetes and cardiovascular diseases in India. This is the first study to show an association of B-vitamins with DR and more controlled prospective studies are warranted to confirm the role of vitamin B12 deficiency in the development of DR.

However, there are no studies that explain the possible roles of vitamin B-12, Mn and Co in the development of DR in diabetic experimental conditions. The present human data may be valuable in understanding the roles of these micronutrients in development of diabetic retinal pathology using experimental animal models. This data can also be used as a tool to develop intervention strategies in prevention of diabetic retinopathy using nutrition intervention or dietary supplements.

7. BIOCHEMICAL AND MOLECULAR BASIS OF TYPE-2 DIABETES-INDUCED CATARACT: EVALUATION OF A SUITABLE ANIMAL MODEL AND ROLE OF DIETARY AGENTS

The statistical facts indicate that the world is facing a growing diabetes epidemic of potentially devastating proportions. Its impact is most severely felt in developing countries. According to the latest WHO estimates currently about 200 million diabetic people are present in the world. It has been estimated that by the year 2025, India will have the largest number of diabetic subjects in the

world. The two basic types of diabetes mellitus are type-1 diabetes mellitus (T1D), which accounts for 5-10% of the diabetic population and type-2 diabetes mellitus (T2D), which is most common type of diabetes, accounts for 90 to 95% of all diabetes. Prolonged exposure to chronic hyperglycemia, without proper management, can lead to various short and long-term chronic complications. Chronic complications of diabetes affect many organ systems and are responsible for the majority of morbidity and mortality. The major mechanism of diabetic complications is the toxic effect of prolonged hyperglycemia, on insulin independent tissues like retina, kidney, peripheral nerve and lens, which results into development of secondary complications of diabetes; retinopathy, nephropathy, neuropathy and cataract respectively.

Cataract is characterized by loss of transparency and is the leading cause of blindness worldwide. Though there are many studies on diabetic cataract and other complications of diabetes in experimental animals to understand pathology and to develop preventive or therapeutic strategies, most of the studies are conducted on T1D animal models. However, majority of the diabetic subjects in population belong to T2D. Excepting some genetic models, there are no studies on T2D- induced complications such as cataract in experimental conditions. Therefore, there is a need to develop or evaluate a suitable experimental animal model of T2D-induced cataract not only to understand the possible mechanisms involved in the development of cataract but also to prevent or delay of diabetic cataract. Hence, the major objective of this study was to evaluate a suitable experimental animal model of T2D cataract for studies on biochemical and molecular basis of T2D cataract and to investigate the role of dietary agents against T2D-diabetes induced cataract.

Experimental design: The following animal experiments were conducted to evaluate a suitable animal model of T2D-induced cataract.

High sucrose or high fat and low-STZ model in SD rats: Adult rats were maintained on AIN-93 diet contained high sucrose (60%) or high fat (24%) for two months followed by low dose of STZ (20 mg/kg) injection and continued for further 4 months.

High sucrose and high fat induced T2D in WNIN rats: Two-month old WNIN rats were maintained on AIN-93 diet alone or AIN-93 diet supplemented with 24% fat and 36% sucrose for a period of six months.

High sucrose induced T2D in WNIN-GR/Ob: Two-month old WNIN/GR-Ob rats were maintained on AIN-93 diet alone or AIN-93 diet supplemented with 60% sucrose for a period of six months.

Neonatal-streptozotocin (n STZ) model: The nSTZ model in three different rat strains (WNIN, SD and WNIN/GR-Ob) by injecting STZ (90 mg/kg in 0.1 M citrate buffer, pH 4.5) to two day old pups intraperitoneally has evaluated. Control pups received only vehicle.

Effect of dietary ginger in nSTZ induced WNIN/GR-Ob rats: nSTZ-induced T2D was produced in WNIN/GR-Ob rat as described above and diabetic rats were sub divided into two groups; a group (nSTZ control) of animals was fed with AIN-93 diet and another group (nSTZ ginger) with dietary ginger (3%) in AIN-93 diet. Whereas vehicle injected (control) animals fed with AIN-93 diet.

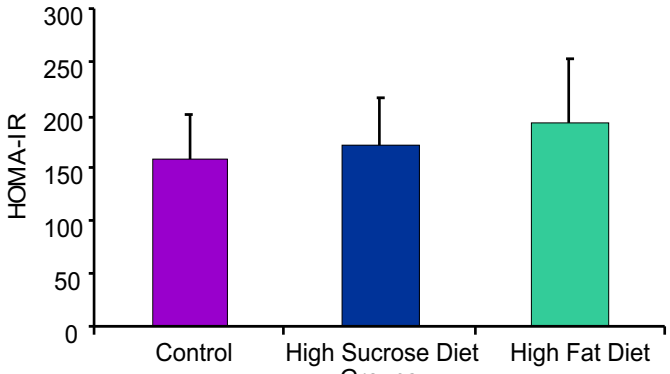
All the rats were fed with their respective diets for a period of six to eight months. During this period animals were monitored regularly for the development of insulin resistance, hyperglycemia and subsequently onset of cataract. Clinical and biochemical estimations were carried out by the standard methods.

RESULTS

Feeding of high sucrose or high fat has resulted in mild insulin resistance at the age of two months in SD rats (Fig. 12). There was an increase in insulin levels and ratio of insulin/glucose in

high sucrose or high fat fed SD rats compared to control, but no hyperglycemia was observed even at the end of six months (Table 43). Although, feeding of high sucrose or high fat has not resulted in frank hyperglycemia, there is initiation of lens opacification after 4 months of experimental period. However, the rate of cataract progression was very slow as it could not mature two months after the initiation.

Fig. 12 HOMA-IR in SD rat fed with high sucrose or high fat for two months



Values are mean ± SD (n=8)

Feeding of high sucrose and fat to WNIN rats for six months resulted insulin resistance/impaired glucose tolerance, but not hyperglycemia. While feeding of high sucrose alone for two months was able to induce to insulin resistance/ impaired glucose in adult WNIN-GR/Ob rats, no hyperglycemia was developed even after six months. Analyses of these lenses suggest that activation of polyol pathway and increased oxidative stress were associated with insulin resistance lens (Table 44).

There was an impaired glucose tolerance and insulin resistance at the age of 2 months in nSTZ WNIN rats. Though nSTZ WNIN rats developed insulin resistance/impaired glucose tolerance (pre-diabetes) at the age of two months, no frank hyperglycemia was observed even at the age of eight months. Biochemical studies indicated that, there was no change either in lens weight or protein content (total protein), but a decline of soluble protein indicating initiation of protein insolubilization in nSTZ WNIN rats (Table 45). Although, aldose reductase (AR) activity was increased marginally, there was a three-fold increase of sorbitol in nSTZ rats when compared to controls indicating the activation of polyol pathway (Fig 13). Similarly, increased MDA levels along with alterations in activities of some antioxidant enzymes indicating there was an increased oxidative stress in nSTZ rat lens (Table 45).

Table 43. Glucose, insulin and insulin/glucose ratios in high sucrose or high fat fed SD rats

	Duration	Control group	High sucrose fed	High fat fed
Glucose	2Months	100.7χ 4.7	96.7χ8.0	95.1χ6.9
	3Months	87.9χ 9.4	92.3χ9.1	69.4χ11.4
	6Months	102 χ 40.8	102 χ 33.3	85.5χ 6.3
Insulin χU/ml	2Months	34.87χ 9.1	39.8χ7.7	45.6χ14.0
	3Months	37.9χ 9.4	72.3χ9.1	59.4χ11.4
	6Months	39.4 χ 11.5	135 χ 72.6	66 χ 43.2
Insulin /Glucose	2Months	0.346χ 0.08	0.410χ0.06	0.479χ0.14
	3Months	0.431χ 0.07	0.783χ 0.09	0.474χ 0.11
	6Months	0.361 χ 0.19	1.433χ 0.79	0.769 χ 0.48

Values are mean ± SD (n=8)

Table 44. Polyol pathway, oxidative stress and antioxidant enzymes in high sucrose and fat fed WNIN & high sucrose fed WNIN/GR-Ob rat lens

Parameters	Control WNIN rats	High fat & sucrose fed WNIN rats	Control WNIN/GR-Ob rats	High Sucrose fed WNIN/ GR-Ob rats
Aldose reductase	20.25±6.22	20.60±2.12	25.09±1.86	28.27±1.73
Sorbitol	351±32.62	437±98	285±22.60	303±95.28
MDA	8.86±0.33	9.68±0.06	6.48±1.28	10.06±1.40
Protein carbonyls	8.40±3.19	9.20±2.82	9.18±0.01	9.36±0.51
SOD	41.2±5.81	43±8.83	57.19±14.19	67±12.14
GPx	21.77±3.43	17.11±1.51	25.80±3.25	27.85±1.05
GST	55.85±1.33	55.85±0.67	54.24±2.42	56.0±5.09

Values are mean ± SD (n=8)

AR activity: µmoles NADPH oxidized/h/100 mg protein; Sorbitol: nano moles/g lens; MDA: nmol/g lens; Protein carbonyls: nmol/mg protein; SOD: units/min/100mg protein; GPx: µmoles of NADPH oxidized/h/100mg protein. GST: µmoles of CDNB-GSH conjugate formed/h/100mg protein.

Table 45. Protein content, oxidative stress markers and antioxidant enzymes in the lens of nSTZ-WNIN rats

Parameter	Control lens	nSTZ lens
Lens weight (mg)	50.6χ5.7	51.6χ1.3
Total protein (mg/g lens)	531χ7	560χ24
Soluble protein (mg/g lens)	357χ14	352χ15
% Soluble protein	67.32	62.78
MDA	10.65χ0.27	12.24χ0.83*
Protein carbonyls	2.25χ0.28	2.102χ0.32
SOD	33.60χ2.32	44.37χ4.81*
GPx	19.56χ1.61	20.16χ2.11
GST	30.70χ2.14	27.43χ2.38
G6PD	11.61χ0.64	12.52χ0.97

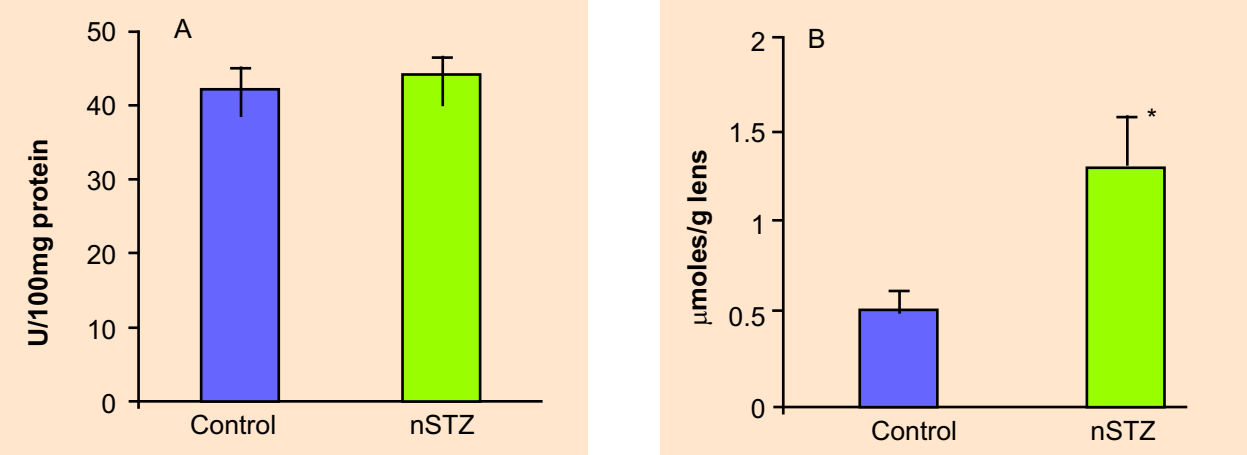
Values are mean ± SD (n=8-14)

MDA: nmol/g lens; Protein carbonyls: nmol/mg protein; SOD: units/min/100mg protein; GPx: µmoles of NADPH oxidized/h/100mg protein; GST: µmoles of CDNB-GSH conjugate formed/h/100mg protein; G6PD: µmoles of NADPH oxidized/h/100mg protein.

High postprandial glucose, impaired glucose tolerance but not insulin resistance was observed in all nSTZ SD rats at two months. Interestingly, some (25%) but not all nSTZ rats developed hyperglycemia (Table 46) and cataract (Fig 14) after three months. However, rate of cataract maturation was very slow.

Since, not all nSTZ SD rats developed diabetes, we have used WNIN/ GR-Ob rat pups for development of T2D. Basically, WNIN/ GR-Ob rats are obese and have the characteristics of impaired glucose tolerance. Injection of STZ to the two-day old WNIN/ GR-Ob pups developed mild hyperglycemia at the age of one month and gradually led to severe hyperglycemia by the age of five months (Fig 15). However, lean animals of WNIN/ GR-Ob rat have not developed hyperglycemia due to STZ. While the onset of cataract due to hyperglycemia was observed in diabetic animals after eight weeks of

Fig 13. Aldose reductase activity (A) and sorbitol levels (B) in the lens of nSTZ WNIN rats



Values are mean ± SD
AR activity was expressed as µmoles NADPH oxidized/h/100 mg protein.
Sorbitol was expressed as µmoles/g lens.

Table 46. Fasting blood glucose (mg/dL) levels of nSTZ SD rats

Groups	Duration (months)					
	One	Two	Three	Four	Five	Six
Control	103±8.77	100±7.54	95.75±5.69	82.0±4.2	83±2.63	82.5±4.03
Diabetic (D)	93±16.23	131±53.83	145±67.05	112±48	109±44	112±38
D+Ginger	100±8.58	122±39.63	160±110	154±97	125±74	160±99
D+Curcumin	91.25±14.1	110±23	128±28	128±48	119±47	128±65

Values are mean ± SD (n=8)

Fig 14. Development of cataract in nSTZ SD rats and the effect of ginger and curcumin

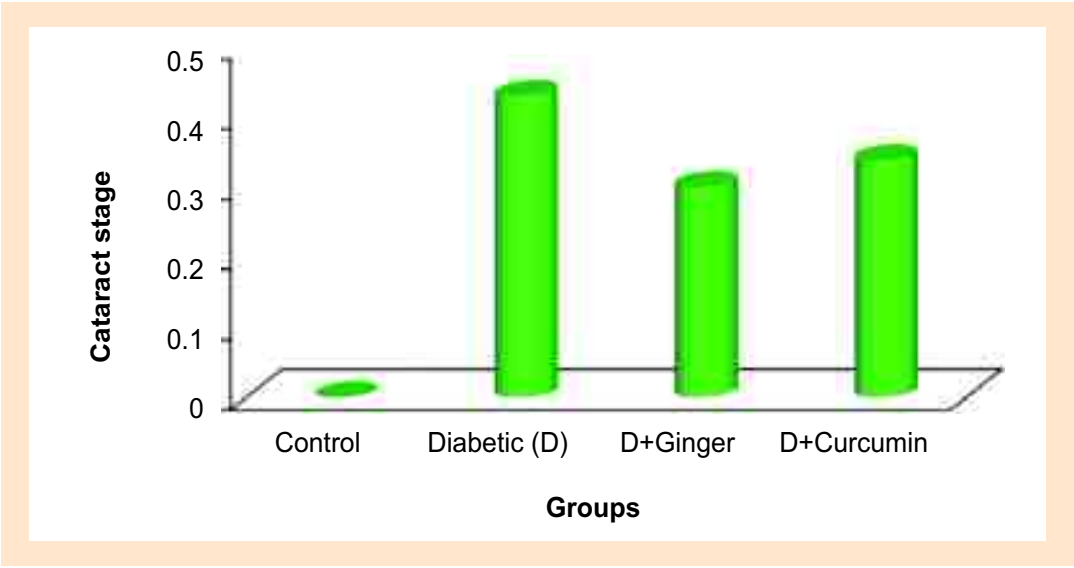


Fig 15. Fasting blood glucose levels in nSTZ WNIN/GR-Ob

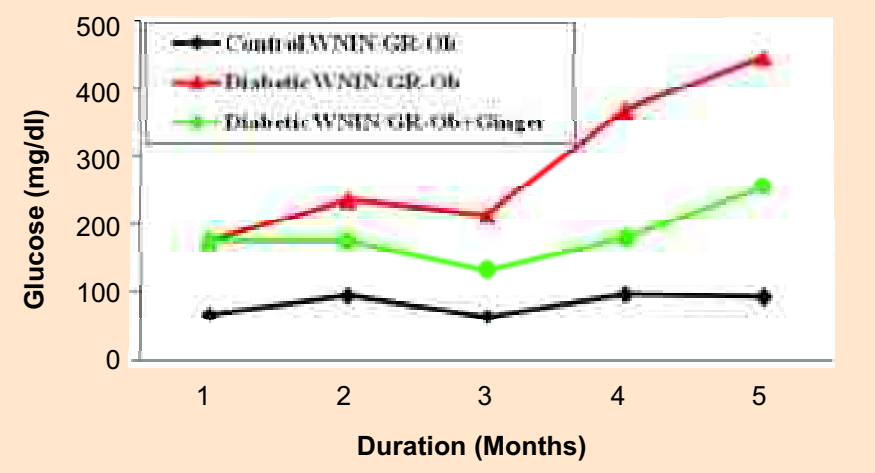
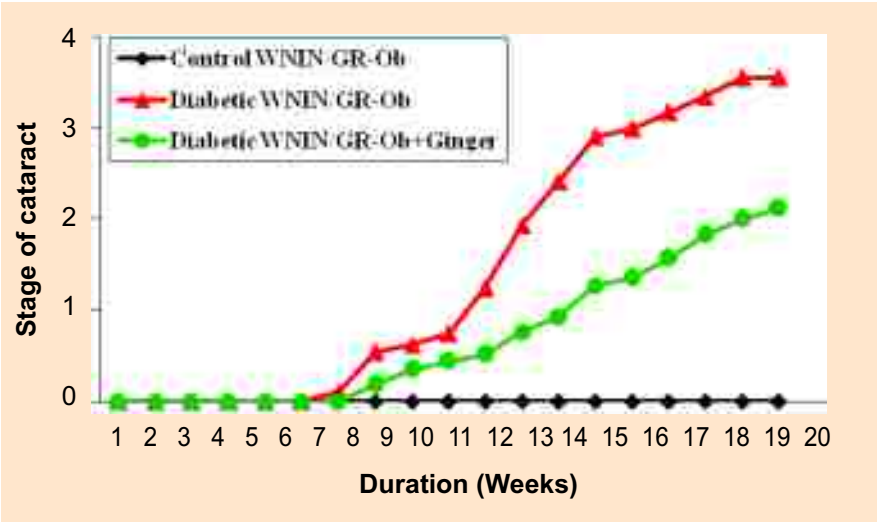


Fig 16. Effect of ginger on onset and progression of cataract in nSTZ WNIN/GR-Ob rats



Values are mean ± SD (n=8)

STZ injection and progressed to mature cataract by 18 weeks in untreated diabetic lenses *(*Fig 16). Irrespective of the rat strains and methods used, insulin resistance/impaired glucose tolerance or pre-diabetes condition activated some of the key pathways like polyol pathway and oxidative stress in the lens which are commonly associated with long-term complications of diabetes. Activation of these pathways could be initiating events for the development of cataract in these animals. Therefore, in the final experiments dietary agents were used (curcumin and ginger) to have AR inhibitory potential and antioxidant activities to prevent or delay the T2D-induced cataract. F*eeding of curcumin* and ginger delayed cataract modestly in nSTZ SD rats *(*Fig 14) but not altered glucose levels (Table 46). While feeding of ginger reduced glucose levels modestly delayed both progression and maturation of cataract significantly in nSTZ WNIN/GR-Ob diabetic rats *(*Fig 15).

Based on these experiments, nSTZ- WNIN-GR/Ob model could serve as a suitable model for studies on T2D induced complications, particularly diabetic cataract and also dietary intervention studies.

8. CHARACTERIZATION OF ACTIVE PRINCIPLES AND MECHANISM OF ACTION OF ALDOSE REDUCTASE (ALR2) INHIBITORS AND ANTIGLYCATING AGENTS FROM DIETARY SOURCES: (II) ALR2 INHIBITORS FROM BLACK PEPPER AND SYNTHESIS OF NOVEL ALR2 INHIBITORS

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. The inhibitory effects of various synthetically and naturally derived compounds on ALR2 in vitro and in different animal models suggest that ALR2 could be a potential target in controlling diabetic complications. Two chemical classes of ALR2 inhibitors (ARI) have been tested in clinical trials. While carboxylic acid inhibitors (zopolrestat and tolerestat) have shown poor tissue permeability and were not very potent *in vivo*, spiroimide (spirohydantoin) inhibitors (like sorbinil) penetrate tissues more efficiently but many have caused skin reactions and liver toxicity. In addition to ALR2, many ARI are also known to inhibit closely related members of ALR2 (such as AKR1A1 and AK1B10) contributing to the poor outcome of ARI clinical trials**.** Thus, intensive research continues to identify and test both synthetic as well as natural products for their therapeutic value to prevent the onset and/or arrest the progression of diabetic complications.

Earlier some dietary sources for their potential to inhibit ALR2 and AGE formation with the ultimate goal to prevent or treat diabetic complications have identified. Black pepper (*Piper sps*) is one among them. This work has been further extended to test extracts of *P. nigrum, P. longum,* and *P. chaba*, for their inhibitory effects on ALR2. Among them, extracts of *P. chaba* showed significant results towards ALR2. The aim of the present study was to identify the active principles from *Piper chaba* that is/ are responsible for ALR2 inhibition. Further, upon chemical transformation of one of the active principles that showed ARI activity, Few synthetically novel compounds have invented through Michael addition which inhibited human recombinant ALR2 and also suppressed sorbitol accumulation in human RBC under high glucose conditions *ex vivo*.

METHODOLOGY

(I) Extraction and isolation: The roots and seeds of *piper chaba* (3 kg) were extracted with hexane at boiling temperature for 72h. This resulted hexane extract which exhibited the ARI activity was subjected to silica gel column chromatography to give five major fractions (F1–F5). Fractions F1-F5 were purified by repeated flash chromatography on silica gel (100–200 mesh) by eluting with EtOAc/ hexane.

