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HIGHLIGHTS

Nutrition related issues pertaining to different geographical regions of the country were probed in detail this year. Uttar Pradesh was surveyed this year to assess the dietary intakes and nutritional status of rural community. North-Eastern India was also the focus of research studies as certain aspects of endemic goitre were investigated and also certain medicinal plants were analyzed for their antioxidant activities. A series of studies on food fortification and trace minerals were carried out as part of basic studies. Also, this year, some new studies were conducted in the areas of cancer-xenobiotics, food safety and extension and training. In the realm of clinical studies, the correlation between women's work and bone health was worked out. Here are the highlights of the research studies carried out during the year.

1. COMMUNITY STUDIES

1.1. Nutrition profile of Indians – A districtlevel survey in Uttar Pradesh

Assessment of nutrition profile of the rural community in 63 districts of Uttar Pradesh revealed that the average intake at the State level of cereals & millets and pulses was satisfactory, while that of milk, sugar & jaggery was less than the recommended dietary allowances. The mean intake of all the nutrients except energy, protein and calcium was less than the RDA. Only about 64% of the households were consuming adequate amount of both energy and protein. In general, about 52% preschool children were undernourished (weight for age < Median – 2SD). The prevalence was >50% in 34 of the 63 districts surveyed. About 42% of adult males and 34% of females had chronic energy deficiency (BMI <18.5).

2. CLINICAL STUDIES

2.1. Women's work and bone health

It is known that bone-loading postures, adopted during exercise, are recommended for bone density improvement. However the stimulation of bone turnover during prolonged bone loading postures in the absence of calcium intakes might prove to be deleterious for bone health.

Earlier studies showed that despite low calcium intakes standing or standing with load bearing/bending postures are better for hip densities when compared to the sitting postures at work. However, in the case of the spine, standing and bending postures significantly reduce bone densities at the spine, but sitting postures do not worsen the spine bone densities. Using the WHO classification for diagnosis of osteoporosis, the onset of bone changes around 40 years of age in the low socio-economic group were seen in spine (-2.27), hip (-1.7), forearm (-1.53) in this order. All the women from the low socio-economic (LSE) group have increased osteoclastic activity (indicated by acid phosphatase), but those with spinal osteoporotic changes are worse off on all the biochemical indicators of bone loss.

2.2. Effect of tamarind on mobilization of fluoride in fluorotic subjects

Tamarind ingestion through diet appears to be helpful in mobilizing deposited fluoride from bone, by enhancing urinary excretion of fluoride after urinary fluoride was stabilized by giving fluoride free water.

3. FOOD FORTIFICATION – TECHNOLOGY DEVELOPMENT

Food fortification is one of the important strategies accepted in the National Nutrition Policy to achieve the goals of controlling anemia in the country. Institute has made considerable progress towards realizing this goal by developing new technology.

3.1. Encapsulated source of iron was found to keep iodine in a stable form in double fortified ordinary common salt while the NIN formulation, required high quality salt for providing acceptable level of iodine.

3.2 A laboratory based technology for fortification of whole-wheat flour 'atta' with iron at 25 and 50 mg/Kg and folic acid at 220 mg/Kg was developed. The two indigenous sources of iron that met the criteria as a chemical source of iron were anhydrous ferrous sulfate and indigenous H-reduced iron powder. The percent bioavailability in adult male human volunteers was three times higher with anhydrous ferrous sulfate compared to H-reduced iron powder.

4. NUTRIENT COMPOSITION OF NEW VARIETIES OF RICE

Eleven varieties of rice obtained from the Directorate of Rice Research, Hyderabad, were analyzed for proximate composition. The protein content in general was higher than 8 g as against 6.8g found earlier (Nutritive Value of Indian Foods). In one of the varieties, it was about 12g. Consumption of such high protein rice could increase the protein intake of individuals by 8 to 9 g/day. This perhaps explains the gradual improvement in PEM status in the country, despite little changes in Indian dietaries intake.

5. NUTRITION AND CHRONIC DISEASES

5.1 Studies conducted on WNIN female rats have shown a correlation between maternal micronutrient status and predisposition of the offspring to insulin resistance in later life.

5.2 In a yet another study on rats, it was found that restriction of both dietary micronuteints as well as calories had adverse effects on fasting glucose & insulin and (Homeostasis model of assessment insulin resistance) HOMA IR index as compared to group with calorie restriction alone. It was also found that antioxidant status (Ferric reducing antioxidant potential - FRAP) was better and oxidative stress (Thio barbituric acid reactive substabces - TBARs) low in rats of 'calorie restricted' group as compared to calorie and micronutrient restricted group. The results of this study confirmed the role of micronutrients in modulating oxidative stress and hyperinsulinemia.

5.3 Postponement of cataractogenesis by diet restriction is in part due to improved antioxidant status and / or enhanced protein editing. The effect of longterm restriction of diet/food, protein and vitamin on the aggregation of crystallins and α-crystallin chaperone activity in rat lens *vis a vis* cataractogenesis was, hence, assessed. Food and protein restriction lowered the susceptibility of α - and γ-crystallins towards aggregation, while vitamin restriction tended to increase the aggregation of crystallins. Interestingly, only by vitamin restriction improved chaperone activity of α–crystallin.

5.4. ARI activity in foods

Diabetes mellitus advances onset and progression of cataract probably by modulating oxidative stress and glycation. Aldose reductase is important in diabetic cataractogenesis. Aldose reductase inhibitors (ARI) are valuable in delaying/preventing it. Screening of extracts of different plant foods indicates significant ARI activity in the extracts of amla, bitter gourd, fruits and Tulsi leaves. Curcumin appears to be the most potent ARI. Curcumin inhibits AR in a non competitive manner. Ongoing studies have shown that extract of black tea - but not green tea - significantly inhibited the glycation (argpyrimidine formation) of purified bovine lens α- crystallin *in vitro*. Significant anti platelet aggregation activity was also observed in water extracts of eight varieties of green leafy vegetables.

5.5. Dietary fatty acids and insulin resistance

Dietary fatty acids are known to affect the risk of chronic diseases particularly of diabetes and CVD. Adequate intake of essential PUFA namely linoleic (18:2n-6) and a-linolenic acids (18:3n-3) and their optimal balance (n-6/n-3 ratio ~ 5-10) has been found necessary for

cardiovascular health. Studies were undertaken to investigate the effects of dietary fatty acids on membrane lipid composition and insulin sensitivity in target tissue in experimental rats.

Dietary saturated fatty acids (SFA) and trans fatty acids (TFA) decreased insulin sensitivity in target tissues (skeletal muscle and adipose tissue). The effects of TFA were more marked than SFA. The current evidence indicates that the atherogenic effects of TFA (increased LDL cholesterol and decreased HDL cholesterol) can be prevented by increasing dietary 18:2 n-6. However, the present studies show that increasing dietary 18:2n-6 did not prevent the TFA induced decrease in insulin sensitivity. On the other hand, increasing dietary ALNA and therefore decreasing n-6/n-3 ratio to 2 increased insulin sensitivity in target tissues in diet induced insulin resistant rat model.

6. RESISTIN AS A MOLECULAR LINK BETWEEN DIABETES AND OBESITY

NIDDM is a result of combination of genetic and environmental factors. Epidemiological observations suggest a strong patho-physiological association of obesity to NIDDM. However, the molecular link between obesity and diabetes remained to be resolved.

Resisitn, a cysteine-rich secretory protein, identified from mouse adipocytes, is down-regulated by anti-diabetic drugs like thiazolidinediones (TZDs). Association of resistin with diabetes and obesity in human system has been controversial as opposed to the murine system. Thus, it appears that mouse and human differ resistens greatly not only in their sequence and structure but in their regulation and thus function. The physiological role of resistin in diabetes and obesity can be better understood with knowledge about its molecular features.

The genomic counterpart of resistin encompassing the transcriptional start and translational stop from mouse and human was amplified and sequenced using ABI automated DNA sequencer. Exon/intron boundries of mouse and human were demarcated using DIALIGN algorithem.

1. The mouse sequence has an additional intron of 2279 bp (intron X), which is absent in human resistin. *In silico* analysis revealed a number of transcription factor binding sites.

2. Of particular importance was the presence of PPAR responsive element (PPRE) along with the binding sites for other transcription factor like Ap1, NFkB, C/EBP.

3. The binding of PPAR/RXR complex to PPRE present on intron X was shown by gel shift assays.

4. Functional relevance of this DNA-protein interaction was demonstrated by transient transfection assays using luciferase as a reporter.

The typical characteristics of the recombinant proteins were studied further.

7. FOOD SAFETY

7.1 Application of Hazard Analysis Critical Control Point (HACCP) to animal products

HACCP is a preventive system to reduce microbial hazards. A study on HACCP was carried out in prawns. The samples were found to be contaminated with pathogenic microorganisms immediately after harvest and also at the market place. However, recipes prepared from prawn were free from pathogenic organisms. While pond water samples were contaminated with microorganisms including *Vibrio* sp., feed and ice samples used for preserving were free from pathogenic organisms. Every step in food chain has to be thoroughly investigated for identifying the source of contamination to ensure food quality and safety. The significance of the present study is that the pond water is the source of contamination. Hence, HACCP need to be practiced during the entire food chain from pond to plate and not only at the export processing units.

7.2. Utilization of mouldy sorghum using Bio-technological means for animal consumption

A study was conducted to assess the feasibility of utilizing lactic acid bacteria, through natural fermentation, to detoxify contaminated sorghum and convert it to a value added animal feed ingredient. The laboratory trial of production of *Lactobacillus* fermentation of mouldy sorghum with addition of locally available resources (e.g. *Casia tora* seeds) was tested at village level by feeding it to cattle. This is a preliminary study, and the acceptability was monitored in terms of feed consumption, which was correlated with milk output.

8. NUTRITION AND CANCER

8.1. In vivo model for genotoxicology

A study was conducted to establish *in vivo* model to assess genotoxicity and evaluate the antimutagenicity of feeding allium through diet. Prior feeding of allium vegetables to rats reduced the DNA damage in tissues and urinary mutagens due to carcinogen treatment.

8.2. Studies on ginger

Non-nutrient components of diet have been shown to inhibit carcinogenic process by enhancing the intracellular levels of xenobiotic metabolism enzymes, which play an important role in detoxification pathway. Spices and their active principles have been shown to possess chemopreventing properties. Study on modulation of xenobiotic metabolism by ginger showed that ginger feeding to rats resulted in stimulation of glutathione-s-transferase enzyme in liver, intestine, lung and kidney suggesting that regular intake of ginger can enhance the detoxification enzymes and thereby afford protection to host against tissue damage induced by exposure to xenobiotics.

8.3. Effect of vitamin restriction and supplementation on rat intestinal mucosal cell apoptosis

50% vitamin restriction increases the oxidative stress, decreasing the anti-apoptotic protein (Bcl-2) expression thereby enhancing the apoptotic rates while vitamin supplementation reverses the changes caused by vitamin restriction. Among the individual vitamins, vitamin E showed maximum decrease in oxidative stress, as well as decrease in apoptotic rates.

9. Training activities in RCH

A total of 186 investigators from different States in the country involved in the district level RCH surveys were trained in 10 batches at Nagpur, Bhubaneswar, Chennai and Hyderabad. Orientation was provided on appropriate techniques using finger prick blood samples for the estimation of haemoglobin levels.

At the request of Food and Nutrition Board, Department of Women and Child Development, Government of India, ICDS functionaries from North Eastern States were given orientation training in organization and establishment of Nutrition Surveillance System.

10. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

10.1 R & D Supporting Service Activities

The Centre was involved actively in the breeding and supply of microbiologically and genetically defined animals to various government and private institutions. Besides supply of animals, blood and blood products were also supplied to different institutions to meet their specific R & D needs. Additionally the Centre was involved in supplying high quality animal feed to different institutes. All these service activities resulted in generating a total income of Rs.31.75 lakhs. The Centre trained a total of 50 candidates in various aspects of laboratory animals care, husbandry, nutrition, diseases, welfare and ethical aspects of experimentation. The Centre also extended technical consultancy in the field of laboratory animal sciences and technology for more than 20 institutions last year.

B. RESEARCH ACTIVITIES

10.2 Effect of satiomem in obese rats

A preliminary project in collaboration with ITRC, Lucknow on the effect of anorexigenic glycoprotein known as "satiomem" isolated from animal and plant membrane was tested in WNIN/Ob rats. The study confirmed the anorexigenic property of the compound shown earlier in normal rats and mice. However, the food intake reduction in these genetically obese rats was only 20%, compared to 40% seen earlier with normal rats. At the level compound is fed to the rats, no apparent toxic effect was seen.

10.3 Molecular analysis of WNIN/Ob rats

The study on the candidate gene involved in obese rats was in collaboration with IISc., Bangalore . It comprehensively showed that leptin receptor gene was not altered in these rats, as per the evidence on coding regions as well as m-RNA levels of various splice variants of the Ob-R gene.

10.4 Genetic typing of obese rats using micro-satellite markers

A project with funding from DBT was initiated for genetic typing of WNIN/Ob and GR-Ob strains using micro-satellite markers. These markers span all the 22 chromosomes (including sex chromes) of the rat and the mutants will be typed along with the parental strain WNIN, related strain WKY and unrelated strain Fischer 344. Out of 100 markers selected for the study (4 markers/ chromosomes), 22 markers were completed and unique markers typical of each strain could be observed.

10.5. PCR based DNA fingerprinting in obese rats

The PCR based DNA fingerprinting using random primers yielded an unique DNA finger profile for homozygous obese rats which is different from its other phenotypes and other standard reference strains. This has been submitted for patenting purposes.

10.6. Age associated oxidative stress in obese rats

Studies on oxidative stress in obese mutant rats showed that lipid peroxidation and protein carboxylation to be high at the age of 3 months in cerebral hemispheres while the other regions of the brain were affected only after 9 months of age.

Some of the research studies initiated during IX five year plan period were continued in the X plan period as they were perceived to be of research significance. New thrust areas were identified and new equipments were procured for the fresh projects during the year. The research endeavours of the scientists and the technicians were the major propellers of the research work.

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I. COMMUNITY STUDIES

1. *Nutrition profile of Indians – A district level survey in Uttar Pradesh*

Earlier, the results of surveys of district nutrition profile in the States of Punjab, Haryana, Himachal Pradesh, Assam, Orissa and West Bengal were reported (NIN Annual Report, 1995-96, 1996-98, 1999-2000, 2001-2002). These surveys, for the first time, provided district level information on diet and nutrition status of communities. Similar survey was carried out in the State of Uttar Pradesh.

General Objective

To assess the dietary intakes and the nutritional status of the rural community at district level in the State of U.P.

Specific objectives

1. To assess the food and nutrient intake of rural population
2. To assess the anthropometric and clinical status of representative segments of population, and
3. To assess the knowledge and practices of mothers on breast-feeding, child rearing and socio-cultural aspects of food consumption in relation to health and disease.

Area of study

The survey was carried out during 2001-2002 in all the 63 districts of the State in collaboration with Institute of Applied Statistics and Development Studies, Luck now (IASDS).

Sampling Design

In each district, twenty villages were selected by systematic random sampling procedure coupled with proportion to population size (PPS), giving due representation to all the blocks/taluks.

In each selected village, twenty households (HHs) were covered. For this purpose, the village was divided into 5 natural groups, based on streets/ *mohalla*/*basti*, of which one group belonged to SC/ST community. From each group, 4 contiguous HHs were surveyed, with a random start.

Investigations

The following information was collected by trained investigators from the state using standard procedures and specially prepared pre-tested schedules.

i) Household socio-economic and demographic particulars.

ii) Anthropometric measurements such as height and weight.

iii) Assessment of household food and nutrients intake in every alternate HH, and food and nutrient intake of individuals in a sub-sample of 5 HHs.

iv) Frequency of consumption of foods in a sub sample of 10 HHs and

v) Prevalence of clinical signs of nutritional deficiency on all the available individuals by using standard techniques.

vi) Knowledge, attitude and practice (KAP) survey of mothers of preschool children with respect to diet during health and disease, nutritional disorders and utilization of services under intervention programmes in the households covered for diet survey.

Results

Coverage

A total of 25,211 HHs were covered for demographic and household particulars and family diet survey was carried out in 12,022 HHs. Among them, about a half of the households were covered for individual diet survey by 24-hour recall method. Nutritional status, in terms of anthropometry and nutritional deficiency signs,

was assessed on 99,850 subjects of different age and sex groups.

Food intakes

The average intake of cereals & millets per CU (533 g) was well above the RDA of 460 g, while the intake of pulses & legumes (39 g) was comparable with the RDA of 40 g. Consumption of income elastic foods such as milk (115 ml), and sugar & jaggery (18 g) was lower than the recommended level.

Nutrient intakes

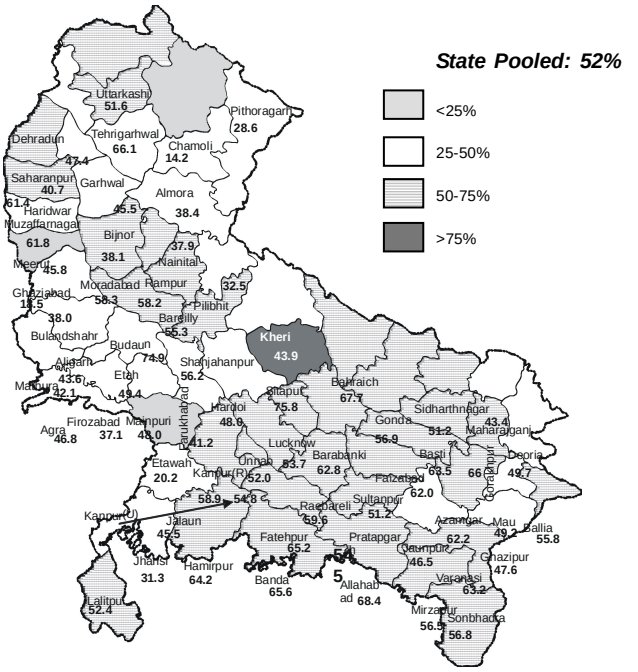
The median per CU/day intakes of energy (2356 kcal), protein (70 g) and calcium (417 mg) were higher than the recommended levels perhaps due to high cereal intake, while the intakes of micronutrients such as iron (24 mg), vitamin A (108 mg) and riboflavin (0.9 mg) were below the RDA. About 64% of the HHs had adequate dietary energy intakes (>Mean-2SD of requirement), while 92% had adequate intake of protein. About 8% of the households had inadequate intake of both protein and energy. Among the preschool children, except for protein and thiamin, the mean intakes of all other nutrients were below the recommended levels. The extent of energy deficit was 28% in case of 1-3 year children and 17% in 4-6 years children. In the case of vitamin A, the deficit was much higher in children of 1-3 years (74%) and 4-6 years (68%). Among the younger adolescents (13-15 years), the intake of all the nutrients, except thiamin and niacin, was below the RDI in both the sexes. Similarly, among the older adolescents (16-18 years), the extent of deficit was more for iron (51% for boys and 31% for girls) and vitamin A (69% for boys and girls) compared to other nutrients. In the case of the adults, except for micronutrients, such as iron, vitamin A and riboflavin, the intake of all other nutrients was above the recommended level. The extent of deficit with regard to mean intake of iron was higher among females (29%) compared to males (4%).

Nutritional Status

The individuals of different age groups were considerably lighter and shorter than the reference population (NCHS). The prevalence of severe undernutrition (<60% weight for age) in preschool children was about 9% and no significant gender differences were observed. Overall prevalence of underweight (< Median-2SD weight for age) was 52%. The extent of undernutrition in different districts is presented in the **Map**. The prevalence of stunting (< Median –2 SD height for age) was 71.5%, while wasting (<Median – 2 SD of weight for height) was 12%.

About 42% of males and 34% of the females had different grades of chronic energy deficiency (CED) as measured by BMI (<18.5). About 56% of males and 61% of the females had normal body mass index (18.5-25.0). The prevalence of obesity (>25) among females was marginally higher (5.1%) than males (2.3%).

Fig 1. Map showing distribution (%) of prevalence of underweight (< -2SD weight for age) – among pre-school children in the state of Uttar Pradesh



Conclusions

Thus, the results indicated that the diet and nutrient intakes, especially micronutrients were grossly inadequate compared to RDA. The undernutrition is also widely prevalent in the State, both among the preschool children and adults.

The data on diet and nutritional status of population, made available for the first time at the district level, would be useful to the planners to prepare strategies and plan of action to combat malnutrition at district level.

II. NUTRITION AND INFECTION

1. Women's work and bone health - A study in an urban slum population

Osteoporosis is a skeletal disease characterised by low bone density and micro-architectural deterioration of bone tissue. This results in increased bone fragility and fractures, a clinical manifestation of osteoporosis. Osteoporotic fractures most commonly occur at the hip, the vertebrae and the distal radius, and are common around 65-70 years (vertebral fractures) and 75-80 years (hip fractures) in Caucasian women. Studies carried out at NIN show that the mean age of hip fractures is at least 10-15 years early in the Low Income Group (LIG) (55-57 years), with vertebral fractures going largely unreported. A large number of factors contribute to this early onset of osteoporotc fractures in Indian women. Some of these may be inadequate intakes of energy, protein and calcium, early meno pause, low body weights and low BMI, etc..

Although calcium is an important nutrient for bone formation, calcium intakes in most women is far below the RDA. High levels of oxalates and phytates in their cereal- pulse diets further impair the bioavailability of even these low levels. Oestrogen is known to maintain bone density by decreasing bone resorption, but the levels of circulating sex hormones in women from the LIG have been shown to be lower than those seen in Western women.

In addition to these factors, the levels of physical activity have an important impact on bone density. Load-bearing exercise is thought to offer protection against bone loss by its direct effect of maintaining lean body mass, and indirectly by stimulating bone turnover due to bone impacting. However, reports indicate when exercise is accompanied by weight loss or on low calcium diets may not result in an increase in bone mineral density but it may actually reduce bone mass.

Based on earlier studies, it may be speculated that bone parameters may deteriorate in the following categories of work:

a) Heavy bone loading activity of construction workers, on low calcium diets may result in bone loss.

b) Activity causing repetitive stress on the musculo skeletal system may worsen the bone status. This may be with bone loading (sweepers) or without (Beedimakers, homebased). Specially, when the subjects are on low calcium diets.

Objectives

To study

1. The ergonomics of women's work i.e. time and posture studies of their work by observation.
2. The effect of bone loading / or repetitive musculo skeletal stress due to women's work on their bone densities and bone health.
3. The effects of the above tasks on serum and urinary biochemical markers of bone turnover.

Study Design

Adult women from a large urban slum (Addagutta) between the ages of 30 and 50, were enumerated for the study. Their occupational groups were recorded. A stratified random sample of 50 women in each occupational group was randomly selected in each of the occupations given below.

They were as follows:

1. Physical load bearers like construction-workers (CW, n-53). Construction-workers perform various tasks, walking or carrying loads of sand and cement etc., as part of their work.
2. Beedi-makers (BM, n-53) sit, often two to three hours or even longer, hunched over their work doing repetitive tasks. Their work is not load bearing.

3. Sweepers (SW, n-52) are involved in load bearing tasks, but also work bent forward as they move for 6-8 hours continuously on a task which causes repetitive stress to the musculo skeletal system. They may be classified as belonging to a group, which has both the impacts.

4. Homemakers (HM, n-69) work, for 5-10 hours a day at housework, childcare and other caring tasks. Their work involves working at more than one task at a time, which is a mix of load bearing and non-load bearing. HM served as controls.

Methodologies

Background information and other routine anthropometric measurements like weight, height and skin-folds measurements at 4 sites were carried out.

The bone densities were measured at three sites, Hip, Spine and Forearm for Bone Mineral content (BMC), Bone Area (BA), and Bone Density (BD). Body composition was obtained by Whole Body Scan using the DEXA.

Dietary intakes were collected by a previous day recall method and intake of calcium calculated.

A fasting blood sample was drawn for Haemoglobin, serum proteins, albumin, bone specific acid phosphatase and alkaline phosphatase which were estimated on the same day.

The serum was processed and preserved for the estimation of Ca, P, 25(OH) D_3, parathormone, urinary Ca, and Creatinine. These reflect various bone specific functions of bone formation and resorption. The durations and postures adopted for housework and occupational work were recorded by observation and recall.

Statistical Analysis

ANOVA and multiple comparison procedure were done to compare the mean values of bone parameter (BP) at various sites by occupational groups. Level of significance was considered as $P < 0.05$.

Results

These may be divided into the following categories:

1. Background information
2. Bone density data
3. Biochemical data

Background information

The mean (±SE), height, weight and BMI were not different between the occupational groups. (Age 41.03±0.505(yrs), Weight (kg) 49.21±0.578, Height

(cms) 149.15±0.323, BMI 22.07±0.234). Their weights and also the mean BMIs were higher than those reported for Indian's by the NNMB.

Bone Parameters

There were no significant difference in bone parameters (BP), that is BMC Area and BMD at neck of femur, hip, spine, forearm and whole body between the occupational groups.

But these values were significantly lower than the values reported for the High Income Group (HIG) and Middle Income Group (Ann.Rep.NIN 2001-2002) and very close to the values of the Lower Income Group (LIG).

However, when the T Scores were arranged in ascending order, it was obvious that the T-Score at the neck of the femur and hip bone were better (Low values) in the occupations with maximum load bearing (CW). Sweepers and the home-makers with less load bearing tasks had T-Scores in between, and the beedi-makers with no bone loading (sitting = 8 hrs). had the highest T Scores at the neck and the hip, though it missed significance. Standing, with load bearing tasks appear to be beneficial to the neck of femur and hip despite low calcium intakes.

Neck	T-score	Hip T-Score (Mean±SE)
CW:	-1.63±0.142	-1.57±0.129
SW:	-1.78±0.151	-1.66±0.126
HM:	-1.88±0.144	-1.77±0.118
BM:	-1.92±0.135	-1.80±0.122

This trend was reversed in the case of spinal BMD, and T-Scores. Workers (BM) sitting for long hours with no load bearing tasks had a better BMD and lower T scores at the Spine, when compared to the CW, SW and HW, who performed tasks which were bone impacting.

	BMD Spine (gm/cm^2)	T-score (Mean±SE)
BM	0.83±0.016	-1.98±0.143
CW	0.79±0.019	-2.31±0.176
HM	0.78±0.017	-2.40±0.156
SW	0.76±0.019	-2.59±0.173

The T-Scores just missed significance (P<06).

The trends suggest that increased standing and bending activities (SW) appear to stress the vertebrae and decreased spine densities.

But sitting postures do not appear to worsen the trabecular bones of the spine.

The results of the T-scores and BMD at the forearm follows the trend of the trabecular bone of the spine.

Trabecular bone is said to be sensitive to hormonal depletion and osteoporotic changes take place at an earlier age. But the hip loses bone later and is said to be due to decreased intestinal absorption of calcium resulting in increased PTH. When the results of the T-Scores were pooled for each site, they are as follows :

Pooled T-scores (Mean±SE) at the Spine-2.27±0.074, Hip -1.7±0.057, and the Forearm-1.53±0.091 (P<0.05).

The order of onset of osteoporotic changes in different bones correspond to the western reports, That is, the Spine is the worst off followed by the Hip.

It is significant that these changes occur 10-15 years earlier in this group of women when compared to Western data.

It is of concern that using the WHO criteria for the diagnosis of osteoporosis, the SW have a mean T-Score which is in the osteoporotic range at the age of 40 years itself.

Biochemistry

The mean Hb, serum protein and serum albumin levels were not different in the 4 occupational groups. However the whole group had high bone specific acid phosphatase, suggesting increased osteoclastic activity.

Serum calcium (mean±SE) was much lower in the sweepers when compared to the others (Ca- 7.82±0.143mg/dl (SW) vs 9.29±0.072mg/dl in the rest). Bone specific alkaline phosphatase was significantly lower (27.53±2.64 vs 30.7±1.092 IU/l) and even the high bone specific acid phosphatase significantly raised in the sweepers (7.04±0.395 vs 5.96±0.159 IU/l).

Urinary Ca by creatinine was raised in the SW (112 vs 92) when compared to the others (P<0.05).

The above biochemical results suggest there is an increased osteoclastic activity in the whole group of women and specially, in the sweepers (low serum calcium, high calcium by creatinine in the urine and increased bone specific acid phosphatase activity).

When this is analysed in relation to bone parameters it appears that work with increased bone loading (CW) benefits the hips BP, without deterioration in the biochemical profile (except high acid phosphatase)

Absence of bone loading appears to benefit the spinal BP (BM), but bone loading with repetitive musculo skeletal stress (HM,SW) appears to worsen

spinal BMD at an early age in women. The sweepers appear to be worse off in relation to spine BMD and T-scores. This is also reflected in the biochemical parameters. It is possible that Biochemical Parameters reflect changes in bone parameters at the spine in this age group.

2. *Immunoglobulins in the vaginal secretions of women with vaginal infections*

Immune mechanisms in the vagina appear to be critically important for the prevention of Reproductive Tract Infections (RTI) and HIV infection. It is recently being recognised that both ulcer and non-ulcer Sexually Transmitted Diseases (STDs) facilitate HIV transmission. Cauci *et al.,* (1998) have shown an impairment of mucosal immune system in patients with Bacterial vaginosis evident from the degradation of IgA and IgM in vaginal washes. Th1 and Th2 cytokines have also been demonstrated in cervico vaginal fluid in normal women. Most women of low socio - economic status (LSES) in developing countries are speculated to have a high load of RTI. They also have multiple nutrient deficiencies, which may influence the immune response.

We reported last year that the Immunoglobulin levels in cervico vaginal secretions (CVS) in women with vaginal infections were comparable regardless of their BMI status. Also, the prevalence of vaginal infections did not differ between the groups with low or high BMI.

This year we looked at the integrity of the immnoglobulins using western blotting and also determined two representative cytokines of Th1 and Th2 viz IL-12 and IL-10 in the cervicovaginal secretions of women with and without vaginal infections

Western Blotting of immunoglobulins

Twenty five samples of CVS (10 controls and 15 BV positive) were concentrated ten fold by lyophilization, electrophoresed on 10% SDS-PAGE and blotted on to PVDF membrane (Millipore). The membrane was blocked with 3% BSA in PBST, washed and incubated with appropriate goat anti human primary antibodies: IgM (1:300) and IgA (1:200). This was followed by washing and incubation with anti goat HRPO conjugate (1: 5000) (Bangalore Genie,India). Immunoglobulins and their degradation products were visualized using DAB (8 mg in 10ml of PBS : pH 7.2) and 5 ml of 30% H_2O_2 .

Measurement of Cytokines by ELISA

Cytokines : IL 12 and IL10 were assayed in samples of CVS, using Elipair reagents (Diaclone Research, France) as per manufacturer's instructions. Monoclonal antibody specific for each cytokine was used as the capture antibody while biotinylated antibody was the detection antibody.

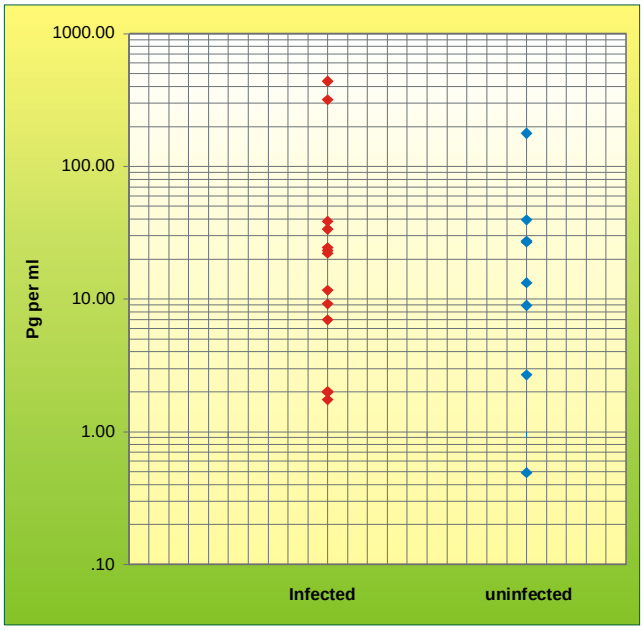
HRP-streptavidin was used for color development. The absorbance was read at 450 nm in an ELISA reader (Lab systems). All samples were run in duplicates and the cytokine concentrations read from the standard curve. The lower limit of detection was 12.5 pg / ml for IL10 and 6.25 pg / ml for IL 12.

Results

Cytokine levels in CVS : IL 12 was not detectable in any of the CVS samples, while IL 10 was detectable in the CVS of about 60% of women with or without vaginal infections. However, higher means and medians were seen in women with infection than those in normals (Figure 1). The values were 71.7 ± 138.9 and 37.09 ± 58.3 pg/ml (mean + SD) in infected and uninfected women respectively and the ranges were 1.7 - 438.9 and 0 .49 - 177.6 pg / ml respectively.

IgM/IgA degradation by Immunoblotting : Among the ten uninfected samples tested only 2 and 3 of them respectively showed degradation of IgA and IgM, whereas of the fifteen BV positive samples tested 15 and 13 samples showed t he degradation of IgA and IgM respectively. Degradation of Ig A and Ig M was also observed in two CVS samples with Nugent score 9 -10.

Figure 1: IL10 levels in the CVS of Indian LSES women



Summary

1. IL 12 (type 1) was not detectable in any of the samples while IL – 10 (type 2) was detected in both the groups, with higher means and medians in the infected women.

IL10 response seen in our women with BV in the present study, with no detectable IL-12, could to be due to the associated anemia and vitamin A deficiency in them. However, studies on a larger sample are required to confirm this.

2. There was a markedly higher degradation of IgA and IgM in the CVS of women with BV as compared to those without BV. The high prevalence of vaginal infection (BV) in these women reported earlier (Annual report 2001-2002) makes these findings significant in view of the mechanism suggested that mucosal impairment due to degradation of Ig A / M may increase HIV transmission in women with BV.

III. MICRONUTRIENTS AND TRACE ELEMENTS

1. *Studies on fortification of wheat flour with iron and folic acid*

Anemia continues to be a public health problem in India and the dietary iron intake is reported to be half of the recommended value. Food fortification is considered as a long-term strategy for alleviating iron deficiency. Selecting whole-wheat flour (atta) as the vehicle (as per PFA specification), the present study was taken up with an aim to find a suitable chemical source of iron in combination with folic acid as fortificant.

Methods

Five potential compounds of iron (anhydrous ferrous sulphate, ferric pyrophosphate, ferrous fumarate, hydrogen reduced iron powder and sodium iron EDTA) were taken with and without equimolar Na_2EDTA (as absorption promoter) to fortify wheat flour at two concentrations of 25 mg/kg and 50 mg/kg. Anhydrous $FeSO_4$ was also tested in combination with SHMP (1%). Fortified wheat flour stored at two conditions of high (35^0C) and normal (24^0C) temperature, with 50% humidity were monitored for organoleptic properties for a period of four months. Typical meals prepared with the fortified and unfortified flour were tested for in vitro iron availability. Two best sources of iron were selected and tested for folic acid fortification at 220 mg/kg. Food products, puri, chapathi and phulka prepared using these flours were subjected to organoleptic evaluation. Storage study and *in vitro* availability studies were repeated as above. An *in vivo* bioavailability of iron using the double sequential method with radioactive sources was carried out in 8 healthy adult male human volunteers.

Results

All the combinations of fortified wheat flour showed insignificant organoleptic change and rancidity compared to the unfortified sample during four month of storage. Addition of SHMP to FeSO4 fortified flour imparted unpleasant taste. In vitro iron availability with FeSO4 (11.7%), NaFeEDTA (10.6%) and H-reduced iron powder (10%) were comparable. Inclusion of Na2EDTA significantly enhanced the availability of iron from FeSO4 (23.2 %) followed by H-reduced iron powder (18.5%). Organoleptic characteristics and acceptability of food items prepared with flour fortified with these two iron sources and folic acid were rated as comparable to unfortified flour. Mean *in vivo* bioavailability of iron from Indian bread (chapathi) prepared with FeSO4 and H-reduced iron powder as iron source and folic acid fortified *atta* was1.63±0.84 and 0.5±0.19%, respectively.

Conclusions

Indigenous sources of anhydrous ferrous sulphate and H-reduced iron powder are good chemical sources for whole wheat flour fortification. Inclusion of equimolar concentration of sodium EDTA in iron fortified wheat flour enhanced *in vitro* availability two times. The *in vivo* iron bioavailabilty with H-reduced iron powder is three times less than that with ferrous sulfate.

In vitro availability of iron from iron fortified meal (n=4)

Fortificant	Percent in vitro availability		Relative availability
	Range	Mean ± SD	
FS	10.7 - 13.2	11.7^{be} ± 1.13	1.00 ± 0.09
FS + SHMP	8.7 - 10.8	9.5^{c} ± 0.93	0.81 ± 0.07
FS + Na_2 EDTA	20.6 - 25.6	23.2^{a} ± 2.28	1.98 ± 0.17
Na - Fe - EDTA	8.2 - 12.7	10.6^{bce} ± 1.89	0.90 ± 0.42
FF	7.6 - 10.1	9.1^{c} ± 1.12	0.78 ± 0.08
FF + Na EDTA	11.6 - 12.7	12.0^{e} ± 0.52	1.02 ± 0.04
HRIP	8.9 - 11.3	10.0^{bc} ± 1.02	0.86 ± 0.08
HRIP + Na_2 EDTA	17.2 - 20.1	18.5^{f} ± 1.10	1.58 ± 0.09
FPP	6.4 - 8.7	7.3^{d} ± 1.08	0.62 ± 0.07
FPP + Na_2 EDTA	10.2 - 12.1	11.4^{be} ± 0.93	0.97 ± 0.07
Basal diet	4.3 - 6.3	5.7^{d} ± 0.95	0.49 ± 0.07

MEANS WITH DIFFERENT SUPERSCRIPT LETTERS ARE DIFFERENT AT P<0.05 BY ONE WAY ANALYSIS OF VARIANCE WITH POST HOC T-TEST.

FS - FERROUS SULPHATE, DEHYDRATED
SHMP - SODIUM HEXA META PHOSPHATE
NA2 EDTA - Disodium Ethylene Diamine Tetra Acetate
Na-Fe-EDTA - Sodium Iron EDTA
FF - Ferrous Fumarate
HRIP - H-reduced iron Powder
FPP - Ferric Pyro Phosphate

2. *Iron absorption promoters in fortification of edible salt*

The impact on anaemia with the existing formulations of double fortified salt (DFS) was not striking as with prophylactic supplement. Therefore, there is a need for finding out an inexpensive salt for (1) the simultaneous fortification of iron and iodine, so that the stability and bioavailability respectively of iodine and iron are good and (2) fortification with compounds such as Na_2EDTA and SHMP.

The objective of the study was to identify an inexpensive quality salt for iodine fortification which can be doubly fortified with or without a stabilizer either in the presence or absence of an iron salt so that iodine and iron are stable and bioavailable.

Based on the recommendations of the experts the project objective was modified to study the stability of iodine and *invitro* bioavailability of iron from DFS prepared and with the following three modified formulations

Methods

DFS containing the following formulations were prepared with 4 types of salt viz.; ordinary common salt with relatively high moisture (2.7%) content (Bull brand, BB), good quality common salt (CS), TATA medium (TM) and TATA high quality salt (TH).

1. NIN-DFS with ferrous sulfate hepta hydrate, potassium iodate and SHMP of 67% P_2O_5 content

2. MI-DFS a premix (15 % iron and 1.06% I) of encapsulated ferrous fumarate and encapsulated potassium iodide, and

3. ILSI-DFS with encapsulated ferrous sulfate (Ferrous sulfate content 48 - 52 %, Balchem encapsulates) and potassium iodate

All the combinations were stored at different temperatures/humidity and checked for iodine stability at 3 and 6 months. The solubility, pH, colour and organoleptic evaluation of fortified salt was tested and compared against unfortified salt. The *in vitro* bioavailability of iron from DFS was also tested.

Results

The initial iodine content in NIN DFS with BB was only 13 ppm and that of all the MI DFS combinations ~80 ppm as aginst 40-50ppm with ILSI DFS. Initial iron content in all the samples was ~ 1000 ppm except in MI DFS combinations which provided ~ 518 -784 ppm.

Stability study

All the combinations had acceptable color on visual rating at the end of 3 and 6 months. However, the MI combination showed optical densities in the range of 0.22-0.26. The rest of the combinations had OD in the range of 0.01-0.1. A 5% solution of NIN DFS resulted in clear solution while ILSI and MI DFS showed lightly colored sediments. The pH of NIN salt formulations was in a narrow range of 3.4-3.8, while the pH ranges for ILSI and MI formulations were 5.7-9.4 and 6.0-9.1, respectively. NIN formulation was more acidic compared to the rest of the combinations. In any combination of salts prepared, TH - DFS yielded higher pH.

The loss of iodine in NIN DFS with BB was highest (iodine level of 1.1-3.2 ppm) followed by NIN DFS with CS, while NIN- TM & NIN-TH had iodine more than 15 ppm. All combinations of MI DFS retained iodine to the initial level of 80 ppm while that with ILSI DFS (encapsulated ferrous sulfate) was around 50 ppm and was close to their initial levels. All the combinations retained the initial level of iron at the end of 3 and 6 months. The *in vitro* iron availability from wheat and rice meals containing DFS respectively were 8.45±0.30 and 10.43±0.42 for NIN, 7.63±0.52 and 9.9±0.79 for MI, 8.95±0.41 and 10.26±0.48 ILSI formulation.

Conclusions

Encapsulated source of iron (ILSI & MI) was found to provide iodine stablity in double fortified ordinary common salt while NIN formulation requires high quality salt for providing adequate iodine content of 15 ppm in DFS. All of them had similar *in vitro* iron availability.

Summary of iodine stability of iron and iodine fortified salt

FORMULATION	Iodine stability (ppm)	
	3 month	6 month
NIN DFS		
BB	3.2	3.2
CS	23.8	19.1
TM	39.6	36.5
TH	42.8	40.5
ILSI - DFS		
BB	49.2	40.7
CS	46.6	43.3
TM	52.9	43.4
TH	54.5	50.8
MI DFS		
BB	76.8	75.6
CS	84.5	84.2
TM	81.8	78.4
TH	84.1	81.5

3. Iron and zinc interactions at the site of absorption in rats

The similarities in some of the chemical and biochemical properties of iron and zinc assume importance in the context of their combined supplementation when the deficiencies of these two micronutrients coexist. We tested the hypothesis that iron and zinc interacts at the site of absorption and impair their intended impact during repletion during deficiency. The following specific objectives have been completed:

1) To assess the sensitivity of intestinal cytosolic aconitase (E.C.4.2.1.3) activity to iron and zinc.

2) To measure the oxidant and antioxidant status during repletion of iron and zinc during iron deficiency.

Methods

i. Sensitivity of intestinal aconitase to iron and zinc

This was tested in iron deficient male WNIN rats (n=18) orally repleted with (n=6) 100, 190, 370 mg of iron/day for 4 wk. Blood hemoglobin (cyanmethemoglobin), serum ferritin (sandwich ELISA) and the duodenal cytosolic aconitase activity (coupled assay isocitrate dehydrogenase reaction monitored at 340 nm for 1h) was measured at the end of repletion period. *In vitro* effect of zinc (0-40 mM) on c-aconitase was evaluated in stock diet fed colony WNIN rat.

ii) The biochemical effects of repletion of iron and zinc during iron deficiency

Twenty four iron-depleted female WKY rats were randomly divided into three groups each of eight animals. They were then fed by gavage either 8 mg of Fe alone (+Fe) or 6.6 mg of Zn alone (+Zn) or a combination of Fe and Zn (+Fe+Zn) for 15 days.

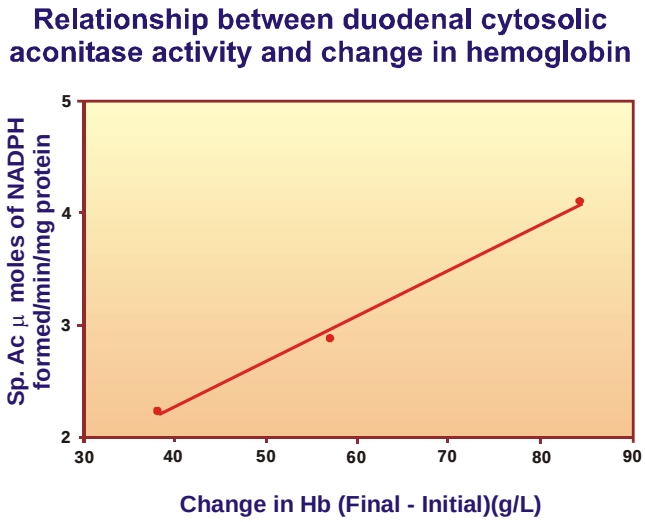
Serum TBARS, ceruloplasmin and a-tocopherol and metallothionein (MT) and protein oxidation and MT in intestinal mucosa (12000g fraction) were estimated. Intestinal Cu, Zn-superoxide dismutase, Mn-SOD (100000 g) and glutathione peroxidase and catalase activity (12000 g) were also measured. Intestinal and serum MT levels were determined by immuno-precipitation followed by western blot analysis using rabbit polyclonal antibodies. One-way ANOVA with post-hoc multiple comparison tests of significant differences among groups were determined.

Results

i) Sensitivity of intestinal aconitase to iron and zinc

Oral repletion with iron resulted in a dose dependent increase in the activity of the duodenal cytosolic aconitase. There was a high degree of linear correlation between the increment in

hemoglobin (r=0.6453) / mucosal ferritin (r=0.8441) and the specific activity of the duodenal cytosolic aconitase (Figure). *In vitro* studies showed that addition of zinc lowered the aconitase activity. The zinc inhibition of c-aconitase was observed to be competitive with a K_i (app) of 28 mM.



ii) Effects of repletion of iron and zinc during iron deficiency on oxidant and antioxidant status

New findings reported are: (i) combined supplementation lowered both iron and zinc status and resulted in better functional integrity of the duodenum compared to individual supplemented groups; (ii) reduction in levels of serum TBARS and duodenal mucosal protein carbonyls in +Fe+Zn compared to +Fe group (Table-1 and Figure); (iii) cytosolic and mitochondrial aconitase activity of duodenal mucosa lowered in +Fe+Zn compared to +Fe group; (iv) activity of antioxidant enzymes (total SOD, mitochondrial Mn-SOD, Gpx, catalase) of duodenal mucosa lowered in +Fe+Zn and +Zn compared to +Fe group; (v) +Fe+Zn and +Zn groups showed higher serum tocopherol and serum and intestinal metallothionein concentration (vi) +Fe+Zn showed lowered ferroxidase activity of serum ceruloplasmin compared to +Fe group.

Fig-2. Serum TBRS and intestinal protein carbonyl during iron and zinc repletion in iron deficient rat

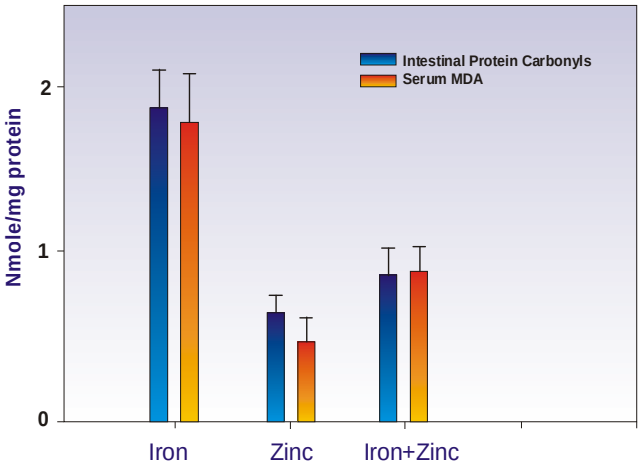


Table 1. Concentrations of iron and zinc in the liver and serum after treatment of the iron deficient rats with doses of iron, zinc and iron+zinc

Group	Liver iron (μg/g wet weight)	Liver zinc (μg/g wet weight)	Serum zinc (μmole/L)
+Fe	220.6^{a} 8.2	30.0^{a} 2.8	24.4^{a} 1.6 (n=7)
+Zn	41.6^{b} 3.7	61.4^{b} 6.3	28.2^{b} 1.4 (n=8)
+Fe+Zn	185.4^{c} 8.6	42.4^{c} 3.0	26.2^{c} 1.3 (n=7)

Conclusions

Intestinal cytosolic aconitase is sensitive to changes in intake of iron and can be competitively inhibited by zinc. Combined supplementation of iron and zinc can reduce the oxidative stress caused due to excess iron during oral repletion in iron deficient rats.

1. *Influence of long-term dietary restriction on chaperone function of α-crystallin and aggregation of β-γ-crystallins in rat lens*

Long-term restriction of calorie intake extends mean and maximum life span of a variety of species and is associated with retardation of many age-related debilities icluding cataract formation in rodents. It is believed that postponement of cataractogenesis by dietary restriction is in part because of improved antioxidant status and/or enhanced protein editing. However, most of the studies so far have focussed on the ultimate change in the lens i.e protein insolubilization/ precipitation in cataract. Eye lens proteins remain in situ for the lifetime of the organism and are subject to extensive posttranslational modifications such as tryptophan oxidation, increased protein-bound browning compounds, fluorophores and protein cross-links finally leading to accumulation of large amounts of insoluble protein. A decreased chaperone-like activity of α-crystallin during aging has also been demonstrated. In this study, we have investigated how long-term diet-food, protein and vitamin- restriction influences the aggregation pattern of crystallins vis a vis α-crystallin chaperone activity in the clear normal rat lens in relation to cataractogenesis.

Methods

Thirty day old Wistar/NIN rats were maintained on (i) regular rodent diet (C), (ii) 50% food restriction (FR), (iii) 75% protein restriction (PR) and (iv) 50% vitamin restriction (VR) diet for 20 weeks. At the end, α-, β- and γ-crystallins were isolated from the lenses of these animals by gel filtration chromatography and subjected to *in vitro* aggregation induced by oxidation, UV irradiation and heat. Aggregation and chaperone activity was assessed by light scattering methods.

Results

(i) Lenses of all the animals were clear and transparent during the course of the study as monitored by slit-lamp biomicroscope.

(ii) The aggregation of β_H-, β_L- and γ-crystallins upon incubation with oxidation reaction mixture for 24 h is significantly less in FR and PR groups (68 and 58% respectively) compared to C group. Aggregation of these crystallins isolated from VR group, however, is markedly higher (132%) than C group (Figure 1). No measurable aggregation was found with α_H- and α_L-crystallins by oxidation.

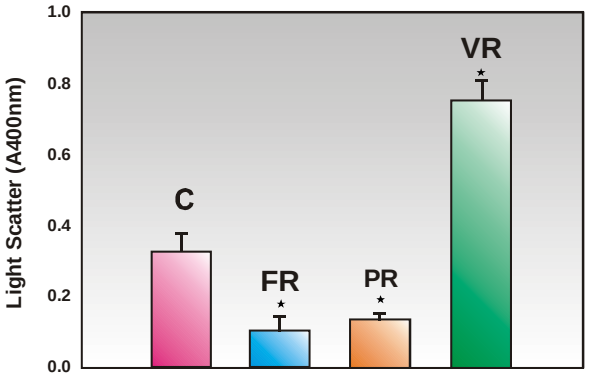


Figure 1. Oxidation-induced aggregation of β_L-crystallin. 0.5 mg/ml β_L-crystallin from control (*C*), food restriction (*FR*), protein restriction (*PR*) and vitamin restriction (*VR*) groups was incubated in oxidation buffer at 37°C for 24 h. Light scattering due to aggregation was monitored at 400 nm. Data are mean ± S. E. of three separate lens extracts each time in duplicates. *Denotes significantly different from control group ($P < 0.05$).