(ii) Michael reaction of piplartine: To a mixture of piplartine and indole, iodine was added and the contents were refluxed in dichloroethane for 12-48 hr. The reaction was monitored by thin-layer chromatography. After complete conversion, the solvent was evaporated and the product was washed with hypo solution and then extracted with chloroform. The combined organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure, purified by silica-gel column chromatography to afford pure product mono adduct (2a-2k) and di adduct (3a-3k).

Chemical and physical properties of piplartine, hydrolysis products and Michael adducts (2a-2k) and (3a-3k) were analyzed.

(iii) Expression and purification of recombinant human ALR2: Recombinant human ALR2 was over-expressed in *E. coli* and purified essentially as described previously.

(iv) 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.

(v) Inhibition studies: For inhibition studies concentrated stocks of all compounds prepared in DMSO were used. Various concentrations of agents 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 compound as 100%. The IC_{50} values were determined by nonlinear regression analysis of the plot of percent inhibition versus log inhibitor concentration.

(vi) *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 Kreb's-ringer bicarbonate buffer, pH 7.4 at 37°C in presence of 5% CO_2 for 3 hr under normal (5.5 mM) and high glucose (55 mM) conditions. The effect of agents on sorbitol accumulation was evaluated by incubating the RBC with different concentrations of agents. At the end of the incubation period, sorbitol content of the RBC was measured by a fluorometric method as described previously using a spectrofluorometer.

(vii). Molecular docking: Molecular docking was done using Discovery (Discover 2.5) package. Crystal structure of human ALR2 was taken from PDB (1PWM) and protein structure was minimized by using charmM force field. All water molecules were removed. Docking was done by discovery ligandfit module. Fitness of each ligand was generated based on the GOLD fitness score.

RESULTS

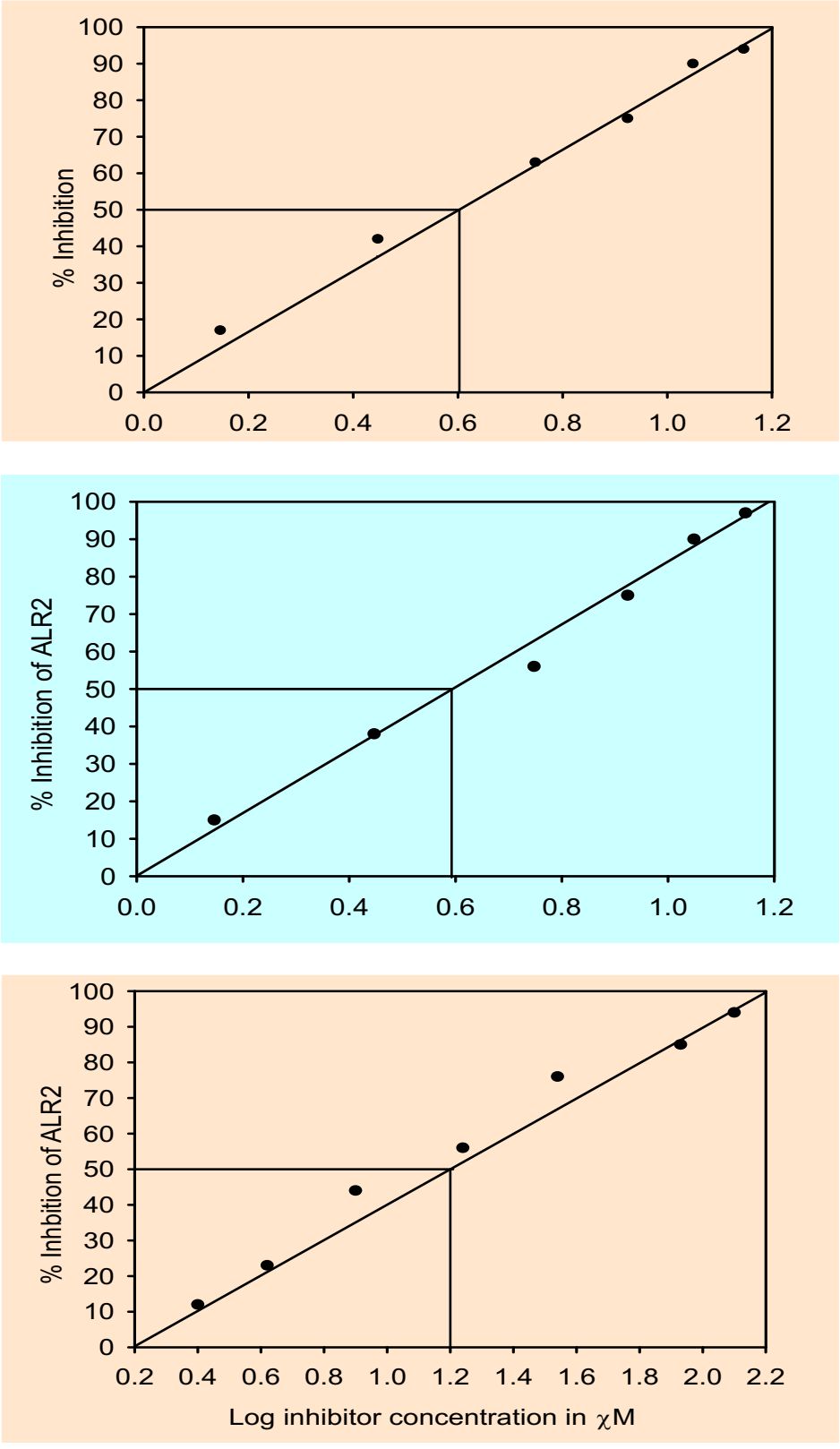
1. The phytochemical investigation on *P. chaba* led to isolation of 15 bioactive compounds consisting of alkamides like pellitorine, piperine, piplartine, guineensine, brachystamide, chingchengenamide, 4,5-dihyropiperlongumine and chabamide, lignans like sesamine, diedudesmine, and some miscellaneous compounds like pipernal and pipataline. All the individual compounds were tested against ALR2.
2. Out of the 15 compounds, piplartine and pipernal have inhibited the human recombinant ALR2 with IC_{50} values 160, and 310 µM, respectively.
3. To improve the efficacy, a series of compounds have synthesized (20 compounds) by Michael addition using different substituted indoles as Michael donors and piplartine as Michael acceptor in the presence of iodine as catalyst. All adducts were tested for their ARI activity against ALR2. From these, adducts 3c and 3e has exhibited the highest and similar ARI activity with an IC_{50} value 4 µM followed by 2j, 2g and 3d with IC_{50} values 8, 15 and, 40 µM, respectively (Fig 17 & Table 47). All the molecules showed a significantly higher inhibition of ALR2 when compared to well-

Table 47. IC-50 values for selected Michael adducts

Sl. No	Compound/Standard	IC-50 (µM)
1	3c	4
2	3d	40
3	3e	4
4	2g	15
5	2j	8
6	Piplartine	160
7	Quercetin	40
8	Sorbinil	8

Data are average of four experimental values

Fig 17. Representative inhibition plots for 3c (top), 3e (middle) and 2j (bottom) against recombinant human ALR2 in vitro

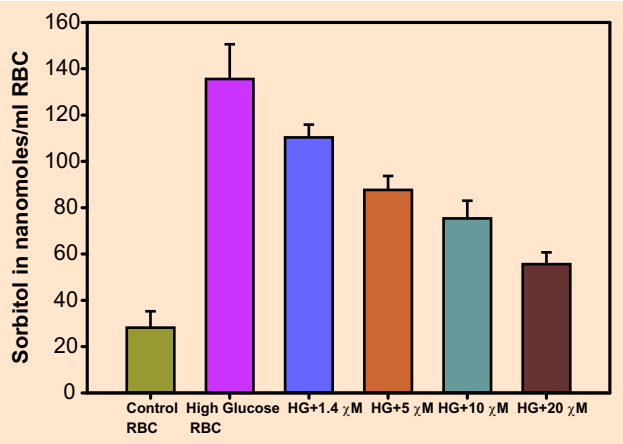


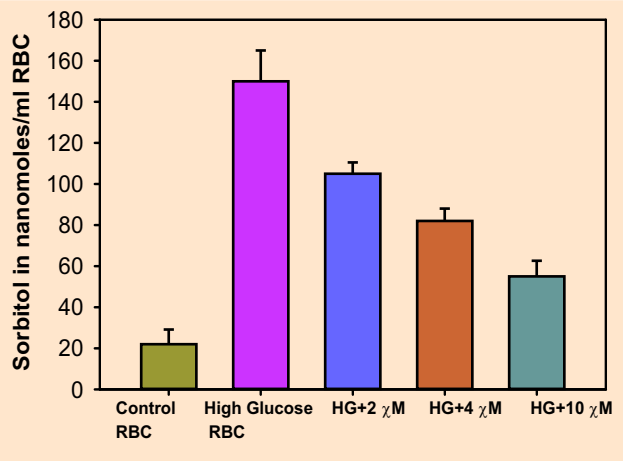
known ARI, quercetin and sorbinol, which inhibited the human recombinant ALR with IC50 value 40 and 8 µM, respectively.

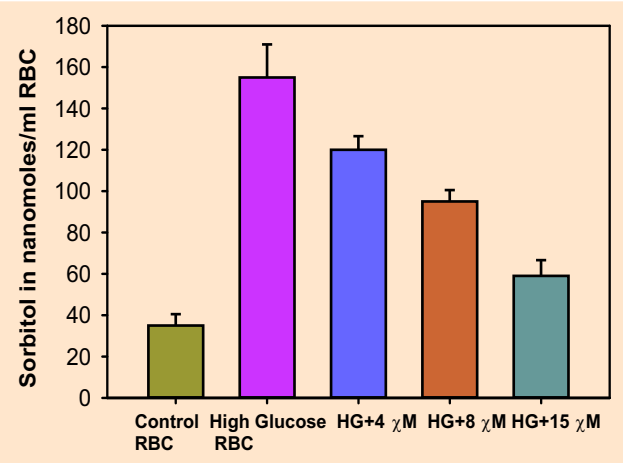
4. *In vitro* incubation of RBC with 55 mM glucose resulted in the accumulation of sorbitol three to four folds higher than the control (Fig 18). Incubation of RBC in the presence of piplartine analogues under high glucose conditions lead to reduction in the accumulation of intracellular sorbitol. Though, degree of inhibition varied according to the IC_{50} values of different analogues, on average there was 40-50% reduction with the concentrations equal to their IC_{50} value of the analogues. These results indicate the significance of ARI potential of these analogues in terms of preventing the accumulation of intracellular sorbitol. Hence, the said compounds, particularly, 3c, 3e and 2j might be useful for the treatment and/ or prevention of diabetic complications.

5. *SAR studies:* All Michael adducts were screened against *in-vitro* ALR2 inhibition. The Michael adducts obtained by addition of indole to piplartine enhanced the activity by 40 folds. Among all adducts, di adducts showed notable activity than mono adducts. Results obtained were encouraging for R_1 substituted with benzyl group (3c, IC_{50} = 4µm) rather than methyl and hydrogen groups. Same results were obtained when R_2 was substituted with phenyl group (3e, IC_{50} = 4µm), rather than methyl group (3d, IC_{50} = 40µm) which also showed good activity. In case of R_3, among all the substitutions, methoxy group exhibited considerable activity (2j, IC_{50} = 8µm), and among the halogens, bromine showed moderate activity (2g, IC_{50} = 60µm). The above results explains that the adduct needs an active methylene group like benzyl at R_1 position and hydrophobic groups like phenyl at R_2 position and electron donating groups

Fig 18. Inhibition of sorbitol formation in RBC under high glucose conditions by analogues 3c (top panel), 3e (middle panel) and 2j (bottom panel)

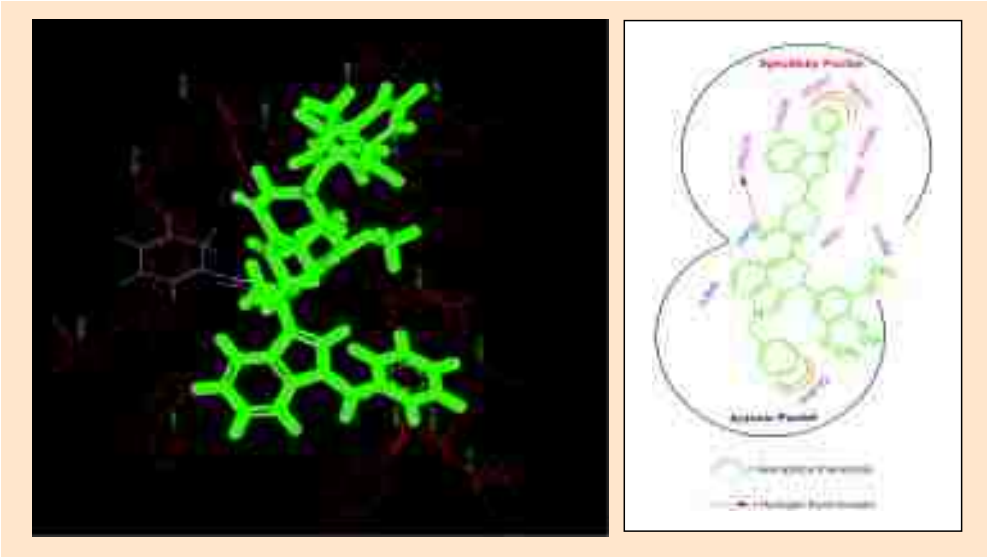






Data represent average of four experiments

Fig 19. Stereo views of ALR2 docked with 3c (Left) and interactions of 3c with active sit and specificity pocket was shown in (Right)



like methoxy at R_3 position are indispensable to show significant activity. However, the hydrolysis products 4, 5 were inactive towards the enzyme inhibition.

6. ***Molecular docking studies:*** The active site of AKR1B1 consists of two major pockets, the so-called "anionic" pocket and the "specificity" pocket. The ligands occupied active site of ALR2 (1PWM) and extended towards the hydrophobic cleft or specificity pocket. Interestingly 3c, 3d and 2j revealed similar binding pattern in that they formed hydrogen bonds with active site residues Trp-20, Tyr-48, His-110, Trp-111, Cys-298, Leu-300, Leu-301, which are involved in catalysis and also with nicotinamide ring (Fig 19). Apart from active site, these inhibitors were also extended towards the flexible hydrophobic site because of indole linked with N-phenyl moiety. Indole linked with N-phenyl moiety of 3c interacted with Phe122 and Trp219 forming the hydrophobic interactions and hydrogen bond formation with Trp20 Pro218, Trp219 and Leu300.

7. Based on these data it is clear that the indole containing phenyl moiety was effective than the well know inhibitor fidarestat. More importantly, the data indicates that the other side phenyl containing indole moiety, which extends into the "specificity pocket", might be the reason for the inhibitors to be more effective than sorbinil and fidarestat. Binding energies calculated in the active site of ALR2 and found that 3e has the highest binding energy among all piplartine analogues due to stable non covalent interactions with (hydrogen bonds and hydrophobic interactions) active site of ALR2 over the sister compounds (3c, 3d and 2j).

CONCLUSIONS

Identified a new natural ALR2 inhibitor from black pepper. A novel method to carry out the Michael addition reaction of indole with natural product piplartine in the presence of iodine, a mild Lewis-acid catalyst under selective solvent conditions was developed successfully. Based on this method, piplartine analogues were synthesized and tested the inhibition of ALR2. Among these compounds 3c, 3d, 2j and 3e showed better potency towards the ALR2 as compared to well know inhibitor sorbinil. These compounds inhibited the sorbitol accumulation in human RBC under high glucose condition which indicates their potential of membrane permeability and inhibition of osmotic stress in insulin independent cells like eye lens, retina neuron and nephrons. Thus, these novel compounds might be useful for the treatment and/or prevention of diabetic complications.

9. IMPORTANCE OF α-CRYSTALLIN HETEROPOLYMER IN THE EYE LENS: TEMPERATURE - DEPENDENT COAGGREGATION OF EYE LENS α- AND β-CRYSTALLINS

Crystallins are the major structural proteins that account for up to 90% of total soluble proteins of the eye lens, whose short ordered arrangement helps in maintaining transparency of the eye lens. Impaired function of the lens due to partial or complete loss of transparency is called 'cataract', a leading cause of blindness worldwide. α-Crystallin belongs to the family of small heat-shock proteins and shown to have chaperone-like activity (CLA). α-Crystallin constitutes about 40% of the total soluble proteins of the vertebrate eye lens. It is composed of two 20 kDa subunits, αA and αB, which share about 60% sequence homology. These subunits self-associate to form a large multimeric complex with an *average molecular mass of* about 800 kDa that contains 30-50 subunits. The ratio of αA to αB subunits in the eye lens varies among species, from 1:3 in dogfish, 9:1 in kangaroo to 19:1 in catfish. However, in most vertebrate lens, the molar ratio of αA to αB is 3:1. While traces of the αA-crystallin are found in some non-ocular tissues, the distribution of αB is ubiquitous. While αB-crystallin is overexpressed in some neuropathological/degenerative conditions, mutation(s) in αB-crystallin are associated with syndromes such as desmin-related myopathy. The apparent difference in the tissue specificity of αA- and αB-crystallins and their involvement in pathology indicate that these two proteins might have evolved to play distinct physiological functions. However, the rationale for the existence of α-crystallin as a heteropolymer with a specific subunit ratio in the eye lens, but not in other tissues, and its physiological significance remains elusive.

Previously, it was demonstrated that although, under physiologically relevant conditions, αB-homopolymer has shown relatively higher CLA, at elevated temperatures CLA of αA-homopolymer or the heteropolymer with a higher αA-proportion (3:1 ratio) was greater. Further, it was explained the dichotomy in the function of α-crystallin heteropolymer in the eye lens, by demonstrating the differences in structural and thermodynamic stability and susceptibility to post-translational modifications between homo- and heteropolymers (with varying ratios of αA *to* αB). Further, despite high sequence homology, αA- and αB-crystallins have shown to behave differently with respect to their chaperone activity, hydrophobicity, structure and other physicochemical properties, particularly with increasing temperature. However, existence of this dichotomy in structural and functional properties of αA- and αB- subunits remained largely unexplained.

Since, αA- and αB-homopolymers have behaved differently structurally and functionally at elevated temperatures In the study, how αA- and αB-crystallins contributes to a light scattering at elevated temperatures (above 65°C) was investigated and how they prevent or augment the aggregation of other lens proteins, mainly β- and γ-crystallins. Recently, a novel point mutation (F71L) was identified in αA-crystallin associated with early onset of age-related cataracts due to defective CLA.

METHODOLOGY

(I) Expression and purification of recombinant αA-, αB- and mutant αA-crystallins: Expression and purification of human αA-, αB- and mutant (F71L) αA-crystallins in the BL21/pET23d cells was carried out as described earlier.

(ii) Preparation of total soluble protein (TSP) and β- and γ-crystallins from goat eye lens: Goat eyeballs were obtained from a local abattoir and the lenses were dissected out. Preparation of the TSP and β- and γ-crystallins from goat eye lens was carried out essentially as described

earlier. TSP was further subjected to ultracentrifugation at 500 000 g for 120 min to deplete α-crystallin from the TSP and TSP devoid of α-crystallin thus obtained was designated as TSP^{-alpha}. TSP^{-alpha} was employed to study the relative importance of αA- and αB-crystallins in suppressing the aggregation of β- and γ-crystallins.

(iii) Size exclusion chromatography (SEC): SEC of goat TSP and TSP^{-alpha} (0.02 mg/ml) was performed on a HPLC system connected to TSKG3000 SWXL 7.8×300 mm column equilibrated with 0.1 M sodium phosphate buffer, pH 6.7, containing 0.1 M sodium sulphate and 0.05 % sodium azide. Thyroglobulin (669 kDa), γ-globulin (160 kDa) and BSA (67 kDa) were used as molecular mass standards.

(iv) Heat-induced aggregation of TSP, TSP^{-alpha}, β-, γ- and α-crystallins: The TSP, TSP^{-alpha}, β- and γ-crystallins were used for heat-induced aggregation studies in the absence and presence of homo- and heteropolymers of αA- and αB-crystallins. Heat-induced aggregation of TSP, TSP^{alpha}, β-, γ- and α-crystallins was monitored as a function of light scattering. Light scattering of the α-crystallin variants (0.3 mg/ml) in 0.05 M sodium phosphate buffer, pH 7.2, containing 0.1 M NaCl was monitored at 85°C in a UV/Vis spectrophotometer at 360 nm. Light-scattering of TSP^{-alpha} (0.30 mg/ml) at 85°C and β- and γ-crystallins (0.15 mg/ml) at 70°C was monitored in the presence of 0.05 or 0.15 mg/ml αA-homopolymer or mutant αA- homopolymer or 3:1 (A to B) heteropolymer or varying concentrations of αB- homopolymer (0.05, 0.1 and 0.15 mg/ml).

(v) Immunodetection of αB-crystallin in the insoluble aggregates: To show the presence of αB-crystallin in the aggregated complex, TSP^{-alpha} in the absence and presence of different α-crystallin variants was subjected to heat-induced aggregation at 85°C for 1 h as described above. At the end, insoluble aggregates were collected by centrifugation at 15, 000 g for 30 min in a refrigerated centrifuge. This insoluble complex was washed twice with 0.05 M sodium phosphate buffer, pH 7.2 and dissolved in SDS-sample buffer. Samples were analyzed by SDS-PAGE using 12% gels and probed with polyclonal anti-αB-crystallin antibodies.

RESULTS

1. While αB- homopolymer displayed highest light scattering at 85°C compared to other α–crystallin variants, the light scattering reduced gradually with increasing proportions of αA in the reconstituted heteropolymer and was lowest with 3:1 (αA to αB) heteropolymer (Fig 20). However, light scattering of mutant (F71L) αA-crystallin at 85°C was higher than wild-type αA-homopolymer indicating the importance of αA-in stabilization of the heteropolymer (Fig 20). These results indicate that αB-homopolymer is more susceptible to aggregation amongst the different α-crystallin variants employed in the study.
2. The elution profiles of goat TSP and TSP^{-alpha} on SEC indicate about 95% removal of α-crystallin from TSP by ultracentrifugation which is further confirmed by immunoblotting using α-crystallin antibodies (Fig 21).
3. As expected, while TSP^{-alpha} has displayed two-fold higher light scattering due to heat-induced aggregation as compared to that of TSP, addition of αA or 3:1-heteropolymer to TSP^{-alpha} reduced light scattering at par with TSP (Figure 3). Interestingly, addition of heteropolymer consisting of mutant αA-F71L to αB- in 3:1 ratio to TSP^{-alpha} could not provide protection but rather showed increased light scattering (Fig 22).
4. However, astonishingly, addition of αB-crystallin to TSP^{-alpha} caused a remarkable increase in the light scattering (Fig 22) indicating coaggregation of αB-homopolymer with TSP^{-alpha} at these temperatures (70-85°C). This increase in light scattering of TSP^{-alpha} upon addition of αB-

crystallin is concentration dependent (Fig 23) and substantiated augmentation of aggregation of TSP^{-alpha} by αB-crystallin.