(iii) UVB-induced aggregation of β_H-, β_L- and γ-crystallins isolated from FR and PR groups is less but slightly higher in VR group compared to C group.

(iv) β_H-, β_L- and γ-crystallins isolated from FR and PR groups have shown significantly lesser aggregation due to heat (at 65°C) compared to C group, whereas it was substantially more in VR group.

(v) Food and protein restriction for 20 weeks did not affect the chaperone activity considerably under native conditions with insulin as substrate. Interestingly, vitamin restriction appears to have improved α-crystallin chaperone activity (Figure 2A).

(vi) Chaperone activity of α-crystallin isolated from FR and PR groups showed a marginal decrease compared to C group against heat-induced aggregation of β_L-crystallin. However, the improved chaperone function of α-crystallin due to vitamin restriction is further confirmed in β_L-crystallin heat-induced aggregation assay (Figure 2B).

Figure 2A. The chaperone activity of α-crystallin as assessed by the suppression of DTT-induced aggregation of insulin B-chain. Aggregation of insulin B-chain in the absence (*trace 1*) or in the presence of 0.5 mg/ml α-crystallin isolated from control (*trace 2*), food restriction (*trace 3*), protein restriction (*trace 4*) and vitamin restriction (*trace 5*) group was monitored by measuring apparent absorption at 400 nm. Data are representative of three such independent assays for three separate lens extracts.

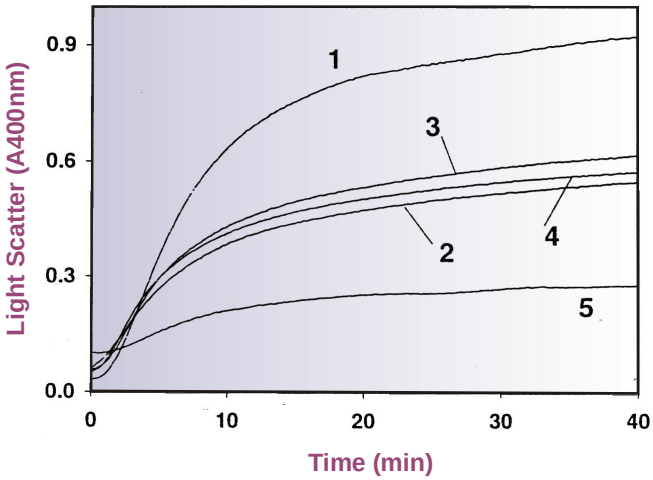
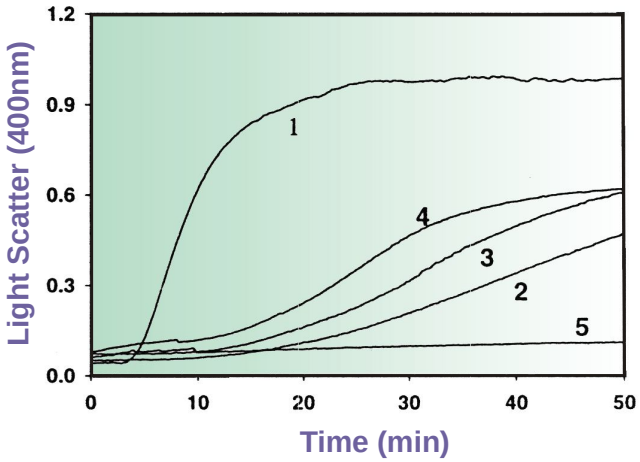


Figure 2B. The chaperone activity of α-crystallin as assessed by the suppression of heat-induced aggregation of β_L-crystallin. β_L-crystallin (0.3 mg/ml in 50 mM phosphate buffer, pH 7.2) was incubated at 65°C in the absence (*trace 1*) or in the presence of 0.5 mg/ml α-crystallin isolated from control (*trace 2*), food restriction (*trace 3*), protein restriction (*trace 4*) and vitamin restriction (*trace 5*) group. Light scattering due to β_L-crystallin aggregation was monitored by measuring apparent absorption at 400 nm. Data are representative of three such independent assays for three separate lens extracts.



Conclusions

These results indicate that while food and protein restriction appear to lower the susceptibility of β- and γ-crystallins towards aggregation, vitamin restriction tends to increase the aggregation. Chaperone activity of α-crystallin is affected (improved) by only vitamin restriction and studies are underway to elucidate the mechanism of increased chaperone activity due to vitamin restriction.

2. Characterization of a new model substrate for investigating the molecular chaperone-like function of α-crystallin

The eye lens small heat shock proteins (sHSP), αA- and αB-crystallins, function like molecular chaperones, both *in vitro* and *in vivo*. In view of the suggested physiological roles of α-crystallin, a variety of substrates and methods have been used in studying the mechanism of its chaperone function of α-crystallin. Except insulin assay, in many aggregation assays, α-crystallin is shown to undergo some structural perturbation due to heat, chemical (like guanidine hydrochloride) or physical (UV light) factors. It is therefore essential

to assess the protective effect of αA- and αB-crystallins under native conditions to enable extrapolation of the results to *in vivo* conditions. Insulin aggregation assay has advantages in that it can be aggregated under native conditions by reducing the disulfide bridges with DTT. However, extrapolating the mechanism(s) based on the binding of insulin B-chain to α-crystallin may not be appropriate due to its small size (3 kDa). Also, many, if not all, putative physiological substrate proteins of α-crystallin are likely to be relatively larger in size (at least to the order of about 7-10 times the insulin B-chain). Therefore, the aim of this study was to characterize a new substrate for understanding the mechanism of chaperone function of α-crystallin and other sHSP under native conditions.

Abrin, a ribosome inactivating protein present in the seeds of *Abrus precatorius*, consists of A chain (30 kDa) and B chain (33 kDa) joined by a single disulfide bond. Reduction of the disulfide link between the two chains of abrin leads to the aggregation of the B-chain. We have investigated whether DTT-induced aggregation of abrin can be suppressed by α-crystallin and if so its utility as a model aggregation assay to understand the chaperone function of α-crystallin.

Methods

(i) Purification of abrin: The protein was purified from the seeds of *Abrus precatorius*. Briefly, the seed were homogenized (10%) in PBS and the crude extract was subjected to 30 and 90% ammonium sulfate precipitation followed by extensive dialysis. The dialysate was loaded onto the Lactamyl-Sepharose affinity column. The bound proteins were eluted with 0.4 M lactose. The lactose fractions were further purified by gel filtration. The fractions corresponding to peak II were pooled, dialyzed extensively and protein concentration was determined using its molar extinction coefficient 100170 M^{-1} cm^{-1}.

(ii) Overexpression and purification of human recombinant αA- and α B-crystallins: Proteins from 1 litre bacterial cultures (BL21 cells containing expression vectors of human αA- and αB-crystallin) were extracted and purified to apparent homogenity according to the procedures described previously (Reddy *et al*, *J. Biol. Chem.* **275**, 4565-4570, 2000). HSP26

protein was a gift from Prof. J. Buchner, Technical University Munich, Germany.

(iii) Abrin aggregation and suppression by αA- and αB-crystallins and HSP26: The aggregation of abrin was initiated by the addition of 30 mM DTT. The extent of B-chain aggregation was measured as a function of time by monitoring light scattering at 400 nm. The suppression of aggregation of abrin B-chain by αA- and αB-crystallins and HSP26 was studied by incubating abrin with the required concentrations of either αA- or αB-crystallin or HSP26 for 10 min. Aggregation was initiated by the addition of 30 mM DTT and the extent of aggregation was measured as mentioned above.

(iv) Gel Filtration: The formation of complex between α-crystallins or HSP26 and the B-chain of abrin was studied by gel filtration chromatography on a 600 x 7.5 mm TSK-G2000 SW column.

Results

(i) Reduction of the disulfide link between the two chains of abrin by DTT leads to the aggregation of the B-chain.

(ii) DTT-induced aggregation of abrin B-chain could be monitored by light scattering similar to that of insulin.

(iii) Importantly, this process could be suppressed by recombinant human αA- and αB-crystallins in a concentration dependent manner (Figure 1), notably by binding to aggregation prone abrin B-chain.

(iv) SDS-PAGE and HPLC gel filtration analysis indicate that there is a soluble complex formation between α-crystallin and abrin B-chain.

(v) Interestingly, in contrast to insulin, there was no significant difference between αA- and

αB-crystallin in suppressing the aggregation of abrin B-chain at two different temperatures (25 and 37 °C) (Figure 2).

(VI) HSP26, an another small heat shock/α-crystallin family protein, was also able to prevent the DTT-induced aggregation of abrin (Figure 2).

Figure 1. DTT-induced aggregation of abrin B-chain and suppression by αB-crystallin at 25°C. Abrin was reduced with 30 mM DTT and the aggregation of the B-chain in the absence (*1*) and presence of 0.10 (*2*), 0.20 (*3*), 0.30 (*4*), 0.40 (*5*), 0.5 (*6*) and 0.60 mg/ml of αB-crystallin (*7*) was monitored by measuring the apparent absorption at 400 nm. The graph is a representative plot of the three individual experiments. Similar results were observed with αA-crystallin(Fig 2).

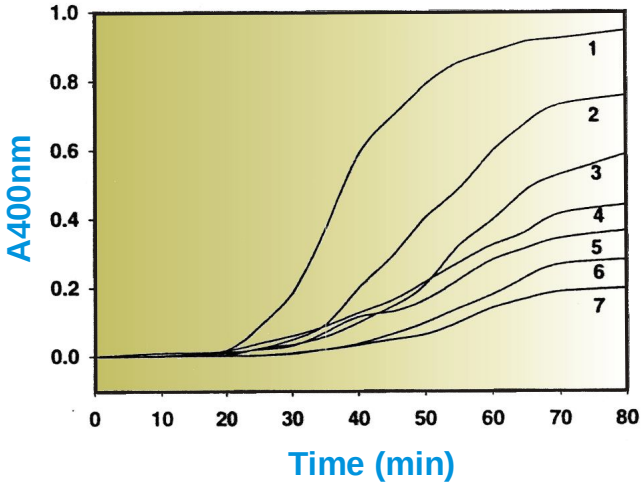
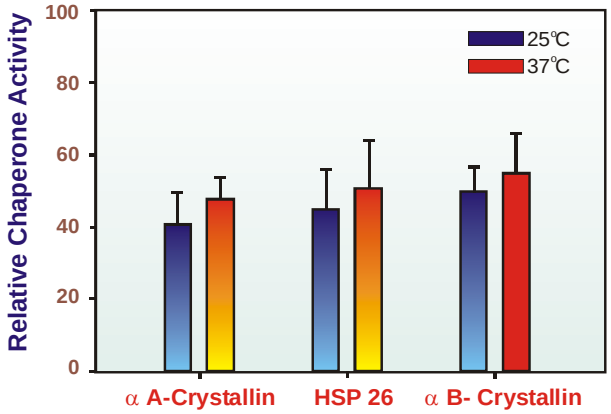


Figure 2. The relative chaperone activity of αA- and αB-crystallin and HSP26 against the aggregation of abrin B-chain at 25 and 37°C. Abrin was reduced with 30 mM DTT in the presence of 0.50 mg/ml of αA-, αB-crystallin or HSP26 and the aggregation was monitored by measuring the apparent absorption at 400 nm. Data represents mean ± S.D. of three independent experiments.



Conclusions

These results suggest that due to relatively larger size of its B-chain (33 kDa), compared to insulin B-chain (about 3 kDa), abrin may serve as a better model substrate for the chaperone studies of α-crystallin and as well as other sHSP. Studies are underway to investigate the effects of various posttranslational modifications on the chaperone function of α-crystallin using abrin aggregation assay in comparison with classical aggregation assays (*e.g.* insulin).

3. Effect of dietary trans fatty acids on insulin resistance, structure and function of adipocytes in rats

Insulin resistance is a characteristic feature of several diet-related chronic diseases such as type 2 diabetes, obesity and hypertension. Both genetic and environmental factors are linked to the development of insulin resistance. Insulin resistance is characterized by hyperinsulinemia dyslipidemia, impaired insulin stimulated glucose uptake in skeletal muscle and adipose tissue and impaired insulin suppression of hepatic glucose production and adipocyte lipolysis. The physical state of membrane lipids (membrane fluidity) is determined by membrane lipid composition, which in turn affects insulin binding and subsequent metabolic responses. It is well known that dietary fatty acids influences both stored lipids (triglycerides) and membrane lipids. It has been shown that skeletal muscle and adipose tissue insulin sensitivity is determined by the phospholipid fatty acid composition. Saturated fatty acids (SFA)

have detrimental effect whereas long chain polyunsaturated fatty acids (LCPUFA) potentiate insulin action. Human studies have shown that although SFA and trans fatty acids (TFA) increase LDL cholesterol, TFA decrease HDL cholesterol and therefore TFA may be more atherogenic than saturated fatty acids. TFA have also been shown to inhibit conversion of linoleic acid (18:2n-6) to LCPUFA, which can be prevented by increasing the dietary level of 18:2n-6.

TFA are formed during partial hydrogenation of vegetable oils and vanaspati contains high level of TFA. In recent years there has been marked increase in intake of TFA due to increased consumption of bakery products and fast foods, which are prepared using vanaspati.

Aims and objectives

To investigate the effect of vanaspati (TFA) at different levels of 18:2 n-6 (2en% and 4en%) on adipose tissue and skeletal muscle lipid composition, fluidity and insulin action in rats.

Methodology

Earlier the effect of dietary TFA (vanaspati) on structural properties of skeletal muscle and adipose tissue was studied at 2en% and 4en% of 18:2n-6 (Ann. Rep. 2000-2002). The results showed that TFA feeding significantly altered plasma triglycerides and cholesterol, adipocyte and skeletal muscle lipid composition. The observed changes were irrespective of 18:2n-6 level in the diet.

During the year, an animal experiment was carried out to study the effects of dietary TFA on adipocyte and skeletal muscle insulin action. 32 weanling WNIN rats were divided into four groups and fed casein-based diet containing 10% fat. Vanaspati was used as the source of TFA.

Group I	Groundnut oil (8en% 18:2n-6 & 4en% saturated fattyacids (SFA)
Group II	Palm oil (2en% 18:2n-6 & 10.5en% SFA)
Group III	Vanaspati + Safflower oil (2en% 18: 2n-6, 3en% TFA & 8.6en% SFA)
Group IV	Vanaspati + Safflower oil (4en % 18: 2n-6, 3en% TFA & 8en% SFA)

Comparison of group I vs II gives the effects of increasing SFA and decreasing PUFA/SFA ratio, group II vs III & IV gives the effects of partial replacement of SFA with TFA at two levels of 18:2n-6 in the diet.

After three months of feeding, blood was collected and animals were sacrificed. Epididymal fat pads and skeletal muscle samples were removed. Adipocytes were isolated by collagenase digestion method and adipocyte plasma membrane was isolated by density gradient centrifugation. The following parameters were studied.

1. *Plasma glucose and insulin after OGTT.*
2. *Plasma triglycerides and cholesterol.*
3. *Adipocyte number and size.*
4. *Norepinephrine-induced adipocyte lipolysis.*
5. *Antilipolytic effect of insulin.*
6. *Adipocyte glucose transport.*
7. *Skeletal muscle (diaphragm) glucose transport.*

The data was analyzed by one-way ANOVA and the results are as follows:

1. Fasting plasma glucose and AUC of glucose after OGTT were similar in all the groups. Compared to groundnut oil, vanaspati and palm oil feeding increased plasma triglyceride and insulin levels. Increasing dietary 18:2n-6 in vanaspati fed groups did not reverse the above changes. Vanaspati and palm oil feeding increased AUC of insulin after OGTT but the values were not significant statistically. Vanaspati feeding decreased plasma cholesterol compared to groundnut oil and palm oil fed groups.

2. Adipocyte cell number and size were similar between the groups.

3. Basal lipolysis was similar in all groups. However norepinephrine stimulated lipolysis was increased in vanaspati and palm oil fed groups compared to groundnut oil group.

4. Compared to groundnut oil, palm oil and vanaspati fed groups showed a decreased sensitivity to antilipolytic effect of insulin which was more marked in vanaspati fed groups

irrespective 18:2n-6 in the diet.

5. Basal adipocyte glucose uptake was similar in all groups. Insulin stimulated glucose uptake was lower in palm oil and vanaspati fed groups compared to groundnut oil fed group. However the decreased glucose transport was more marked in vanaspati fed groups irrespective 18:2n-6 in the diet.

6. Basal skeletal muscle glucose uptake was similar in all groups. Compared to ground nut oil and palm oil fed groups, TFA fed groups showed diminished insulin stimulated glucose uptake irrespective of 18:2n-6 in the diet.

The results of the present study confirm that dietary TFA and SFA causes insulin resistance as evidenced by increased plasma insulin and triglycerides. Both SFA and TFA altered adipocyte insulin sensitivity as evidenced by impaired insulin stimulated glucose transport and antilipolytic effect of insulin. However TFA have more deleterious effect on adipocyte

insulin sensitivity than SFA. Increasing 18:2n-6 in the diet did not prevent the deleterious effects of TFA. These studies therefore suggest that replacement of part of the dietary visible fat with vanaspati may contribute to insulin resistance, which in turn increases the risk of diet related chronic diseases. However studies are needed to evaluate the contribution of TFA (vanaspati) to increased prevalence of type 2 diabetes and its sequelae in Indian population.

4. Biochemical and metabolic studies with sesame lignans

The higher oxidative stability of sesame oil is attributed to the lignans (sesamol, sesamin and sesamolin) The antioxidant properties of individual lignans were studied in *in vitro* enzymatic (cumene hydroperoxide) and non-enzymatic (iron-ascorbate) lipid peroxidation systems (Ann. Rep. 1996-2000). The results showed that though all the lignans inhibited lipid peroxidation in the enzymatic system, their effects were less marked than BHT and tocols. The inhibitory effects of sesamin or sesamolin may be either due to inhibition of cytochrome P450 (BBRC 2000, **227** 531) or due to oxidation of methylene dioxy groups by microsomes (Mol Pharmcol 1992, **42,** 695). Lignans in combination with varying levels of tocols showed enhanced inhibitory effects suggesting synergism with tocols. Further, the antioxidant potentiating effects of lignans was confirmed in rats fed sesame oil diets.

Since sesamin and sesamolin inhibited lipid peroxidation only in the enzymatic system (microsomes), the effects of these compounds was studied in microsomes using the non-enzymatic system. Liver microsomes pre-incubated with 40 or 100 nmoles of sesamin or sesamolin at 37^0C for 60 minutes, followed by induction of oxidative stress with Fe^{2+}-ascorbate or ABAP showed inhibition of lipid peroxidation whereas with boiled microsomes they did not inhibit lipid peroxidation. These observations corroborate our earlier findings that sesamin and sesamolin do not inhibit lipid peroxidation in rat liver mitochondria. Therefore, it appears that the metabolites of sesamin and sesamolin may confer antioxidant properties to these compounds. The mechanism of synergistic effects of lignans and tocols would pave way to better understanding of nutrient-non-nutrient interactions in the prevention of chronic diseases.

5. Effect of dietary alteration of n-6 and n-3 polyunsaturated fatty acids on insulin resistance, structure and function of adipocytes and skeletal muscle (DST)

Insulin resistance is a common metabolic abnormality associated with obesity, type 2 diabetes and hypertension. The major target tissues for insulin action are skeletal muscle and adipose tissue. Binding of insulin to the target tissues results in autophosphorylation of the receptor and activation of tyrosine kinase, which in turn phosphorylates several intracellular substrates resulting in transport of glucose. One of the possible mechanisms of insulin resistance could be altered insulin binding and signal transduction. The physical state of membrane lipids (membrane fluidity) is determined by membrane lipid composition, which in turn affects insulin binding and subsequent metabolic responses. It is well known that

dietary fatty acids influences both stored (triglycerides) and membrane lipids. It has been shown that skeletal muscle and adipose tissue insulin sensitivity is determined by the phospholipid fatty acid composition. Saturated fatty acids have detrimental effect whereas long chain polyunsaturated fatty acids (LCPUFA) potentiate insulin action. Since n-3 PUFA inhibits factors, which have atherogenic effects and increases factors, which have antiatherogenic effects, an optimal intake of n-3 PUFA (n-6/n-3 ratio) is recommended for reducing the risk of coronary heart disease.

Aims and objectives

To investigate the effects of increasing dietary 18:3n-3 or LCn-3 PUFA and therefore alterations of n-6/n-3 ratio (18:2n-6/18:3n-3 or 18:2n-6/LCn-3 PUFA) on adipose tissue and skeletal muscle lipid composition, fluidity and insulin action in i. diet induced (sucrose) and ii. genetic (WNIN/GR-Ob)-insulin resistant rat models.

Methodology

To study the effects of dietary increase in 18:3n-3 and therefore alterations in 18:2n-6/18:3n-3 ratio, two experiments (I & II) were conducted in diet (sucrose) induced insulin resistant rats.

Experiment I

Fifty six weanling WNIN rats were divided into four groups (14 animals in each group) and fed casein-based diet containing 10% fat. Insulin resistance was induced by replacing starch with sucrose (55% of total diet). Vegetable oil blends used as dietary fat source furnished: saturated fatty acids ~6 en%, monounsaturated fatty acids ~8.5en%, PUFA (18:2n-6 + 18:3n-3) ~ 6.7 en% and P/S ratio ~ 1.1. The ratio of 18:2n-6 and 18:3n-3 in various groups are as follows:

Group I : Starch (18:2n-6/18:3n-3 ratio = 200)
Group II : Sucrose (18:2n-6/18:3n-3 ratio = 200)
Group III : Sucrose (18:2n-6/18:3n-3 ratio = 10)
Group IV : Sucrose (18:2n-6/18:3n-3 ratio = 2)

After three months feeding, blood was collected after overnight fasting and animals were sacrificed. Epididymal fat pad and skeletal muscle (diaphragm) were removed. Adipocytes were isolated from epididymal fat pads and plasma membrane was prepared from adipocytes by density gradient centrifugation. The results on food intake, body weight gain, epididymal fat weight, plasma parameters insulin, glucose, triglyceride and cholesterol were reported (Ann. Rep. 2001– 2002). The results showed that compared to starch, sucrose feeding induced insulin resistance as evidenced by increase in plasma insulin, area under the curve for insulin following OGTT and triglycerides. Decreasing 18:n-6/18:3n-3 ratio to 2 reversed the above changes. During the year the analysis of structural parameters of adipose tissue and skeletal muscle were done and the results are as follows:

The results were analyzed by one-way ANOVA.

1. Adipocyte plasma membrane phospholipid (P) content was similar in all groups. However cholesterol (C) content and cholesterol to phospholipid (C/P) molar ratio were higher in sucrose fed group compared to starch fed groups. Decreasing 18:2n-6/18:3n-3 ratio to 2 in sucrose fed groups decreased the membrane cholesterol and C/P ratio suggesting that sucrose induced alterations were reversed. Adipocyte plasma membrane phospholipid fatty acid composition was similar in all groups except that LC n-3 PUFA was high in groups fed 18:2n-6/18:3n-3 ratios 10 and 2.

2. Compared to starch, sucrose feeding decreased adipocyte plasma membrane fluidity. However decreasing 18:2n-6/18:3n-3 ratio to 2 in sucrose fed groups normalized the fluidity.

3. Skeletal muscle triglyceride content was similar in all groups.

4. The data on skeletal muscle phospholipid fatty acid composition showed that, decreasing dietary 18:2n-6/18:3n-3 ratio increased incorporation of LC n-3 PUFA (20:5n-3, 22:5n-3 and 22:6n-3) with a concomitant decrease in LC n-6 PUFA (20:4n-6).

Experiment II

To study the functional properties of adipocyte and skeletal muscle, 40 weanling WNIN rats were divided into five groups and fed casein-based diet containing 10% fat. The design and duration of the second experiment was same as experiment I expect that an additional group which furnished 18:2n-6/18:3n-3 ratio of 50 was included.

After three months feeding the following parameters were studied.

1. Adipocyte number and size.
2. Norepinephrine induced lipolysis.
3. Antilipolytic effect of insulin.
4. Adipocyte glucose transport.
5. Skeletal muscle (diaphragm) glucose transport.

The results were analyzed by one-way ANOVA.

1. Adipocyte number and size were similar in all the groups.

2. Both basal and norepinephrine induced - lipolysis were higher in sucrose fed group compared to starch fed group. Decreasing 18:2n-6/18:3n-3 ratio in sucrose fed group from 200 to 50,10, or 2 decreased both basal and norepinephrine - induced lipolysis to the same extent.

3. Sucrose feeding resulted in decreased adipocyte sensitivity to the antilipolytic effect of insulin. Decreasing 18:2n-6/18:3n-3 ratio in sucrose fed groups from 200 to 50, 10 or 2 significantly increased the adipocyte sensitivity to the antilipolytic effect of insulin to the same extent.

4. Basal glucose transport was similar in all groups. Compared to starch, sucrose feeding decreased insulin stimulated glucose transport. Decreasing 18:2n-6/18:3n-3 ratio in sucrose fed groups to 2 improved the adipocyte insulin sensitivity as evidenced by increased glucose transport.

5. Sucrose feeding did not alter skeletal muscle glucose transport. However there was a significant increase in insulin – stimulated glucose transport in sucrose fed group with 18:2n-6/18:3n-3 ratio of 2 compared with the groups fed dietary oils with higher ratio.

The results of the present study confirm that sucrose feeding induces insulin resistance as evidenced by increased plasma triglycerides, insulin, AUC of insulin after OGTT. Sucrose feeding also altered adipocyte plasma membrane lipid composition, fluidity and insulin sensitivity (glucose transport and antilipolytic effect of insulin). The above changes were reversed by decreasing the 18:2n-6/18:3n-3 ratio to 2 suggesting that sucrose induced insulin resistance can be prevented by decreasing 18:2n-6/18:3n-3 ratio to 2. Decreasing the ratio to 2 also improved skeletal muscle insulin sensitivity as evidenced by increased glucose transport.

6. Effect of copper and molybdenum on development of skeletal fluorosis in rabbits

Epidemiological studies show that prevalence of genu valgum in fluorosis endemic areas was significantly higher in the population. Among these, people with a staple diet of sorghum showed higher incidence of the disease as compared to those on a staple diet of rice (4% and 1% respectively). High molybdenum ingestion leads to secondary copper deficiency and copper is known to have a role in the maturation of collagen and it is also evident that sorghum contains higher amounts of molybdenum than rice. Also, copper deficiency leads to osteoporosis, in cattle, which habitually graze on copper deficient pastures or on pastures containing high molybdenum. Currently, it is not known whether the effect of molybdenum on copper is exaggerated during high fluoride intake or high fluoride coupled with copper deficiency in bone leads to genu valgum type of deformities.

Objective

To investigate the role of copper and molybdenum in the development of skeletal fluorosis in rabbits and also their interactions.

Methodology

Thirty rabbits aged between 2 to 3 months were grouped into 5 groups and casein based semi-purified diet was given for six months

Group	No of animals	Treatment
Control	6 No.	Basal diet + distilled water
Fluoride	6 No.	Diet + Fluoride (150ppm) water
Fluoride + Molybdenum	6 No.	Diet containing molybdenum (0.1%)+ Fluoride (150ppm) water
Fluoride-Copper	6 No	Diet devoid of copper + Fluoride (150ppm) water
Fluoride + Molybdenum + Copper	6 No	Diet containing molybdenum Copper (0.1%) + Fluoride 150 ppm water.

Biochemical Analysis

Fluoride content in urine, serum, and bone was estimated by ion selective electrode. Calcium, Cu, Zn, Mg and P in urine, serum and all bone and Mn content only in ribs was estimated by atomic absorption spectrophotometer. Body composition was analyzed using TOBIC Model SA 3000 COILID-3152 meant for small animals.

Results

Diet intakes in F+Mo group were significantly lower than all other groups. Weight gain, lean body mass (LBM) and body fat were lower in F+Mo group than all other groups. There were no significant differences in the hematological parameters (PCV, total leukocyte count TLC, Hb% and differential leukocyte counts DC) except lymphocytes which were significantly lower in F-Cu and F+Mo+Cu than other groups. ESR (1H) in F+Mo+Cu group was also found to be higher ($P<0.05$) than control group.

Percent ash content in different bone

Ribs

Per cent ash content in ribs was significantly low ($P<0.05$) in control group as compared to all experimental groups ($P<0.01$). Percent ash content in ribs of F group is significantly less ($P<0.05$) as compared to F+Mo+Cu group.

Per cent ash content in femur bone of control group was significantly lower ($P<0.01$) than F+Mo, F-Cu and F+Mo+Cu groups while that of F+Mo+Cu group was significantly higher ($P<0.01$) than all other groups.

Skull

Per cent ash content in control group was significantly lower ($P<0.05$) than F group as well as F+Mo and F+Mo+Cu ($P<0.01$). While in F+Mo+Cu group it was significantly higher than F group ($P<0.05$) and F–Cu group ($P<0.01$). Ash content in F+Mo was significantly higher ($P<0.05$) than F–Cu group.

Mineral content in bones

Ribs

Manganese (mg/g) content in ribs of control group was significantly less ($P<0.01$) than all other groups and thus there were no significant differences among the experimental groups. Phosphorus (mg/g) content in F+Mo+Cu group was significantly higher ($P<0.01$) than all other groups including control. Fluoride (mg/g) content in control group was significantly less ($P<0.01$) than all other groups. Whereas fluoride content in group F+Mo+Cu was significantly lower ($P < 0.05$) than F and F-Cu groups. There was no significant difference in Ca, Zn, Cu, and Mg among the groups.

Femur bone

Copper content (mg/g) in femur bone of group F+Mo F-Cu and F+Mo+Cu were significantly higher ($P<0.05$) as compared to control group. Magnesium (mg/g) in group F+Mo was significantly higher ($P < 0.05$) as compared to all other groups. Fluoride (mg/g) content in control group was significantly lower ($P < 0.01$) than F and F+Mo groups .It was also seen that Fluoride in F+Mo is significantly more ($P < 0.05$) as compared to group F+Mo+Cu group. There was no significant difference in Ca, Zn, Mg and P content between the groups.

Serum

Serum Cu content in control, F and F+Mo groups were significantly lower ($P<0.01$) than F-Cu group .As expected, copper content in F+Mo+Cu group was significantly higher ($P<0.05$) than control, F and F+Mo groups.Serum fluoride(mg/ml) levels in control group was significantly lower ($P<0.01$) than F, F+Mo, F-Cu,where as fluoride levels in F+Mo+Cu group was significantly higher ($P<0.01$) than all other groups. There was no significant difference between the groups in serum calcium, magnesium and zinc content.

Twenty four hour urinary excretion parameters

Urinary excretion of fluoride was significantly ($P < 0.01$) higher in all the groups as compared to control group and calcium was significantly higher ($P < 0.01$) in group F + Mo than control F and F-Cu groups. Calcium excretion was also significantly higher ($P < 0.05$) in group F+Mo+Cu as compared to control, fluoride and F-Cu groups.

Copper excretion through urine was significantly higher ($P < 0.05$) in group F+Mo+Cu as compared to F+Mo, F-Cu and control while excretion of hydroxyproline was higher ($P < 0.05$) in F and F-Cu groups as compared to control group.

There was no significant change in 24 hr. urinary excretion of zinc and magnesium in different groups.

Parathyroid hormone (PTH)

Parathyroid hormone (pg/ml) in control group was significantly lower ($P<0.01$) than group F+Mo and F+Mo+Cu .PTH in F+Mo group was significantly higher ($P<0.01$) than control, fluoride and F-Cu groups whereas significantly lower ($P<0.05$) than F+Mo+Cu group.PTH levels in F+Mo+Cu group was significantly higher ($P<0.01$) than control, fluoride, F-Cu groups.PTH levels in F-Cu group was significantly higher ($P<0.05$) than fluoride group.

Conclusions

Based on above results, it can be concluded dietary molybdenum, favors increase in fluoride deposition in bone and supplementation of copper along with molybdenum does not help in decreasing fluoride toxicity.

7. Effect of tamarind on mobilization of fluoride in fluoritic subjects

Skeletal fluorosis is widely prevalent in endemic areas of Andhra Pradesh, India and there are no known drugs or medicines to treat this disorder. Our earlier experiments in dogs and humans indicated a beneficial effect of tamarind ingestion by promoting increased urinary excretion of fluoride. Hence we undertook the present study on humans to examine the effect of tamarind on mobilization of bone fluoride in fluorosis endemic areas.

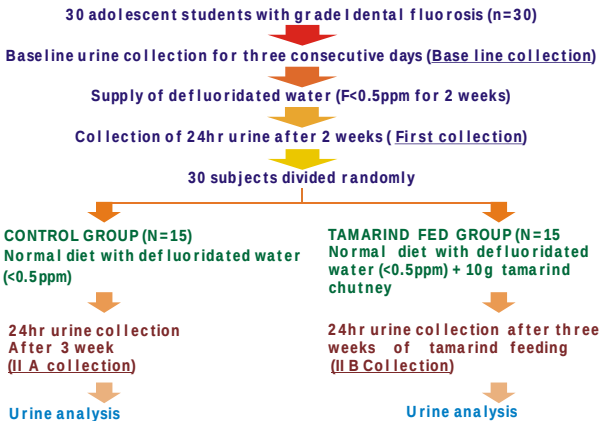
Methodology

Ten social welfare boys' hostels from Nalgonda district of Andhra Pradesh, India, were surveyed for fluoride levels in drinking water. All surveyed hostels had bore wells as the source of drinking water. In all hostels, the fluoride content in water was less than 2ppm while in Choutuppal hostel, fluoride content was 2.77ppm.The students of 8^{Th} and 9^{Th} class residing in social welfare boys hostel, Choutuppal, were screened for dental fluorosis. Though prevalence of dental fluorosis from grades I to III were seen, the students with grade I dental fluorosis only were selected for the experiment and to maintain uniformity.

After obtaining clearance from Institutional Human Experiment, ethics committee and informal consent of subjects, thirty healthy, male, adolescent children, aged 13.6 ± 0.5 years (mean ± SD) with Body Mass Index (BMI) of 17.10 ± 1.53 (in kg/m^2) and fat fold thickness of 9.2±2.64 mm were selected and recruited for the study after obtaining informed consent. All the selected subjects were clinically normal except for the presence of dental fluorosis. (Grade I).

Baseline urine collection was done for three consecutive days to get data in all 30 students. Defluoridated water (F< 0.5 ppm) for cooking and drinking was provided to the all students for 2 weeks and 24h urine was collected on the last day. This *first collection* was to get data after stabilization on defluoridated water. The students were then divided into two groups of 15 each. Control group received normal diet with defluoridated water for cooking and drinking for another 3 weeks. Tamarind fed group received normal diet with defluoridated water plus 10 g tamarind chutney every day and at end of 3 weeks, 24h urine collection was made and this second collection *(II A and II B collection*) was to find out the effect of defluoridated water and tamarind intake on urinary constituents, in the respective groups.

Study design



Sample Analysis

Fluoride content of water and urine samples was estimated using an Orion expanded ion analyzer and dietary as well as urinary calcium, magnesium and copper were estimated by atomic absorption spectrophotometer. Protein content and fat content of food was analyzed according to standard AOAC method. Carbohydrate and energy contents were calculated by standard procedures to get the total kilocalories of each food. Blood parameters were studied by standard procedures for estimation of Hb%, PCV, ESR, total leukocyte count and differential counts to know the general physical status of the subjects.

Results

Diet

The diet analysis data, indicates that the protein (43.8±4.76 g) and energy (1721.3±184.34 Kcal) intake of the subjects was less by 37% and 30% respectively and Ca intake (233.7 ± 25.34 mg) was 60% less than RDA.

Water

Calcium, Mg and Cu levels in drinking water of study area were very low and conductivity was very high when compared to municipal (normal) drinking water.

Hematological parameters

Hb%, PCV, ESR, total WBC count and differential counts were within the normal range.

Changes in urinary constituents before and after 2 weeks on defluoridated water ingestion (baseline and first collection)

The urinary constituents (total urinary volume, pH, F, Ca, Cu and Mg) of the subjects before and after two weeks on defluoridated water were measured and compared. There was significant decrease in urinary fluoride and magnesium ($P<0.01$) levels and also copper ($P<0.05$).

Urinary parameters were compared after randomly dividing the 30 students into two groups of 15 each. There was no difference in urinary constituents of two groups and thus they were homogeneous.

Changes in urinary constituents after defluoridated water ingestion for 5 weeks and defluoridated water for 2 weeks (first collection) and 5 weeks (II A collection)

There was significant reduction ($P<0.05$) in urinary pH and significant increase and copper excretion after defluoridated water ingestion for five weeks as against two weeks values. There was no change in urinary total volume , F, Ca and Mg levels on defluoridated water ingestion for 5 weeks as compared to values obtained after two weeks ingestion of defluoridated water.

Changes in urinary constituents after tamarind ingestion for 3 weeks (II A collection and II B collection)

After three weeks of tamarind ingestion there was significant increase ($P < 0.01$) in urinary pH and fluoride and significant decrease ($P < 0.01$) in calcium and copper ($P < 0.05$). There was no change in volume and magnesium as compared to controls (taking defluoridated water without tamarind supplements).

Conclusions

Tamarind intake appears to be helpful in mobilizing deposited fluoride from bone, by enhancing urinary excretion of fluoride when urinary fluoride was stabilized by giving fluoride free water.

8. Resistin : A molecular link between type 2 diabetes and obesity

Diabetes, a major cause of morbidity and mortality, is a consequence of loss of pancreatic β cell function leading to lack of insulin secretion and target tissue resistance to insulin. Insulin resistance and type 2 diabetes is a combination of genetic and environmental factors. Of the known risk factors namely high fat diet, insufficient exercise and obesity, the latter is the most common and extensively studied. Epidemiological observations suggest a strong patho physiological correlation of obesity to type 2 diabetes, however, the molecular link between these two conditions remained to be solved. Recently, resisitn, a cysteine rich secretory protein, which is down regulated by anti-diabetic drugs like thiazolidinediones (TZDs), has been implicated as the link between NIDDM and obesity. Immunoneutralization of resistin with anti-resistin antibody increased insulin sensitivity whereas direct administration of resistin protein lowered the glucose tolerance threshold and impaired insulin activity. This discovery, while trying to establish a molecular link between obesity and type 2 diabetes, has raised several unanswered questions., Several human studies have demonstrated a lack of correlation between levels of resistin and obesity. Levy *et al* reported no correlation of resistin in etiology of insulin resistance in Fischer 344 rats. Association of resistin with diabetes and obesity in human system has been controversial as opposed to the murine system. Thus it appears that mouse and human differ greatly not only in their sequence but also in their structure.

Results

The resistin gene was amplified by RT-PCR from total RNA isolated from human subcutaneous adipose tissue. The cDNA corresponding to mature polypeptide was subcloned into pQE30 vector to obtain a N-terminal Histidine tag fusion protein. The protein was over expressed in *E. coli* M15 cells. The over-expressed recombinant resistin was purified to homogeneity from inclusion bodies, after solubilization in 8 M urea, using metal affinity column. While MALDI-TOF mass spectrometric analysis of the purified protein generated a single peak corresponding to the estimated 11.3 kDa size, the protein displayed a concentration dependent oligomerization as evident from size exclusion chromatography. The oligomeric structure was SDS-insensitive but ß-mercaptoethanol sensitive pointing to the importance of disulfide linkages in resistin oligomerization.

Fluorescence analyses revealed that, resistin is unusually stable protein, since, the intrinsic fluorescence remained unchanged in presence or absence of 8M urea. However, a significant shift was observed in presence of 20% ß-mercaptoethanol. These results indicate that cysteine residues involved in disulfide bond formation are stabilizing the native structure even in presence of 8 M urea, and the protein can be completely unfolded only under strong reducing conditions.

This observation was further strengthened by CD spectra of recombinant resistin, which remained essentially same in presence or absence of 8 M urea. CD spectrum also revealed the concentration dependent shift from alpha helical to beta sheet structure, which was very interestingly found to be reversible. Measurement of SH groups using NBD-Cl assay showed a

concentration and time dependent decrease in free cysteines.

9. *Understanding the mechanism of action of PPARg in regulation of glucose metabolism*

Pertoxisome proliferator activated receptor (PPARs), members of nuclear hormone receptor super family known to transduce a wide variety of signals like, nutritional, environmental or inflammatory events into a defined set of cellular responses at a level of gene transcription. PPARs form obligate heterodimers with retinoid X receptor and bind to defined PPAR elements (PPRE) in the regulatory region of target genes. The PPARs exist in three closely related isoforms, PPAR alpha, gamma and delta. Each member displays a tissue selective expression pattern. PPARg serves as a key regulator for adipocyte differentiation and promotes lipid storage in mature adipocyte by modulating the expression of several genes in the pathway. Various types of fatty acids, some of the eicosanoids (derivatives of aracidonic acid metabolism) and prostaglandin are effective ligand of PPAR. TZDs, a new class of anti-diabetic drug are known to enhance target tissue sensitivity of insulin by acting as a high affinity ligand for PPARg. Identifying the genes that are regulated by PPARg is likely to shed some light on the mechanisms of glucose homeostasis.

Results

Genomic DNA from mouse (Swiss mice) and human was isolated. Primers were designed from published mRNA sequence encompassing the transcriptional start and translational stop signal. Amplicons were cloned into pGEMT easy vector and sequenced using Big-Dye terminator sequencing mix onto ABI automated DNA sequencer. The genomic DNA of mouse resistin was deposited in GenBank (Accession number: AF480491). This sequence when BLASTed against the nucleotide sequence at the NCBI BLAST server, a match with the chromosome 8 contig of C57/BL6/J mice was found. The cDNA of mouse resistin was aligned with the genomic sequences by using DIALIGN algorithem to demarcate the exon/intron boundries. Similarly, human resistin was also aligned with its cognate chromosome 19 contig. The mouse sequence has an additional intron of 2279 bp (intron X), which is absent in human resistin. *In silico* analysis revealed the presence of a number of transcription factor binding site in this intron. Of particular significance was the presence of PPAR/RXR heterodimer binding site termed as IntX-PPRE along with several other transcription factor binding site like Ap1, NFkB, C/EBP *etc.* We synthesized oligonucleotides corresponding to this PPRE sequence and compared its ability to bind PPAR/RXR heterodimer by gel shift assay along with a known PPRE of Acyl CoA oxidase gene. The functional relevance of this DNA-protein interaction was demonstrated by transient transfection into 3T3 L1 cells using luciferase reporter construct with IntX-PPRE cloned upstream into a promoter less vector pGL3basic (B53) or SV40 driven pGL3 control plasmid (C23).

Conclusion

The mouse genomic resistin is almost three times bigger than its human counterpart. At mRNA level the mouse and the human resistin exhibit 64.4 % homology whereas at genomic level it had a greatly reduced hology showing only 46.7 %.

The mouse resistin has an additional 2279 bp intron after the translation stop codon, which is absent form human resistin. A high degree of conservation is observed in donor and acceptor region of both human and mouse resistin. In both cases the start and stop codon are located in second and fourth exons respectively. However, the polyadenylation signal is well separated in mouse by intron X.

Fig: 1. Genomic organization of mouse and human resistin.

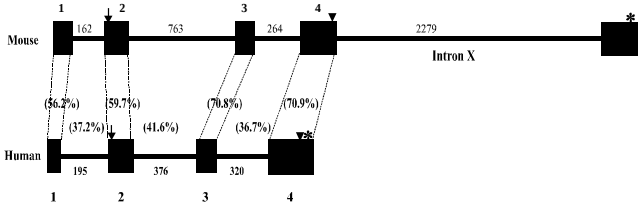
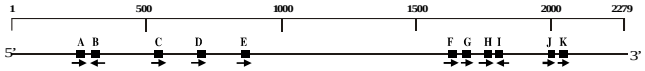


Fig 2. Putative regulatory elements present in IntronX.



A) UUCUCU intronic silencer motif (239-244)
B) ApI binding site (297-307)
C) ApI building site (527-535)
D) C/EBP binding site (690-707)
E) [(A/U)TGG]9 intronic enhancer repeats (S49-1628)
F) UUCUCU intronic silencer motif (1623-1628)
G) NFI-B binding site (1675-1684)
H) ApI binding site (1751-1761)
I) PPAR/RXR binding site (1793-1812)
J) IRFI/IRF2 binding site (1988-2000)
K) HNF3b binding site (2034-2048)

Fig 3. Gel shift assay with Int-X PPRE and Aco-PPRE

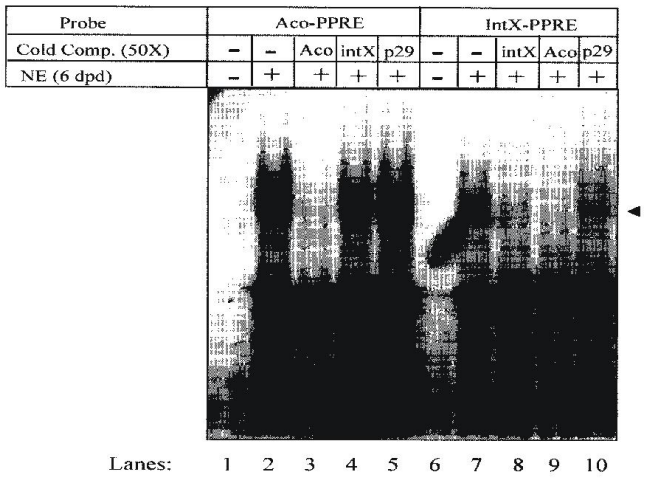
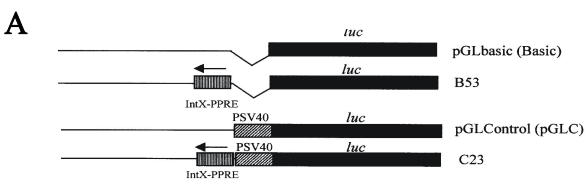


Fig 4. Functional modulation of luciferase expression by IntX-PPRE



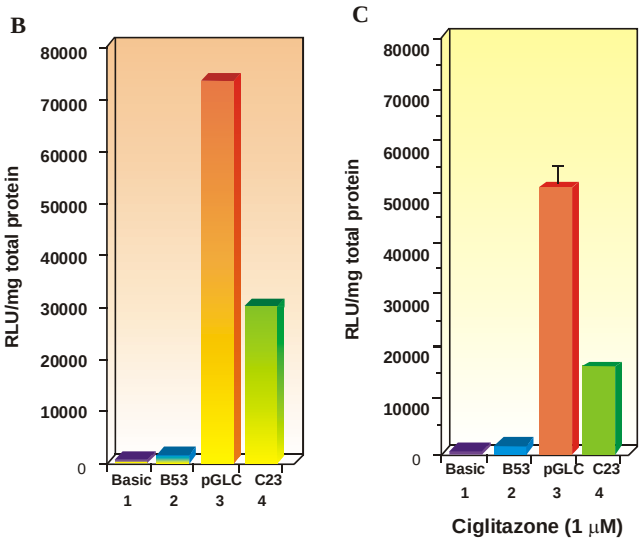
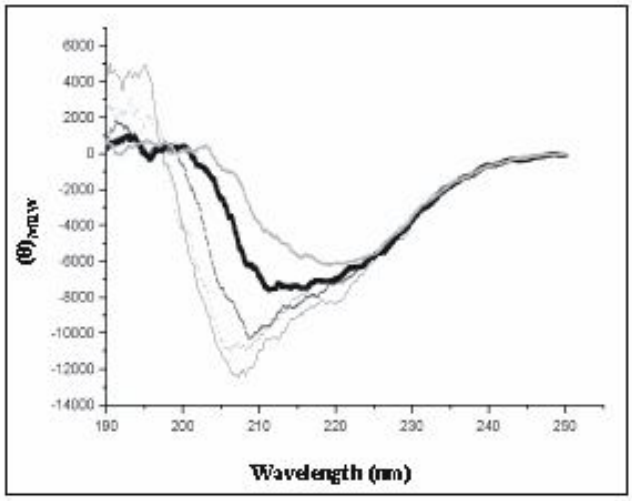


Fig 5. CD-spectra of purified human resistin at different concentrations



10. Etiology of endemic goitre in Northeast India : Role of environmental goitrogen

Northeast India falls under the goiter endemic sub-Himalayan belt. Although salt iodization programme has been introduced in the region more than two decades back, the impact of the programme on iodine deficiency disorders (IDD) in the region has not been evaluated. In the etiology of endemic goiter, the predominant factor is iodine deficiency but environmental goitrogens are also known to act in conjunction with iodine deficiency or as in some cases alone

to peicipitate the conditions. The population in Northeast India consumes a variety of foods, some of which are known/suspected to be goitrogenic. Investigations are necessary to determine whether the consumption of such foods has any impact on iodine status of the population. In addition, although sporadic studies are available on goiter prevalence, there appears to be no systematic study on IDD in the region as a whole. Therefore, the present study has been undertaken with the following objectives to cover the entire region state-wise.

Aims and objectives

1. To assess the iodine nutriture of the population.

2. To evaluate the implementation and impact of salt iodization programme, and

3. To assess the intake of goitrogenic foods as well as its impact on IDD in northeast India.

Methodology

Selection of villages/towns was carried out by the thirty cluster sampling method (PPS) as recommended by WHO/UNICEF/ICCIDD (1992). Sample size was worked out at an estimated prevalence of 10%, confidence level of 95%, relative precision 8% and design effect of factor 3. In each cluster, 100 households were covered for KAP studies and clinical examination of goiter. Urine and salt sampling was carried out from every tenth households.

In the previous year data on Sikkim and Meghalaya have been presented (*Ann. Rep 2001-02*). The study was undertaken in the states of Arunachal Pradesh and Mizoram during this year.

Results

Arunachal Pradesh

1 A total of 4540 households comprising 12687 individual (6,020 females and 6,667 males) were covered in the study. Majority of the population practiced animistic religion (59.5%) namely Dony polo followed by Christianity 23.2% and Buddhism 9.0%.

2 KAP studies (Head of household being the respondent) showed that 67.0% were aware of goitre and 33.7% knew that the use of iodized salt prevents goitre. Iodized salt were used by 99.6% of the households for more than 5 years.

3 Salt purchase pattern showed that 68.5% of the households purchased salt once a month and 23.5% once a fortnight. Salt was stored mainly in plastic/glass bottles (55.8%), and in bamboo jars (41.4%), and they were mostly covered by 73.5% of the households. Powdered salt was used by 83.6% of the household surveyed.

4 Mean ± SD iodine content of the household salt (n=194) was 30 ± 13.4ppm. Taking 15 ppm as the cut off level it was observed that 88.1% of the household salt were effectively iodized.

5 The main source of drinking water was tap (83.3%) and spring (8.1%) water. Analysis of drinking water samples (n=201) collected from household showed low mean ± SD iodine of content 0.5±1.4µg/l, ranging from 0.0 to 14.6µg/L confirming environmental iodine deficiency in the state.

6 The total goitre rate (TGR) in Arunachal Pradesh was 1.9%. Frequency distribution of goitre by age showed TGR of 0.1% in 1-5 years, 0.15% in 6-12 years, 1.18% in 13-18 years and 4.04% in adults. Goitre prevalence among females was found to be 2.7%, much higher than the males (0.9%)

7 A total of 235 goitre cases (147 grade 1,88 grade II) detected were mostly adults of grade 1,116 cases and grade II, 86 cases.