5. Detection of αB-crystallin in the insoluble aggregates of TSP^{-alpha} by immunoblotting using αB-crystallin specific polyclonal antibodies (Fig 24) provided a direct evidence for coaggregation of αB-crystallin with lens protein complement devoid of αA-crystallin.

Fig 20. Heat - induced aggregation of α-crystallin variants. Light scattering of α-crystallin variants (0.3 mg/ml) at 85°C was monitored at 360 nm in a spectrophotometer: B (trace 1), 1:3 of A to B (trace 2), A-F71L (trace 3), 1:1 of A to B (trace 4), A (trace 5) and 3:1 of A to B (trace 6). Data are average of three independent experiments

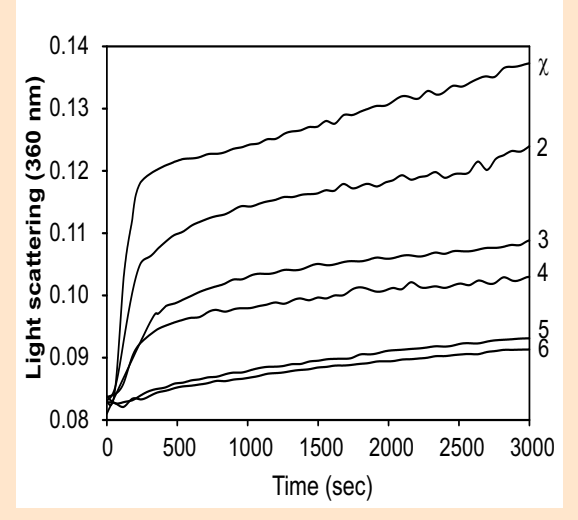
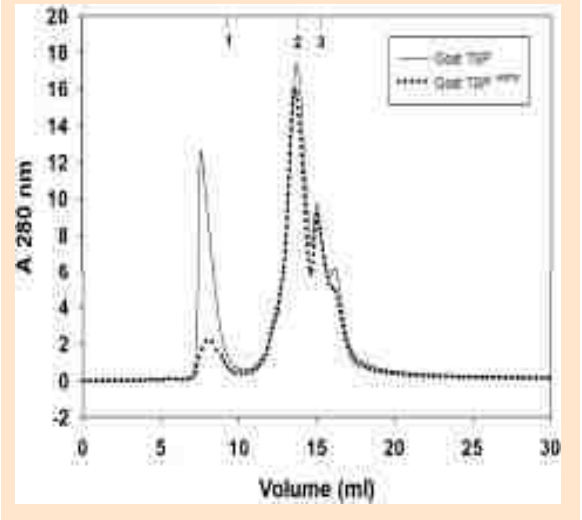


Fig 21. HPLC profile of goat TSP and TSP^{-alpha} on TSKG3000SWXL column. Elution positions of standard molecular weight markers, thyroglobulin (650 kDa; position-1), ovalbumin (160 kDa; position-2) and BSA (67 kDa; position-3) are indicated on the top



6. Since, β- and γ-crystallins are the major components of TSP^{-alpha}, we next determined whether αB-crystallin - mediated coaggregation at and above 70°C involves either both β- and γ-crystallins or specifically either of the two. Heat-induced aggregation of γ-crystallin at 70°C, although relatively higher than β-crystallin, was not augmented but rather suppressed significantly in the presence of αB-crystallin (Fig 26). In contrast to γ-crystallin, light scattering of β-crystallin at 70°C was enhanced by two folds in the presence of αB-crystallin (Fig 26).

7. These observations bring out an interesting facet of αB-crystallin that it co-precipitates with other lens proteins, mainly β-crystallin, in the absence of αA-crystallin, which might have a significant bearing on the selection of α-crystallin as a heteropolymer with a specific ratio of αA to αB subunit**.** Although, previously we have reported that αA-homopolymer is a better chaperone compared to αB-homopolymer in protecting β- and γ -crystallins at elevated temperatures, we have not observed any coprecipitation of αB- and β-crystallins at 60-65°C. It is interesting to note opposite effects of αB- but not αA-crystallin on β-crystallin with further increase of a mere 5-10°C temperature just following their transition temperatures.

Fig 22. Heat-induced aggregation of goat TSP and TSP^{-alpha}. Light scattering of goat TSP^{-alpha} (0.3 mg/ml) at 85°C was monitored at 360 nm in the absence (*trace 1*) and presence of 0.15 mg/ml of αA-homopolymer (*trace 2*), heteropolymer with 3:1 αA to αB ratio (*trace 3*), heteropolymer with 3:1 A-F71L to αB ratio (*trace 5*) and αB-homopolymer (*trace 6*). Light scattering of TSP alone (0.3 mg/ml) is also shown (*trace 4*). Data are average of three independent experiments

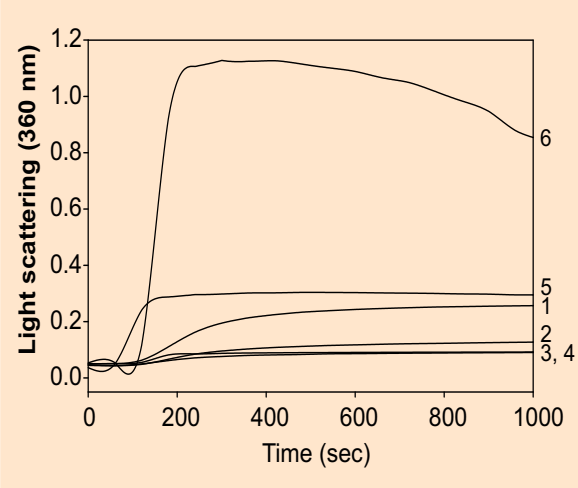


Fig 23. Coaggregation of αB-crystallin and TSP^{-alpha}. Light scattering due to aggregation of goat TSP^{-alpha} (0.1 mg/ml) at 85°C was monitored at 360nm in the absence (*trace 1) and* presence of 0.05 (*trace 2*), 0.10 (*trace 3*) and 0.20 mg/ml (*trace 4*) αB-crystallin. Data are average of three independent

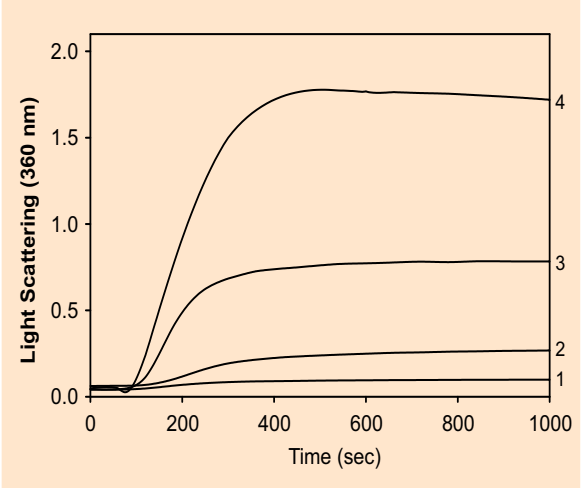
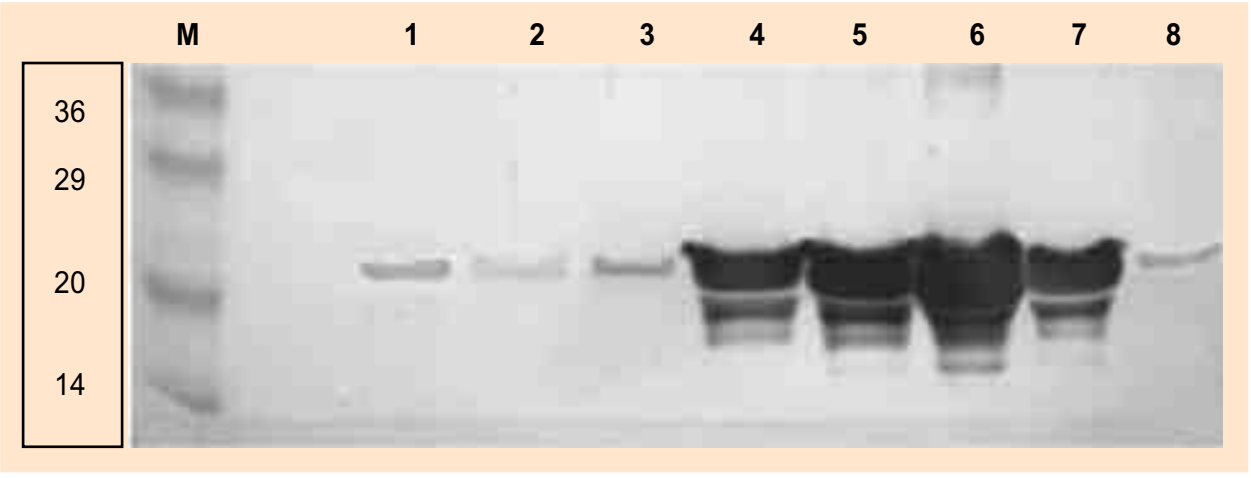
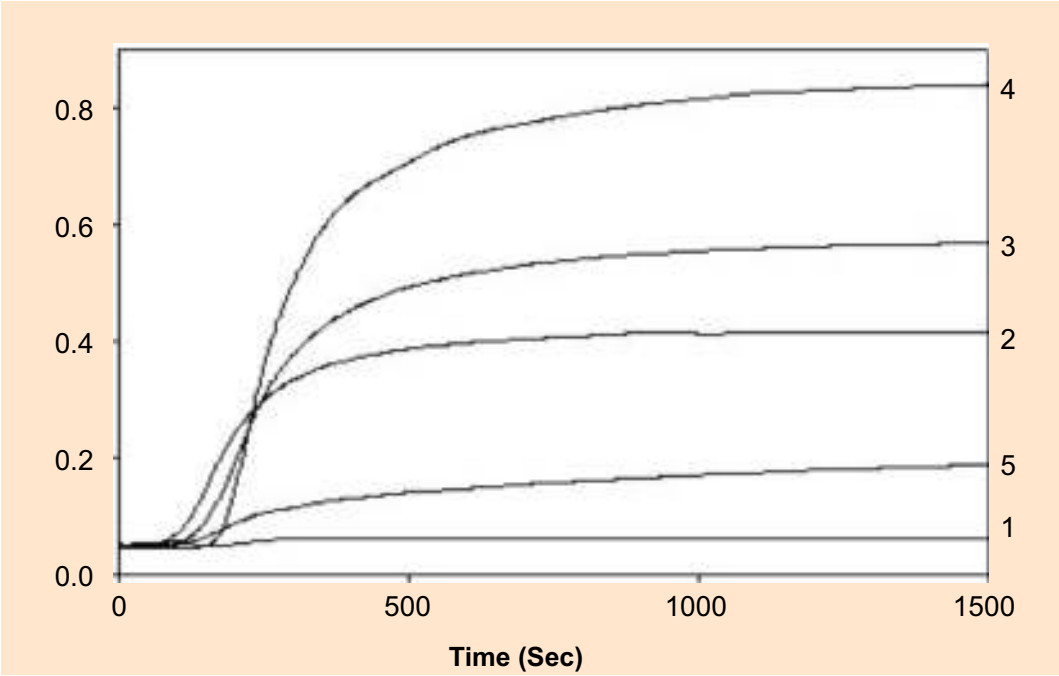


Fig 24. Immunodetection of αB-crystallin in insoluble aggregates. Insoluble aggregates were collected after heat treatment of samples at 85°C and analyzed by SDS-PAGE using 12% gels and probed with polyclonal anti-αB-crystallin antibodies. Molecular weight markers (*Lane M*), TSP (*Lane 1*), TSP^{-alpha} in the absence (*Lane 2*) and presence of 0.10 mg/ml A-homopolymer (*Lane 3*), 0.05 (*Lane 4*), 0.1 (*Lane 5*) and 0.20 mg/ml B-crystallin (*Lane 6*), 0.20 mg/ml heteropolymer with 1:1 A to B ratio (*Lane 7*) and 0.20 mg/ml heteropolymer with 3:1 A to B ratio (*Lane 8*).



Data are representative of three independent experiments

Fig 25. Heat-induced aggregation of goat β- and γ-crystallin in the presence of αB-crystallin. Light scattering of 0.05 mg/ml recombinant αB- crystallin (*trace 1),* 0.15 mg/ml goat β-crystallin (*trace 2*), 0.15 mg/ml goat γ-crystallin (*trace 3*), 0.05 mg/ml recombinant αB- crystallin and 0.15 mg/ml goat β-crystallin (*trace 4*) and 0.05 mg/ml recombinant αB- crystallin and 0.15 mg/ml goat γ-crystallin (*trace 5*) at 70°C was monitored at 360 nm.



Data are average of three independent experiments.

CONCLUSIONS

Despite the critical role of α-crystallin in many tissues, little is known regarding structural and functional significance of the two sub-units. At high temperatures (>70°C), not only αB-crystallin aggregates, but also enhances the aggregation of other lens proteins. Intriguingly, αB-crystallin-mediated coaggregation at and above 70°C involves β- but not γ-crystallin. Further, αA-crystallin, but not a mutant (F71L) αA-crystallin, prevented aggregation of αB-crystallin and also reduced coaggregation of αB- and β-crystallin. These studies explain the rationale for the existence of α-crystallin heteropolymer with αA subunit as a major partner that is vital for lens transparency and provide insights into αB-crystallin-induced co-aggregation which may have a bearing in some pathological conditions where αB-crystallin is over-expressed. Taken together, these findings highlight the physiological importance of αA on the overall stability and chaperone potential of the α-crystallin heteropolymer in an environment of low protein turnover. In addition, these results throw a light on molecular basis of some neurodegenerative diseases wherein αB-crystallin is over-expressed.

10. ROLE OF MICRONUTRIENTS IN CAUSATION OF TUBERCULOSIS

Current anti-TB therapy is effective, but it has severe limitations including drug's adverse effect, long treatment period, and emergence of drug resistant strain. Mtb resistant to multiple drugs has been defined as MDR-TB for an isolate that is resistant to two potent drugs (e.g., Isoniazide and rifampin) or XDR-TB for isolates with a wider resistance. MDR-TB prevalence has reached 4.6% globally.

Although malnutrition is frequently observed in patients with pulmonary tuberculosis but their nutritional status, especially of micronutrients, is still poorly documented. Many studies reported that patients with active pulmonary tuberculosis are malnourished as indicated by reductions in visceral proteins, anthropometric indexes, and micronutrient status. Available data, mostly obtained from animal models, underlines the important role of micronutrients in immunity against tuberculosis; However, their relevance to humans must be interpreted cautiously. It is known that deficiencies of zinc, Ca, Se, Mg, Fe, vitamin D, vitamin A and vitamin C, can cause profound impairment of immunity. Therefore, it was aimed to investigate the influence of dietary deficiencies of these micronutrients on tuberculosis etiology.

Since, the bacilli is a macrophage resident and macrophages are considered as the first line of defense, it is likely that inflammation plays a key role in determining the outcome of an infection. We demonstrated that proteins like human resistin which is predominantly expressed in the macrophages can trigger a proinflammatory response. Therefore, it will be interesting to study the roles of resistin and adiponectin in the *M. tuberculosis* pathogenesis. Nonetheless, the human resistin is secreted by macrophages, which is the main site of infection of *Mycobacterium tuberculosis.* Therefore, it is tempting to speculate a role for resistin in the pathogenesis of the bacteria. Resistin is shown to be a proinflammatory molecule therefore it will be interesting to investigate the role of resistin in the pathogenesis of tuberculosis.

AIMS AND OBJECTIVES

1. To establish a correlation between *M. tuberculosis* pathogenesis and micronutrient status in patients and to evaluate the effect of supplementation in sputum conversion rate.
2. To evaluate the effect of micronutrients on the ability of *M. tuberculosis* to infect macrophages.
3. To study the role of resistin as a marker for sterilization
4. To understand the mechanistic action of resistin in innate immune pathway.

RESULTS

A clinical trial to study the effects of multimicronutrients supplementation in pulmonary tuberculosis patients was done and the correlation between Mtb pathogenesis and micronutrients status was started. The effect of micronutrients in sputum conversion rate with antituberculosis treatment was investigated. In the study, a significant lower value of micronutrients (Ca, Mg, Zn and vitaminD) in patients as compared to healthy control was seen. However, no effect of micronutrients on sputum conversion rate was observed. But during treatment, it was found that, micronutrients like calcium levels were significantly reduced after 2 months of treatment and again significant rise in the level of micronutrients was observed. In Zinc also the same pattern was found but it was not statistically significant. In case of magnesium, no change was found in the level during treatment. In case of vitamin D, the level in patients were significantly lower than healthy control but in case of TB patient, the vitamin D level was in deficient group and in case of healthy control the vitamin level was in insufficient group.

Fig 27. Resistin levels show significant decline as a function of anti-tuberculosis treatment (ATT) in TB patients. Comparison of the circulating resistin levels in TB patients at T_0= serum sample was taken before the start of treatment; T_2= serum sample taken 2 months after the start of treatment; T_4= serum sample taken after 4 months; T_6= serum sample taken after 6 months; the healthy controls and TB patient's contacts using the box plot analysis.

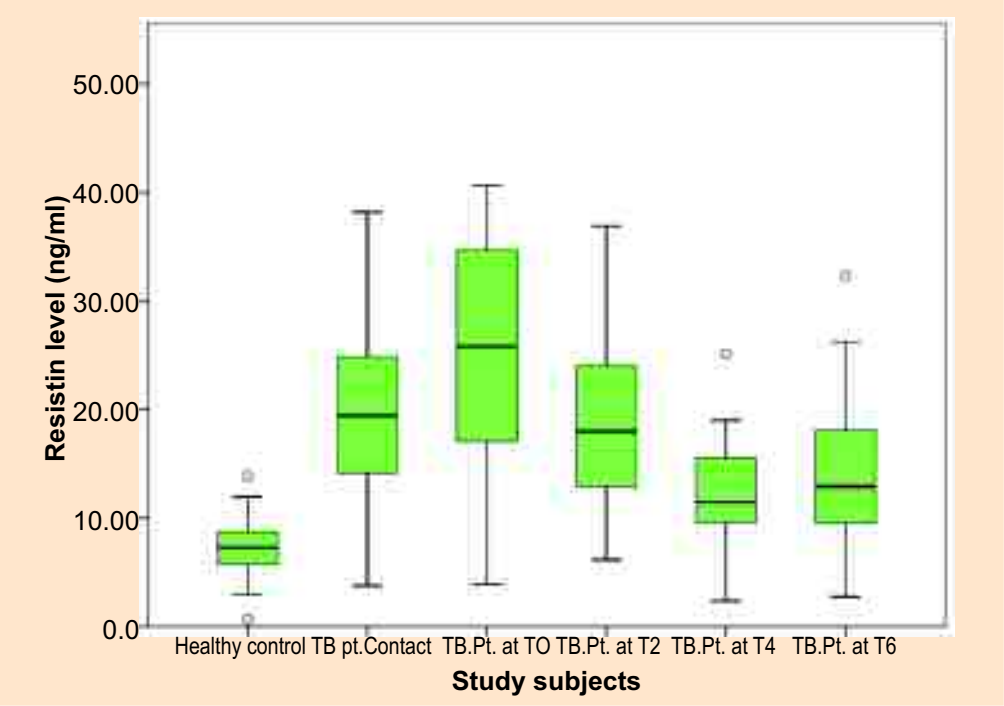
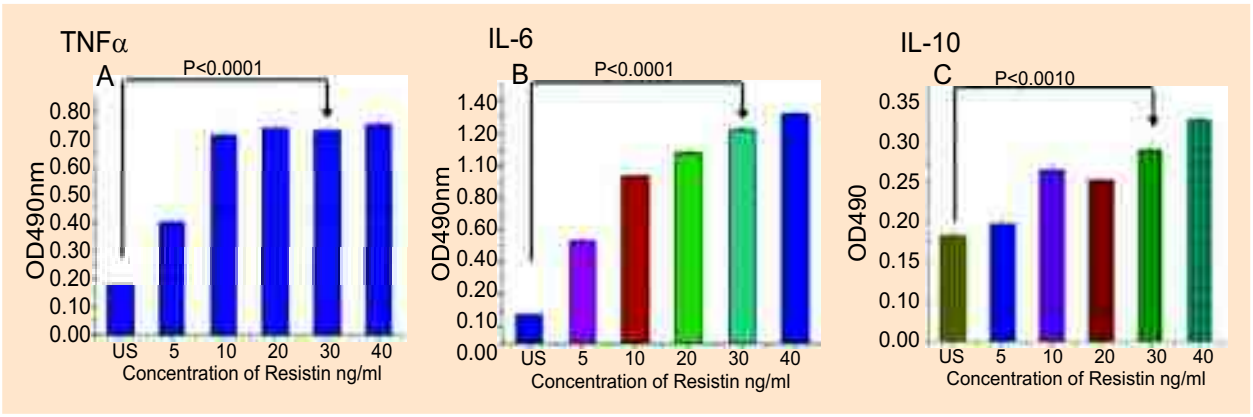


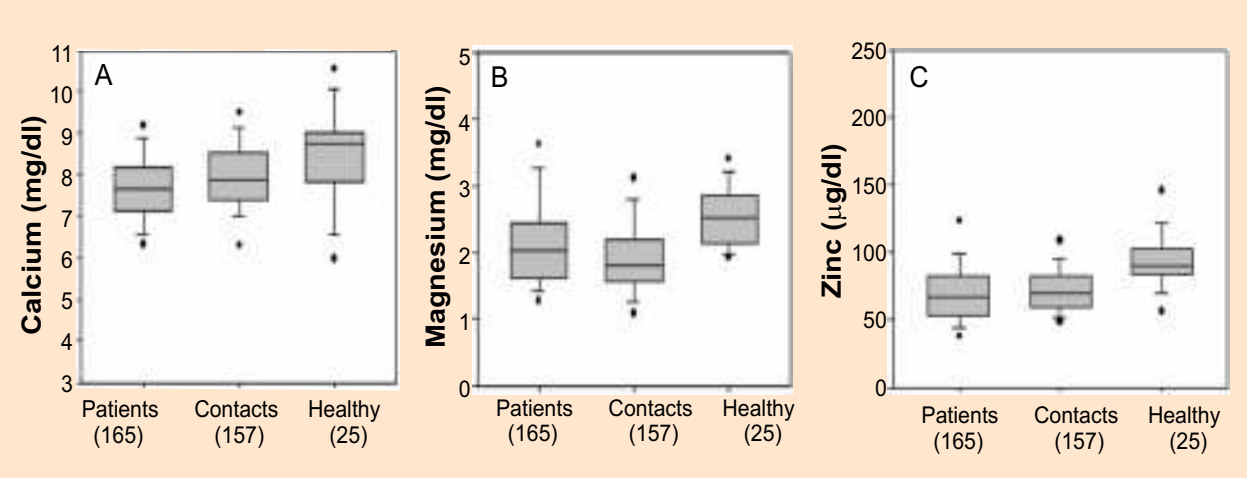
Fig 28. Resistin induces the secretion of pro and anti-inflammatory cytokines in human macrophage (THP-1) cells. PMA differentiated THP-1 cells were incubated for 48 hours in the absence (US) or stimulated in the presence of increasing concentration of conditioned medium human resistin. Levels of TNF-α (A), IL-6(B) and IL-10(C) in the culture supernatant of PMA differentiated THP-1 cells were then scored by sandwich ELISA. The levels of TNF-α(A), IL-6 (B) and IL-10(C) increased as a function of concentration of resistin.