8 Cluster wise breast milk (n=375) iodine content was in the range of 3.6-27.3 µg/l with a mean ± SD of 12.4 ± 12.4µg/l.

9 Median urinary iodine excretion (n=2533) in

Arunachal Pradesh was 81.3μg/l ranging from 1.27 to 624.84 μg/l. Frequency distribution of urinary iodine excretion showed that 39.5% were excreting adequate iodine (100 μg/l), 31.0% showed moderate deficiency (50-99.9 μg/l), 20.7% showed mild deficiency (20-49.9 μg/l) and 8.8% showed severe deficiency (<20 μg/l),

10 Significant but weak correlation (r=0.392, p<0.05) was observed between TGR and urinary iodine excretion.

11 Mean ± SD thiocyanate excretion (n=2533) was 1.84 ± 1.4 mg/L (range 0.1 to 14.2 mg/L) with 1.6 excreting above 6 mg/l. The present study showed that 4.5% of the samples had I/SCN ratio below normal of which 1.5% were below the critical threshold of 3 when goitre develops due to thiocyanate overload.

12 On the whole there appears to be non uniform adequate iodine supply as the effect of thiocyanate can be overcome by adequate iodine. Therefore there is a need for surveillance programme to improve the iodine nutriture of the population in order to eradicatie goitre from the state.

Mizoram

1. The study covered 3029 households and 13890 individuals of which 6588 were males and 7302 were females.

2. KAP studies conducted on the head of households showed that 95.6% were aware of goiter and 90% knew that the use of iodized salt prevents goiter.

3. Salt purchase pattern showed that 91% of the households purchased salt from the village itself, mostly once a month (93.7%). The majority of the population used powder salt (77.5%) stored in plastic glass jars mostly covered (98.9%).

4. The mains source of drinking water were tap (71.3%) and rain water (18.6%).

5. Iodine content of drinking water (n- 201) was mean ±SD μg/L 0.52±.36 confirming environmental iodine deficiency.

6. Mean± Sd iodine content of crystalline salt (n=63) was 19.1±9.8 and that of powder salt (n=146) 48.7±12.7. Taking 15 ppm as the cutoff level it was observed that 85% of the household salt was effectively iodized.

7. Median iodine content of breast milk (n=179) was 10.0 mg/dL.

8. A total of 1619 goitre grade I and 255 grade II cases were detected in Mizoram. Females accounted for 1134 grade I and 221 grade II cases, of which adult females comprised of 953 grade I and 220 grade II goiter cases.

9. Total goiter rate (TGR) in Mizoram was 13.3% (range 4.8-24.6%). Frequency distribution of TGR by age showed TGR of 0.4% in 0-5 years, 5.2% in 6-12 years, 9% in 13-18 years and 20.1% in adults.

10. Median urinary iodine excretion (n=2737) in Mizoram was 178 μg/L (range 2.5 – 137 μg/L). Frequency distribution of urinary iodine showed that 72.1% were excreting adequate iodine, 15.8% showed moderate deficiency, 9.6% mild deficiency and 2.9% severe deficiency.

11. A significant correlation (r=-0.481 P<0.001) was observed between TGR and urinary iodine excretion.

12. Mean± SD urinary thiocyanate excretion (n=2685) was 2.93±1.64 mg/L with 6.1% of the samples excreting above 6 mg/L. The mean iodine/thiocyanate ratios (μg/mg) were well above the normal level of 7. However, 1.3% of the individuals were having I/SCN ratios below 6 and 1% below 3 the critical threshold when goiter develops due to dietary thiocyanate.

Urinary iodine excretion patterns shows that the population is excreting adequate iodine however TGR of 13.3% indicates uneven distribution

of adequate iodine supply compounded with dietary dietary thiocyanate load and other goitrogenic substances not accounted by urinary thiocyanate for the prevalence of endemic goiter in the state of Mizoram.

11. Nutrient composition (proximate and trace mineral content) of new varieties of rice

Human body needs wide range of nutrients to perform voluntary and involuntary metabolic functions. For the last two decades, the scientific basis of nutrition has expanded extensively, particularly on the role of diet in the development of chronic degenerative diseases such as cardiovascular diseases, hypertension, auto immune diseases and other chronic disorders.

Reliable data on the nutrient composition of foods used for human consumption are critical for many areas of endeavor including health assessment, the formulation of appropriate institutional and therapeutic diets, and for planning by several agencies.

Food composition databases are widely used to evaluate the nutrient content of foods and specific diets. Hence, food composition tables become an integral part of the knowledge required to understand the roles played by the nutritional environment in human health and also prevention of disease.

The food composition tables presently available in India have large gaps both in terms of the food items and the food constituents. Current data available is old and needs update. Hence, there is a need for accurate data in food composition tables.

Therefore, present studies were undertaken with the objective to analyse nutrient composition (proximate and trace mineral content) in eleven authentic rice varieties obtained from Directorate of Rice Research (DRR), Rajendra Nagar, Hyderabad and 3 samples of rice sold in fair price shops.

The analytical results of the study are as follows:

1. The moisture content of these rice varieties were in the range minimum of 9.0g% in Tulasi variety to a maximum 11.0g% in Sona masoori variety.

2. The protein content was low in Yamini variety (5.3g%) and high in Triguna variety (11.9g%). But majority of the rice varieties had protein content about 8.0g% and above, which showed the improvement in protein content over those reported earlier.

3. Fat content was very low in all the rice varieties, but Kasturi (1.47g%) and Yamini (1.45g%) had high fat content among the rice varieties analysed.

4. Calcium content among the rice varieties was in range from 5.6mg/100g (fair price shop variety) to 13.5mg/100g(Mugad sugandha).

5. Phosphorus (256 mg/100g) and iron (3.6 mg/100g) contents were high in Yamini variety than other varieties.

6. Among all the rice varieties Zinc content was high in Tulasi (3.30mg/100g), Aditya (3.20mg/100g) and Mugad sugandha (3.10mg/100g).

7. B-Complex vitamins such as riboflavin and niacin content of these rice varieties were in the range 0.13-0.19 mg/100g and 0.46-0.99 mg/100g respectively.

The above analytical results indicate that the hybrid varieties i.e Triguna (11.9 g%), Suraksha (11.2g%) Swarnadhan (11.1g%), Kasturi (10.6g%), Aditya (10.5g%), Mandhya vijaya (10.4g%), Tulasi (10.3g%) and Mugad Sugandha (10.2g%) are good sources of protein content, while the results reported in earlier studies showed a range of 6 to 8g protein per 100g.

Proximate Composition of New Varieties of Rice

Sl. No	Name of the rice variety	Moi-sture	Pro-tein	Fat	Ash	Crude fibre	Carbo-hydrate	Energy (Kcal)	NA	B_2
		g/100g							mg/100g	
1	Fair price shop	10.4	7.7	0.11	0.40	0.05	81.3	357	0.58	0.15
2	Super market	9.9	7.8	0.18	0.40	0.02	81.7	360	0.46	0.19
3	General stores	9.5	8.8	0.16	0.50	0.03	80.5	358	0.65	0.18
4	Sona massori	11.0	9.0	0.30	0.30	0.08	79.3	356	0.77	0.14
5	Aditya	10.0	10.5	0.92	0.60	0.08	78.0	362	0.95	0.15
6	Triguna	9.5	11.9	0.72	0.50	0.03	77.3	363	0.72	0.15
7	Tulasi	9.0	10.3	0.99	0.80	0.03	78.9	366	0.95	0.14
8	Kasturi	9.1	10.6	1.47	0.60	0.02	78.2	368	0.76	0.13
9	Swarna-dhan	9.1	11.1	0.76	0.30	0.04	78.7	366	0.60	0.13
10	Surak-sha	9.4	11.2	1.02	0.50	0.03	77.8	365	0.52	0.16
11	Mandya vijaya	9.0	10.4	1.00	0.40	0.07	79.1	367	0.48	0.15
12	Yamini	9.3	5.3	1.45	0.80	0.07	83.1	367	0.99	0.14
13	Mugad sugan-dha	9.2	10.2	1.01	0.40	0.08	79.1	366	0.68	0.16
14	Jaya	9.6	7.8	0.78	0.50	0.09	81.2	363	0.80	0.14

Trace Minerals Contents of New Varieties of Rice

Sl.No	Name of the rice variety	Ca	P	Fe	Zn	Cu	Mg	Mn
		mg/100g						
1	Fair price shop	5.6	130	0.8	1.1	0.3	48	0.4
2	Super market	6.4	114	0.8	1.1	0.2	36	0.4
3	General stores	7.6	131	1.1	1.1	0.2	48	0.6
4	Sona massori	5.5	125	0.5	1.2	0.1	19	0.5
5	Aditya	7.2	217	2.2	3.2	0.3	76	0.5
6	Triguna	8.3	199	2.2	1.7	0.1	57	0.8
7	Tulasi	12.7	245	3.4	3.3	0.2	85	0.8
8	Kasturi	10.8	190	1.5	2.0	0.2	93	0.5
9	Swarnadhan	8.5	114	0.9	2.0	0.1	49	0.5
10	Suraksha	8.5	199	1.5	1.8	0.2	74	0.4
11	Mandya vijaya	12.2	149	1.0	1.2	0.1	45	0.3
12	Yamini	7.0	256	3.6	2.4	0.1	84	0.8
13	Mugad sugandha	13.5	182	2.0	3.1	0.2	61	0.8
14	Jaya	7.5	166	0.6	1.5	0.1	124	0.4

V. PATHOLOGY

1. *Effect of vitamin restriction and supplementation on intestinal mucosal cell apoptosis*

Apoptosis is an evolutionarily conserved cell suicide process executed by caspases and regulated by Bcl-2 family proteins. It is one of the tissue homeostatic mechanisms, and dysregulation results in pathology. Vitamins are known to alter apoptosis and our previous studies also showed that 50% vitamin restriction had high impact in altering mucosal cell turnover compared to food and protein restriction. With this background, the animal studies were planned to look into the mechanism involved in this alteration and to check whether these changes can be reversed, and to study the role of individual vitamins in the causation of the same.

Objectives

1. To study the effect of 50% total vitamin restriction and vitamin supplementation on rat intestinal mucosal cell apoptosis.

2. To look into the mechanism involved in this alteration

Methodology

Twenty one days old WNIN rats having an average body weight of 30 - 40g were divided into two groups. Control group having 12 animals was fed on normal AIN-93G diet. Vitamin restricted group having 42 animals received a 50% vitamin restricted diet for 20 weeks. At the end of the feeding regimen, 6 animals were sacrificed from each group, small intestines collected and the oxidative stress and apoptosis parameters were estimated in them, while the other animals continued through the experiment.

Of the remaining 36 animals in the vitamin restricted group, they were divided into 6 groups and fed with 6 different diets; control, riboflavin supplemented, folic acid supplemented, vitamin E supplemented, Multiple vitamin supplemented and 50% vitamin restricted diet for a further period of 2 weeks. 6 animals in the control group were continued on the same diet. At the end, these animals were sacrificed and the above parameters were studied. Body weights (weekly), hemoglobin and serum protein levels were also recorded at the end.

Methods followed

1. *Plasma vitamin levels:* Riboflavin, a-tocopherol were measured by HPLC and Folic acid by using a kit supplied by Trivitron Diagnostics.

2. *Morphometry:* Apoptotic cells were counted in Haematoxylin and Eosin stained intestinal sections. 1000 cells in each section were counted and apoptotic index (%) was calculated.

3. *Agarose Gel Electrophoresis*: DNA extracted from mucosal cells, was resolved on agarose gel for ladder pattern which is the landmark feature of apoptotic cells.

4. *Antioxidant status of the cell*: Antioxidant enzyme activities like SOD, Catalase, GPx and levels of GSH, TBARS and protein carbonyls (oxidative stress markers) were measured in tissue extracts following standard protocols.

5. *Immunoblotting*: Anti-apoptotic protein(bcl-2) and pro-apoptotic protein (Bax) expression was checked by using western blot technique

6. *Functional Integrity*: Lys-ala- dipeptidyl amino peptidase and Alkaline phosphatase activities were measured as markers of mucosal cell functional integrity.

Results

Effect of vitamin restriction

1. Body weights, Hemoglobin and serum protein levels were not significantly different between groups.

2. *Plasma vitamin levels*: Riboflavin, folic acid

levels decreased to 50% and α-tocopherol levels decreased to 40%.

3. *Apoptotic rates*: Apoptotic index and Caspase-3 activity increased with 50% vitamin restriction. Ladder pattern was observed in experimental animals on DNA gel electrophoresis.

4. *Oxidative stress:* Levels of TBARS, protein carbonyls and antioxidant enzyme activities were very high; and GSH levels were low

5. *Bcl-2 expression (Anti-apoptotic)* : No expression of Bcl-2 with 50% vitamin restriction was seen.

6. *Bax expression (Pro-apoptotic):* Increased expression of Bax was observed with 50% vitamin restriction

7. Functional Integrity: altered

Effect of vitamin supplementation

1. *Plasma vitamin levels*: Improved with supplementation.

2. *Apoptotic rates* : Decreased with multiple vitamin supplementation, control diet (having all vitamins) and individual vitamin supplementations (riboflavin, folic acid, vitamin E); maximum decrease was observed with vitamin E and multiple vitamin supplementations

3. *Oxidative stress:* There was significant decrease in oxidative stress with control (having all vitamins) as well as individual vitamin supplemented diets, more so with vitamin E and the group which were supplemented and rehabilitated with all vitamins. GSH levels were highest with *vitamin E and multiple vitamin supplementations*.

4. *Bcl-2 expression (Anti-apoptotic):* Very high expression was observed in vitamin E and multiple vitamin supplemented groups.

5. *Bax expression (Pro-apoptotic):* Decreased with vitamin supplementation and it was not significantly different between the supplemented groups.

6. *Functional Integrity:* Was regained with supplementation and same as control.

Conclusions

The results show that 50% vitamin restriction increases the oxidative stress, decreasing the anti-apoptotic protein (Bcl-2) expression and there by enhancing the apoptotic rates.

Vitamin supplementation could reverse the changes caused by vitamin restriction. Among individual vitamins, vitamin E showed maximum decrease in oxidative stress, as well as decrease in apoptotic rates and an increase in the GSH levels and enhanced Bcl-2 protein expression. This experiment demonstrates the importance of vitamins in modulating apoptosis but stresses more on the significance of vitamin E.

2. Scanning electron microscope studies on effect of copper and molybdenum on development of skeletal fluorosis in rabbits

Around the globe, 23 nations including India are affected with fluorosis due to consumption of fluoride contaminated water. Reports say that intake of an optimum dose has no effects on human health. Intake of a high dose of fluoride has effects on skeleton called “Skeletal fluorosis” and on teeth called “dental fluorosis”. The present study is aimed to study the role of copper and molybdenum in the development of skeletal fluorosis in rabbits. Animals were divided in to 5 groups including controls. In each group copper, molybdenum and fluoride were given to rabbits in combination with fluoride water. After six months rabbits were sacrificed, tibial bones were separated. These samples (bones) were cut in to required sizes by bone cutter, Fat was

removed by passing through ether and dried by ethyl alcohol series. These samples were placed on SEM stubs endosteal surface facing upwards, with double sided adhesive tape coated with Gold and Palladium (300 A^{o}) in HUS 5-GB vacuum evaporator and scanned by Hitachi. S-520

scanning electron microscope operated at 10 to 15 kV. Pictures were taken at appropriate magnification and printed in required size. In the control rabbit tibial bone endosteal surface shows an array of collagen bundles with calcification or mineral deposition. In fluoride treated animals, mineralisation is not normal in between the collagen bundles. In fluoride and Molybdenum treated rabbit collagen bundles are not seen, and mineralisation is disrupted. In fluoride and copper treated animals collagen bundles are seen with more cross linkings, which appear like a sieve. In fluoride, molybdenum and copper treated animals situation of fluoride and molybdenum appear to repeated without collagen bundles, mineralisation process is completely disrupted and haphazard without cementing the collagen bundle with minerals. In Fluoride treated animals, bone mineralisation occurred but structurally different, in copper treated animals mineralisation and ossification taken place significantly because maturation of collagen is based on lysyl oxidase which is copper dependent enzyme. Thus, the strength of the bone is effected in such groups of rabbits, which are treated with molybdenum along with copper and fluoride.

3. SEM stdies of vegetables and spices

Scanning electron microscope as an wide application in food and nutrition, because of its resolving power and magnification one can study structure of food samples upto cellular and molecular level. It is possible to study processed as well as natural food samples. Thus we selected few vegetables and spices to study their ultrastructure by Scanning Electron Microscope. Selected vegetables were sweet potato (*Ipomoea batatas Lam*), potato (*solanum tuberosum L*), Radish (*Rhaphanus sativus L*), Edible arum (*Colacasia antiquorum schott*), Beet root(*Beta vulgaris L*) and spices Turmeric (*Curcuma domestica Val*) and Cloves (*Syzygium aromaticum*). These samples were processed by fixing and drying by freeze dry method or lyophilization. Samples were placed on SEM stub with double adhesive tape and coated with gold (300Aº) in a vacuum evaporator. Samples were scanned by SEM at 10-15kv operating voltage Pictures were taken at appropriate magnification and pictures were printed in required size. Few raw curry vegetables like sweet potato, potato, and Edible arum. Showed the storage of nutrients such as Sugars and Starch in the parenchymatous cells other raw salad vegetables like radish and beetroot have showed cellular pattern with out any significantly stored nutrients. Spices like turmeric and cloves revealed the woody xylem elements and glandular secretary cells in the cortex region respectively. It is also suggested to study cooked vegetables to know the loss of nutrients by scanning electron microscope.

VI. EXTENSION AND TRAINING

1. ICMR Annual Day Celebrations

As part of the ICMR Annual Day Celebrations, the Institute organised a series of events from Nov. 15 – 27, 2002. These included special talks, extension activities and Open Day.

Guest lectures

Experts from various fields were invited to deliver guest lectures. The topics included: (i) Water as a source of food security (ii) Nutrition research in India – Past glory; Future prospects, and (iii) ICMR – Industry interaction for human welfare.

Quiz and Essay competition

An inter-school quiz competition on "Biomedical Sciences, ICMR and its institutes' was organised for high school students on November 20, 2002. An Essay Writing Competition on *Medical Research in India – Present Scenario and Future Challeneges* for students of medical colleges of Andhra Pradesh was also held.

Open Day

An "Open Day" which was organised on November 27, 2002, at the Institute had attracted about 2500 visitors from various parts of the twin cities. A large number of school children, students of Home Science Colleges, senior citizens, doctors, teachers and people from different walks of life thronged the institute. The people visited various sections of the Institute and obtained first-hand information from the scientists on various activities and achievements of NIN during a special poster display session. The scientists of the Institute also conducted free health and nutrition check-up including measurements of heights and weights, blood sugar, blood grouping, haemoglobin assessment and diet counselling for the visitors. Video films on the activities of NIN and various nutritional disorders were also screened for the benefit of the visitors.

Open Forum

The event culminated in an open forum wherein experts in relevant fields clarified several doubts of people, collected through special drop-boxes. The queries covered a multitude of topics ranging from diabetes, heart diseases and obese rat model developed at the Institute and blending of edible oils, bone health and health problems in various age groups, nutritive value of foods and healthy diets.

A series of radio talks were also broadcast by scientists of the Institute between 25-30 November, 2003, on topics like nutrition, fruits, fat soluble vitamins etc. as part of the celebrations.

2. Workshops and Seminars

2.1 A Workshop on "Strengthening Food Safety and Food Quality Systems in India", was organised at the Institute on 28-29 June 2002. The Workshop was sponsored by FAO, CIA, Ministry of Health and Family Welfare, Govt. of India and NIN. Various issues related to Codex Alimentarius and its applicability in India were deliberated upon in the Workshop. Over 60 delegates from various parts of the country participated.

2.2 An Inter-Country Workshop on *"Feeding Minds, Fighting Hunger"*, jointly sponsored by FAO/UNESCO was organised at the Institute from 27th to 29th August 2002. The Workshop was attended by 50 participants from both education and nutrition sectors from Bangladesh, India, Indonesia, Nepal, Philippines, and Sri Lanka as well as representatives of FAO Headquarters, Rome, Regional Office for Asia–Pacific in Bangkok, UNESCO, New Delhi, and Paris. Scientists from the Institute and ICMR took part in the workshop and NIN's Initiative in implementing the Feeding Minds, Fighting Hunger Programme in India was presented at the workshop.

2.3a.two-day interactive programme on "Research Methodology" for prospective

research guides of the NTR University of Health Sciences, Andhra Pradesh was organised on March 21-22, 2003. Over 50 participants comprising Professors, Associate Professors of various disciplines from medical colleges of AP attended the programme.

3. Extension Activities

3.1. Publications

The four quarterly periodicals, viz. Nutrition (English), Poshan (Hindi), Poshana (Telugu) and Nutrition News, covering popular articles of public interest on nutrition were published. These periodicals have been well received by the public as reflected in their letters with queries and quest for more information.

During the year, a revised edition of the publication "A Manual of Laboratory Techniques" was published. In addition, the following popular publications were reprinted:

1. Nutritive Value of Indian Foods
2. Nutrient Requirements and Recommended Dietary Allowances for Indians
3. Some Common Indian Recipes and their Nutritive Value
4. Nutrition for Mother and Child
5. Some Therapeutic Diets
6. Menus for Low-Cost Balanced Diets and School Lunch Programmes (Suitable for South India).

The publications of the Institute continued to be popular among the public and during the year, a total amount around **Rs.5,26,000/-** was generated through their sales.

3.2. Exhibitions

The division's staff participated in the following exhibitions:

i. Fourth Low-Cost Science Exhibition at VDP High School, Falaknuma, Hyderabad organized by Confederation of Voluntary Association (COVA) in association with Andhra Pradesh State Council of Science and Technology during 2nd and 3rd November 2002.

ii. "IDEAS 2002 - Educational Exhibition" sponsored by St. Ann's High School, Bolarum, Hyderabad during 8th and 9th November, 2002.

iii. "Pride of India 2003 Science EXPO" as part of 90th Indian Science Congress held in Jnana Bharathi Campus, Bangalore University, Bangalore during 3rd - 7th January, 2003.

iv. "Heritage Health Festival" organized by AP Tourism and Culture and Health, Medical and Family welfare Departments, Govt. of Andhra Pradesh held from 7th to 9th February 2003 at Hyderabad.

v. The division organised a sales counter of NIN publications at the IXth Asian Nutrition Congress held in New Delhi between 23rd Jan. and 2nd Feb. 2003.

3.3. Popular Talks

Popular talks were delivered on nutritional themes for the benefit of visitors comprising high school and college students and paramedical

staff from all over the country. Besides, the following popular talks were also delivered to diverse groups at different places:

i. A popular talk on 'Nutrition and Health' for the supervisors of ICDS project, Department of Women and Child Welfare, Govt. of Andhra Pradesh, on 16th April 2002.

ii. A popular talk on nutrition for adolescent girls organised by an NGO "ANKURAM - Women and Child Development Society", Hyderabad, on 9th May 2002.

iii. A series of eight lectures were delivered to the youth of slums at the summer camps organised by Confederation of Voluntary Associations (COVA) in the old city of Hyderabad.

iv. A popular talk on 'importance of nutrition for school children' in a summer workshop organised for school children by an NGO Sampoorna Yoga Sadhana, Hyderabad on 26th May, 2002.

v. A lecture on 'Health, Nutrition and Sanitation' to the students of Diploma in Public Health and Sanitation Technology, at Hyderabad on 4th June 2002.

vi. A popular talk on 'Nutrition to the farmers' in a seminar organised by Janagaon Milk Chilling Centre in collaboration with SADHANA an NGO at Janagaon, on 17th June 2002.

vii. A talk on "Approaches Towards Teaching Science" for biological teachers of Kendriya Vidyalaya Sangathan, Hyderabad, on 4th July 2002.

viii. A popular talk on 'Nutrition, Health and Dietary Habits' to the executives of Motorola India Electronics Ltd. Hyderabad, on 30th July 2002.

ix. A lecture on 'Importance of Nutrition and Balanced Diet' for two batches of Constables and Head Constables of Civil Police Force on 23rd October 2002 at the Andhra Pradesh Police Training College, Hyderabad.

x. An orientation lecture on 'Nutrition for Women and Children' to the nursing students of St. Theresa Hospital, Hyderabad, on 13th December, 2002.

3.4. Radio Talks

The staff of the division delivered a series of radio talks on nutrition, including fat and water soluble vitamins, fruits etc. These talks were delivered in Telugu and were broadcast on All India Radio in the morning programme "Udayatarangini" from 25th-30th November, 2002 and 26-28th December, 2002.

4. Special Events

4.1. National Nutrition Week (1-7th September 2002)

i. Extension lecture on 'Nutrition and Women's Health', for ICDS supervisors, at Acharya N.G. Ranga Agricultural University, Hyderabad.

ii. Nutrition education camp for school children, Government High School, Moulali, Hyderabad.

iii. Nutrition awareness camp for adolescent girls of Mukaramjah High school, Purani Haveli, Hyderabad, in association with COVA, Hyderabad.

iv. Nutrition education camp for health functionaries at Kodangal village, Mahboobnagar district, jointly organised with Food and Nutrition Board, Hyderabad.

v. Nutrition Extension Programme for Tribals in two hamlets of Madhapuram village in Nalgonda district.

4.2. National Technology Day (11th May 2002)

An awareness camp on 'Nutrition and Health' was organized for schol children to commomerate National Technology Day in association with Confideration of Voluntary Associations (COVA)

4.3 World Food Day (16th October 2002)

A Workshop was organised for teachers and heads of schools from Hyderabad in association with COVA. This Workshop was conducted to create awareness about Feeding Minds Fighting Hunger (FMFH) Project of the Institute.

5. Public Relations

The Nutrition Museum continues to attract students from school and colleges, health workers and NGO groups from all parts of the country. Lecture-cum-video programmes on various nutrition themes were conducted for these visitors in batches. Reporters from several newspapers interacted with the scientists of the Institute and published their research highlights. In addition, articles from the Institute's periodicals were also picked up by various newspapers in different Indian languages.