Interestingly, we found that resistin could also stimulate expression of TLR2 in macrophages (Fig 28A). A significant increase in the levels of TLR2 was observed in cells treated with resistin (100 ng/ml). However, no significant induction of TLR2 was observed in these cells (Fig 28B).

Vitamin D levels were categorized into three groups such as deficient, insufficient and sufficient group, and it was found that 90% patients were in the deficient group at the time of recruitment and 50% of healthy controls were in deficient group. Surprisingly, in case of vit. D found significant rise in the level of vit. D after six months of supplementation as we did not find difference in placebo group. Although the rise in supplemented group, mean value of vit. D were in deficicient group. This gives an insight about the high dose of vit. D to see the effect during treatment. No difference in Vit. D level between Muslim female and Hindu female although female muslim are burqa (veil) clad are suppose to be less exposed to sunlight (Fig 26).

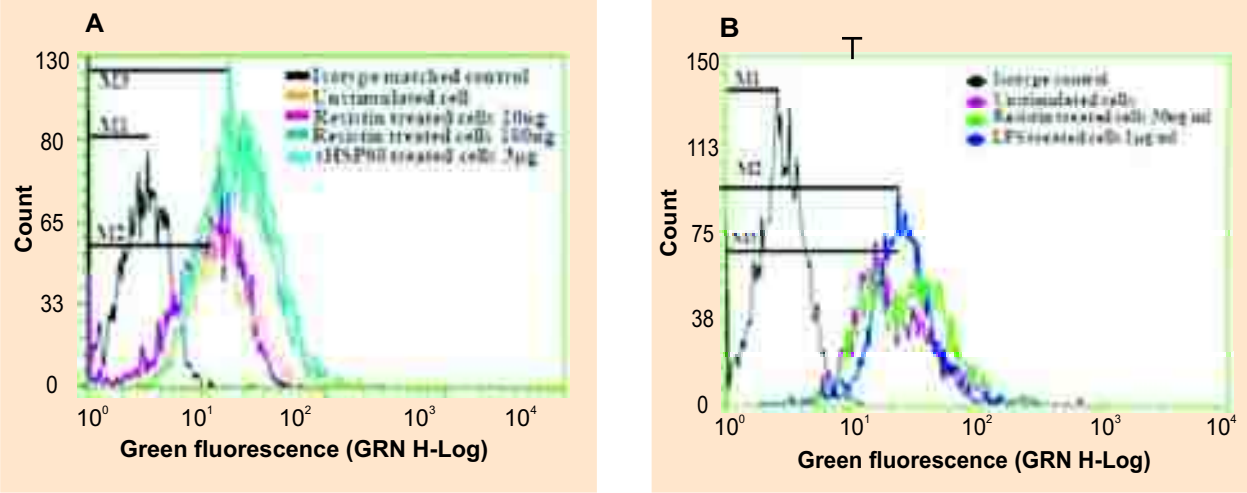
Fig 26. Distribution of different micronutrients in active tuberculosis patients, contacts and healthy controls. (A) serum calcium; (B) serum magnesium; (C) serum zinc. Significance of difference was tested with independent sample t test. Calcium levels were significantly lower in patients than healthy ($p<0.001$), between contacts and healthy ($p<0.05$), no significant difference between patients vs contacts ($p=0.05$). In case of magnesium and zinc, significantly lower value of magnesium and zinc in patients and contacts in comparison to healthy ($p<0.001$), even patients have significantly lower value of magnesium than contacts($p<0.05$), but no significant difference in the level of zinc between patients and contacts ($p=0.45$)



Next, the role of resistin, a host proinflammatory molecules, and a significant higher level of resistin was found before the start of treatment (at time T0) as compared to healthy control. It is for the first time such study was carried out in pulmonary TB patients. Estimation of resistin levels was done at different time points (T0, T2, T4 and T6 months) during treatment. Interestingly, a significant decreases in the levels of resistin was found during the treatment (Fig 27). Therefore, resistin may be used as a surrogate marker for early detection as well as endpoint for treatment for pulmonary tuberculosis.

The role of resistin in innate immune pathway and role in apoptosis also studied. Interestingly, resistin was found to stimulate the expression of TLR2. Earlier it was shown that resistin can stimulate expression of TNF-α and IL-12 through NF-Kb activation. However, in the study stimulation of TNF-α, IL-6 is NF-κB dependent but NF-κB activation is TLR2 independent and also found that resistin could stimulate the expression of IL-10 which Th2 cytokines. Therefore, resistin was involved in the stimulation of both Th1 and Th2 cytokines (Fig 28).

Fig 28. Resistin induces expression of TLR2. PMA differentiated THP-1 macrophages were treated with different concentration of human resistin (10, 100ng/ml) or *M.tb* rHsp60 (positive control) for 24 hours. The fluorescence signals were measured by flow cytometry. A significant increase in the expression of TLR2 as compared to unstimulated cells is observed (A). No effect of resistin on TLR4 expression was observed (B).



In conclusion, this study reveals many interesting findings. Micronutrients deficiency may be an effect of disease not the cause *per se*. The data also reveal that use of high dose of these micronutrients may have some therapeutic effect. Another important finding is that resistin could be used as a surrogate marker for treatment of pulmonary tuberculosis. Resistin also shows pleiotropic effects on our immune system, as it stimulates the secretion of both the Th1 and Th2 cytokines. Another interesting role in anti-apoptosis which is TLR2 dependent was found. The data speculates that the role of resistin in regulation of T cell (Treg) which is most important part of cellular immunity against *M. tuberculosis.* So, it would be very interesting to study in detail resistin's role in innate and adaptive immune pathway.

11. ISOLATION, CHARACTERIZATION AND MAINTENANCE OF PRIMARY CULTURES OF THE PANCREATIC ISLET CELLS FROM NIN WISTAR MUTANT OBESE RATS

The advantages of cell cultures are characterization of heterogeneous cell populations, flexible methods/protocols, investigation of proliferation status. Understanding the sub lethal and lethal effects the exogenous compounds and recovery of the cells and measurement of basal and cytotoxicity leading to death of the cells predictable. Primary cell cultures are direct descendents of the cells *insitu* and provide continuation of the *insitu* cell lineage. They also provide a continuous supply of biological material for the study. Techniques such as immobilization of cells (mixed cultures, multiple trans genes) and organotypic-3-D reconstructed tissues in cultures (eg. skin and eye) are the more recent technological developments in the field of cell culture. The mutant rats provide an opportunity to screen anti-diabetic drugs both synthetic and natural *in vivo*. Pancreatic

cell lines are useful for the basic study of pancreatic biology and for the possible application to cell transplantation therapies for diabetes. The objective of the project was to develop primary cultures of pancreatic islet cells from the obese mutant and also to elucidate the mechanism of hyper insulinemia in these cultures. These *in vitro* systems can eventually replace the *in vivo* system, if the attempts succeed.

AIMS AND OBJECTIVES

1. Isolation, purification and development of primary cell cultures of islet cells from WNIN/Ob & WNIN/GR-Ob
2. To elucidate the mechanism of hyper insulinemia in the primary Islet cell cultures of WNIN/Ob & WNIN/GR-Ob isolation.
3. Development of cell lines from the insulin sensitive target tissues

METHODOLGY

Islet isolation and primary cell cultures

The islets were isolated under sterile conditions as per the protocol of Banerjee and Bhonde (1), except for the collagenase con which was reduced to 0.5mg/ml.briefly,the pancreatic tissues were collected under aseptic conditions, minced3 into very fine pieces and digested with collagenase type V at 37°C for 10 minutes. The islets were washed in RPMI 1640, seeded into the medium containing RPMI 1640 supplemented with 10% FBS and antibiotics (penicillin 200 U/mL and streptomycin 0.2 mg/mL) in culture grade flasks at 37C and 5% CO_2 for 48 hour-6 days in culture. Briefly,the pancreatic tissues were collected under the sterile conditions, collagenase digested, centrifuged and the pellet consisting mostly of the islet fractions were suspended in the RPMI with 10% FCS. The viability was assessed by TBE and also by DTZ staining of the islets.

Islet Viability– (MTT) assay

The primary cultures of the islets were assessed for their viability by the Thiazolyl blue tetrazolium bromide (MTT) assay. Briefly, MTT reagent was added to the cell suspension and incubated for a period of two hours at 37°C. The islets were sedimented; isopropanol was added and incubated at room temperature for 20 minutes to solubilize the formazan formed. The purplish blue colour of the supernatant was measured at 532nm and the values are expressed as percentage viability compared to WNIN wistar as the control islets.

Hemotoxylin and Eosin staining

The method of tissue fixation was by standard method wherein briefly, 6 m thick sections were stained with hematoxylin and eosin dyes to assess the integrity of pancreatic tissue in the WNIN/Ob, lean and control as a function of age.

Ultra Structure

The primary cultures of the islets were processed for the TEM and SEM by the standard procedures. For TEM, the samples were fixed directly Reagents: Karnowsky's fixative, Phosphate Buffer, Acetone, Toluene and Resin mixture (Epoxy resin, Hardner DDSA and DMP-30) and for SEM Reagents: Karnowsky's fixative, Phosphate Buffer, Acetone, Toluene and Resin mixture (Epoxy resin, Hardner DDSA and DMP-30).

Immunolocalisation for Insulin /Glucagon and Insulin /Glu-2

From WNIN/Ob, lean and contol , a portion towards the splenic region was fixed in formaldehyde. After repeated washings in PBS, followed by dehydration in ethanol tissues were embedded in paraffin. A series of 6 m thick sections were mounted on to poly L lysine coated slides and dried overnight at 37°C. Briefly, after blocking with 4% Horse serum the samples were incubated with primary antibody overnight at 4°C Insulin1:500, Glucagon (SantaCruzi: 200) and Glu-2 (1:200). The samples were treated with secondary antibody tagged with appropriate fluorescent dyes for Insulin, Glucagon and Glu-2 aii procured from molecular probes. The fluorescence images were captured using the confocal microscope, Leica SP5 series and were appropriately corrected with their controls.

Insulin secretion

The insulin secretion assay was carried out by taking 250 islets each from WNIN/Ob, lean and controls were washed twice in Krebs-Ringer bicarbonate buffer (pH 7·4) supplemented with 10 mmol/l HEPES and 1 mg/ml bovine serum albumin (KRBH). To determine the effect on basal insulin secretion, islets from all the groups were incubated in KRBH containing 5.5 mM glucose at 37°C for 1 hour and the supernatant was collected. The insulin responsiveness on stimulation with high glucose was carried out on the same islets by incubating with KRBH containing 16.5 mM glucose for 1 hour. All supernatants were stored at –80°C until measurement. Insulin was estimated using INS-EASIA kit and expressed as IU /ml.

Total Intracellular insulin content

Total Intracellular insulin content was estimated from the acid ethanol extract of the whole pancreas. The whole pancreas isolated from the animals was incubated in [75% Ethanol, 1.5 % 10N HCl]. The supernatant was collected and stored at –80°C until estimation.

Measurement of serum malondialdehyde (MDA)

Lipid peroxidation was estimated by measuring the thiobarbituric acid reactive species (TBARS) using 1,1,3,3, tetra methoxypropane**.** Briefly, 80 of serum from the control and experimental groups were mixed thoroughly with freshly prepared thiobarbituric acid and heated at 90ºC for 30 minutes in a water bath, cooled to RT and centrifuged. The pink colored product which developed after the addition of 100 n-butanol was measured at 532 nm for thiobarbituric acid reactive species. The MDA content was expressed as nanomoles per litre.

RT- PCR Analysis

Total RNA was extracted using Trizol reagent. A reverse transcription reaction was carried out using total RNA (2ug) at 50ºC for 30 minutes. The PCR conditions were 30 cycle of 94ºC (1minute), annealing temperature (45 seconds), 72ºC (1 minute) (Eppendoff Thermocycler RT-PCR system). The final extraction was at 72ºC for 10 minutes. The respective sequences of PCR primer, the annealing temperatures and the expected product size from the mRNA of each gene were as follows:

Insulin:	PDX1
Forward: caatcatagaccatcagcaagc	Forward: tacaaggacccgtgcgcatt
Reverse: tcaagttgagcatcactgcc	Reverse: tcaagttgagcatcactgcc

β -Actin

Forward: ccccattgaacacggcatt

Reverse: ggtcatcttttcacggttggc

RESULTS

- WNIN/Ob rats demonstrated an increase in plasma insulin, tissue insulin, islet hyperplasia and hypertrophy which was evident with onset of age (35 >6 months >12 months).
- The hyperinsulinema in the Ob/Ob rats were observed immediately after weaning and were able to demonstrate by 35 days (H.E).
- Ultra structure analysis showed degranulation in the islets of the Ob/Ob rat pancreas as compared to its counterpart lean and parenteral control.
- Thus, it showed that large islets were less efficient secretors of insulin when compared on a volume basis, although the total amount of insulin released was larger.
- The WNIN/Ob rats demonstrated showed increase in TBARS both in tissue and plasma (TBARS method).
- IHC showed that colocalisation of insulin/glucagon ratio was relatively unaltered.
- Pdx-1 expression was upregulated in WNIN/Ob rats as compared to its lean and control.
- The islet isolation protocol was standardized by modifying the collagenase digestion method (differential digestion).
- An increase in the islet yield recovered and integrity from the WNIN/Ob rats.
- The primary cultured islets were maintained for a peroid of 48-96 hours with islet cell integrity and insulin secretion function (DTZ and viability).
- However, beyond >96 hours the cells lost their viability as assessed by TBE and MTT assay.
- Interestingly, insulin secretion with challenge was not observed unlike that in the parenteral controls. This was more seen >9 months of age (Insulin secretion assay).

Salient findings

Hyperinsulinemia induced stress was appreciable in the pancreatic tissue with age. Chronic insult showed impaired insulin secretion of both at basal and with high glucose challenge. Interestingly ultra structural analysis demonstrated dispersed and degranulation of insulin secreting cells in 12 month of obese rats. These observations are significant as obesity reflects a state of imbalance between oxidant stress and anti-oxidative defense mechanism(s) and beta cells are unique as they are weak in their antioxidant system compared to other organs. These mutant obese rats provide an excellent opportunity to study the cellular, biochemical and molecular mechanism(s) underlying obesity and associated degenerative disorders and opens up for testing the antioxidant supplementation to manage the tissue stress.

12. DIETARY OILS (COCONUT AND VIRGIN COCONUT) AND THEIR HEALTH IMPLICATIONS

Quantity and quality of dietary fat in terms of fatty acid composition greatly impact IR and associated disorders. In general, saturated fats increase and polyunsaturated fatty acids (n-6 and n-3 series) mainly linoleic and α-linolenic acid mitigate the problem of athero-thrombogensis by altering important metabolic pathways. Dietary fats alter the fatty acid composition of membranes, which is determined by the delicate balance between the metabolic conversion of 18:2 n-6 (linoleic) and 18:3 n-3 (α-linolenic acid) fatty acids due to competition for the same enzymes, which in turn, modulate wide range of non-eicosanoid and eicosanoid–dependent physiological functions.

In India, fat consumption varies not only with respect to the quantity but also in terms of the type of the oil consumed (quality of fat). The latter is mainly due to regional, cultural preferences, culinary practices and varied agronomic conditions. Among the various oils that are consumed in India, coconut and palm oil possess high amounts of saturated fatty acids and are known as tropical oils. These oils were not promised as 'healthy' by media. However, Palm oil has come out of the bad press by extensive research program carried out by PORIM (Malaysia). On the other hand, there are not many studies wherein the health effects of coconut oil and virgin coconut oil are thoroughly evaluated. Therefore, the present study has been designed to address the health concerns arising out of coconut oil consumption and also evaluate the possible beneficial effects (if any) of virgin coconut oil and the following objectives have been formulated for the study.

OBJECTIVES

1. To evaluate the impact of consumption of diets prepared with coconut oil/virgin coconut oil on lipid metabolism, insulin resistance and inflammatory parameters of young adult healthy human volunteers/obese subjects.
2. To study the effect of chronic consumption of coconut oil/virgin coconut oil on lipid and energy metabolism/obesity and development of insulin resistance in Wistar as well as WNIN/Ob rats.

Experimental design

Human metabolic study was planned to examine the effects of coconut oil and virgin coconut oil on health. For this purpose, healthy male volunteer with normal body mass index (BMI) and over weight volunteers were recruited into the study. Initially, the normal weight volunteers were kept on coconut oil diet and overweight volunteers were kept on virgin coconut oil diet for a period of eight weeks. At the beginning and end of eight weeks of dietary regimen, fasting blood samples were drawn, plasma was separated and kept at -80^{0}C for analysis. Before initiation of the study thorough medical examination was done and the volunteers were explained about the purpose of the study and written consent was obtained from them for their willingness to participate in the study. The study plan was approved by Institutional Human Ethical Committee.

All the three meals was prepared at metabolic kitchen and care was taken that all micro and macro nutrients were kept constant except the source of visible fat and provided to the volunteers. After the study period of eight weeks, again all the biochemical and body composition by bio-impedance and anthropometric parameters were recorded. In the second phase, all the normal and overweight volunteers were kept on control diet (groundnut oil) after a washout period of five weeks. Before initiation and at the end point, again all the biochemical and body composition by bio-impedance and anthropometric parameters were measured.

In the third phase of the study, all the volunteers with normal body mass index were given virgin coconut oil diet after a washout period of five weeks. Again in the third phase, initial and final

measurements were made with respect to biochemical parameters, and body composition through bio-impedance and anthropometry were measured.

METHODS

- Using commercially available kits, the following parameters were determined in plasma such as total cholesterol, esterified cholesterol, lecithin: cholesterol acyl transferase (LCAT), APO A, B and Lp (a).
- Statistical analyses were done by student's t-test and paired t-test.

RESULTS

Impact of various oil (coconut/virgin, coconut/groundnut) based diets on plasma parameters was studied. No significant changes were observed in any of the oil-based diets in normal subjects. Though similar trend was seen in over-weight subjects, plasma free cholesterol levels were increased at the end of groundnut oil based diet consumption (Table 50a & 50b).

Table 50a. Impact of various oil (coconut/virgin coconut/groundnut) based diets on plasma parameters of normal subjects

Plasma Parameters (mg/dL)	Virgin coconut Oil (n=8)		Groundnut Oil (n=8)		Coconut Oil (n=6)	
	Initial	Final	Initial	Final	Initial	Final
Total cholesterol	186χ14.9	199χ21.0	194χ18.9	196χ17.9	202χ21.0	1968χ23.6
Free cholesterol	79χ9.4	94χ13.4	85χ10.4	83χ11.7	95χ15.6	98χ18.0
Esterified cholesterol	107χ8.1	104χ9.5	108χ9.4	112χ7.5	107χ8.25	100χ7.29
LCAT-Activity#	1.45χ0.02	1.43χ0.03	1.34χ0.01	1.37χ0.03	1.34χ0.03	1.35χ0.03
LP (a)	20.5±6.49	11.2±5.27	17.8±7.6	22.6±7.8	27±11.6	16±5.79
APO A	83±3.4	81±2.79	88±4.13	83±7.02	90±7.82	86±4.06
APO B	46±5.4	50±6.45	49±7.8	45±8.4	44±5.2	42±7.6

Table 50b. Impact of various oil (coconut/virgin coconut/groundnut) based diets on plasma parameters of over-weight subjects

Plasma Parameters (mg/dL)	Virgin Coconut Oil (n=8)		Groundnut Oil (n=8)	
	Initial	Final	Initial	Final
Total cholesterol	168χ11.9	176χ8.4	178χ10.5	182χ12.2
Free cholesterol	67χ8.40	78χ7.8	76χ9.2	89χ9.07*
Esterified cholesterol	100χ6.9	98χ8.03	102χ7.4	93χ5.9
LCAT-Activity#	1.32χ0.03	1.39χ0.02	1.3χ0.03	1.32χ0.02
LP(a)	8.7±2.8	9.0±2.5	9.1±2.0	13.0±3.2
APO A	70±7.4	68±5.9	69.0±1.5	66.0±2.7
APO B	35±4.9	43±3.8	36.0±2.6	43.0±11.3

*# LCAT-activity is the ratio of emission intensity at 470/390nm. Values are mean ±SE, n=number in parentheses indicates the number of samples. * Statistically significant at p<0.05 by Paired "t" test.*

CONCLUSION

Consumption of coconut or virgin coconut oil-based diets had no adverse effect on the concentration of plasma cholesterol and its components (free and esterified cholesterol) and apolipoproteins.

V. FOOD CHEMISTRY

1. STABILITY OF ADDED IODINE IN DIFFERENT INDIAN COOKING PROCESSES

Food is the major source of total iodine intake by man, with water and beverages contributing small amounts. In general, grain crops are poorer sources of iodine than vegetables. Green leafy vegetables contain higher levels of iodine than other vegetables but fish and seafood are the richest natural source in foodstuffs. Thus, the proportion of each type of food consumed determines the dietary iodine exposure.