Technical information was provided to the general public on nutrition and health-related aspects and dietary counselling was offered to the needy general public.

6. Training Programmes

The four regular training programmes of the Institute, viz., (i) M.Sc (Applied Nutrition) (ii) Post-Graduate Certificate Course in Nutrition (iii) Annual Training Course in Endocrinological Techniques, and (iv) Techniques for Assessment of Nutritional Anaemia were conducted. This year, a total of 33 candidates attended these courses and among them, 21 were in-service candidates from various medical and home science colleges in different States.

In the training courses, the participants have been exposed to the latest topics in the field of nutrition through lectures, practical sessions and demonstrations by the Institute's as well as expert guest faculty. Community visits were also arranged to give the participants a holistic prospective.

In addition to the regular training courses, the following ad-hoc training programmes on various aspects of nutrition were organised:

a) Sixteen Investigators of RCH Programme from West Bengal had undergone training-cum-standardisation in methodology of collection of blood samples for haemoglobin estimation (May 6-8, 2002).

b) One month adhoc training programme for Ms.Sangeetha Kumari of RMRC, Port Blair (March 28 – June 27, 2002).

c) Training programme in the field of 'Community Nutrition' for two WHO Fellows from Bhutan (Aug 5. – 12 Sept, 2002).

d) Training programme in the field of 'Maternal Nutrition' for two WHO Fellows from Srilanka (Oct 7. – 18, 2002).

e) Adhoc Pre-PhD training programme for the registered scholars of NTR University of Health Sciences was held during 16-27 December, 2002. A total of 20 participants took part in the programme.

f) Adhoc training for a WHO Fellow from Myanmar in the field of Planing and Management of Nutrition Programmes (Jan. 20-31, 2003).

g) Adhoc training for three WHO Fellows from Myanmar in the field of Public Health (Feb. 3-14, 2003).

VII. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

A. FOOD SAFETY

1. Aflatoxin contamination in Walnut

Aflatoxins have been reported to occur in a variety of agricultural commodities like cereals, oilseeds, and treenuts. Occurrence of aflatoxins in treenuts such as walnut is gaining considerable significance in recent times because of their economic value.

A study has been undertaken to assess aflatoxin contamination in walnuts produced in India with the following objectives:

1. To study the mold contamination of walnuts available in the market.
2. To study the extent of aflatoxin contamination in walnuts.
3. To observe the post-harvest practices and storage of walnuts.

Methodology

Walnut samples were collected from major walnut growing areas in Kashmir Valley, namely Baramulla and Pulwama. From these areas, samples were procured from field, household and wholesale and retail shops. A total of 54 walnut samples from Kashmir Valley and 10 walnut samples from Hyderabad markets were collected for aflatoxin analysis. Samples were subjected to physical examination, mycoflora analysis and aflatoxin analysis by HPLC method.

Results

Physical examination of the walnuts showed that majority of the nuts had mean weights ranging from 8.0-13g for in-shell and 2.4-5.2g for kernel. The samples obtained from Kashmir were light amber to brown in colour while those from Hyderabad markets had black colour in 76% of the walnuts examined. Most of the black coloured samples were visibly mouldy. Examination of mycoflora in walnut samples showed that fungi belonging to *Penicillium* and *Rhizopus* species were predominant while *Aspergillus* sp. was less frequently observed.

The result of aflatoxin analysis showed that out of a total of 54 samples analysed from Kashmir, aflatoxin was detected in 9 samples with levels ranging from 0.1-1.5 mg/kg. Aflatoxin was not detected in any of the Hyderabad samples. Observation of post harvest practices in processing of walnuts in Kashmir showed that the process of harvesting, hulling, washing and sun drying are critical points that can have considerable influence on mold and aflatoxin contamination.

2. Application of Hazard Analysis Critical Control Point (HACCP) to animal products

There is an increased concern about the safety of animal products from microbial contamination, as foods of animal origin are high risk commodities. Hazard Analysis Critical Control Point (HACCP) approach is a preventive system of microbial hazards and is an action oriented programme to identify and reduce the problem of foodborne diseases. HACCP has been made compulsory for all sea food exports and many importing countries are insisting on HACCP certification.

A study on application of HACCP in shrimps from pond to plate was carried out during the year.

An HACCP on brackishwater pond cultured tiger prawn *Peraeus moudon* was initiated to identify hazards and critical control points. The study was carried out by selecting six ponds located near Kavali town of Prakasam District. Based on preliminary observations, the samples were collected immediately after harvesting at the ponds, after being transported to markets of Hyderabad city and after the prawn recipes were prepared at the house of consumer. The samples were anlaysed for physical and microbiological quality. In addition, the pond water, feed, and mud samples of the pond and the ice used for packing the prawns were also analysed for microbiological contaminants.

The microbiological analysis of prawns revealed that all the samples were contaminated with faecal coliforms, *Staphylococcus* spp., *Salmonella* spp., and *Vibrio* spp., both at the pond, immediately after harvesting and after transporting to the markets of Hyderabad city.

However, the recipes prepared out of prawns were free from contamination with pathogenic organisms, except one sample which is contaminated with *Staphylococcus* spp. None of the samples is contaminated with *Listeria* spp.(Table 1).

Two of the pond water samples were contaminated with faecal coliforms, and one with *Vibrio* spp. Three of the mud samples were contaminated with *Vibrio* spp. All the feed and ice samples were free from contamination with pathogenic organisms (Table 2).

Results of the physical contaminants revealed that all the prawn samples collected at the pond site after harvesting and after reaching the markets of Hyderabad City contained sand particles ranging from 0.1g to 0.2g. Only two (each) samples at the pond site and after reaching the markets of Hyderabad city contained 2-4 pieces of scales and none of the recipes prepared from prawns contained the physical contaminants.

It can be concluded from the present study that the prawn recipes were free from contamination with pathogenic organisms suggesting that the traditional method of cooking followed were safe from consumption point of view as well as from food safety point of view.

However, the presence of pathogenic microorganism in the prawns are of concern. The study emphasizes that the HACCP process should not only be confined to processing unit but need to extend to the entire food chain from pond to plate.

Table 1: Microbiological Quality of Prawns (HACCP from Pond-to-Plate) Range of organisms cfu/g

Type of organism	At Pond after Harvesting N = 6	After reaching the markets N =6	RecipeN = 6
Total aerobic plate count	4.5×10^3-3.3×10^4	3×10^3- 1.4×10^5	3×10^2 7.5×10^3
Total coliforms	2×10^3 - 4×10^4	1×10^3- 1.5×10^4	Nil
Staphylococus spp.	1×10^3 - 4×10^4	3×10^3- 9×10^4	2.1×10^3
Salmonella spp.	2×10^2 - 6×10^2	2×10^2- 4×10^2	Nil
Vibrio spp.	3×10^4- 3.5×10^4	4×10^4- 5×10^4	Nil
Listeria spp.	Nil	Nil	Nil

Table 2: Microbiological quality of Pound water, mud, feed and ice (cfu/g)

Type of organism	Pond Water (N = 6)	Mud (N = 6)	Feed (N = 6)	Ice(N = 6)
Total aerobic plate count	3×10^4 - 4×10^5	ND	ND	ND
Total coliforms	1×10^4 2×10^5	ND	ND	ND
Staphylococus spp.	Nil	ND	ND	ND
Salmonella spp.	3×10^4	4×10^5	Nil	Nil
Vibrio spp.	3×10^4	4×10^5	Nil	Nil

ND = Not done. Only pathogenic organisms such as *Salmonella spp*. and *Vibrio spp*. were checked.

B. CANCER AND XENOBIOTICS

1. *Preclinical toxicity of r-DNA Anti-Rabies Vaccine (DRV) & Combination Rabies Vaccine (CRV)*

Rabies, a highly fatal viral disease of the central nervous system, is caused by bites of warm-blooded animals such as dogs and wolves. Apart from the human death toll in developing countries (approx. 40,000 cases/year in India) loss of animal wealth is reported to be substantial.

The conventional treatment of Rabies comprises of post bite prophylaxis with cell culture vaccine, whose manufacture involves technological problems and is not economically viable. The r-DNA Anti Rabies Vaccine (DRV) claimed to be the Worlds first DNA-Rabies Vaccine was developed by Indian Institute of Science, Bangalore in

association with Indian Immunologicals Limited under the 'Jai Vigyan' Programme of Department of Biotechnology, Ministry of Science and Technology, GOI. The present vaccine is not only cost effective but can be manufactured by recombinant technology. The combination of DRV with inactivated tissue culture rabies antigen (CRV) has been noted to potentiate antibody production. Therefore, the proposed r-DNA Anti Rabies Vaccine alone and in combination with tissue culture rabies antigen needs to be tested for its safety in rodent and non-rodent (Primates) (according to the International guidelines and schedule 'Y' of Drug and Cosmetic Act 1940, Govt. of India) before its therapeutic use in humans and animals.

Aims & Objectives

The preclinical toxicity tests of r-DNA ANTI RABIES VACCINE (a product developed by INDIAN INSTITUTE OF SCIENCES, Bangalore) alone and CRV was tested in rodent and non-rodent (Primates) per the requirement of schedule 'Y' of Drug and Cosmetic Act 1940, Government of India.

Methodology

Test compound:

1. r-DNA anti Rabies Vaccine (DRV)
2. Combination Rabies Vaccine (CRV)

Dosage form : Injectable (Vials)
Route of administration: Intramuscular
Test doses : Therapeutic doses (DRV (1X)& CRV (1X)) (TD)
: Average dose (DRV (2X) & CRV (2X)) (AD)
: Highest dose (DRV (10X)) (HD)

Test System:

1. Mice (Swiss albino)
2. Monkeys (*Macaca Mullatta)*

Test details:

Test groups	Experimental details					
	Pretesting *		Sub-acute @		Chronic +	
	Mice	Monkey	Mice	Monkey	Mice	Monkey
VC	-	-	(10M + 10F)	(1M + 1F)	(10M + 10F)	(2M + 2F)
DRV (1X)	-	-	(10M + 10F)	(1M + 1F)	(10M + 10F)	(2M + 2F)
CRV (1X)	-	-	(10M + 10F)	(1M + 1F)	(10M + 10F)	(2M + 2F)
DRV (2X)	-	-	(10M + 10F)	(1M + 1F)	(10M + 10F)	(2M + 2F)
CRV (2X)	-	-	(10M + 10F)	(1M + 1F)	(10M + 10F)	(2M + 2F)
DRV (10X)	(5M + 5F)	(2F)	(10M + 10F)	(3M + 1F)	(10M + 10F)	(2M + 2F)

( ) No of animals M = Male F = Female

*Pretesting : Single dose exposure of DRV (10X) and observed for lethality in 15 days.

@ Subacute test :

Dosage Schedule (Experimental): Three repeated doses consecutively at various concentrations and combinations as prescribed in schedule 'Y' Drug and Cosmetic Act 1940, GOI.

+Chronic toxicity test: The following five doses as per the intended clinical use of test compound was administered in mice & monkeys .

Days	1	4	7	14	28
Dose schedule	+	+	+	+	+

The animals were selected, conditioned, and exposed to the test compounds (with DRV and CRV) through intramuscular route at various concentrations.

Observations

Routine examination Physical, Physiological, Haematology, Clinical Chemistry, Gross necropsy, Histopathology, Immunopathology, (Anti-ds-DNA antibodies, Anti nuclear antibodies) and Residual DNA test.

Statistics

Data compiled and analysed for significance using appropriate tests using SPSS 10.0 version.

Results

The results of this study are as follows :

- No pre-terminal deaths,
- No significant treatment related effect on

food intake, body weight, clinical signs and behavioural activity etc.

- No significant changes in haematological parameters and clinical chemistries
- No specific test compound-induced pathological changes in the various organs were observed.
- No evidence of any Immunotoxicological effects
- No anti-ds-DNA or Anti nuclear antibodies
- The results of PCR analysis of residual DNA in monkeys was negative in all except one monkey (10X DRV) after 90 days at site of injection (one femtogram)

Conclusion

No specific abnormalities in physical, physiological and clinical chemistry/haematological/pathological/immuno-toxicological profiles were recorded in mice & monkeys exposed to the test compound at various dose levels under the experimental conditions. The report has been released to the sponsoring agency.

2. *Antioxidant activities of certain medicinal plants from Northeast India*

Plant medicines are of late gaining its importance all over the world. They are considered less toxic free from side effects and have been reported to have beneficial effect for many degenerative diseases. We have earlier carried out studies on the hepatoprotective activities of some extracts in a paracetamol induced liver injury rat model and demonstrated their beneficial effect (Ann. Rep. 1999-2000). Last year, we have reported on the antioxidant principles and polyphenol contents in these extracts (Ann. Rep. 2001-2002). The study is now completed with the present report on the functional characteristics of these extracts. We are presently reporting on the reducing and radical scavenging activities of these extracts. Antioxidant performance of any herbal extract is mediated through its reducing or radical scavenging potential.

Materials and Methods

Organic and aqueous extracts of the following plants were prepared and subjected to reducing, radical scavenging activities assays by standard procedures. The results are given in Table 1.

Table 1. Radical scavenging and reducing abilities of North Eastern Plant Extracts

S.No.	Plant	Solvent	Scavenging Activity (%)	Reducing Power (O.Ds)
1	Leucas	Methanol	51.2	0.335
		Petr. Ether	14.3	0.189
		Chloroform	4.6	0.109
		Aqueous	31.8	0.278
2	Cessampetos perera	Methanol	35.5	0.441
3	Vitex negundo	Chloroform	3.4	0.105
		Methanol	36.1	0.298
4	Cajanus cajan	Petr. Ether	20.7	0.202
		Chloroform	18.1	0.213
5	Sid cordifolia	Aqueous	13.8	0.189
		Chloroform	18.0	0.184
		Petr. Ether	3.9	0.093
6	Glycosmis pentaphyla	Methanol	18.4	0.323
7	Costus speciosus	Methanol	1.9	0.049

Results and Conclusions

Scavenging activity : Among the organic extracts, it was observed that the methanolic extracts of plants appeared to have higher scavenging activity compared to any other solvent. Methanolic extract of Leucas had the highest scavenging activity (51.2%) followed by Vitex, Cessampetos, Glycosmis. Costus species has the lowest activity (1.9%). The other extracts namely chloroform, petr. ether, aqueous showed considerably lesser activity compared to methanolic ones.

Reducing power : The reducing power activity carried out by the method of Yildirim et al showed a similar picture to what was earlier observed in the scavenging activity assay. Increased absorbance of the reaction mixture in this case indicates increased reducing power which is beneficial. Methanolic extracts of Cessampetos showed the highest reducing activity (O.D = 0.44) followed by Leucas, Glycosmis and Vitex negundo. The other extracts with chloroform, petr. Ether and water exhibited 30 – 50% of methanolic activity.

In conclusion, it is observed that

methanolic extracts of certain plants of North Eastern India demonstrate good scavenging and reducing activities which indicate their antioxidant potential.

3.Development of in vivo model for genotoxicity

Many nutrients in diet are reported to possess antioxidant and other health promoting properties. Earlier reports from NIN (Ann. Rep. 1996, 1999) have shown that the allium compounds possess antimutagenic properties. This study was taken up to assess the *in vivo* antigenotoxicity due to allium feeding.

Aims and objectives

To establish *in vivo* model to assess genotoxicity and evaluate the antimutagenicity feeding allium through diet.

Methods

Allium vegetables (garlic 0.1% + onion 1%; garlic 0.5% + onion 5%) were fed for one month to wistar/NIN rats aged 4 weeks. Half of the allium fed animals received Benzo (a) Pyrene (BP) 5 mg ip injection at the end of one month. One group received neither alliums norhcarcinogen and another received only BP. Blood and other tissues of interest were collected one week after carcinogen treatment for assessment of DNA damage (Ann. Rep. 2001-2002). Urine samples were collected from all animals for 24 hrs immediately from the time of carcinogen treatment, frozen and stored at –20°C until analysis. During the year under report, urinary mutagens were recovered and tested for mutagenicity using bacterial salmonella test.

Outcome

The results are as follows :

1. Allium vegetables fed groups did not show any adverse effects.
2. There was reduction in the number of revertants in TA 98 and TA 100 strain of salmonella typhimurium in carcinogen treated rats prior fed with alliums suggesting protective effect of alliums against DNA damage and mutagenicity.
3. These results suggest that daily intake of allium vegetables may be helpful in warding off genotoxic effects of harmful chemicals.
4. Allium vegetables (garlic and/or onion) were fed at different levels as showin Table 1.

Table 1. Reduction in urinary mutagen excretion in allium fed rats

Treatment N	Mean Revertants (±SD) S. typhimurium	
	TA 98 (S9)	TA 100 (S9)
Control	16 ± 3	136 ± 10
B (a) P	43 ± 7 [a,b]	487 ± 28 [a,b]
Garlic 0.1 % + Onion 1%	18 ± 2	133 ± 25
Garlic 0.1 % + Onion 1% + B.P.	16 ± 3 [a]	218 ± 19 [a]
Garlic 0.5 % + Onion 5 %	10 ± 2	168 ± 40
Garlic 0.5% + Onion 5% + B.P.	16 ± 3 [b]	234 ± 30 [b]

Values are Mean ± SD of 6 animals in each group.
Values bearing same superscripts are different
a, b = P < 0.05

4. *Modulation of xenobiotic metabolism of Zingiber officinale (ginger)*

Herbs and spices are the most important sources of antioxidants. They are natural components of diet and hence are safe. Some spices like turmeric, ginger, garlic and onion which form an integral part of various Indian and continental cuisines are known to contain certain bioactive phytochemicals in them. The rhizome of ginger (*Zingiber officinale*) is used as a spice and food seasoning due to its sweet aroma and pungent taste. Many phytochemicals are known to stimulate the drug metabolizing enzymes in the host tissues and help in the process of detoxification.

Aims and objectives

To study the effect of ginger feeding on drug metabolizing enzymes in rats.

Study design

Animal model : Male wistar/NIN rats
No. of rats : 24

Treatment groups : Four, 6 rats/group
Duration of the experiment :One month

Ginger powder incorporated in the diet at 0.5%, 1% and 5% levels was fed to male wistar/ NIN rats respectively for a period of one month. At the end of the experiment the animals were sacrificed and the organs namely liver, lung, kidney and intestine were taken for the estimation of drug metabolizing enzymes such as NAD(P)H quinone reductase, GST, AHH and UDPGT.

Results on NAD(P)H quinone reductase assay have been reported in the previous Annual Report; and it showed significant activity in liver and intestine in the ginger fed groups. The activities of other enzymes are presently reported

Results

1. AHH activity was not altered by ginger feeding either in the liver or in the other extra hepatic tissues such as intestine, kidney and lung (Table 1).

2. UDPGT activity was also found to be similar in all the groups (Table 2).

3. GST activity was significantly elevated in the liver ($P < 0.001$) at 0.5%, 1% and 5% levels of ginger feeding (Table 3) compared to controls.

4. In intestine, lungs and kidney GST activity was significant in 1% and 5% ($P<0.05$) ginger fed groups (Table 3).

Table 1. Effect of ginger (G) on AHH activity in rat tissues

Treatment	Liver	Lung	Kidney	Intestine
Control	1.10 ± 0.273	0.40 ± 0.218	0.46 ± 0.127	0.50 ± 0.120
0.5 % G	1.26 ± 0.181	0.40 ± 0.244	0.47 ± 0.212	0.58 ± 0.312
1.0 % G	1.41 ± 0.325	0.42 ± 0.213	0.51 ± 0.208	0.64 ± 0.160
5.0 % G	1.28 ± 0.269	0.51 ± 0.221	0.63 ± 0.253	0.74 ± 0.246

Values are Mean ± SD
Values are expressed as n moles/mg protein/min
No. of observations= 6

Table 2. Effect of ginger (G) on UDPGT activity in rat tissues

Treatment	Liver	Lung	Kidney	Intestine
Control	5.10 ± 1.343	0.45 ± 0.382	1.55 ± 0.903	0.88 ± 0.920
0.5 % G	6.20 ± 1.830	0.62 ± 0.160	2.29 ± 1.102	1.14 ± 0.706
1.0 % G	5.65 ± 2.372	0.56 ± 0.167	2.22 ± 1.503	1.82 ± 0.822
5.0 % G	6.93 ± 2.077	0.64 ± 0.185	2.52 ± 0.575	1.22 ± 0.394

Values are Mean ± SD
Values are expressed as n moles/mg protein/min
No. of observations= 6

Table 3. Effect of ginger (G) on GSHT activity in rat tissues

Treatment	Liver	Lung	Kidney	Intestine
Control	533.2 ± 66.51 [a]	105.9 ± 11.28 [a]	108.0 ± 14.16[a]	125.4 ± 26.73 [a]
0.5 % G	682.2 ± 133.8 [b]	122.4 ± 21.48 [b]	141.6 ± 41.07 [a,c]	143.9 ± 41.82 [a,c]
1.0 % G	773.2 ± 53.58 [b]	145.5 ± 36.42 [b]	209.4 ± 50.19 [b,c]	193.9 ± 14.73 [b]
5.0 % G	774.0 ± 78.39 [b] P < 0.001	167.4 ± 44.82 [b] P < 0.05	223.5 ± 82.51[b] P < 0.01	209.4 ± 31.41 [b,c] P < 0.001

Values are Mean ± SD
Values are expressed as CDNB units conjugated/min/mg protein
No. of observations = 6
Values with different superscripts are significantly different by ANOVA

Conclusions

GST group of enzymes play a major role in the detoxification pathway and help in the conversion of reactive chemicals to non reactive polar compounds which can be excreted from the body. Increase in GST levels of rat tissues due to ginger feeding suggests that regular intake of ginger through diet can enhance the activity of phase II detoxification enzymes, and thereby afford protection to host against tissue damage as a result of exposure to xenobiotics.

5. Ethno pharmacological validation of biodynamic compounds in traditional medicine

Natural and synthet`ic antioxidants play vital role in protecting cells and tissues against oxidative damage caused by free radicals. The natural anti-oxidants are receiving greater attention in view of their low toxicity and wider therapeutic potentials. Therefore a, study has been planned to evaluate herbal medicines, prescribed by ayurvedic physician to prevent and treat the diseases such as arthritis, asthma, cancer etc,

for their antioxidant activity.

Aims and Objectives

1. To evaluate plants used in traditional system as potential antioxidants using battery of tests.

2. To compare antioxidant potential of the plant preparations prepared by modern and traditional methods.

3. To assess the antioxidant / therapeutic efficacy of two potent preparations using invivo models.

4. To study the co-relation between antioxidant activity and pharmacodynamic action of the selected preparations.

Methodology

a) Selection of plants

The following coded herbal medicines were selected in consultation with an expert ayurvedic practitioner. The raw material of the respective plants as per the season were procured with the approval of taxonomist.

Plant No	Plant/Extract Code	Therapeutic activity
1	1206, 1105, 1301	Asthma
2	2202, 2102, 2304	Rheumatoid Arthritis
3	3107, 3311	Rheumatoid Arthritis
4	4212, 4109, 4308	Rheumatoid Arthritis
5	5213, 5110, 5322	Rheumatoid Arthritis

c) Extraction procedure

The procured raw material was made into fine particles by grinding/chopping and were soaked in solvents viz. water, methanol, water and methanol mixture (50:50). Extracts were prepared as per the standard procedures followed by the traditional ayurvedic physicians. All the extracts were made into powder form by flash evaporation/ lyophilisation.

d) Evaluation of antioxidant activity

Evaluation of antioxidant activity has been evaluated by the following established methods and compared with vitamin 'C' as a standard antioxidant.

i. Estimation of antioxidant activity:

The oxidation of linoleic acid is measured by modified TBA method.

ii. Free-radical scavenging activity:

The free-radical scavenging activity is determined by Colorimetric assay with DPPH,

iii. Mitochondrial peroxidation assay:

Inhibition of the peroxidation initiated by Fe^{+2} – H_2O_2 system in rat liver mitochondria was measured by the assay of MDA.

The above methods were standardised at least at three concentration levels and IC_{50} values were established for comparison with Vit-C for each plant extracted in three different solvents.

Results

The above Table indicates the activity fold of each extract with reference to the standard.

a) The potential inhibitory activity on the initiated lipid peroxidation as measured by LA assay was found between 0.0012-0.229 mg/ml concentration for the various plant extracts. There was nearly 4-5 fold inhibitory activity in almost 60-65% of the plant preparations compared to vitamin C.

b) Scavenging activity as measured by DPPH assay showed that majority of extracts exhibited such activity ranging from 0.003-0.193 mg/ml concentration.

c) Inhibitory activity of the extracts was observed in the peroxidation initiated by Fe^{+2} – H_2O_2 system in rat liver mitochondria. The antioxidant activity in this system was found to be in 70% only. The other 30% showed no activity.

This assay also determines the effectiveness of the extracts in biological system.

d) Among the various extracts screened the maximum inhibitory activity, scavenging activity was found to be in 4308 and 5322 which are extracted in W+M.

Conclusions

a) The plant preparations [4308, 5322] are found to have maximum antioxidant activity as evaluated by the battery of tests.

b) The aqueous/organic extract in all the plants has indicated potential antioxidant activity as compared to water extract potency mentioned in the traditional preparations.

c) Majority of the extracts prepared confirm antioxidant activity in different tests. However only two plants showed potential antioxidant activity in all the battery of tests.

d) The conventional procedures of dispensing the herbal products by ayurvedic physician as traditional medicine indicate the efficacy of herbal products as potent antioxidants.

Work Planned

(i) Studies will be further extended to evaluate the antioxidant potential of the screened plant extracts using explant culture system. Biochemical parameters will be also estimated.

(ii) *In vivo* animal experimentation using rat as an experiment model (comparision with a known antioxidant) is planned for antioxidant activity evaluation. The *in vivo* studies include the effect of plant extracts on CCl_4 induced hepatotoxicity (a free radical mediated damage). Estimation of antioxidant enzymes, oxidative damage of proteins and histopathological evaluation of hepatic tissue will be undertaken.

VIII. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES (NCLAS)

A.. SERVICE ACTIVITIES

1. Supply of Animals

With the strict implementation of receiving confirmed orders with advance payment for supply of animals, there has been a 40% reduction in the breeding and 30% in the supply of animals. While 43,763 animals were bred and 36,167 animals were supplied during the previous year, 26,111 animals were bred and 25,160 animals were supplied during 2002-03. The income generated from this activity amounted to 19.4 lakhs and this was also 25% less compared to last year.

The mortality (as percentage of total number of animals available in each species and strains) varied from 0.2% in BALB/c mice to 7.7% in Fischer 344 N rat colony in Barrier Maintained colony and was within the normal range. However the mortality was high (24%) in N:NIH(S) nude mice colony. A breeding nucleus of C57BL/6J nude mice was brought from National Institute of Virology, Pune as they had problem with its breeding. After maintaining a small colony for 2 years, the breeding was stopped since there was no demand for them and the old age animals were dying. The homozygous nude mice [N:NIH(S)] which are used for breeding develop abscess as they aged and subsequently die due to emaciation. In order to ensure quality, the supply of nude mice was suspended for a short period and investigations were taken up to identify the cause of mortality. Breeding outside the isolators has also been suspended in order to minimize any spread of infection. Subsequently this problem has been overcome. In the conventional facility the percentage of natural death varied from 2.3% in Swiss mice colony to 9.8% in hamster colony. It was high in mutant colonies; 20.2% in WNIN Ob/Ob, 35% in WNIN Gr/Ob and 40% in wild white rat colony. It has already been reported in the earlier annual reports that these mutant rat strains harbour opportunistic microorganisms and they have shorter life span. The percentage of animals disposed off are also high in mutant colonies. While propagating the obese mutant rats normal counter parts are also propagated as littermates. Since only obese rats are being used for experimentation the other counterparts have to be disposed off. Further some of these mutant animals also develop spontaneous tumors and hence have to be disposed off (Table 1 and 2).

TABLE 1. DETAILS OF BREEDING AND SUPPLY OF DIFFERENT SPECIES AND STRAINS OF LABORATORY ANIMALS (BARRIER MAINTAINED COLONY) DURING THE PERIOD FROM 01-4-2002 TO 31-03-2003

Sl. No.	Species	Strain or Breed	Total Number of Animals								
			Stock								
			Stock as on 1.4.2002	Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied	Died	Disp	Balance as on 31-3-2003
1	Mouse	BALB/c An. N (inbred)	637	7740	8377	100	7374	7474	13 (0.2)	-	890
		C57BL/6J (inbred)	1092	1533	2625	5	2158	2163	100 (3.8)	101 (3.8)	261
		N:NIH(S) Nude (athymic) (inbred)	274	658	932	71	403	474	233 (25.0)	33 (3.5)	192
		C57 BL/6 nude (athymic)	15	-	15	-	-	-	12 (80.0)	-	3
2	Rat	Wistar/NIN (inbred)	528	4245	4773	168	4196	4364	49 (1.0)	-	360
		SD (Sprague Dawley) (Outbred)	319	1002	1321	139	802	941	58 (4.4)	-	322
		Fischer 344 N (inbred)	96	124	220	26	16	42	17 (7.7)	-	161
3	G. Pig	N:HART (Hartley)	128	320	448	2	309	311	6 (1.3)	-	131
		Dunkin (Hartley)	144	236	380	24	202	226	19 (5.0)	-	135
		N:NIH (Coloured)	92	164	256	3	120	123	17 (6.6)	-	116
4	Rabbit	New zealand white	61	94	155	13	72	85	11 (7.1)	2 (1.3)	57
	TOTAL		3386	16 116	19502	551	15652	16203	535 (2.7)	136 (0.7)	2628

Percentage of animals supplied to other Institutions: 80.3 %, NIN : 2.8 %(

( ) Values are percentage of number of animals available in each species.

TABLE 2. DETAILS OF BREEDING AND SUPPLY OF DIFFERENT SPECIES AND STRAINS OF LABORATORY ANIMALS (CONVENTIONAL COLONY) DURING THE PERIOD FROM 1.4.2002 TO 31.3.2003

Sl. No.	Species	Strain or Breed	Stock As on	Total Number of animals							Balance as on
			01.04.02	Bred During the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied	Died	Disp.	31.3.03
1	Mouse	Swiss (inbred)	677	4097	4774	16	3670	3686	112 (2.3)	90 (1.9)	886
2	Rat	WNIN (inbred)	1606	4341	5947	188	4547	4735	274 (4.6)	216 (3.6)	722
		WNIN/Ob-Ob (inbred)	807	245	1052	70	-	70	213 (20.2)	272 (25.9)	497
		WNIN/GR-Ob	784	444	1228	50	-	50	430 (35.0)	165 (13.4)	583
		Wkyoto (inbred)	169	184	353	34	-	34	27 (7.6)	109 (30.9)	183
		CFY/NIN (inbred)	63	64	127	-	-	-	10 (7.9)	18 (14.2)	99
		Holtzman (inbred)	109	146	255	-	-	-	19 (7.5)	35 (13.7)	201
		Wild White	53	22	75	-	-	-	30 (40.0)	-	45
3	Hamster	Golden (inbred)	160	452	612	-	368	368	60 (9.8)	60 (9.8)	124
4	Monkey	Macaca mulatta (Rhesus)	34	-	34	10	-	10	-	-	24
5	Sheep		1	-	1	-	-	-	-	-	1
6	Goat		4	-	4	-	4	4	-	-	-
		TOTAL	4467	9995	14462	368	8589	8957	1175 (8.5)	965 (6.7)	3365

Percentage of animals supplied to other Institutions: 59.4 %, NIN: 2.5 %
( ) Values are percentage of number of animals available in each species.

2. Supply of animal feeds

During the reporting period, apart from meeting the needs of the facility, 20420 kg of rat and mice feed and 1929 kg of guinea pig and rabbit feed were supplied to other institutions. There has been a marginal increase, 2.7%, in the supply of animal feed and 23.8% in the income generated from the sale of feed (Rs.12.5 lakhs in 2002-2003 compared to 10.1 lakhs in the previous year).

3. Supply of blood and blood products

During the reporting period a total volume of 797 ml of blood, sera and plasma were supplied to 10 different institutions including the hoste institute on 49 occasions. A sum of Rs.25,325/- was realized by sale of blood and blood products.

4. Health Monitoring

During the reporting year 480 samples were collected from various sources for parasitological, microbiological and virological screening as detailed below in Table 3.

Results

Parasitology

Few of the Swiss mice (Conventional) and BALB/c were found to have etoparasite mites (Myobia musculinus). This was eradicated by using appropriately autoclaved paddy husk as bedding. Among the animals screened, the conventional mice and rats were found to have Syphasia Obvaleta in ceacum. Few of the SD rats from the barrier-maintained colony were found to have liver cysts (tapeworm - Taenia taeniformis).

Microbiology

While WNIN rats maintained in the barrier colony were found to have sporadic presence of E.coli, *Proteus Sp. Staphylococcus Sp. Streptococcus Sp, Micrococcus Sp.*, and *Listeria* monocytogenes, the rats from conventional colony were found to have additionally, Corynebacterium *kutcheri* and Klebsiella *pneumoniae*. The BALB/c colony was found to be free of Klebsiella pneumoniae whereas the Swiss and C57 BL/6J showed the presence of this organism in few of the animals screened.

Table 3

Conventional Colony			
S.No	Species Strains	Number Screened	Age
1	WNIN Rats	25	15 >6 10 <6
2	Swiss Mice	54	<6 months
3	Hamster	01	>6 months
Barrier Maintained colony			
1	WNIN Rats	38	6 <6 months 32 >6 months
2	SD	93	>6 months
3	Fischer 344	02	<6 months
4	BALB/c Mice	42	6<6 36>6 months
5	Nude Mice	44	<6 months
6	C57BL6/J	44	02<6 months 42>6 months
7	G.pigs	40	>6 months
8	Rabbits	43	>6 months
Isolator colony			
1	SD Rats	02	>6 months
Other Samples			
Personnel		09	--
Water		10	--
Diet		11	--
Bedding		10	--
Cage, Canopy etc		12	--
Total		480	

Virology

Presence of Sendai virus was detected in obese mutant colonies (in all the phenotypes, WNIN Gr-Ob –6 , WNIN Ob/Ob – 6). Pneumonia virus of mice (PVM) was observed to be present in 21 obese mutant rats, 2 BALB/c, 16 N:NIH(S) nude and 6 Swiss mice. All other samples tested were found to be negative for PVM.

In order to strengthen the routine health-monitoring programme a project has been proposed under future projects.

5. Human Resource Development

As part of human resource development the Centre organized the following training courses:

1. 35th Annual Laboratory Animal Technicians' Training Course- Seven candidates attended and successfully completed.

2. 23rd Annual Laboratory Animal Supervisor's Training Courses was conducted during September – November and 12 candidates successfully completed the course.

3. Orientation in animal experimentation and welfare was imparted to 12 Ph.D. students of the Institute.

4. As part of pre-Ph.D. Course of NTR Medical University, Vijayawada, 24 medical personnel were taught on ethics, welfare and planning of animal experimentation.

5. Two postgraduate students of microbiology were trained in health monitoring of laboratory animals.

6. Ad hoc training for a week was imparted to two persons from Intervet India Ltd., Pune, one person from National Brain Research Centre, New Delhi and one person from Reddy Laboratories Ltd., Hyderabad.

6. Technical consultancy

During the reporting period one of the Senior Officer of the Center extended technical advise to the following institutions either for renovation of the existing animal facilities or for establishing new facilities by visiting these institutions:

1. National Institute of Virology, Pune.
2. National Aids Research Institute, Pune
3. National Institute for Research in Reproductive Health, Mumbai.
4. National Institute of Occupational Health, Ahmedabad.
5. National Brain Research Center, Gurgaon, Delhi.
6. Central Jalma Leprosy Research Institute, Jalma, Agra.
7. University of Delhi, South Campus, Delhi.
8. Center for Cellular & Molecular Biology, Hyderabad.
9. Center for DNA Fingerprinting & Diagnostics, Hyderabad.
10. Regional Medical Research Center, Bhubaneswar.
11. Regional Research Laboratory,Jammu

12. Industrial Toxicology Research Centre, Lucknow.
13. Institute for Immunohaematology, Mumbai
14. Bharat Biotech, Hyderabad.

In response to several queries from different institutions information pertaining to the care, breeding, procurement, nutrition, disease, and genetics of animals was also furnished.

7. Preclinical Toxicological Work

An advanced centre for Preclinical Toxicology Evaluation of new drug molecules and vaccines was established at the Institute based on the active participation and contribution of the staff from NCLAS, FDTRC and NIN.

During the year, the NCLAS facilitated to carry out animal experimentation pertaining to the pre-clinical toxicity evaluation of re-combinant anti-rabies vaccine and combination rabies vaccine involving the use of Swiss mice and Rhesus monkeys. The study was supported by the Department of Biotechnology. The final report on this study was submitted to the DBT this year.

8. New Initiative

Establishment of a National Animal Resource Facility for Biomedical Research at Genome Valley, Turkapally, Hyderabad

The Government of Andhra Pradesh had approached the National Centre for Laboratory Animal Sciences (NCLAS) for taking initiative for establishing a National Animal Facility, for safety evaluation of various new drug molecules, vaccines, biotech and herbal products, cosmetic etc., in the Biotech Park, Genome Valley at Turkapally, Hyderabad. During the initial interaction with the representatives of the Government of AP, it was indicated that allocation of land required for establishing such a facility free of cost would be possible.

Accordingly a proposal to establish a centralized National facility was mooted by the NCLAS with the approval of DG, ICMR in 2001. This centre is envisaged to have all modern state of the art facilities to ensure screening of all products as per the internationally acceptable standards and prevailing statutory requirements for global acceptance and marketing of the products.

As desired by the DG, ICMR, a brain storming meeting of the representatives from the local Pharma and Biotech industries was organized on 23rd January 2003 to consider a draft proposal prepared by the NCLAS on the need and feasibility of establishing such a centre. This meeting was also attended by representatives from ICMR and the National Institute of Nutrition and the National Centre for Laboratory Animal Sciences. The general consensus was that there is an urgent need for such a centre and a formal draft proposal was prepared and sent to the DG, ICMR for his perusal and approval. A brochure highlighting the major facility to be created and the activities proposed to be undertaken by this National facility was also prepared. The formal proposal was forwarded to the Secretary, Industries & Commerce Dept, Govt. of A.P. The Govt. of Andhra Pradesh welcomed the proposal and was kind enough to announce the allocation of a plot of land measuring 102.69 acres free of cost to the Indian Council of Medical Research, Ministry of Health & Family Welfare, Govt. of India, during the inauguration of the Biotech Invest Meeting held on 31st January, 2003. The Hon. Chief Mister of A.P., Sri N.Chandrababu Naidu announced the free allocation of the land and released the brochure on this proposed facility.

This new facility is envisaged to be:

1. A joint venture among the Private Industry (Pharma & Biotech), the Government of India (through Ministry of Health & Family Welfare) and Govt. of A.P.

2. A joint venture with appropriate representation through a Board of Directors from the Government of India, Govt. of Andhra Pradesh, Research organizations and the pharma and biotech industries.

3. A world class facility for breeding and housing large animals such as primates, canines and other specialized models such as transgenic

and knockouts, required for testing of various products and other R & D activities.

4. A complementary centre to the existing initiatives already taken by ICMR to provide laboratory animals for research, development and testing of new products and processes.

5. A centralized organization to train and generate adequate human resources needed for the specialized needs (drug development, safety evaluation etc.) of the industry.

6. A nodal point for establishing a net work of laboratories and facilities for co-ordinating the activities of animal resource facilities already existing within the country, with focus on the present and future needs of the industry.

7. A nodal point for establishing collaboration with international agencies, through appropriate linkage.

Since this proposed Centre is envisaged primarily to cater to the needs of Pharma & Biotech Industries, it is only appropriate to seek the views of the major pharma and biotech industries, with regard to the requirements of the industry, the facilities to be created, the mode of its function and the financial implications. A questionnaire to get the feed back is under preparation.

B. RESEARCH ACTIVITIES

1. Effect of "SATIOMEM" an anorexigenic membrane glycoprotein in WNIN/Ob rats - A preliminary study

Industrial Toxicology Research Centre (ITRC), Lucknow, has purified and characterized a anorexigenic glycoprotein from animal as well as plant membranes. This bioactive substance known as 'Satiomem' is a 45 Kda glycoprotein containing more than 65% carbohydrate consisting of mannose, glucose, glucosamine and galactosamine. This was found to cause significant loss of appetite (upto 45%) in normal laboratory mice and rats following parenteral administration. The biological effect was found to remain for more than 24 hrs and by 48 hours, the food consumption was found to return to normal levels. It has no rebound effects and gets metabolized by the end of 48 hrs of its administration without any toxic effect. It was felt that it would be worthwhile to administer and validate the effect of 'satiomem' in WNIN/Ob rats to explore the true nature of this compound.

Experimental design and methodology

Thirty-five days old WNIN/Ob female rats were used for the study. The animals were divided into control and experimental groups, each group consisting of six animals. The experimental group received 'Satiomem' in saline every alternate day i.p. at a dosage of 1.5 mg/kg body weight for 5 weeks followed by 3 mg for 2 weeks and 4.5 mg for the next two weeks. The control rats received saline alone. Food intake was measured every day and body weights were taken at weekly intervals. Blood samples were taken at 5, 7 and 9 weeks for lipid analysis (Triglycerides and cholesterol), clinical chemistry and for haematological parameters at the end of the experiment. The animals were sacrificed at 9th week and major organs were checked histopathologically for any toxic effects.

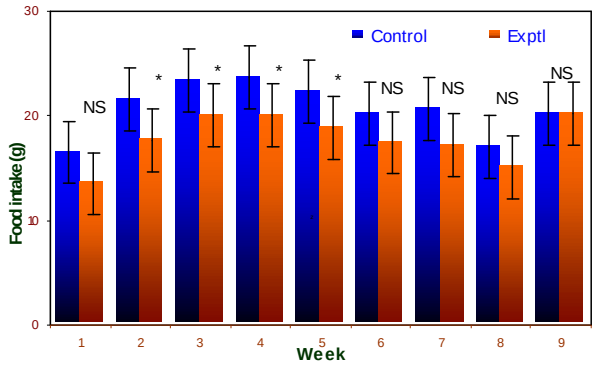
Results

1. Food intake was reduced by 20% in the experimental group, which was significant at 2nd, 3rd and 4 weeks ($P < 0.005$) (Fig. 1).
2. Increase in dosage of satiomem (3 mg and 4.5 mg/kg) did not bring about further reduction and on the contrary, the reduction observed earlier vanished at the end of the experiment (Fig 1).
3. The experimental group showed a marginal reduction in body weight, though the reduction was not statistically significant (Figure 2).
4. Haematological parameters, lipid profile and clinical chemistry parameters did not show any difference between control and experimental groups.
5. All the organs showed normal histopathology.

Conclusion

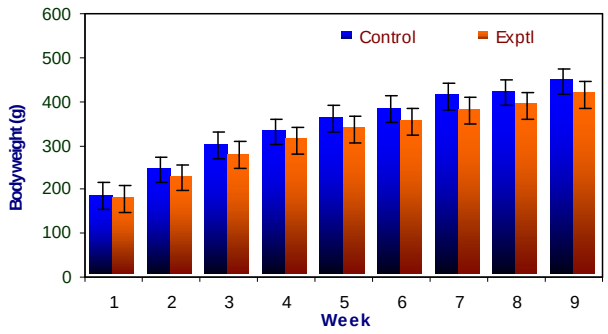
The preliminary study confirmed the anorexigenic effect of 'satiomem' observed earlier in normal mice and rats. But unlike in the earlier study, where the reduction was found to be 40%, in the present study the reduction was not more than 20% and the effect wore off after 9 weeks, even after increasing the dosage. The difference in the response of this drug between normal rats and the WNIN/Ob rats may be due to the genetic difference of the latter.

Fig 1. Effect of Satiomem Injection on Food Intake of WNIN / Ob Rats



* : p 0.05 : Values are Mean SD of six rats.

Fig 2. Effect of Satiomem Injection on body weight of WNIN / Ob Rats



No significant difference. Values are Mean SD of six rats

Satiomem Dose : IP Injections : 1 to 5 weeks :1.5 mg/Kg b.wt in salin
6 & 7 week : 3 mg/Kg b.wt in saline
8 & 9 week : 4.5 mg/Kg b.wt in saline

2. *Study of haematological parameters and some receptors involved in lipoportein metabolism*

Obese rats of WNIN/Ob strain are hyperinsulinemic, hyperleptinemic, hyperlipidemic (hypertriglyceridemic and hypercholesterolemic). The increase in plasma total cholesterol was mainly due to an increase in HDL-C fraction. Interestingly, feeding of high but not toxic levels of vitamin A and polyunsaturated fatty acid rich safflower oil instead of groundnut oil in the diet could effectively reduce the HDL-C. In addition, some of the haematological parameters also showed certain changes, which could be due to abnormal lipoprotein metabolism.

3. *An experimental study to identify the source plant of 'Lakshmana' with reference to its activity in female infertility - A preliminary screening*

Euphorbia pilurifera, Solanum xanthocarpum and Ipomea sepiaria are three important medicinal plants described in Ayurveda literature having phytoestrogenic activities. In the ancient Ayurvedic text there is a mention of one preparation called 'Lakshmana' which is used for treatment of female infertility. There are claimants from different Ayurvedic schools regarding the source of 'Lakshmana', and all the three plants listed above are projected to be source of 'Lakshmana'.

The current study is planned to identify the true claimant of Lakshmana out of the three plants described above by testing the effects of the extracts of these plants in WNIN/Ob female rats which have estrogen deficiency resulting in abnormal estrous cycles and stunted growth of its reproductive structures. It was decided to test the efficacy of these plant extracts in reversing the infertility seen in these rats and thereby identify the true 'Lakshmana' amongst them.

Study design and methodology

The female WNIN/Ob rats at the weaning age were selected for the study. There were four experimental groups consisting of six animals. Group I receiving normal laboratory chow, group II plant I extract (Euphorbia fusifornis) group III receiving plant II extract (Solanum xanthocarpum) and group IV receiving plant III extract (Ipomea sepiaria). The plant extracts were given to the animals through diet at 1% level. Animals were observed for the day of vaginal opening and estrus cycle stages. At the end of

90 days, animals were sacrificed and reproductive organs along with kidney and liver were examined for histopathology.

The study design was modified slightly with respect to the number of animals and the phenotypes used for the study subsequently as most of the animals selected at 21 days turned out be heterozygous carriers (+/-), and only one homozygous obese (-/-) animal was available per group. The experimental groups thus consisted of 6 heterozygous carriers (+/-) and one homozygous obese animal (-/-), and a total number of 28 animals were used for the study.

Results

1. There was no difference in the days taken for the opening of the vagina in experimental groups (II, III and IV), compared to control (group I).

2. In the experimental group, 83% (5/6) of group II showed regular estrus cycle (4-5 days cycle with all 4 stages), compared to 66% (4/6) in group II, 33% (2/6) in group IV and 16% (1/6) in control group.

3. There was a significant difference in the weight of the uterus in experimental group II (0.54+0.03), compared to control I (0.46+0.02), III (0.47±0.03) and IV (0.44+0.02) groups.

4. The length of the uterine horn also slightly increased in-group II (3.52+ 0.020), as compared to group III IV and I (3.18+0.09, 3.3+ 0.23 and 3.4+0.24) respectively.

5. The weight of the ovary was also found to be increased significantly ($P<0.05$) in group II (0.68+0.11) compared to group I (0.54 +0.09), III (0.50+0.08) and IV group (0.52+0.09) respectively.

6. The effect was more dramatic in homozygous obese rats of the experimental groups especially in-group II, compared to the control group. In female obese rat of group II and III the days for opening of vagina decreased by several days, (group II – 45 days vs group III - 59 days, group IV - 77 days, group I - 78 days). The group II obese showed normal estrus cycle and the weight of the uterus in the animal was 0.40 gm and the length 4.2 cm compared to 0.30 gm and 3.5 cm in the control. The ovaries also showed an increased weight compared to control.

7. All the experimental animals showed normal histopathology compared to control.

Conclusion

It would have been ideal to have homozygous obese female (-/-) only for the study, but unfortunately most of the animals selected on the basis of BMI at 21 days turned out to be carrier animals and only few were homozygous obese animals. Inspite of this major draw back, the study still enabled us to draw proper conclusions.

As per the literature survey, the local plant Euphorbia fusifornis surmised to be the real source of "Lakshmana". The above study also supports this above view. Only this plant extract showed maximum phytoestrogenic effects, by way of increased weights in ovary, uterus etc. and also in correcting the oestrus cycles, both in carrier as well in the obese animals.

INSTRUMENTATION SERVICES

The instrumentation division forms the backbone of all research activities of the Institute. It is involved in the procurement, installation, maintenance, servicing of equipments so that they are always in working condition for the benefit of the scientists. Wellestablished procedures developed over a course of time and adopted by various other reputed all India institutions are followed for this purpose. There are varieties of scientific equipments belonging to the categories of the most sophisticated electronic, electro-mechanical and refrigeration based. These instruments are constantly in use and need to be attended to, both as part of preventive maintenance as well as repairing them in times of failure. The following table indicates the number of complaints received during the period mentioned above and also the action taken in addition to providing information on the new installations carried out during the period.

Divisions of Department	Equipment Installed	Equipment Complaint received	Equipment Repaired	Pending
Electronics	72	185	175	10
Electro-Mechanical	13	148	130	18
Refrigeration & Air-Conditioning	22	168	168	--
Total	107	501	473	28

Training

The staff of the department take part in various training programmes of the institute as members of the faculty to deliver lectures and also arrange for demonstration of various instruments with standards for calibration and explain the various features. The following are the training programmes of the Institute in which our staff have participated:

1. M.Sc (Applied Nutrition).
2. Endocrionological Techniques.
3. Certificate Course in Nutrition.
4. Assessment of anemia.
5. Research Scholars of our institute
6. Research Scholars from the NTR University of Health Sciences
7. Various adhoc programmes.

Extra activities

The services of the department were also utilized by the RMRC, Bhuvaneswar for servicing of a wide range of equipment at their institute on the approval of the Director. Mr.A.K.V. Rajamouli, Technical Officer and Mr.B.V.Prasanna Kumar, Technical Officer were deputed by the Institute.

Mr. B. Ramulu, *Technical Officer*, was on deputation to Blue Star, Pune, for a two days training session on maintenance of screw type compressors. He was closely associated with the installation of the Air-conditioning plant for the Primate Facility and was made responsible for monitoring the progress of the work and report the matter periodically.

He was also nominated by SRAMIKA VIDYA PEETH, Panjagutta, Hyderabad, as an external examiner on their Board.

The following members of staff under went training on First-Aid .

1. Mr. Mohd. Younus
2. Mr. K. Srinivasa Raju
3. Mr. G. P. Narender

The instrumentation department of the institute has been identified as a centre for providing Inplant Training to the participants undergoing a course in Medical Electronics and Process Control Instrumentation at the Advanced Training Institute at Ramanthapur, Hyderabad as part of Man power building activity and supported by the Union Ministry of Labour . Every year not less than four candidates will be provided the necessary training.

Special efforts were taken to organise lectures on various topics of interest involving the latest instrumentation techniques and their

applications in biological and nutritional research studies. Some of them are given below:

1. The techniques of GC-MS and its applications in biological research - Perkin Elmer.

2. The techniques of ICPMS and its applications in biological research -Varian.

3. The techniques of LCMS and its applications in biological research -Shimadzu.

4. The techniques of Flow Cytometry and its applications in biological research -Beckman Coulter.

The following seminars were attended by the staff of the department.