Iodine deficiency is not only of nutritional and public health concern but also a major impediment to human and economic development. Elimination of Iodine deficiency is thus a global health priority and the only way to eliminate IDD is to make iodine available to the deficient population. From the public health point of view, universal salt iodization programme is recognized as an economical, convenient and effective means of preventing IDD.

The actual availability of iodine from iodized salt at consumer level can vary widely due to a number of factors including the variability in the amount of iodine added during production, uneven distribution within batches or bags produced due to poor mixing and loss during transportation, storage and meal preparation. The WHO/UNICEF/ICCIDD (2001) suggested that there might be 20% loss of iodine through cooking and food preparation practices. Detailed information on the retention of iodine during the process of cooking is necessary to ensure adequate supply of dietary iodine to the population and to satisfactorily manage the programme on the control of IDD. While urinary iodine level provides a good measure of the iodine nutriture of the population, iodine content of ready to eat recipes is also an important parameter to estimate iodine intake by the population. Therefore, this study was undertaken to investigate the stability or retention of iodine from iodized salt when added to a selection of the most commonly used Indian recipes under normal cooking conditions.

Study area and sample collection

The study was carried out in 23 states covering 48 districts located in different regions of the country. In each district, two households were identified on the basis of their willingness to participate in the study. Information on the cooking method which was typical of those used in domestic Indian kitchen was collected by personal interview with the help of a schedule. From the interviews, recipes most commonly consumed in daily diet were selected for investigation and the house lady was requested to prepare the recipe. Salt to be used for the recipe preparation was tested by rapid testing kit to determine the presence of iodine. In case any household was using non iodized salt then there was provision to supply iodized salt to the house lady for preparing the recipes. Prior to cooking all individual ingredients used were weighed separately and recorded in order of addition to each recipe preparation. All samples of raw food items used in the preparation of the recipes was collected from each of the households. The cooked sample was homogenized and packed in air tight polypropylene jars. The samples were then transported to the laboratory at NIN in an icebox to avoid spoilage and analyzed for iodine content. Each recipe was replicated in the laboratory at NIN using the same food items collected from the surveyed household and analyzed for iodine content. Samples of salt and water were also collected separately in poly bags and plastic bottles respectively. Iodine content in water and salt samples were analyzed in the laboratories of

the participating centres in Hyderabad, Bhubaneshwar, Pantnagar, Udaipur and Nagpur. Salt samples were analyzed for its iodine content by potentiometric titration. All food and water samples were analysed in triplicate by the kinetic colorimetric method of Moxon & Dixon (1980). The method was validated with the use of Certified Reference Material NIST 1549. Casein was used as in house quality control material with every batch of analysis. Recovery analysis was also performed with samples spiked with iodide equivalent to 1 mg/kg with every batch of analysis.

RESULTS

Iodine content in drinking water and household salt samples

Household drinking water samples (n = 82) showed a wide variation of iodine content between the states with mean iodine content ranging from1.3µg/L in Meghalaya to 23.2µg/L in Tamil Nadu varying appreciably from place to place within the states. Frequency distribution of iodine content in drinking water showed that 65.8% had iodine content less than 10µg/L, 24.4% between 10.1-20µg/L and 9.8% more than 20 µg/L. In general, water samples from or near the coastline had significantly higher water iodine content than other regions. State-wise mean iodine content of the household salt samples (n=116) ranged from 11.3 ppm in Bihar to 55 ppm in Jammu and Kashmir. Eight states namely Gujarat, Orissa, Assam, Sikkim, Meghalaya, Chattisgarh, Haryana and Jammu and Kashmir had mean salt iodine content > 40ppm. Himachal Pradesh and West Bengal had mean iodine content between 15-30 ppm while <15 ppm was found in only one state viz; Bihar. A very small number of salt samples (10.4 %) contained < 15 ppm iodine. These substandard samples were found in Bihar (2), West Bengal (3), Haryana (1), Uttarakhand (4) and Himachal Pradesh (2). None of the households surveyed was using non-iodized salt. The Mean±SD iodine content of household salt was 31±16 ppm. The wide variation in the iodine level of household salt may be due to the different level of iodization by various salt manufacturers and loss during transportation and storage. However, the quality of the salt used at household levels with respect to iodine content was generally good reflecting the wide acceptance, availability and use of iodized salt throughout the country.

Retention of iodine in recipes prepared using iodized salt

Retention of iodine in recipes during cooking procedures carried out both in the field and in the laboratory was found to be comparable which essentially indicates that the same cooking procedure if followed by even different individuals and at different locations would result in similar retention of iodine for the same recipe. The retention of iodine was studied in 140 recipes comprising of 8 non vegetarian and 132 vegetarian preparations. The retention of iodine in the 140 recipes ranged from 5.5% in shallow fried okra to 97.1% in Puri (deep fried Indian bread) with a mean of 60.2% ± 21.2 %. Frequency distribution of 139 recipes showed low retention (<20%) of iodine in 5.7% of the recipes, moderate retention (20%–40%) in 15.7% while very high retention (>80%) was observed in 20% of the recipes. More than half of the recipes studied retained iodine in between 41 – 79% which indicates efficient supplementation of iodine through iodized salt. Analysis of the data showed significant correlation ($r = -0.194$ $P<0.05$) with the time of addition of the iodized salt to the recipe and iodine retention. Significant correlation ($r= 0.796$, $P<0.01$) was observed between retention of iodine and duration of cooking after salt addition.

Iodine content of recipes prepared with iodized salt

The mean±SD iodine content of all the cooked recipes (n=140) was 32.1µg/100g 24.1µg/100g which indicates a very wide variation in the iodine content of the recipes prepared and consumed throughout the country. Frequency distribution showed 37% of the recipes had iodine content <20µg/100g sample, 32% had in the range of 20 to 40µg/100g and 31% had >40 µg/100g. Of the

total recipes analyzed 18% had iodine content <10 µg/100g and 9% had iodine content >70µg/100g.

Effect of recipe preparation methods on iodine retention

Each of the recipes were grouped according to the major cooking methods used in their preparation and the data thus compiled were analyzed to determine the association of the mean iodine retention with respect to each cooking method. The retention of iodine was observed to be minimum in shallow frying with oil (52% ± 23%) as well as in boiling (53% ± 27%). Frying with boiling also resulted in almost similar iodine retention (55% ± 23%). Increased iodine retention was observed with shallow frying without oil (63% ± 16%), boiling with oil seasoning (65%± 13%), steaming (68% ± 16%) shallow frying followed by cooking with water (69% ± 10%) and pressure cooking followed by shallow frying (69% ± 17%). The maximum retention of iodine was found in deep frying (77.8% ± 20.7%) and pressure cooking (82.2% ± 6.2%).

CONCLUSION

The Mean iodine content of household salt and retention of iodine in 140 recipes was 31±16ppm and 60±21% respectively. The data on the 140 cooked recipes revealed a wide variation in the iodine content with a mean of 32.1µg / 100g 24.1µg/100g. The data on iodine content of the cooked recipes provide an estimate of iodine availability and intake by the Indian population through the diet which will be useful for modeling the impact of strategies to increase iodine exposure via the use of iodized salt. Fortification of salt with iodine has resulted in the desired effect in the Indian population and the fortification level (30 ppm) seems reasonable at present. However, this level of fortification needs to be monitored specially in the light of the WHO recommendation for reduced salt intake.

VI. EXTENSION AND TRAINING

Service activities

1. PUBLICATIONS

The popular publication "Recommended Dietary Allowances" which was under revision for quite some period was revised and reprinted. The other publications which were reprinted, on popular demand include Count What You Eat, Dietary Tips for the Elderly, Low Cost Nutritious Supplements and Fruits.

2. TRAINING PROGRAMMES

Regular Training Programmes

This year a total of twenty six candidates have attended the regular training programmes of the Institute viz. (i) MSc (Applied Nutrition) II Batch 2010-11 – 15 participants (ii) Post-Graduate Certificate Course in Nutrition - 4 participants (iii) Training Course on Assessment of Nutritional Anaemias - 7 participants.

3. EXTENSION ACTIVITIES

3.1 Exhibitions

- Bharat Nirman Public Information Campaign, organized by Press Information Bureau, Government of India, Hyderabad, at Maheshwaram, Rangareddy District. (Aug. 3-5, 2010)
- Fifth Uttarakhand State Science and Technology Congress, held at Dehradun. Served as a Jury for assessing scientific papers for a session on "Food, Nutrition and Child Development". (Nov. 10-12, 2010)
- Science and Technology Exhibition, and erection of ICMR Pavilion as a part of 98th Indian Science Congress, held at SRM University, Chennai. (Jan. 3-7, 2011)
- Bio-Asia Conference at HITEX, Hyderabad whereabout 200 delegates from 12 countries of Asia had participated. More than 3,000 people visited NIN stall (Feb. 22-24, 2011).

3.2 Popular Lectures/ Awareness Camps

- Conducted a series of eight awareness programmes and popular lectures on "Nutrition and Health" for groups such as adolescent boys, girls, sport persons and their coaches, during summer camps organized by The Confederation of Voluntary Associations (COVA), at various schools, playgrounds and community centers of old city of Hyderabad. (May 1- 20, 2010)
- Radio talk on "HIV and Nutrition", broadcast on All India Radio, Hyderabad and Vijayawada. (May 21, 2010)
- Lecture on Nutrition and Diet for the children aged 2-5 yrs, at Bachpan, Play School, Nagaram, Hyderabad. Parents of about 60 school children participated in the programme. (Aug. 14, 2010)

- Lecture on “Nutrition, Health and Physical Activity”, at the Andhra Pradesh Police Department (Aug. 16 & 18, 2010).
- Awareness session on “Nutrition during adolescence” at Paragon High School, Vattepally. About 70 school students and their teachers participated (Aug.16, 2010).
- Popular talk on “Nutrition and reading food labels” at Sharada Vidyalaya, Shamsheer Gunj, Hyderabad. About 150 students of degree colleges and their lecturers participated (Aug. 18, 2010).
- Lecture on “Nutrition and Health” to the students of Babu Jagjeevan Ram Government Degree College, Nampally, Hyderabad. About 200 students from various colleges and 60 teachers attended the programme (Aug. 19, 2010).
- Nutrition awareness session and distributed some communication material to the volunteers of the National Service Scheme at NSS Camp organized by St. Francis College of Women, Hyderabad in at Pasumamula Village, RR District of AP. As many as 100 student volunteers and about 20 village self help group (SHG) women were sensitized (Nov.10, 2010).
- Extension lecture on “Nutrition and Health” to the district police personnel, organized by Intelligence Department of A.P. Police, Hyderabad (Nov. 23, 2010).
- Extension lecture on “Nutrition and Health” to the students of P.G. Diploma in Theology, organized by an NGO BLESS, Hyderabad. (Nov. 23, 2010).
- Lecture on “Nutrition and Health” to the police personnel of the Central Industrial Security Force, Shaikpet, Hyderabad. About twenty Inspector General's attended the lecture. (Dec. 17, 2010)
- Extension lecture on “Nutrition and Health” to the members of the International Association of Lions Club, Hyderabad. About 40 members of the Lions Clubs will attend the lecture. (Dec. 29, 2010)
- About 75 students of Farah High School, Baba Nagar, Hyderabad were sensitized on 'importance of nutrition during adolescent phase' through a lecture. (March 3, 2011)
- Nutrition extension lecture at Khulq memorial High School, Misrigunj, Hyderabad in a programme jointly organized by local NGOs viz., COVA and STARS. About 75 students of Urdu and English media participated. (March 29, 2011)

3.3 Development of Communication Material

Educational films were developed on the following subjects in association with Educational Multi-media Research Centre (EMMRC), Hyderabad:

“You are what you eat!” – A film on simple dietary tips and need for physical activity

“How to read food labels?” – A discussion programme

What is Junk Food' - A discussion programme

4. SPECIAL EVENTS

National Nutrition Week Celebrations (1-7 September, 2010)

- Awareness session on “Nutrition and dietary guidelines” was organized for the girl students of Govt P.G. & Degree College Hussaini Alam, Hyderabad as part of the National Nutrition Week. Over 125 students participated (Sept. 1, 2010).

- Popular talk on “Dietary guidelines” for the students of Government Degree College for Women, Hussainialam, Hyderabad in a nutrition awareness camp jointly organized with voluntary organizations - STARS and COVA (Sept. 2, 2010)
- One-day symposium on this year's theme – Nutrition Promotion for a Stronger Nation, at NIN in association with Food and Nutrition Board, Govt. of India. (Sept. 6, 2010)
- Popular lecture on nutrition for the benefit of 200 boys and girls as part of the National Nutrition Week Celebrations at Sharada Vidyalaya School, Shamsheer Gunj (Sept. 7, 2010).

5. ACTIVITIES OF SECRETARIAT FOR WHO SOUTH-EAST ASIA (WHOSEA) NUTRITION RESEARCH-CUM-ACTION NETWORK

The Institute has been serving as the Secretariat for the WHOSEA Nutrition Research-cum-Action Network since 2004. This year, the status of the Institute has submitted the annual report as the 'WHO collaborating centre in nutrition and primary health care' 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. As per the APW, the number of issues per year, has been scaled up to three instead of two. The January 2011 issue of the newsletter was printed and sent to all the network members and the next issue is under preparation.

VII. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

1. WNIN/Ob MUTANT RAT MODEL TO STUDY DNA DAMAGE AND MUTAGENICITY TESTING

NCLAS at NIN has established two obese mutant rat models- WNIN/Ob and GR/Ob, former with euglycemia and the later with hyperglycemia. These animals show distinct physical, physiological and biochemical indices of obesity and age faster than the normal wistar rats. Apart from obesity, these rats shows incidence of tumors (60%), cataract (10%), opportunistic infections (100%) and kidney abnormalities (80%). Carcinogenic process is known to be preceded by damage to DNA. This is known to induce alterations in cellular genome and altered gene expression. Accumulations of such mutations are associated with ageing and other mutation based degenerative diseases like cancer, diabetes, cataract etc. Since these mutant rats shows obesity and obesity related degenerative chronic disorders, it is possible that they harbour large proportion of damaged DNA & accumulation of age related end products.

Therefore, it was thought worthwhile to study DNA damage in various organs of these animals and estimate the levels of enzymes in liver and Kidney.

AIMS AND OBJECTIVES

a) To assess DNA damage in blood of mutant rats

b) To study the levels of Quinone reductase, Glutathione S-transferase and Glutathione Peroxidase

METHODOLOGY

Animals

WNIN/Ob, WNIN/GR-Ob and WNIN rats aged 1 year were obtained from the National Centre for Laboratory Animal Science (NCLAS) and housed in the animal facility where the temperature was maintained at 24-25°C with 12-hr dark/light cycle. 3 males and 3 females were taken from each strain. The animals were fed with standard pellet diet. All the animals were euthanised by placing them in a chamber containing carbon dioxide (CO_2) and liver and kidney were collected. Whole blood was collected from the orbital plexus prior to euthanisation and used for the comet assay.

Groups		Number of rats
WNIN/Ob	Obese	6
	Lean	6
WNIN/GR-Ob	Obese	6
	Lean	6
WNIN		6

The Comet Assay

Single cell suspension was made using cell dissociation technique and the resulting cell suspension was taken for the Alkaline Comet assay.

Enzyme Assay

NAD(P)H Quinone reductase (QR): QR was estimated in liver and kidney.

Glutathione S-transferase (GST): GST was estimated in liver and kidney cytosol using 1-chloro-2,4, dinitrobenzene (CDNB).

Glutathione Peroxidase (GSHPx): GSHPx in the cytosolic fraction of liver was determined by a modification of the coupled assay procedure.

Estimation of Protein: Protein concentrations were estimated.

Statistical Analysis

Statistical Package for Social Science (SPSS) windows version 15.0 was used for statistical analysis. Mean and SD values were calculated for all the variables and groups. Mean values were compared by one way ANOVA. Paired 't' test was used for comparison of mean differences of dependent samples for each group.

RESULTS

The data revealed no significant differences in mean levels of DNA damage in OTM & TL parameters in the blood of various groups (Table 51).

The QR and GST levels in WNIN/Ob and WNIN/GR-Ob were not significantly different than WNIN. But GSHPx levels in Lean animals of Ob and Gr-Ob strain were significantly different than WNIN, WNIN/Ob and WNIN GR-Ob animals. The data revealed no significant differences in GSHPx levels of WNIN, WNIN/Ob and WNIN GR-Ob groups (Table 52).

Table 51. Comet Assay in blood

Group		Tail % DNA	Olive Tail moment (OTM)	Tail length (TL)
WNIN/Ob	Obese	8.3±5.53	0.3±0.16	0.6±0.33
	Lean	3.3±1.47	0.2±0.06	0.4±0.15
WNIN/GR-Ob	Obese	13.0±9.78	0.5±0.25	0.8±0.34
	Lean	10.9±7.31	0.4±0.20	0.8±0.39
WNIN		13.9±8.11	0.5±0.25	0.8±0.42

All values are mean±SD

Table 52. Enzyme levels in tissues

Groups	QR *(nmoles/min/mg protein)*		GST *(CDNB U conjugated/ min/ mg protein)*		GSHPx *(Cytosol oxidised/ min/ mg protein)*
	Liver	*Kidney*	*Liver*	*Kidney*	*Liver*
WNIN/Ob	111.3±19.61	119.0±15.00	526.1±28.69	105.1±18.60	248.6±24.49[a]
Ob-Lean	120.4±16.64	124.7±17.94	567.0±30.65	131.1±21.56	225.4±17.39[b]
WNIN/GR-Ob	110.5±11.57	119.5±17.25	531.7±29.85	107.6±19.27	242.5±22.25[a]
GR-Ob-Lean	124.1±16.53	130.0±22.52	558.2±25.30	128.3±12.77	212.6±19.28[b]
WNIN	118.0±11.88	125.9±16.06	534.3±35.10	112.9±21.78	238.3±21.25[a]

Values are mean±SD of 6 rats/group.
Means in the same column with different superscripts differ significantly ($p<0.05$)

CONCLUSION

The results of the study showed that, there was no significance in the antioxidant status of DNA damage and antioxidant enzymes in obese and lean rats compared to the WNIN rats as they did not exhibit any induction in the strand breaks in the blood tested as evidenced by the alkaline comet assay.

2. ASSESSMENT OF ENVIRONMENTAL LEAD EXPOSURE ON INFECTION AND IMMUNITY

Lead, the ubiquitous environmental pollutant causes sub-clinical organ system damage specially haemopoietic, renal and nervous systems. Undernutrition *per se* may aggravate the lead toxicity. Current evidences suggest that elevated lead levels alter immune functions by enhancing lymphocyte proliferation and possibly increase severity of infectious diseases. Micronutrient deficiency specially of Fe may hamper immune function.

HYPOTHESIS

Elevated levels of lead and micronutrient deficiency may alter immunity and enhance lead induced cytotoxicity.

OBJECTIVES

1. To assess the immune function in Pb exposed iron deficient animal model.
2. To determine the effect of oral Pb exposure on intestinal microflora in iron deficient rats.
3. To evaluate the protective effects of thiamine on Pb induced inhibition of *Lactobacill* and *E. coli*.

METHODOLOGY

In vivo

The experiment was carried out in weaning Sprague Dawley (SD) rats (n=64 equal number of males and females), which were divided into two groups (n=32 with equal number of males and females) and supplemented with normal or iron deficient diet (AIN-93G) for a period of 4 weeks. The hemoglobin and serum iron content of animals in each group were estimated at various time points to confirm iron deficiency. After establishing the iron deficiency, the animals in each group were further divided in to 2 sub-groups (n=16 with equal number of males and females). The first sub-group served as the control and the second one was exposed to Pb (25 mg /Kg body weight) for a period of 4 weeks. At the end, hemoglobin, serum iron, blood and urine Pb levels were estimated. This was followed by TT vaccination to half of animals in each sub-group, the remaining half of the animals served as control of each sub-group. The percent CD4+ and CD8+ lymphocytes were estimated using Flowcytometer. The mucosal IgA, serum TT specific and total IgG and IgM levels were estimated by ELISA method. The *in vitro* splenocyte proliferation index was assessed by H thymidin incorporation followed by counting the radioactivity in Liquid Scintillation Counter. Rat bone marrow micronucleus test was used to assess the extent of cytotoxicity caused by Pb exposure in iron deficiency. The fecal samples were analysed for *Lactobacilli*, *E. coli* and yeast using the selective medium Lactobacilli MRS agar, Endo agar and Saborauds agar respectively in order to enumerate micro flora present in intestine.

In Vitro

Bacterial cultures were processed to observe their morphology under scanning electron microscopy after treatment with thiamine, Pb, Thiamine + Pb control was untreated. The cells were fixed with 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 1.0 h at room temperature. After two washes with phosphate buffer, cells were post-fixed for 30 min in 1% OsO_4 in the same buffer, washed three times with phosphate buffer, dehydrated in a graded series (30, 50, 70, 80, 90, and 100%) of ethanol, dried under vacuum overnight, mounted and coated with gold sputtering (Hitachi, Ion sputter E-1010, Japan). The specimens were examined using Hitachi scanning electron microscope (S3400N, Japan) at a magnification of x 15,000.

RESULTS

In Vivo

1. The mean Hb levels and serum iron levels (66.2±13.904 µg/dL) of the rats fed with iron deficient diet was significantly ($p<0.001$) lower than normal diet group (210.5±44.23 µg/dL).
2. Mean blood lead levels (BLL) were significantly ($p<0.001$) high in Pb exposed rats fed on iron deficient diet.
3. The CD4/CD8 ratio was increased in Pb exposed rats regardless of iron status.
4. Mucosal IgA levels were decreased significantly in rats exposed to Pb and fed on iron deficient diet.
5. Serum TT specific IgM levels were decreased ($p<0.048$) in Pb exposed iron deficient rats compared to control rats.
6. Significantly ($p<0.025$) higher splenocyte proliferation index was observed in iron deficient and Pb exposed rats as compared to normal group.
7. There was an increase in Micronucleated cells in Pb exposed iron deficient rats.

In Vitro

1. Lead induced inhibitory effects were mitigated by thiamine when *Lactobacilli* or *E. coli* were cultured in presence of thiamine.
2. Scanning Electron Microscopic (SEM) studies had revealed a continuous dividing, chain like structures of *Lactobacilli* grown in absence of Pb. The Pb treated *Lactobacilli* appeared as elongated rod like structures. Bacterial cultures in presence of Pb plus thiamine had shown normal binary fission identified by a notch at regular interval with cells appearing as continuous chain like structures under SEM.