1. LC-MS and its application in biological research- Perkin Elmer.

2. ICP-MS and its applications. - Perkin Elmer.

Conferences & Training Courses attended

Mr. V. Satish Babu, T.O.

1. ISMAS Silver Jubilee Symposium 2003 on Mass Spectrometry from 27-1-2003 to 31-1-2003

2. Training programme on Basic Trouble shooting and maintenance of GC 6890HP at Agilent Technologies,Singapore from 24-2-2003 to 26-2-2003.

Mr. R. Chaugule, T.O.
Mr. K. Sreenivasa Rao, T.A.

Advanced Service Training Course on HPTLC(CAT WIN) from 4-6-2002 to 6-6-2002 at Mumbai.

List of Equipment Installed 2002-03

S.No.	Name of the Equipment	Make	Model
1.	Pulse Oximeter and Cardiac Output Device	BCI International	Auto Corr
2.	Vertical dual cooled Electrophoresis(Without power pack)	Hoefer	SR 600
3.	Vertical Electrophoresis	Hoefer "	SE 260 Mighty Small II SE 250 Mighty Small II
4.	Electrophoresis(Vertical)	Hoefer	SE600
5.	Electrophoresis Power Pack	Bio-rad	3000
6.	Electrophoresis Power Pack	Amersham	EPS 601
7.	Capillary Electrophoresis	Bio-Rad	Bio-Focus 3000

8.	Hybridization oven/ Shaker	Amersham Pharmacia	RPN 2510
9.	Microwave Oven	BPL	800G
10.	Transilluminator	UVP Inc.	LMS20E
11.	UV Cross Linker	Hoefer	UVC 500
12.	Gel Drying System with vacuum system	Bio-Rad	
13.	Gel Drying System	Bio-rad	583
14.	Densito meter with scanner and printer	Bio-Rad	GS-800
15.	Table Top Centrifuge -2nos.	Heraeus	Biofuge pico
16.	Micro Centrifuge	Eppendorf	5415D
17.	Table Top Refrigerated Centrifuge	Sigma	3K30
18.	Super speed Centrifuge .	Beckman Coulter	J-25 AVANTI
19	Incubator-Shaker-4nos	Heidolph	
20.	Trinocular Research Microscope	Nikon	E-800
21.	Trinocular Research microscope	Leica	DMLB + DC100
22.	Inverted Microscope	Nikon	TS 100
23.	Tissue Processor	Shandon	Citadel 2000
24.	Polygraph	Biopac	MP 100
25.	Activity Monitor	Columbus	--
26.	Feed in take and Water in take measuring system with software	Columbus	--
27.	Plethysmometer	Panlab	LE 7500
28.	Implantable Animal ID System	Stoelting	
29.	GC-MS	Perkin Elmer	Turbomass
30.	UV-VIS Spectrophotometer	Thermo Spectronic	330
31.	UV-VIS Spectrophotometer	Perkin Elmer	EZ201
32.	Spectrophotometer -2nos.	Thermo Spectronic	Aquamate
33.	Nitrogen Generator	Domnick Hunter	UHPN1500
34.	Hydrogen Generator	Claind	2600
35.	PCR Machine	Applied Biosystems	9700

36.	PCR Machine	Eppendorf	Master cycler 5333
37.	Auto sampler for HPLC	Shimadzu	SIL-10DVP
38.	TLC Plate Coater	Camag	
39.	CO_2 Incubator	Thermo Forma	Steri Cycle
40.	CO2 Incubator	Thermoforma	381
41.	Variable Temp.Incubator	Heraeus	
42.	Low Temp. Water Circulator	Julabo	F-12MP
43.	Liquid handling system	Thomas Scientific	202350
44.	Micro Balance	Sartorius	BP145D
45.	Balance	"	BL 610
46.	Balances -3nos.	Sartorius	BLC
47.	Balances -6nos.	Essaeteraoka	
48.	Balances -3nos.	Denver	XP 3000
49.	Balance	Mettler	AX504
50.	UPS Systems3KVA -5nos.	Consul	
51.	UPS systems 2kVA-1no	Consul	
52.	RIA Software for Auto Gama Counter	Hewlett Packard	Cobra II
53.	Digital Copy Printer	Gestetener	5308B
54.	Water Purification System	Labconco	90007-03
55.	PH meter	Orion	420A+
56.	Ultrasonic Water Bath	Branson	B1510E
57.	Water Purification System	Millipore	ELIX10 , Millique Biocell, Surepro

LIBRARY AND DOCUMENTATION SERVICES

The Library of NIN has continued to cater to the documentation and information needs of the Institute and other ' research organizations, besides helping the Home Science and Medical Colleges ' for their information needs. It has also played a key role in the area of reference activities by providing functional and informative resources such as MEDLINE searches, ProQuest searches and other On-line retrieval activities using the LAN network. Participation in exchange of information amongst ICMR Libraries was another major activity of the Library and for this purpose the URL < http://Groups.com/Group/ICMR Librarians > was actively used.

The Library automation activity for Cataloguing the print-collection has progressed satisfactorily. The ISIS and the LIBRIS softwares were used extensivzely during the year and in the process, over 15000 bibliographical records which were reconverted using the LIBRIS package, were made accessible over the LAN Nodes using the URL < http://Library4/NIN >.

The Library actively participated in developing in the ICICI-Knowledge Park sponsored Website <www.Jccc-vic.informindia.co.in > wherein a Library Consortium consisting of seven (7) National Libraries are involved. With the help of this Website, we are now able to access bibliographical information from over 500 biomedical Journals and all this data can be accessed by using the **User-ID** along with **Password ' nin ' .**

The other important support activity carried out by the Library was to help Scientific, Technical / Administrative staff and outside clientele with excellent quality and instant photostat facility. Resource - sharing and User - Education Programmes were also under taken actively. We are also happy to report that the Institute's Papers slated for publication in national / international journals are regularly routed through the library. Several of the published papers have also been made accessible to our users through the data-base which in turn provides them the information through our on-line services. Data pertaining to journals availability at NIN are also made accessible through the URL < http://uncat.nic.in>.

The Library has contributed substantially to the development of " A Draft Manual of Procedures for Management of Libraries and Information Centres of ICMR ". Incidentally the library also has undertaken the task of training the Librarian of RMRC (ICMR) Port Blair, this year.

The following Journals (Nine in number) have been added to the existing subscription list of 2002.

1. Antioxidants & Redox Signaling
2. Bone
3. Calcified Tissue International
4. Diabetologia
5. Fitoterpia: The Journal for the study of Medicinal Plants
6. Internat J Obesity
7. J Clinical Endocrinology & Metabolism
8. J. Ocular Pharmacology & Therapeutics
9. Nature Medicine

The Library has taken a Corporate Membership from ' Universities Federation for Animal Welfare, UK ' for 2002.

The following library services were expanded as detailed below.

1. NEW ADDITIONS

Books	*267*
Reports	*470*
Journals(New Subs.)	*9*
Thesis / Dissertations	*2*
Microforms	*27*
CDROMS (MEDLINE)	*12*

2. OTHER ACTIVITIES

Journals Bound	*307*
Visitors using the Library	*6,127*
Circulation of Books/Journals etc.	*2,937*
MEDLINE Abstracts provided	*7,090*
No. of E-mails sent outside	*204*

No. of E-mails received	*966*
Photocopying (No. of pages)	*3,84,977*
Number of Annual Reports mailed	*625*
No. of Books/Journals received on Inter Library Loan	*300*
No. of Duplicate Journals sent out	*200*
No. of INTERNET Searches provided	*200*
No. of Reprints sent	*228*
ProQuest Full Text Database Searches provided	*50*

3. TOTAL LIBRARY COLLECTIONS

Books	*15,129*
Journals (Bound Volumes)	*26,508*
Journals subscribed for 2002	*237*
Journals received (Gratis/Exchange)	*285*
Microforms (Microfiche)	*1017*
Slides	*277*
Reports	*10,373*
Reprints	*3,07,476*
Theses & Dissertations	*334*
MEDLINE CDROM Discs	*128*
Current Contents on Diskettes with Abstracts	*664*
ProQuest (Ful Text E-Journals) on CDROMS	*326*

4. BIBLIOGRAPHIES COMPILED

1. A Catalogue of Books & Serials on ' Laboratory Animal Science'
   Available at NCLAS (Library Documentation Bull.Sr.4.Suppl.2) 2002.
2. A Select List of Journals in Nutrition Science (Documentation Bulletin Sr.105), 2002.
3. A Select List of useful URL's in Nutrition Informatics (Documentation Bulletin Sr.106), 2002.
4. List of Subscription Journals Received (Daily - Chart).
5. NIN Library Holdings, 2002.
6. List of NIN Scientific Publications, 2002.

SCIENTIFIC PUBLICATIONS OF NIN FOR 2002 (DIVISION - WISE)

Sl. No	Name of the Division	In Peer Reviewed Jls.	In Conf. Reports	Books/ Reports	Popular Articles
1	Clinical Division	4	-	1	1
2	Pathology	2	-	-	1
3	Biochemistry Division	4	1	-	-
4	Molecular Biology	3	-	-	1
5	Biophysics	4	2	-	3
6	Food Chem-Analytical	1	-	-	-
7	Endocrinol. & Metabol.	4	-	-	1
8	Field Division (NNMB)	7	-	2	1
9	Statistics	7	1	-	-
10	Education & Training	-	-	-	5
11	Food Toxicology	3	1	-	1
12	Drug Toxicology	6	-	-	1
13	NCLAS	-	-	-	2

PH.D PROGRAMMES

Ph.D. Awardees

RESEARCH SCHOLAR/STAFF	UNIVERSITY	YEAR	TITLE OF THESIS
1. Chennaiah S.	Osmania	2002	Isolation and characaterisation of calcinogenic principle from Solanum melongena leaves.
2. Hemalatha S.	Osmania	2002	Biochemical and metabolic studies with sesame lignans

RESEARCH SCHOLARS REGISTERED FOR PH.D.

RESEARCH SCHOLAR/ STAFF	TITLE OF THE PROJECT	GUIDE
1. Anil Kumar Dube (1993)	Nutritional education for urban adolescents: Use of social marketing principles in communication	Dr. Mohan Ram, M.
2. Rajendraprasad. M.P. (1997)	Nitrosamines and its relevance to cancer in India	Dr.Kamala Krishnaswamy
3. Radhika, M.S. (1998)	Effect of food based vitamin A supplementation during pregnancy on maternal and child health	Dr. Bhaskaram, P.
4. Nirmala, K. (1999)	Plant constituents as - chemopreventive agents	Dr. Kalpagam Polasa
5. Pratima Rao (1999)	Multicentric study on intake of food colours	Dr. Ramesh Bhat, V.
6. Vijayalakshmi, A (2000)	Role of nutrition in modification of apoptosis	Dr. Raghunath, M./ Dr. Sesikeran, B.
7. Saravanan N. (2000)	Effects of dietary alteration of n-6 and n-3 polyunsaturated fatty acids on insulin resistance, structure and function of adipocytes	Dr.Ghafoorunissa
8. Sreedhar B. (2000)	Iron and zinc interactions at the site of absorption	Dr. Madhavan Nair, K.

RESEARCH SCHOLAR/ STAFF	TITLE OF THE PROJECT	GUIDE
9. Jayakumar S. M. (2000)	Studies on food intake regulation and obesity in WNIN/Ob and WNIN/GR-Ob rats	Dr. Vajreswari, A.
10. Rita Saxena (2000)	Role of food processing on antioxidant activity and development of recipes with high antioxidant activity	Dr.M.Raghunath
11. Venu L. (2001)	Foetal metabolic programming for insulin resistance: Identification of causative maternal nutritional factors (Micronutrients)	Dr. Raghunath, M.
12. Krishna Kumari Menon (2001)	Positive Deviance in child nutrition	Dr.Vijayaraghavan, K.
13. Manjula T. (2001)	Ethno-pharmacological validation of biodynamic compounds in traditional medicine	Dr. Dinesh Kumar, B.
14.Satish Kumar (2001)	Molecular chaperone function of alpha crystalline	Dr. Bhanuprakash Reddy G
15. Ms. Aruna, B. (2002)	Biophysical characterisation or resistin	Dr. Nasreen Z Ehtesham
16. Haseeb A. (2002)	Understanding the mechanism of action of PPAR? as a link molecule between obesity, type 2 diabetes and CHDs	Dr. Nasreen Z Ehtesham
17. Uma Devi A. (2002)	Study of energy metabolism in WNIN obese rat mutants	Dr. Giridharan NV
18. Kiran Kumar B (2002)	Genetic typing of WNIN/Ob and WNIN/GR-Ob strains using microsatellite markers	Dr. Giridharan NV
19. Anil Kumar (2002)	Molecular chaprone function of alpha crystallin under hyper glycemic condition : Modulation by antiglycating and aldose reductase inhibitory potential of dietary factors	Dr. Bhanuprakash Reddy G

RESEARCH SCHOLAR/ STAFF	TITLE OF THE PROJECT	GUIDE
21. Megha Saraswat	Screening of aldose reductase inhibitors and anti-glycating agents from dietary sources and assessing their anticarcinogenic potential	Dr. Bhanuprakash Reddy G
22. Mrudula T	Characterization and significance of a novel fatty acid elongase of the eye lens	Dr. Bhanuprakash Reddy G

PARTICIPATION OF SCIENTISTS IN VARIOUS MEETINGS/SEMINARS/SYMPOSIA/WORKSHOPS

2002

April 6 — **Dr. Kamala Krishnaswamy**: ICSU National Committee Meeting of Indian National Science Academy, New Delhi.

April 10-11 — **Dr. Kamala Krishnaswamy**: Scientific Advisory Committee on Cancer Research, at ICMR Hqrs, New Delhi.

May 5-10 — **Dr. G.Bhanuprakash Reddy**: Annual Meeting of Association for Research in Vision and Opthalmology (ARVO), held at Fort Lauderdale, Florida, USA.

May 5-8 — **Dr. Ghafoorunissa:** 93rd AOCS Annual Meeting and Expo, at Montreal, Quebec, Canada.

May 7-11 — **Dr. Ghafoorunissa**: Conference of ISSFAL 2002 – Dietary fats and health, at Montreal, Quebec, Canada.

June 8 — **Dr. B.Sesikeran**: Seminar on "CME-Oral Pathology"' at Osmania Medical College, Hyderabad.

June 8-15 — **Dr. K.Vijayaraghavan**: Meeting of the Expert Committee on "Standardization of health indicators, daily requirement of food and nutrition and analyzing the nutritive value of the diet of the Jarawas", at Regional Medical Research Centre, Port Blair.

June 10-14 — **Dr. B.Sivakumar**: Meeting of the Expert Group on "Medical research and drug regime in respect of Jarawa tribes", at Regional Medical Research Centre, Port Blair.

August 1 — **Dr. B.Sesikeran**: All India Workshop on WHO Project on "Development of an Atlas of Cancer in India", at Indian Institute of Science, Bangalore.

Sept. 13-15 — **Dr. B.Sesikeran** and Dr. **P. Uday Kumar**: XXI Association of Pathologists and Microbiologists Conference (A.P. State Chapter), at Guntur medical College, Guntur.

Oct. 21-22 — **Dr. V.Jagadeesan**: National Seminar on "Electro and Magneto Ceramics, Devices and Systems", at skanharrao Mohite Mahavidyalaya, Maharashtra.

Oct. 17 — **Dr. T.C.Raghuram**: Meeting of Nutrition Foundation of India, New Delhi.

Nov. 22-28 — **Dr. P. Uday Kumar** and **Dr. K.V.Radhakrishna**: ICMR Workshop on "Clinical pharmacology in traditional medicine", Mumbai.

Nov. 24-29 — **Dr. G. Bhanu Prakash Reddy**: Symposium on "Current excitement in Biology", at Centre for Cellular Microbiology, Hyderabad.

Dec. 15 — **Dr. B.Sivakumar, Dr. K.Vijayaraghavan, Dr. Veena Shatrugna** and **Dr. Shahnaz Vazir**: Symposium on Micronutrient Supplementation in Health and Disease, organised jointly by National Institute of Nutrition and Centre for Research on Nutrition Support Systems, at India International Centre, New Delhi.

2003

Jan. 3-7 **Dr. K.V.Rameshwar Sarma, Dr. D.Raghunatha Rao and Mr. G.M.Subba Rao**: Science and Technology exhibition "Science Expo-2003 – Pride of India", organized as a part of the 90th Session of the Indian Science Congress, Bangalore.

Jan. 10-12 **Dr. M.P.Rajendra Prasad** : ICMR/ WHO Workshop on "Strategies for prevention and control of diabetes mellitus", New Delhi.

Jan. 30-31 **Dr. Ahmed Ibrahim** and **Dr. P. Uday Kumar**: First winter Symposium on "Cell biology and molecular medicine", at CMC, Vellore.

Feb. 23-27 **Dr. Ghafoorunissa, Dr. Ramesh V.Bhat, Dr. B.Sivakumar, Dr. K.Vijayaraghavan, Dr. G.N.V.Brahmam, Dr. B.Dinesh Kumar, Mr. S.Ghosh, Mr. T.Longvah, Dr. K.Madhavan Nair, Dr. K.V.Ramehwar Sarma, Dr. M.P.Rajendra Prasad, Dr. B.Sesikeran, Dr. Shahnaz Vazir, Dr. M.Vishnuvardhan Rao, Dr. D.Raghunatha Rao, Dr. S.Vasanthi, Dr. Arjun L.Khandare, Mrs. Krishna Kumari Menon, Dr. M.Raghunath, Dr. P.Ravinder, Mrs. Rita saxena, Dr. L.Singotamu, Dr. D.Sreeramulu, Dr. Y.Venkataramana** and **Dr. C.Vijayakumar Reddy**: Participated and presented papers/ posters in the IX Asian Congress on Nutrition, sponsored by Federation of Asian Nutrition Societies, Nutrition Society of India and Nutrition Foundation of India, held at New Delhi.

WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT THE INSTITUTE

1. Methodology of collection of blood samples for haemoglobin estimation. Sixteen investigators of RCH Programme from West Bengal underwent training-cum-standardisation (May 6-8, 2002).

2. FAO-DGHS-NIN sponsored Workshop on "Strengthening food safety and quality systems in India" (June 28-29, 2002).

3. The 35th MSc (Applied Nutrition) Course (17th June 2002 to 14th March 2003).

4. Training programme in medical Librarian-ship for Ms. Sangeetha Kumari, from RMRC, Port Blair (May 28 – June 27, 2002).

5. Meeting of the NNMB Steering Committee (July 12, 2002).

6. Meeting of the Pre-SAC and Scientific advisory Committee of NIN/FDTRC/NCLAS (Aug. 8-9, 2002).

7. FAO-UNESCO Inter-Country Workshop on "Feeding Minds, Fighting Hunger, Malnutrition and Food Insecurity" (Aug. 27-29, 2002).

8. Adhoc training programme for two WHO Fellows from Bhutan, in the field of Community Nutrition (Aug.5 – Sept.12, 2002).
9. XXXI Annual Training Course on Endocrinological Techniques and their Applications (Aug.12 – Sept.27, 2002).

10. National Nutrition Week celebrations (Sept. 1-7, 2002).

11. ICMR Annual Day celebrations (Nov. 2002)

12. A Training Course on "Techniques for Assessment of Nutritional Anaemias" (Dec. 2-13, 2002).

13. Ad-hoc Pre-PhD training programme for the registered scholars of NTR University of Health Sciences (Dec. 16-27, 2002).

14. XXXX Post – Graduate Certificate Course in Nutrition (Jan.1 – March 15, 2003).

15. Essay writing competition for school children and Debate competition for Inter-College students in connection with National Science Day Celebrations (Feb. 2003).

16. Adhoc training programme for three WHO participants from Myanmar in Public Health (Feb. 3-14, 2003).

17. Two day interactive programme on Research Methodology for prospective guides in medical colleges of AP, organised by NIN and NTR University of Health Sciences, Hyderabad (March 21-22, 2003).

SERVICES RENDERED TOWARDS INCOME GENERATION FOR THE INSTITUTE

1. Technical Service

Analytical Chemistry Department of the Institute undertaken the analysis of the samples sent by Government and Non-Governmental Organizations under the following three categories:

i) Proximate composition
ii) Trace mineral analysis
iii) Amino acid analysis

An amount of Rs.1,00,322/- was generated during the year on analysis of the samples.

2. Pathology Services

A sum of Rs.2,29,875/- was generated during the year on toxicology services (Histopathology) and diagnostic services provided by the Pathology Division.

3. Training Programmes

By admitting 12 unsponsored private candidates to the three regular training courses, Rs.86,000/- was generated.

SCIENTIFIC PUBLICATIONS

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Bamji MS, Neelam: Environment and nutrition-effects of interactions and interaction with heavy metals. Proc. Indian Nat. Sci. Acad. B68 (5):401-414, 2002.

2. Bhanu Prakash Reddy G, Narayanan S, Yadagiri Reddy P, Surolia I: Suppression of DDT-induced aggregation of alpha a and alpha b-crystallins: A model aggregation assay for alpha crystallin chaperone activity *in vitro*. FEBS Letters. 522 (1) :59-64, 2002.

3. Bhanuprakash Reddy G, Yadagiri Reddy P, Vijayalaxmi A, Satish Kumar M, Suryanarayana P, Sesikeran B: Effect of long-term dietary anipulation on the aggregation of rat lens crystallins: Role of alpha-crystallin chaperone function. Molecular Vision. 8:298-305, 2002.

4. Bhaskaram P: Micronutrient malnutrition, infection and immunity: An overview. Nutrition Rev. 60 (5) :S40-S45, 2002.

5. Bhat, RV, Sudershan RV: The agreement on the application of sanitary and phytosanitary measures and its relation to technical barriers to trade. Packaging India. 35(5): 73-74, 2002.

6. Ghafoorunissa, Vani A, Laxmi R, Sesikeran B: effects of dietary alpha-linolenic acid from blended oils on biochemical indices of coronary heart disease in indians. lipids. 37(11): 1077-1086, 2002.

7. Hemalatha R, Bhaskaram P, Balakrishna N, Saraswathi I: Association of tumor necrosis factor alpha & malnutrition with outcome in children with acute bacterial meningitis. Indian J Med Res. 115 (2) :55-58, 2002.

8. Kamala Krishnaswamy, Lakshmi AV: Role of nutritional supplementation in reducing the levels of homocysteine. J. Assoc. Physicians of India. 50 (5S) :36-42, 2002.

9. Kamala Krishnaswamy, Naidu AN, Prasad MPR, Reddy GA: Fetal malnutrition and adult chronic disease. Nutrition Rev. 60 (5) : S35-S39, 2002.

10. Khandare AL, Rao GS, Lakshmaiah N: Effect of tamarind ingestion on fluoride excretion in humans. Europ J. Clin. Nutr. 58 (1) : 82-85, 2002.

11. Krishnaiah YSR, Satyanarayana V, Dinesh Kumar B, Karthikayan RS: Studies on the development of colon-targeted delivery systems for celecoxib in the prevention of colorectal cancer. J. Drug Targetting. 10 (3) :247-254, 2002.

12. Krishnaiah YSR, Satyanarayana V, Dinesh Kumar B, Karthikeyan RS: *In vitro* drug release studies on guar gum-based colon targeted oral drug delivery systems of 5-fluorouracil. Euro. J. Pharmaceutical Sci. 16 (3): 185-192, 2002.

13. Krishnaiah YSR, Satyanarayana V, Dinesh Kumar B, Karthikeyan RS, Bhaskar P: *In vivo* evaluation of guargum-based colon targeted oral drug delivery systems of celecobix in human volunteers. Euro. J. Drug Metab. Pharmakokinetics. 27(4): 273-280, 2002.

14. Kumar KSD, Kumar A, Shiva Prakash, Swamy K, Jagadeesan V, Jyothy A: Role of red cell selenium in recurrent pregnancy loss. 22(2): 181-183, 2002

15. Laxmaiah A, Mallikharjuna Rao K, Brahmam GNV, Sharad Kumar, Ravindranath M, Kashinath K, Radhaiah G, Hanumantha Rao D, Vijayaraghavan K: Diet and nutritional status of rural preschool children in Punjab. Indian Pediatrics. 39 (4) :331-338, 2002.

16. Mallikharjuna Rao K, Balakrishna N, Hanumantha Rao D, Bhaskara Rao B: Nutritional status of pre-school children in different agro-economic regions of Andhra Pradesh. J. Human Ecol. 13(5):363-367, 2002.

17. Mujeeb-Ur-Rahman, Visweswara Rao K: Pattern of food consumption and nutrient adequacy - A case study of adults by region. Indian J Nutr Dietet. 39 (4) :160-172, 2002.

18. Nasreen Z. Ehtesham: Does resistin resist insulin. Current Science 83(10): 1190-1191, 2002.

19. Pratima Rao, Bhat RV, Naidu AN: Situation analysis of the availability of foods with added colours in Hyderabad vis-a-vis the PFA act. Indian Food Industry. 21(4):41-43, 2002.

20. Radhika MS, Bhaskaram P, Balakrishna N, Ramalakshmi BA, Savitha Devi, Sivakumar B: Effects of vitamin A deficiency during pregnancy on maternal and child health. Brit. J Obst & Gynaecol. 109 (6) :689-693, 2002.

21. Raghu P, Bhanu Prakash Reddy G, Sivakumar B: Inhibition of transthyretin amyloid fibril formation by 2,4-dinitrophenol through tetramer stabilization. Arch Biochem Biophys. 400 (1) :43-47, 2002.

22. Ranganathan S, Someswara Rao M, Nagaratnam A, Mishra UC: Internal ^{40}K radiation dose to Indians. Radiation Protection and Environment. 25(2): 66-71, 2002.

23. Shahnaz Vazir: Behavioral aspects of development of eating behavior and nutritional status. Nutrition Rev. 60 (5) :S95-S101, 2002.

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