CONCLUSIONS

- Chronic Pb exposure even at low levels can reduce the immune functions in iron deficiency.
- The *in vitro* bacterial culture studies had shown protective effect of thiamine against Pb induced bacterial inhibition.

3. EVALUATION OF HERBAL AND NUTRACEUTICAL PRODUCT FOR ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY

Atherosclerosis is a progressive result of chronic inflammation, oxidation and lipids accumulation within macrophage cell in the walls of arteries. Key determinant of atherosclerosis is macrophage foam-cell formation by activated monocyte and Vascular Cell Adhesion Molecule-1 adhesion, intake of ox-LDL via CD-36 receptors on macrophages and Reactive oxygen species (ROS) generation. In view of this, the present study was undertaken to evaluate the herbal and nutraceutical products which can prevent or stabilize the atheroma.

HYPOTHESIS

Herbals and nutraceuticals with potential antioxidant, anti-foam cell formation and anti-inflammatory activities can act as anti-atherosclerotic agent.

AIM

Evaluation of the herbal and nutraceutical formulations meant to prevent or stabilize the atherosclerosis.

OBJECTIVES

1) Screening of the selected herbals and nutraceuticals extracts for potential anti-oxidant and anti-inflammatory activity.
2) Qualitative characterization of chemical constituents of extracts using various analytical techniques.

METHODOLOGY

Identifying the test materials:

The Herbals (*Terminalia arjuna, Cyprus rotundus*) and Nutraceuticals (Garlic, Turmeric, Ginger) were obtained from *Dabur* Research and Development center, Ayur, Ghaziabad (UP) India. Crude test materials and respective hydro-alcoholic [Ethanol (1): Water (1)] extracts were qualitatively tested as per Indian Pharmacopoeia (IP) & WHO monographs so as to maintain the standards.

Anti-oxidant activity *(in-vitro)*

The extracts of individual test materials and in various combinations were analyzed for anti-oxidant activity using DPPH free radical scavenging activity and anti-lipid peroxidation in rat liver mitochondria. The IC_{50} value was established and compared with Vit-C and Vit-E.

Anti-inflammatory activity *(in-vivo)*

Acute anti-inflammatory activity was performed by Carrageenan induced paw oedema animal model in WNIN rats (0.2mL, 2%w/v) and C57BL/6 mice (300 µg/paw). The Carrageenan induced paw oedema was measured at various time points (1 to 7, 24, 48, 72, 96 hr) before and after administration of test material using plethysmometer. Dose response curve was established to determine ED_{50}.

Chronic anti-inflammatory activity was performed by implanting 10 mg sterile cotton pellet (Dipped in 1% Carrageenan and dried) to induce granuloma pouch in C57BL/6 mice. Test compound extracts were administered in three doses for seven days to record interleukin levels in blood. In addition, cotton pellet was removed, dried and weighed to determine localized activity.

RESULTS

1 The gram percentage yield of *Terminalia arjuna, Cyprus rotundus,* Garlic, Turmeric and Ginger was found to be 29.12%, 24.18%, 23.32%, 20.16% and 4% respectively.

2 Anti-oxidant activity *(in-vitro)*

Among all extracts, Hydro-alcoholic extract of all the test materials was found to be potent anti-oxidant. PHN (Poly-Herbal Nutraceutical) a combination (1:1:1:1) of each lyophilized test materials was found to have potent anti-oxidant activity at low concentration than individual test materials.

3. The IC_{50} of DPPH free radical scavenging activity of test materials were at follows: *T. arjuna* = PHN > *C. rotundus* > Turmeric > Ginger > Garlic (Table 53) while lipid peroxidation was PHN > Garlic > *T. arjuna* > Ginger > Turmeric > *C. rotundus.* (Table 53).

Table 53. Comparative anti-oxidant activity of PHN and test materials

Sl. no	Test plant	In-vitro anti-oxidant IC_{50} Con. (µg/mL)			
		Free radical scavenging activity	R^2	Lipid peroxidation	R^2
1	Vitamin- C	6.6 + 0.22	0.991	-	-
2	Vitamin- E	-	-	31.1 + 1.45	0.974
1	*T. arjuna*	7.5± 0.76	0.999	34.6 ± 0.88	0.981
2	Ginger	64.6± 0.27	0.994	38.1 ± 2.26	0.999
3	Turmeric	42.1 ± 0.99	0.998	83.7 ± 1.21	0.993
4	Garlic	110.2 ± 0.08	0.986	16.9 ± 1.35	0.982
5	*C. rotundus*	9.8 ± 1.04	0.999	186.9 ± 2.04	0.987
6	PHN	7.6± 0.34	0.999	14.5± 2.46	0.987

Values are mean ± SEM

4. Acute anti-inflammatory activity *(in-vivo)*

All the test material significantly inhibited carrageenan induced mice and rat paw oedema. According to percent inhibition PHN was found to be potent anti-inflammatory combination as compared to aspirin in term of duration and reduction in mice paw oedema (Fig 29 & 30). The PHN activity was observed at both immediate and late phase (Biphasic) of inflammatory response.

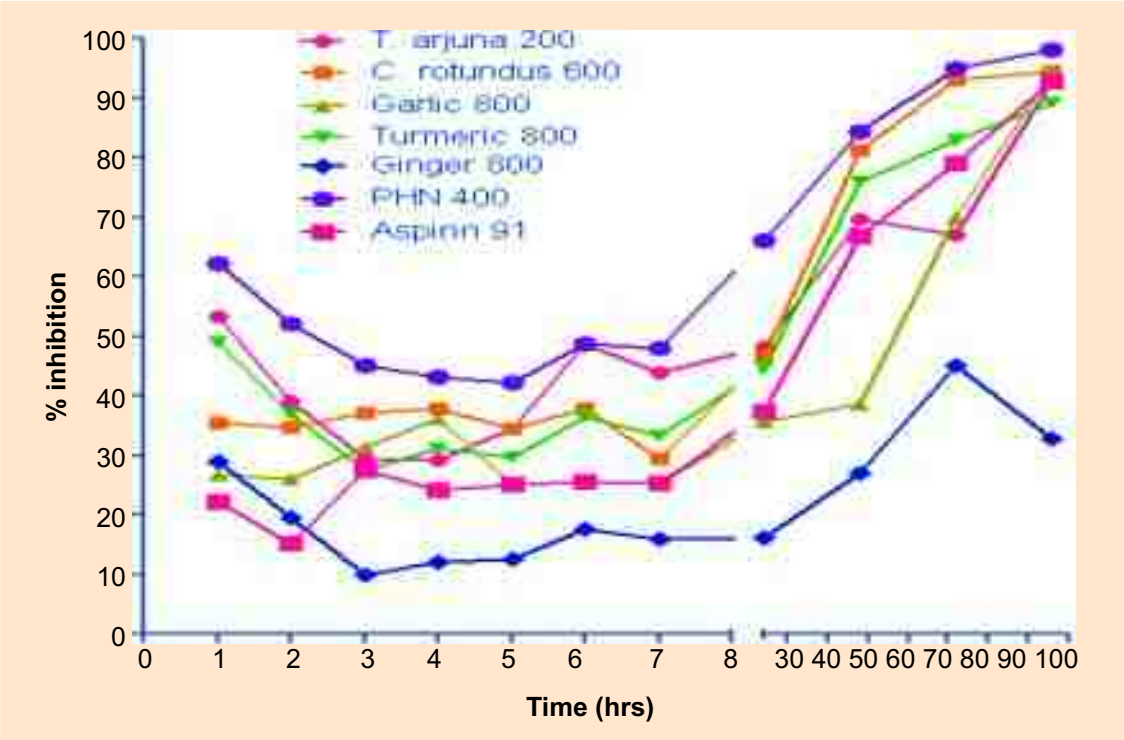
5. Chronic anti-inflammatory activity

It was observed that the hydro-alcoholic extract of all the herbals and nutraceuticals were exhibited a significant and dose-related inhibition of the dried weight cotton pellet (Fig 31 & Table 54). The reduction in weight of pellet was: PHN > *T. arjuna* > *C. rotundus* > Garlic > Turmeric > Ginger. Interleukin profile is being investigated.

Table 54. Percent inhibition of test materials over chronic inflammation in mice

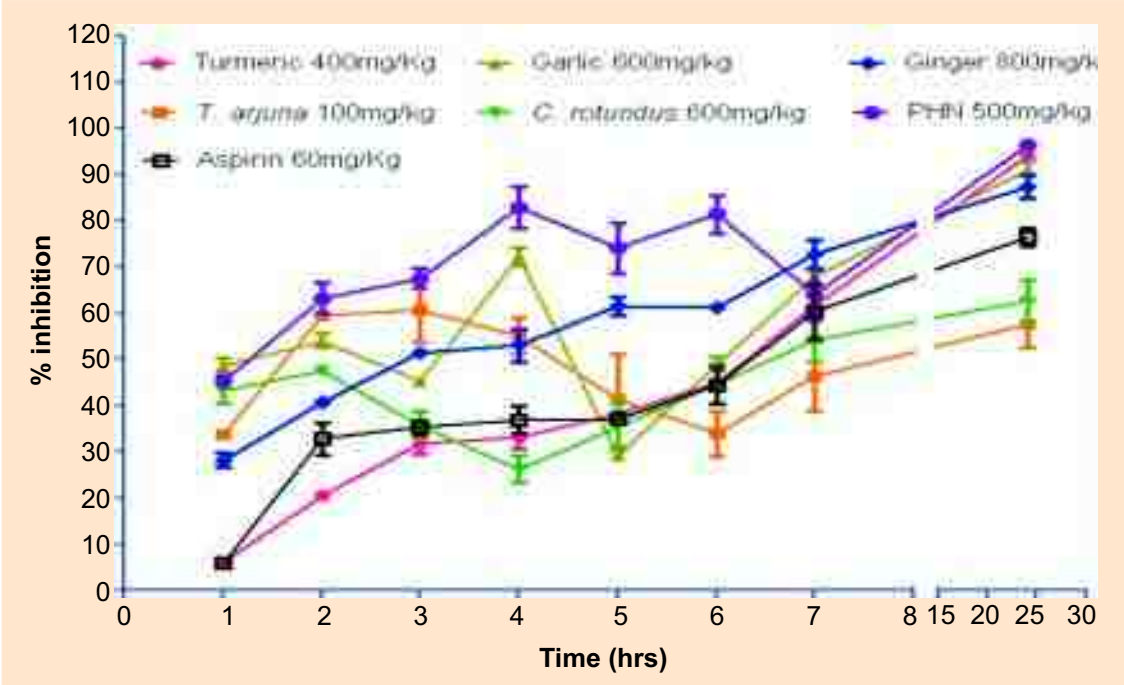
Sl. no	Groups	Doses mg/kg	Inhibition (%)
1	*T. arjuna*	50	16.597
		100	28.51
		200	34.507
2	*C. rotundus*	150	31.207
		300	35.418
		600	38.321
3	Garlic	200	25.898
		400	36.541
		800	40.720
4	Turmeric	200	16.427
		400	21.812
		800	41.823
5	Ginger	200	18.911
		400	33.756
		800	35.46
6	PHN	100	37.992
		200	44.599
		400	50.844
7	Aspirin	91	27.88

Fig 29. Effect of Herbals and Nutraceuticals hydro-alcoholic extracts in carrageenan induced mice paw oedema



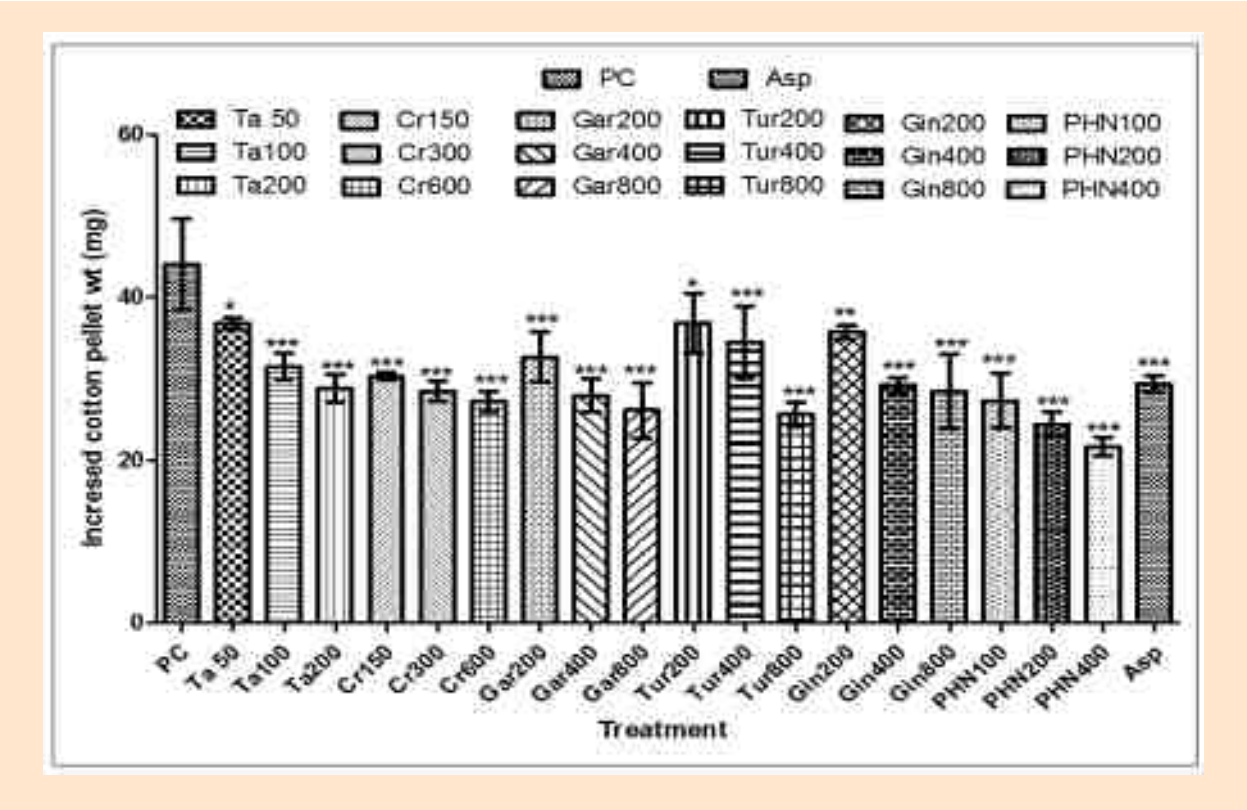
Values are mean ± SEM, n=6 in each group

Fig 30. Effect of Herbals and Nutraceuticals hydro-alcoholic extracts in carrageenan induced rat paw oedema



Values are mean ± SEM, n=6 in each group

Fig 31. Effect of Herbals and Nutraceuticals hydro-alcoholic extracts on % inhibition of *cotton pellet induced granuloma* pouch mice



*Values are mean ± SEM, n=6 in each group. One-way ANOVA (P<0.05) followed by Tukey- Kramer multiple comparison post-hoc test * P<0.05, **P<0.01, ***P<0.001 significantly different from control*

CONCLUSION

Among all the test materials extracts, PHN was observed to have potent anti-oxidant and anti-inflammatory activity which can be considered for further evaluation to determine anti-atherosclerosis potential.

VIII. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

A. SERVICE ACTIVITIES

1. Breeding and supply of animals

During the 12 months period, 36,048 animals were bred and out of which 29,341 animals were supplied for research including its supply within the parent institution. There was an increase of 7% in the supply of animals compared to last year, and proportionately the income generated also went up by 5-6 lakhs. The details of individual strains bred and supplied are shown in Tables 55 & 56.

2. Supply of animal Feed

a. Stock Animal feed

Apart from the stock feed of 45.05 kg for the animals under our care, an additional, 32.629kgs of animal feed (31,338 kg of rat/mouse feed; 7291 kgs of g.pig/rabbit feed) was supplied during the period generating an amount of Rs.37.54 lakhs, which is 15% more than last year, in terms of supply as well as the income.

b. Experimental Animal Feed

Custom made experimental animal feed was continued as in last year, and 460 kgs, were supplied which is a marginal increase of 1 to 2% compared to last year. The details of experimental diet supplied are given in Table 57.

c. Blood and Blood products

During this period, a total of 802 ml of Plasma / Serum and 252 ml of blood were supplied to 14 different institutions on 40 different occasions and an amount of Rs 1, 21,573 has been collected. In addition, tissues and organs from hamsters were supplied to 2 institutions and a sum of Rs. 500 was realized.

3. Health Monitoring

Microbiological Monitoring

During the period, a total of 598 samples were subjected to microbiological monitoring. Among these a total of 230 samples belonged to different strains of mice (BALB/c 48, C57/6J 48, Swiss 46, Hetero NIH 8, Nude NIH 42, FVB 28, Hetero NCI 8 and Nude NCI 2) and a total of 368 samples belonged to different strains of rats (WNIN 68, SD 64, Fischer 64, Holtman 24, CFY 48, Kyoto 20, GRObb 40, Ob Ob 40). They were tested for Mycoplasma, Sendai and Hantaan viruses and Claustridium piliformis (CPIL), Helicobacter, Celia Associated Respiratory Bacillus (CARB) bacteria by using ELISA method. The conventional microbiological methods were used as the laboratory is under renovation. The results are furnished in the Tables 58 & 59.

About 21% of mice showed positive for the presence of antibodies to Mycoplasma and all mice strains have been found to be negative for Sendai and Hantaan viruses and CARB and CPIL bacteria. About 36% of rats showed antibodies to Mycoplasma and 62% showed the presence of Helicobacter. Hantaan virus that was tested as part of the investigation for hematuraia was found in 2 SD rats and one WNIN rat. Sendai virus was present in 6% of rats tested. Interestingly, there were no CARB respiratory bacillus and CPIL bacteria in all the rats tested.

Table 55. Details of breeding and supply of different species and strains of laboratory animals During the period 1.4.10 to 31.3.11

S. No	Species	Strain or Breed	Stock As on 1.4.10	Total Number of animals								Balance as on 31.3.11
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Total Supplied	Died	Disp. Old age	Sick	
1	Mouse	BALB/c An. N (inbred)	418	2253	2671	24	1309	1333	426	600	-	312
		C57BL/6J (inbred)	1177	5926	7103	111	4276	4387	1604	-	-	1112
		N:NIH(S) Nude (inbred)	186	280	466	-	218	218	33	-	-	215
		NCr. Nude	137	373	510	-	152	152	16	50	-	292
		FVB/N (in bred)	182	103	285	-	10	10	10	157		108
		Swiss (in bred)	2029	5837	7866	120	4620	4740	463	320		2343
2	G. Pig	N:HART (Hartley)	432	1385	1817	15	1335	1350	111	-	-	356
		N:NIH (Coloured)	126	807	933	10	678	688	30	-	-	215
3	Rabbit	New Zealand white	55	147	202	36	67	103	35	-	-	64
4	Monkey	Macaca mulatta (Rhesus)	24	-	24	-	-	-		-	-	24
	TOTAL		4766	17111	21877	316	12665	12981	2728	1127	0	5041

Table 56. 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 1.4.10	Total Number of animals							Balance as on 31.3.11
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disp.Old Age/ Sick	
1	Rat	CFY/NIN (inbred)	60	92	152	-	-	-	9	46	97
		Fischer 344 N (inbred)	111	222	333	8	200	208	5	-	120
		Holtzman (inbred)	120	55	175	-	-	-	29	58	88
		SD (Sprague Dawley) (Outbred)	1157	5193	6350	729	3788	4517	307	650	876
		Wkyoto (inbred)	108	27	135	-	-	-	8	65	62
		WNIN (inbred)	2565	10936	13501	239	9924	10163	112	675	2551
		WNIN/GR-Ob	916	772	1688	258	-	258	69	435	926
		WNIN/Ob-Ob inbred)	805	740	1545	278	-	278	51	290	926
2	Hamster	Golden (inbred)	883	900	1783	-	936	936	55	405	387
3	Sheep		1	-	1	-	-	-	-	-	1
Total			6726	18937	25663	1512	14848	16360	645	2624	6034
Total (1+2)			11492	36048	47540	1828	27513	29341	3373	3751	11075

Percentage of animals supplied to other Institutions: **94%**

Apart from above, 144 samples from 24 monkeys were tested during the period, for 6 different organisms' virus, bacteria and protozoa (B virus, Simian T lymphotophic virus (STLV), Simian Retrovirus (SRV) Rabies, Tuberculosis (TB) and Malaria as a part of the health screening prior to selection of the animals for conducting experiments related two different projects approved by DBT. The results furnished in the table 60 show that the animals were free from antibodies to all the 6 tested organisms, indicating that the animals were never exposed to the listed pathogens.

Table 57. Experimental feed supplied from 1.4.2010 to 31.3. 2011

S. No.	To whom supplied	Type of diet	Quantity (Kgs)
1	Vimta Labs, Hyd.	60 Kcal fat diet	40
2	S.K.University, Ananthapur	Fructose diet	15
3	S.K.University, Ananthapur	High Fat diet	104
4	CCMB, Hyderabad	Maltodextrine	69
5	Hamdard University	High Fat diet	47
6	Sastra University, Thanjavur	High Fat diet	115
7	NBRC, Haryana	Iron Def. diet	5
8	IICT, Hyd.	71% Fat diet	5
9	Chitramet, Kerala	Cal. Def. diet	20
10	JNTU, Hyd	High Fat diet	15
11	Kolkata Univ.	High Fat diet	25
TOTAL			460

Genetic Monitoring

Genetic Monitoring is meant to keep the genetic integrity of the inbred strains maintained at animal facility. Rodents used in today's biomedical research must not only be free of disease that may influence experimental results, but also be well-defined in terms of their genetic makeup. This is especially true with the increasing use of transgenic rodents in most areas of biomedical research.

The genetic monitoring program has been primarily designed to:

1. Construct genetic profiles for each strain/line maintained in the breeding colonies and provide routine genetic surveillance of the various strains for compliance to their profiles
2. Provide for rigid enforcement of proper genetic management procedures within the breeding colonies. The genetic profile of a particular strain is composed of a set of genetic markers, which uniquely defines the strain and differentiates it from other inbred strains

DNA based tests to facilitate the periodic genetic monitoring of laboratory rodents, was in vogue recently and have not lagged behind in this aspect. The rat strains maintained here and monitor as much as 11 different loci located on different chromosomes of rat. This was done utilizing PCR techniques, generating strain specific markers. The genotyping work of the following rat strains had initiated: SD, Fisher 344, CFY, WKY, WNIN, WNIN Ob/Ob (Obese, Carrier and lean) WNIN

Table 58. Results of testing of Mice during April 2010 to March 2011

S. No	Virus / bacteria	No. of samples Tested 230	Mice strains								Result & % Positives
			BALB/c 48	C57/6J 48	Swiss 46	HeterNIH 8	NudeNIH 42	FVB 28	Heter NCI 8	Nude NCI 2	
1	Mycoplasma	46	3/8	3/8	1/6	0/8	0/6	----	2/8	1/2	10/46 Positives 21%
2	Sendai Virus	46	0/10	0/10	0/10	---	0/9	0/7	----	----	All negatives
3	Hantaan	46	0/10	0/10	0/10	---	0/9	0/7	----	----	All negatives
4	CAR Bacillus	46	0/10	0/10	0/10	---	0/9	0/7	----	----	All negatives
5	CPIL	46	0/10	0/10	0/10		0/9	0/7	----	----	All negatives

Table 59. Results of testing of Rats during April 2010 to March 2011

S. No	Virus / bacteria	No. of samples Tested 368	Rat strains								Result & % Positives
			WNIN 68	SD 64	Fisher 64	Holtz 24	CFY 48	Kyoto 20	GROb 40	ObOb 40	
1	Mycoplasma	46	1/8	6/8	4/8	1/8	3/6	2/8	----	----	17/46 Positives 36%
2	Sendai Virus	46	1/8	0/8	1/8	----	1/6	----	1/8	0/8	3/46 positives 6%
3	Hantaan	92	1/20	2/16	0/16	0/16	0/12	0/12	----	----	3/92 positives 3%
4	Helicobacter	46	6/8	8/8	4/8	----	5/6	----	7/8	1/8	31/46 Positives 67%
5	CAR Bacillus	46	0/8	0/8	0/8	----	0/6	----	0/8	0/8	All Negatives
6	CPIL	92	0/16	0/16	0/16	----	0/12	----	0/16	0/16	All Negatives

GrOb/Ob (Obese carrier and Lean) strains. Genomic DNA was prepared from rat tails and subjected for genotyping with SSLP primers using PCR for amplification and genotyping. Among the 11 primers, three primers (R117, R 148, R 196) were found to be very promising as they show differences amongst the different strains. Identification of strain specific markers with validation of more number of genomic DNA samples of each strain have to be screened in coming days for the development of strain specific DNA markers.

Table 60. Results of testing of Monkeys during April 2010 to March 2011

S. No	Virus/ Bacteria/ Protozoan	No. of samples Tested 144	Non Human primate Rhesus Monkeys		Result
			Males (12)	Females (12)	
1	B virus	24	0/12	0/12	All negatives
2	STLV	24	0/12	0/12	All negatives
3	Rabies	24	0/12	0/12	All negatives
4	SRV	24	0/12	0/12	All negatives
5	Malaria	24	0/12	0/12	All negatives
6	TB	24	0/12	0/12	All negatives

4. Human Resource Development

Under Junior level training course (LATTC), 12 participants were trained and under senior supervisory training course (LASTC) level, 11 participants were trained. Ad-hoc training ranging from 1 week – 3 weeks was given to 12 candidates from January to April.

The centre conducted a mini symposium entitled “Animal models developed by Indians in India' on April 24, as part of the World Laboratory Animal Day celebrations. This was sponsored by ICMR, CPCSEA and several pharma companies. There were 12 oral presentations which showcased India's advancement in the field of animal model development as mutants, transgenics and knock outs. During this occasion, 5 prominent scientists (Dr. Buduk Dr.H.G.Sen, Dr.S.N.Naik, Dr.N.K.Goverdhan, Dr.K.R.Bharadwaj and Mr.S.Hariharan) who contributed to the growth of laboratory animal sciences in India were honoured.

5. Research Support

Main laboratory including Pre Clinical Toxicology (PCT)

During the period, 25 research projects was approved by IAEC for implementation. Out of these, 4 were completed, 18 are in progress and 3 are yet to be initiated.

B. RESEARCH ACTIVITIES

1. EFFECT OF *MUCUNA PRURIENS* ON WNIN OBESE RATS

Konch or velvet bean is a medicinal herbaceous plant of the genus Mucuna and species pruriens (*Mucuna pruriens* Linn*)* used mainly in Unani medicine as an aphrodisiac. The crude extract of the seeds of this plant is a rich source of L-DOPA and has an additional capability of reducing the body fat, plasma glucose and lipids. The present study was aimed to study the effect of the seed extract on an unique inbred obese rat model established at National Institute of Nutrition, India, for its anti obesity, hypoglycemic and hypolipidemic activity. *M. pruriens'* dried seeds were procured from Lala Dawasaz, Charminar, Hyderabad and the ethanol extraction was carried out using soxhlet apparatus. Ethyl alcohol used as solvent after confirming the maximum yield (2.4% gm) of active compound by HPTLC method. Hand made pellets were prepared in the quantities of normal dose (ND) 0.5 gm and high dose (HD) 1 gm, containing 6 mg and 12 mg of the drug respectively in 20% protein powder diet.

METHODOLOGY

Eighteen obese male rats with their 18 lean littermates were taken and divided in to three groups and were fed with the herbal seed extract for 45 days. The meal feeding pattern/training for the experimental rats was carried out as per the standard protocol. The experimental and control rats were fasted for 4 hours in a day from 9.00 hours to 13.00 hours and thereafter fed with 0.5 gm and 1.0 gm hand made pellet of 20% protein stock powder diet mixed with the extracted drug. After ensuring the complete ingestion of the pellet, both the group of animals were fed 20% stock diet pellets *ad libitum*. After the initial acclimatization for the training programme, the training schedule was continued for a period of 45 days.

Body composition of the treated and control rats was analyzed for lean body mass, body fat and extra cellular fluids like total body sodium and potassium by Total Body Electrical Conductivity (TOBEC). The circulatory levels of plasma glucose, cholesterol, triglycerides and reproductive hormones were measured using standard kits and histology of liver and testis was also studied in both treated and control rats.

RESULTS

The major observation using the *M.pruriens* seed extract is the overall improvement in the body composition of the animals during the course of the study. The supplementation of *M.pruriens* seeds ethanol extract improved the lean body mass content and decreased the total body fat in the ND and HD treated rats of both obese as well lean counterparts. There is also a significant increase in the FFM and TBNa and TBK levels in the treated group rats. The normal dose regimen of 6 mg/ per rat seems to be quite effective in controlling blood glucose, cholesterol and triglycerides in these mutants. The higher dose of 12 mg may have long-term effect especially in controlling lipids in these mutant rats. The reason for differential dose effects may be due to the differences in the absorption and metabolic clearance of the drug at different doses, and there by the availability of the drug at any given time. It was also observed that within 15 days of seed extraction treatment, the ND and HD treated obese rats had become more active and they resumed their grooming behaviour as well. This overall improvement in their general well being in terms of increased activity may be due to the increased utility of the metabolic fuel, as witnessed by the correction in glucose homeostasis.

Accessory glands weights in control obese males are lower than ND and HD treated obese rats. There was no significant difference were seen in the weights of testis and other accessory glands in

mutant rats and their lean littermates. The results of sperm count in response to the seed extract treated at ND and HD level shows a significant increase in treated obese rats compared to control obese rats *(P<0.001)*. The increase in sperm count were found to be more in HD group compared to ND group *(P<0.001)*. However, no significant differences were seen in the sperm count of extract treated and control lean rats.

The effect of the *M.pruriens* seed extract treated at ND and HD levels on gonaidal index of obese and lean rats showed a significant effect compared to control obese and lean rats (P<0.05). In general, the LA muscle weights were increased in obese and lean rats treated with the seed extract. There was an increase of 22.22% in the LA muscle total weight in ND and HD group compared to control group of both obese and lean rats. The circulatory reproductive hormone levels testosterone, LH, FSH and prolactin levels were significantly increased with the increase in the dose in both obese and lean experimental rats compared controls. In seed extract supplemented obese rats the testosterone levels were significantly high in HD group followed by ND and control group (P<0.05). But, in the case of lean littermates ND treated rats had significantly higher testosterone than HD and control groups (*(P<0.001).* LH and FSH levels were significantly increased in both ND and HD treated rats (P<0.001). The seed extract did not show any increase in the prolactin levels of obese rats belonging to control and treated groups. However, a significant increase in prolactin levels was seen in lean control and treated groups (*(P<0.001)* .

IX. PRE-CLINICAL TOXICOLOGICAL STUDIES

1. PRE CLINICAL TOXICITY EVALUATION OF EX VIVO CULTURED HUMAN ADULT MESENCHYMAL STEM CELLS (MSCS)

Human Mesenchymal Stem Cells (MSCs) are present as a rare population of cells in bone marrow, representing 0.001 to 0.01% of the nucleated cells. They can rapidly grow and expand in culture to more than a million fold without losing their stemness. MSCs can differentiate into mesodermal lineage such as myocardium, bone, cartilage and skeletal muscle and also into other lineages like neurons and endothelial cells, both *in vivo* and *in vitro*. Many studies have used allogenic MSCs without getting rejected by the host. Therefore, Stempeutics Research Pvt. Ltd., India is making an attempt to explore the efficacy of allogenic MSCs as a therapeutic modality in acute myocardial infarction and critical limb ischemia.

In view of promoting such preparations for clinical therapy, the safety evaluation is one of the regulatory pre-requisite. The proposed therapy is a innovative and there are no guidelines both at national and international levels to evaluate it safety. The DCGI and ICMR have suggested the Centre for advanced studies to develop the appropriate protocol to evaluate the pre-clinical safety profile of such compounds.

METHODOLOGY

1. **The Acute toxicity Test** with 10 and 20 times of intended therapeutic dose (TD) in Fischer rats, Rabbits was undertaken by intramuscular and intravenous route.

2. **The Sub – Chronic Toxicity Test** (90 days) was conducted in Sprague Dawley rats, Rabbits was undertaken by intramuscular and intravenous route after establishing the safety during acute toxicity test. The dosage levels includes Vehicle control (VC), Therapeutic dose (TD), Average dose (5 X TD) and High Dose (10 X TD). The test compound (Frozen mesenchymal stem cells stored in cryo bags) was reconstituted in Plasmalyte – A (containing 5% human serum albumin and 10% DMSO). (SOP No: NIN / PHARM / SATC –01) and was administered in a constant volume by subcutaneous/ IV routes. All the animals were monitored bi-weekly for live phase, cage side, physical and neurological parameters etc. The hematological profile was assessed in whole blood and in plasma for clinical chemistry at various time points viz. 48hr, 5th day, 30th day, 60th day and on 90th day after test compound administration. The animals were euthanized (necropsy) on two occasions i.e. on 15th day and 90th day (Final) after test compound exposure, to collect all organs for gross necropsy and histopathological examination of all major organs. Genotoxicity potential was also investigated by May – Gruenwald stain followed by Giemsa stain.

RESULTS

Acute Toxicity in Rat & Rabbit (14 days)**:** No lethality was recorded in rats after exposure to single dose of 20XTD where as 40% mortality was observed in rabbits after exposure to 20XTD single dose of body weights by IV route of administration.

Sub-chronic Toxicity – Rat (IV, IM): No mortality was recorded in animals through out the experimental period of 90days after exposure to different concentrations of test compound by IV route whereas by IM route, one female animal (2%) in therapeutic dose group died on 4th day of test

compound exposure. However, histopathological observation indicated that lethality was not related to test compound.

Sub-chronic Toxicity – Rabbit (IV, IM): 4.16 % mortality was recorded (i.e one male animal in high dose group during test compound administration) in animals which received IV route. There was 8.3% mortality recorded (i.e one female animal in high dose group on 12th and one male animal in vehicle control group on 44th day after test compound exposure) in IM route.

CONCLUSION

No abnormalities were noticed in physical, physiological, neurological, hematology, clinical chemistry parameters in rats and rabbits by intramuscular administration of test compound (Ex-vivo cultured human allogeneic adult mesenchymal stem cells) by IV & IM in Mice, Rats and Rabbits under the experimental conditions. Necropsy findings were also within normal range.

2. PRE CLINICAL TOXICITY EVALUATION OF VNJN – 21

VNJN-21, a synthetic peptide comprised of central 15 amino acids, is developed by Vanjan with an intention to promote it as the effective agent in treatment of AIDS. The significant decrease in viral status after treatment with test material in HIV challenged {with Simian Human immunodeficiency Virus (SHIV)} chimpanzee and macaques. VNJN – 21 is a synthetic peptide derived from V3-loop region of GP120 protein*. It is expected that VNJN – 21 will induce limited cross – resistance to currently approved anti–retroviral and fewer toxicities due to its novel mechanism of action and cellular target. Since, this product has to be introduced for clinical use generating pre-clinical safety data as per the regulatory requirements is mandatory. The present investigation was undertaken with an objective of evaluating the safety profile of the product.

METHODOLOGY

1. The Acute toxicity test: Mice were exposed to three different concentrations viz. (10XTD, 20XTD and 50XTD), rats were exposed to two different concentrations viz. (10XTD and 20XTD) and rabbits at 10XTD dose, once by intravenous route. This was followed by observation for lethality and activity daily for 14 days.

2. The Sub – Chronic Toxicity Test (60 days) was conducted in Sprague Dawley rats, rabbits by intravenous route at three dose levels viz., therapeutic dose (TD), average dose (5 X TD) and high dose (10 X TD) after establishing the safety during acute toxicity test. The test compound was administered intravenously on 0^{th}, 1^{st}, 3^{rd},7^{th}, 11^{th}, 14^{th}, 21^{st} and 28^{th} day in various dose concentrations. The saline was used for vehicle control group of animals. All the animals were monitored bi-weekly for live phase, cage side, physical and neurological parameters etc. The hematology, clinical chemistry profile in blood/ serum samples was undertaken on 48hrs of 4^{th} exposure, last exposure (28days). In recovery group the above investigations were carried out on 15^{th} day and 30^{th} day of post exposure. The Gross necropsy, histopathology, Genotoxicity was conducted on 48 hrs (50% animals), 15^{th} day (25%) and 30^{th} day (25%) of last exposure.

RESULTS

Acute Toxicity in Rat & Rabbit (14days): Pre terminal deaths 10%, 50%, 30% mortality in mice which received test compound by intravenous route once in a dose of 10XTD, 20XTD, 50XTD

respectively. There was no mortality in Rats and Rabbits which received 20XTD & 10XTD dose respectively.

Sub-chronic Toxicity – Rat (IV): No mortality was recorded in animals through out the experimental period of 60days after exposure to different concentrations of test compound in IV route. There was no significant changes in body weight gain, food intake, physical and physiological activities as compared to control group. The hematology, clinical chemistry and histopathlogy profile was found to be normal.

Sub-chronic Toxicity – Rabbit (IV): No mortality was recorded in animals through out the experimental period of 60days after exposure to different concentrations of test compound in IV route. There was no significant changes in body weight gain, food intake, physical and physiological activities as compared to control group. The hematology, clinical chemistry and histopathlogy profile was found to be normal.

CONCLUSION

No abnormalities were found in physical, physiological, neurological, hematology and clinical chemistry parameters in rats and rabbits by intravenous administration of test compound rats (18mg/kg) and rabbits (9.3mg/kg) under the experimental conditions. Necropsy findings were also within normal range.

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 dessimination services like MEDLINE Searches, Proquest Medical Library Full Text Database of journals and other online retrieval activities using the LAN Network of the Institute. Library continued to participate in exchange of data, journals and information using the URL<http://Groups.yahoo.com/group/ICMR Librarians>.

The library has continued to provide an excellent Photostat support to the scientists, technical as well as to the administrative staff. Resource sharing and user education programmes etc are continuously being undertaken by the library. 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).

MODERNIZATION 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 3607 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 1739 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 24,133 e-journals with links to full text at publisher sites and provides free access to full-text of 1913 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 1778 Journals.

d) Online subscription of 5 core journals such as BMJ, LANCET, NATURE, NEJM, SCIENCE has been renewed by ICMR and it is also accessible.

e) The following equipments were procured for the library.

I)	HP PC	-	4
ii)	UPS	-	4
iii)	Steel Almarah	-	4

NEW JOURNALS ADDED

I. Indian Journals

1. Annual Subscription of Gazette Notification on Drugs & Cosmetics Act & Rules & Drugs
2. Annual Subscription of Gazette Notification on Food Safety / Standards Act Prevention of Food Adulteration Rules
3. Annual Subscription of Gazette Notification on Ministry of Environment & Forests
4. Annual Subscription of Prevention of Food Adulteration Cases (FAC)
5. Drug Cases (Narcotics)
6. Indian Journal of Veterinary Pathology

II. Foreign Journals

7. Biochemistry
8. Biometals
9. Cell
10. Circulation
11. EMBO Journal + EMBO Reports (combined Subscription)
12. FEBS Letter
13. Journal of Adolescent Health
14. Journal of Electron Microscopy
15. Journal of Ethnopharmacology
16. Journal of Experimental Medicine
17. Journal of Trace Elements in Medicine and Biology
18. Medicine and Science in Sports and Exercise
19. Modern Pathology
20. Molecular Pathology (Incorporated with Journal of Clinical Pathology)
21. Proceedings of the National Academy of Sciences
22. PsychoNeuroendocrinology
23. Scanning
24. Toxicology and Applied Pharmacology
25. Vitamins and Hormones: Analysis and applications

JOURNALS DELETED

Indian Journals

1. Advance Drug Review

Foreign Journals

2 American Journal of Veterinary Research
3 Animal (Formerly Reproduction Nutrition Development)
4 Animal Technology and Welfare
5 Animal Welfare
6 Current Contents (Life Sciences)
7 Mycopathology
8 Nutrition Abstracts & Reviews Series B
9 Society and Animals

The following library services were expanded as detailed below:

1. NEW ADDITIONS

Books	430
Reports	280
Journals (New Subs.)	25
Thesis / Dissertations	6

CDROMs		111
PC Quest CD's	10	
General CD's	101	

2. OTHER ACTIVITIES

Journals Bound	1,015
Visitors using the Library	1,811
Circulation of Books/Journals etc.	1,366
No. of E-mails sent outside	2,932
No. of E-mails received.	13,283
Photocopying (No. of pages)	3,24,386
Number of Annual Reports mailed	478
No. of INTERNET Searches provided	263
No. of Reprints sent	172
Proquest Full Text Database searches provided	3,607

3. TOTAL LIBRARY COLLECTIONS

Books	17,624
Journals (Bound Volumes)	33,735
Journals subscribed for 2010	356
Journals received (Gratis/Exchange)	315
Microforms (Microfiche)	1,080
Slides	280
Reports	12,916
Theses & Dissertations	378
MEDLINE CDROMS Discs	383
Current Contents on Diskettes with abstracts	664
Proquest (Full Text E-Journals) on CD ROMS	495
General CD's	194

Ph.D PROGRAMMES

PhD Awardees

S. No	Research Scholar	Title of the thesis	Award Year	University
1	Padmavathi I.J.N	Role of maternal chromium status in the development of insulin resistance in the offspring	2010	Osmania
2	Sheril Alex	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	2010	Osmania
3	Mrudula T	Characterization and significance of a novel fatty acid elongase of the eye lens	2010	Osmania
4	Sreenivasulu K	CaCo-2cell as a model to study bioavailability, mechanism of absorption and cytoprotective effects of zinc	2010	Osmania
5	Naga Bala Shankara Srinivas P.	Studies on the significance of α-crystalline heteropolymer in the eye lens	2010	Osmania
6	Ms. Manjula Talluri	Ethnopharmacological validation of biodynamic compounds in traditional preparations	2010	Osmania
7	Vasuprada I	Studies on the response and interactions of iron and zinc in CaCo - 2 cells	2010	Osmania
8	Satyanarayana.B	Biological significance of phytoferritins	2010	Osmania
9	Y Srinivasa Reddy	Effect of environmental lead exposure on infection and immunity in under nutrition	2011	Osmania
10	Prashant A	Impact of various doses of vitamin A on obesity and lipid metabolism in WNIN/Ob rats	2011	Osmania

Research Scholars Registered for PhD

S. No	ResearchScholar (Year of joining)	Title of the thesis	Supervisor
1.	Md. Naseeruddin (2004)	Understanding the role of micronutrients in pathogenesis of *Mycobacterium tuberculosis*	Dr. Nasreen Z Ehtesham
2.	Shashikiran G (2005)	In vitro regeneration of the insulin secreting cells from the adult pancreatic ductal epithelial cells (progenitors/stem cells) – the role of specific nutrients	Dr. Vijayalakshmi V
3.	Rajukumar D (2005)	Characterization and differentiation of pancreatic progenitor/ stem cells (Nestin positive cells) to insulin secreting cells - the role of specific nutrients	Dr. Vijayalakshmi V
4	Manisha Ganeshan (2005)	Foetal origins of adiposity and insulin resistance : role of peri/postnatal manganese status	Dr. Raghunath M
5.	Vara Prasad SSS (2005)	Exploration of basal glucocorticoid and levels and their possible role in obesity and insulin resistance using WNIN/Ob and WNIN/GR-Ob rat models	Dr. Vajreswari A
6	Sainath PB (2005)	Insulin, insulin receptor and its signaling mechanism(s) in the brain and insulin sensitive target organs in the WNIN-Ob and WNINGR-Ob rats	Dr. Raghunath M
7	Pratibha B (2005)	Immune status of WNIN mutant rats with reference to leptin and obesity	Dr. Giridharan NV
9	Sreevani M (2005)	Understanding and dissecting the role of resistin in the etiology of insulin resistance using obese rat model	Dr. Nasreen Z Ehtesham
8	Sylvia Fernandez Rao (2006)	Effect of Maternal Self-Esteem and Locus of Control on the Receptivity to Intervention on Responsive Feeding and their Impact on Growth and Development of Rural Infants	Dr.Shahnaz Vazir
10	Anand Kumar K (2006)	Maternal vitamin B_{12} restriction induced changes in body adiposity, hyperglycemia and insulin resistance in WNIN rat off spring : Molecular basis of the changes	Dr. Raghunath M
11	Priyanka Shankar (2006)	Studies on high fluoride and low calcium on bone metabolism in rats: Biochemical mechanisms	Dr. AL Khandare
12	Little Flower Augustine (2007)	Stress allostatic load and micronutrient status among higer secondary students: Impact of dietary advice	Dr. Madhavan Nair K

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor
13	P Muthenna (2007)	Characterization of active principles of aldose reductase and antiglycating agents in functional foods	Dr. Bhanuprakash Reddy G
14	A. Satyanarayana (2007)	Biochemical, molecular and nutritional aspects of diabetic retinopathy	Dr. Bhanuprakash Reddy G
15	Agatha Betsy (2008)	Assessment of dietary exposure to select contaminants and dietary intake of select nutrients among the various socioeconomic sections of Hyderabad	Dr. Kalpagam Polasa
16	Prathipati Vijayakumar (2008)	Role of polyunsaturated fatty acids (PUFAs) on regulation of obesity using an obese mutant rat model (WNIN/Ob)	Dr. Vajreswari A
17	Soundarya (2008)	Establishment of propoyable cell lines from adult adipose tissue of WNIN mutant rats (WNIN GR-Ob and WNIN Ob/Ob)	Dr. Vijayalakshmi V
18	Swarnim Gupta (2008)	Dietary diversification of Indian vegetarian diet to improve the iron bioavailability: Studies using CaCo-2 cell model	Dr. Madhavan Nair K
19	Deethu Sara Varghese (2008)	Assessment of body composition in Indian females using different techniques	Dr. Venkatramana Y
20	B ShankarAnand (2009)	Role of T cells and secreted cytokines in insulin resistance and obesity	Dr. Sudeep Ghosh
21	Ramesh Athe (2009)	Meta analysis approaches on “Micronutrient food fortification and its effect on health, social and economic factors”- A statistical model building	Dr. Vishnuvardhana Rao M
22	Chetan C. Nimgulkar (2009)	Evaluation of herbs/nutraceutical products for anti atherosclerotic agent	D Dinesh Kumar B
23	Mehraj-Ud-Din Bhat (2009)	Role of UPP in vitamin D deficiency induced muscle atrophy and hypoinsulinemia	Dr. Ayesha Ismail
24	A. Gandhi Nayak (2009)	Vitamin A metabolism in obesity	Dr. Vajreswari A
25	Anupama Tyagi (2009)	Anti-inflammatory potential of n3 PUFA in experimental ulcerative colitis: Biochemical and molecular study	Dr. S. Ahmed Ibrahim
26	V. Sudhakar Reddy (2009)	Role of small heat shock proteins in diabetic complications	Dr. Bhanuprakash Reddy G
27	Pallavi Namburi (2010)	Regulatory role of zinc in Hepcidin mediated iron metabolism	Dr. Madhavan Nair K
28	N Bindu (2010)	Studies on the anticancer properties of *Murraya Koenigii* leaves	Dr. Ayesha Ismail

S. No	ResearchScholar (Year of joining)	Title of the thesis	Supervisor
29	Jitendra Kumar (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
30.	Anju Elizabeth Thomas (2010)	Fetal programming for neuro-muscular skeletal development in the rat offspring-role of antenatal and perinatal Mg deficiency.	Dr. Raghunath M
31	Himadri Singh (2010)	Establishment of propagable cell lines from pancreas (Ductal Epithelial Cells) from WNINGR-Ob rats	Dr. Vijayalakshmi V
32	G Kishore Kumar (2010)	Characterization of retinal degeneration in a novel obese rat model	Dr. Bhanuprakash Reddy G
33	Sarin Sarah Jose (2010)	Diabetic complications: Dietary intake and blood levels of nutrients	Dr. Bhanuprakash Reddy G
34.	Anil S (2010)	Modulation of adipose tissue inflammation by dietary n-3 PUFA	Dr. S. Ahmed Ibrahim
35.	Y. Sravanthi (2010)	Effect of prenatal iron supplementation on iron-zinc homeostasis and placental zinc transporters. Studies in pregnant women and in iron/zinc depleted Be Wo cell line.	Dr. Madhavan Nair K
36	Mr. Chalamaiah (2010)	Fish Egg Protein Hydrolosis as Nutraceuticals /Health food in promotion of immuno-modulatory activities	Dr. B. Dinesh Kumar
37.	P Ravindranath (2011)	Purification characterization and primary elucidation of human milk factor that enhances iron absorption	Dr. P. Raghu
38.	A.Vijayendra Chary (2011)	Molecular link between Cd23 and Cd21 expression and role of infant nutrition in young children with allergic asthma	Dr.B.Dinesh Kumar
39.	Mr.Rachit Badolia (2011)	Effect of fructo-oligosaccharides (FOS) coated probiotics dietary intervention on fetal immune programming and other effects on immune system in mice	Dr.N.V.Giridharan
40	Mr. Nagabhushan Reddy (2011)	Anti diabetic effects of some simple amino acid-chromium complexes and their probable mechanism of action	Dr. M. Raghunath

Institutional Staff Registered for PhD

S. No	Name of the Staff	Title of the thesis	Supervisor
1.	Dr. A Laxmaiah (2008)	Assessment of prevalence of overweight/obesity, hypertension and type II diabetes among 20-60 year urban population in Hyderabad	Dr. B Sesikeran
2.	Mr. Yadagiri Reddy P (2007)	Molecular studies on obesity-induced cataractogenesis	Dr. Bhanuprakash Reddy G

AWARDS/ HONOURS CONFERRED ON SCIENTISTS

Name of the Scientist	Award/ Honour recived
Dr.V.Sudershan Rao, Mr.G.M.Subba Rao and Dr.Kalpagam Polasa	Won the Platinum City Strategist Award 2009 from Foundation for Futuristic Cities for their strategy on "Making Hyderabad the Street Food Capital of India".
Mr.G.M.Subba Rao	Received the IAMCR Travel Grant Award 2010 for participation in the Conference of the "International Association of Media and Communication Research (IAMCR)", 18 - 22 July 2010 at Braga, Portugal.
Dr.V.Vijayalakshmi	Awarded ICMR International Fellowship for Senior Bio-Medical Scientists 2010-11
Dr.K.Madhavan Nair	Elected as a "Fellow of the Andhra Pradesh Akademi of Sciences (FAPAS)", Hyderabad
Dr.P.Suresh	Appointed as the AAALAC International Adhoc specialist and Site visitor from India to accompany AAALAC Council members for inspecting the facilities for accreditation of laboratory animal centres in the Pacific Region.
Ms.Little Flower Augustine (SRF)	Awarded the NSI Young Scientists Junior Award in Community Nutrition for her paper titled "Development of a valid and reliable questionnaire for testing knowledge on micronutrients among adolescent students", at the 42nd National Conference of the Nutrition Society of India, organized by Mumbai Chapter, Mumbai during 19-20 November 2010
Ms.Deethu Sara Varghese (SRF)	Awarded the NSI Prize for the best oral presentation for her paper titled "Waist-stature ratio and waist-circumference as better indicators of fatness in young women", at the 42nd National Conference of the Nutrition Society of India, organized by Mumbai Chapter, Mumbai during 19-20 November 2010.
Ms.Komilla Pareek (JRF)	Awarded the "IDA Organizing Committee Award" for the best poster for paper titled "Micronutrient composition and iron availability from representative regional Indian diets", in the 43rd Annual National Conference of Indian Dietetic Association, held at NIN, during 3-4 December 2010

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

Date	Name of the Scientist	Conference/ Meeting/ Workshop/ Seminar/ Training
2010		
March 1, 2010 – Feb. 28, 2012	Dr.C.Suresh	Visiting Research Scientist Fellowship, at Savannah State University, USA
June 14 -17	Dr.P.Suresh	As a Specialist & Site Visitor on behalf of the AAALAC International for Accreditation of Laboratory Animal Facilities in the Asia Pacific region, attended the AAALAC International Orientation Seminar organized during the FELASA SCAND - LAS meeting, at Helsinki, Finland
June 14-17	Dr.N.Harishankar	11th FELASA and 40th Scand-LAS meeting, at Helsinki, Finland. Presented a paper on "Body composition of small laboratory animals - Non-invasive vs conventional methods" in the platform session of the conference
June 14-16	Dr.G. Bhanuprakash Reddy	Meeting of Indo-EU collaborative project on “Functional Foods”, at Stockholm, Sweden
June 30, 2010 – March 31, 2011	Dr.Sanjay Basak	BOYSCAST Fellowship programme in the area of “Reproduction Technology”, at Department of Nutrition, Institute for Basic Medical Sciences, University of Oslo, Norway
July 18-22	Mr.G.M.Subba Rao	Conference of the “International Association of Media and Communication Research (IAMCR)”, at Braga, Portugal. Presented a paper on “Communicating nutrition information in community settings – a critical examination of some institutional approaches in India”, in the Working Group on Health Communication and Change
July 18-22	Dr.S.Vasanthi	Workshop on “Safety Assessment of Genetically Modified Foods”, organized by the South Asia Biosafety programme (SABP), held at BRAC, Centre for Development and Management, Bangladesh. Served as Faculty and Workshop leader on the topic entitled “Assessing the potential allergencity of foods derived from genetically modified plants”

Date	Name of the Scientist	Conference/ Meeting/ Workshop/ Seminar/ Training
July 25 – Sept. 2	Dr. Kalpagam Polasa	Food Safety Laboratory Training Programme, to be held at Michigan State University, Michigan, USA
Aug. 15-28	Dr.K.Madhavan Nair	Meeting of the Indo-US Project on "Bioavailability of iron and zinc in representative Indian and US diets/ Enhancing dietary iron and zinc bioavailability in Indian children (MHR RO3 grant) and to prepare the study materials, at Baylor College of Medicine, Houston, Texas, USA.
Oct. 10-16	Mr.T.Longvah	SAARCFOODS Meeting, organized by SAARC Food Data Systems, at Colombo, Sri Lanka.
Nov. 3-5	Mr.T.Longvah	International Scientific Symposium on "Biodiversity and Sustainable Diets" organized by FAO, Rome
Nov. 7-12	Dr.N.V.Giridharan	2010 ICLAS Governing Body Meeting with the 4th Asian Federation of Laboratory Animal Science held at Taipei, Taiwan
Dec. 4 -19	Dr.V.Vijayalakshmi	ICMR International Fellowship for Senior Indian Biomedical Scientists for the year 2010-11 for training in the Division of Diabetes and Endocrinology, Karolinska Institute Laboratory for Molecular Immunogenetics, Karolinska Hospital, Stockholm, Sweden
Dec. 20 -21	Mr.S.Sreedhar (JRF)	Fasting and Sustainable Health Conference 2010, held at Penang, Malaysia
2011		
Jan. 7-Feb.26	Dr. P. Uday Kumar	Training in "Allergencity assessment of genetically modified foods" under Normal E Borlaug International Agricultural Science and Technology Fellowship Programme – 2010, at University of Nebraska, Lincoin, USA
Jan. 24-28, 2011	Dr.N.Harishankar	6th Small animal imaging workshop, held at Tubingen University, Germany
March 14 – June 5, 2011	Dr.B.Dinesh Kumar	Research Programme under Norman E Borlaug International Agricultural Science and Technology Fellowship Programme 2010, at Pennsylvania University, Harrisburg and USDA Agricultural Research Service, Beltsville, USA

Date	Name of the Scientist	Conference/ Meeting/ Workshop/ Seminar/ Training
March 21-25	Dr.Sukesh Kumar Sinha	5th Session of Codex Committee on Contaminants in Foods (CCCF), held at The Hague, Netherlands
March 15-17	Dr.D.Sreeramulu	8th International Conference on "Functional foods for chronic diseases: Science and practice", held at University of Neveda, Las Vegas, USA. Presented a paper on "Natural antioxidant activity of commonly consumed foods in India and effect of heat treatment of green leafy vegetables"
March 15-17	Dr.D.Sujatha	8th International Conference on "Functional foods in the prevention and management of chronic diseases: Metabolic syndrome", held at University of Neveda, Las Vegas, USA. Presented a paper on "Association of hyperhomocysteinemia with insulin resistance in apparently healthy middle aged urban men"

WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN

- In connection with the World Laboratory Animal Day, a One day Symposium on “Animal models developed by Indians in India”, is being organized by National Centre for Laboratory Animals Sciences (NCLAS), NIN in association with Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA), Animal Welfare Board, Ministry of Environment & Forests, Government of India, New Delhi. (April 24)
- Meeting of the Scientific Advisory Committee of NIN/FDTRC/NCLAS. (Aug. 11-13)
- NIN Foundation Day will be celebrated on 27th September 2010. Dr.V.Jayaraman, Director, National Remote Sensing Centre, Hyderabad will deliver the Foundation Day lecture on “Use of Space Technology in Promoting Agriculture, Food Security and Health in India”. Dr.V.M.Katoch, Secretary to Government of India, Department of Health Research & Director General, ICMR, was Guest of Honour.
- The Second batch of two year MSc (Applied Nutrition) Course 2010-2011 was commenced on September 1, 2010. Fifteen students were admitted under the State and Central quota.
- A one day symposium was organized on this year's theme – Nutrition Promotion for a Stronger Nation, at NIN in association with Food and Nutrition Board, Govt. of India. (Sept.6)
- 30th Laboratory Animal Supervisors' Training Course, organised by National Centre for Laboratory Animal Sciences (NCLAS). (Sept. 1- Nov. 30)
- In connection with the World Food Day celebrations, a one day Symposium was organized on “United Against Hunger” in association with Association of Food Scientists Technologists, Hyderabad Chapter. (Oct. 16)
- Training programme for the officials of Food and Nutrition Board, Ministry of Women and Child Development, Government of India, New Delhi. (Oct. 4-8)
- NIN-WHO Workshop on “Total Diet Study Dissemination”. (Nov. 16)
- A Public Forum was organized on “Nutrition and Health” to mark the ICMR Centenary celebrations. Students from various colleges and general public participated in the forum. (Nov. 16)
- Pre Conference Workshops on “Theory to practice for students and upcoming dietitians” and “Train the trainer for senior dietitians and nutritionists” as part of the 43rd Annual National Conference of Indian Dietetic Association. (Dec. 2)
- 43rd Annual National Conference of Indian Dietetic Association on the theme “Dietetics – Widening Horizons”, jointly organized by Indian Dietetic Association, AP Chapter in association with NIN. (Dec. 3-4)
- 43rd Annual Conference of Indian Pharmacological Society (IPS), India and International Conference on Concept: "Pharmacology and Translational Research", organized by National Executive Committee 2010 - IPS in association with Food and Drug Toxicology Research Centre, NIN. Dr.APJ. Abdul Kalam, Hon'ble former President of India, delivered the valedictory address. (Dec.13– 16)
- 48th Post-Graduate Certificate Course in Nutrition. Four candidates were participated in the training programme. (Jan.5-March 18)
- Annual Training Course on Assessment of Nutritional Anaemias. Seven candidates were participated in the course. (March 21-31)

SERVICES RENDERED TOWARDS INCOME GENERATION

1. PATHOLOGY SERVICES

During the year, a total income of Rs.2,08,820/- was generated from various projects of Institute's preclinical toxicology and surgical pathology and cytology samples.

2. TRAINING PROGRAMMES

I. An amount of Rs.6,00,000/- was generated from the tution fee collected from the first and second year participants of 2 year MSc (Applied Nutrition) course [1st and 2nd year candidates – 15 each] .

2. An amount of Rs. 61,000/- was generated from eleven private candidates admitted to the regular training programmes viz., Post Graduate Certificate Course in Nutrition (4) and Training Course of Assessment of Nutritional Anaemias (7).

INSTRUMENTATION SERVICES

LIST OF EQUIPMENTS PROCURED DURING THE FINANCIAL YEAR 2010 -11		
S.No.	**Name of the equipment**	**Principal**
1.	Automated solvent extraction system	M/s. Buchi Labortechnik AG Switzerland / M/s. Buchi India Pvt. Ltd, Mumbai
2.	Atomic Absorption Spectrometer with accessories	M/s. Shimadzu Asia Pacific Pte Ltd, Sinagpore / M/s. Toshwin Analytica, Scbd
3.	Maldi Tof-Toff – MS MS with spares and consumables	M/s. AB Sciex PTE, Ltd, Singapore/ M/s. Labindia Instruments Pvt. Bangalore
4.	Electroretinogram (ERG)	M/s. LKC Technology, IN, USA/ M/s. Bio Med Healthtec Pvt. Ltd, Mumbai
5.	-80*C Ultra Low temperature Upright Deep freezer	M/s. Scimed Asia Pte, Ltd, Singapore/ M/s. Care Bio systems Pvt. Tld, Sec'bad
6.	CO2 incubator with optional accessories	M/s. Thermo Fisher Scientific (Hong Kong) Ltd, Hong Kong / M/s. Pinnacle Biosolutions, Secunderabad
7.	Molecular Imager	M/s. Synoptics Ltd, UK, / M/s. Biodigital Pvt. Ltd, New Delhi
8.	Co2 Incubator with accessories and open air shaker	M/s. Thermo Fisher Scientific (Hong Kong) Ltd, Hong Kong, / M/s. Pinnacle Bio solutions, Secunderabad)
9.	Inverted Microscope with essential accessories	M/s. Olympus Singapore Pvt, Ltd, Singapore, / M/s. DSS Imagetech, Sec'bad
10.	Gradient PCR Machine	M/s. Bio-rad Pacific Ltd, Hongkong M/s. Bio-Rad Laboratories (I), Hyderabad
11.	CO2 Incubator with optional accessories	M/s. Thermo Fisher Scientific (Asheille), LLC, USA / M/s. Pinnacle Bio solutions
12.	Cutting Mill	M/s. Retsch GmbH, Germany/ M/s. Inkarp Instruments, Pvt. Ltd, Ahmadbad
13.	Refrigerated Micro Centrifuge (Sorvall Legend Micro 21R)	M/s. Thermo Electron LED GMBH, Germany / M/s. Pinnacle Bio Solutions
14.	Mini Protein Tetra Cell (Mini Vertical Electrophoresis unit)	M/s. Bio-Rad Pacific Ltd, Hong Kong, M/s. Bio-Rad Laboratories (I), Hyderabad
15.	SYRING PUMP	M/s. Harvard Apparatus Inc, USA / M/s. Marsap Services, Pvt. Mumbai
16.	Gas Chromatograph	M/s. PerkinElmer Singapore pte, Ltd, Singapore, / M/s. Smart Labtech Pvt. Ltd, Hyderabad
17.	GM 9D Glucose analyser	M/s. Analox Instruments Ltd, UK, M/s. Smatech, New Delhi

S.No.	Name of the equipment	Principal
18.	Portable RMR measuring equipment / portable resting metabolic analyser	M/.s Microlite Medical Home Solutions, Inc, USA
19.	Organic Flash purification system	M/s. Teledyne Isco Inc, USA / M/s Septec Marketing India Pvt. Ltd, Mumbai
20.	Protein Purification System	M/s. Bio-Rad Pacific Ltd, Hong Kong M/s. Bio-Rad Laboratories pvt. Ltd, Hyd
21.	Binary Gradient Fast HPLC System with PDA & FLD, ELSD,PC & Printer – 3Nos.	M/s.Dionex India Pvt. Ltd., Mumbai
22.	Binary Gradient HPLC with PDA 7 FD	
23.	High Performance Liquid Chromatography	
24.	Quaternary Gradient HPLC with PDA & FLS Detector	
25.	Amino Acid Analyser	M/s.Icon Analytical Equipments Pct. Ltd., Mumbai
26.	IN VIVO Imaging System	M/s.Imperial Life Sciences Pvt. Ltd., Gurgoan
27.	Robotic Spot Picker	M/s.DSS Imagetech Pvt. Ltd., Secunderabad
28.	Liquid Scintillation Analyser	M/s.Perkin Elmer India Pvt. Ltd., Hyderabad
29.	Gas Chromatography with Head Space with Auto sampler – 2nos.	M/s.Smart Labtech Pvt. Ltd., Hyderabad
30.	Nitrogen Generator with oil free compressor	M/s.Stiring Cryogenics India Pvt. Ltd., New Delhi
31.	Digital Rotary Flash Evaporator	M/s.Genevac Limited, UK
32.	Ultra Purification Water System	M/s.Elga Labwater, UK
33.	-80C Vertical Deep Freezer	M/s.Cryogenic Systems Pvt. Ltd., Hyderabad
34.	2D Page (IEF CELL)	M/s.Bio-Rad Pacific Limited, Hongkong
35.	Body Composition Analyser	M/s.Maltron International Limited, UK
36.	Digital Rotary Flash Evaporator	M/s.Heidolph Instruments , Germany
37.	Thermal Cycler	M/s.Astec Co.Ltd., Japan
38.	Real Time PCR with High throughput	M/s.Bio-Rad Pacific Limited, HongKong
39.	Indirect Ophthalmoscope wireless binocular	M/s.Shree Enterprises, Nagpur
40.	Inverted Microscope – 2nos.	M/s.Olympus Singapore Ptd. Ltd., Singapore
41.	Tissue Homogenizer	M/s.Omni International USA

S.No.	Name of the equipment	Principal
42.	Microscope with PC and Camera	M/s.Speed fair Co., Ltd., Hong Kong
43.	Gel Documentation System	M/s.Expert Vision Labs Pvt. Ltd., Mumbai
44.	IVC Systems with Double Decker for obese rats	M/s.Tecniplast Spa, Italy
45.	Water Purification System	M/s.Siemens Pvt. Ltd., Singapore
46.	Table Top Refrigerated Centrifuge	M/s.Andreas Hettich GmbH, Germany
47.	CO2 Incubator	M/s.New Brownswic scientific Co., USA
48.	Video Tracking & Monitoring System	M/s.Ain Corporation, Hyderabad
49.	Individually ventilated animal cages for mice	M/s.Citizen industries, Ahmadabad
50.	Fat Extractor – 2nos.	LECO
51.	High speed gas chromatography – 2nos.	Agilent
52.	Fully automated Kjeldahl nitrogen analyzer-2nos	Foss
53.	Microwave digestive system	Cem
54.	Binary gradient HPLC with PDA detector- 4nos	Dionex
55.	Binary gradient HPLC with PDA & FLD – 2nos.	
56.	Binary gradient HPLC with PDA & ELSD-2nos.	

NIN SCIENTIFIC PUBLICATIONS - 2010

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

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B. PAPERS PUBLISHED IN PROCEEDINGS/ BOOKS

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