I. COMMUNITY STUDIES

1. A RAPID SURVEY ON DIET AND NUTRITIONAL STATUS OF SAHARIA - A PRIMITIVE TRIBE IN RAJASTHAN

The 'Saharia' is one of the primitive tribes of the State of Rajasthan, inhabiting mostly the Shahbad and Kishanganj blocks of Baran district. It is economically most backward tribe. Baran is one of the chronically drought prone districts of Rajasthan. Recurrent droughts have adverse effects on the household food security leading to high prevalence of undernutrition of the tribe (Ann.Rep.2003-2004). There were reports in the media regarding starvation deaths among Saharia tribal community in Kishanganj block of Baran district. A rapid survey was therefore, carried out among the Saharia tribe during October-November 2004, to assess their nutritional status and to investigate the incidence of starvation deaths, if any.

Objectives

- To assess food and nutrient intakes at the household level among the Saharia tribe in Shahbad and Kishanganj blocks of Baran district.
- To assess the nutritional status of <5 year children and adults in the community, in terms of anthropometry and prevalence of clinical signs of nutritional deficiency and
- To collect information on the cause of deaths if any, that had occurred during previous six months, by conducting verbal autopsy.

Methodology

Keeping in view the quick nature of the survey, it was decided to cover a total of 200 children for the nutritional assessment among <5 year children @ 40 children in each group of 0+ to 4+ years. With an expected coverage of 25 children per village, it was proposed to cover a total of 8 viallges selected randomly from the two blocks namely Shahbad and Kishanganj of Baran district @ 4 villages/block.

In each village, starting from the north east corner of the Saharia colony, the survey was carried out in the contiguous households (HHs) till the targeted number of 25 children of 0-5 years was covered for nutritional assessment. Family diet survey was carried out in a sub-sample of 5 households, having atleast one pre-school child. The information on infant and child feeding practices was also collected from the house wife in all the HHs covered for diet survey.

Coverage

A total of 314 households from 8 villages from two blocks were covered. A sub-sample of 40 households was covered for family diet survey, as well as for the assessment of knowledge and practices of women on breast-feeding and complementary feeding. Nutritional anthropometry and clinical examination was carried out on 238 children of 0-5 years and 422 adults. History of morbidity during the previous fortnight was also collected on all the individuals covered for anthropometry. Verbal autopsy was done in the Saharia households where deaths were reported during the past six months to assess the probable cause of these deaths.

Results

Household particulars

A majority (70%) were nuclear families with average family size of 5.6. Many male heads of the HHs (81%) and their spouses (96%) were illiterate. Forty two percent of the households surveyed were possessing varying extent of land. Major occupation of head of the 82% households was agricultural labour or other labour. Bore wells formed the major source of drinking water in about 75% of the HHs; while in the rest, draw well was the main source. About 50% of the HHs surveyed had to travel a distance of >10 km. to reach nearest primary health centre.

Food and Nutrient intake

The average intake (per CU/day) of various foods except cereals & millets were lower than the RDI. The intakes of protective foods such as pulses, green leafy vegetables, milk and that of sugar & jaggery were less than 40% of the RDI, while the consumption of roots & tubers, other vegetables and fats & oils was to the extent of 45-50% of RDI.

The average daily intake of protein, calcium, iron, thiamin and niacin was comparable to the recommended allowances, while that of other nutrients were below the RDA. The extent of deficit in the intakes compared to RDA was relatively more with respect to vitamin A (62%) followed by vitamin C (43%), total fat (35%), free folic acid (22%), riboflavin (14%) and energy (9%). In general, the nutrient intakes among Saharias were marginally better as compared to that observed in general population in the drought affected areas of Rajasthan State (NIN Annual Report-2003-04). This could be attributed to availability of employment opportunities at the time of survey, which was the harvesting season and also availability of wheat through PDS at highly subsidized rate under "Saharia Special Component Programme". The frequency distribution according to consumption of various nutrients expressed as percent of RDA indicated that the proportion of HHs with intakes of less than 50% of RDA was relatively higher with respect to vitamin A (75%) and vitamin C (55%). None of the HHs, at the time of survey were found to be consuming energy below 50% of RDA, as against a figure of 5% reported for the drought affected areas of the State.

Nutritional Status

Clinical signs of nutritional deficiency

About 4% of infants were emaciated, while an equal proportion had conjunctival xerosis. About 1% of 1-5 year children had oedema (suggesting Kwashiorkor) and 5.7% were emaciated. The prevalence of vitamin A deficiency signs such as conjuntival xerosis (17.6%) and Bitot spots (8.3%) was much higher in these children than that reported earlier for the State of Rajasthan (0.3%) (DWCD 1998). The prevalence of nutritional deficiency signs was observed to be negligible among adults.

Anthropometry

Children (0-5 years)

About 72% of the 1-5 year children were underweight (weight for age < median-2SD of NCHS standards), while the prevalence of severe underweight (weight for age < Median-3SD) was to the extent of 24%. The prevalence of underweight was relatively higher (72%) than that reported during the drought survey (66%) as well as the figures reported (48%) for Rajasthan in District Nutrition Profile Survey (DWCD, GOI, 1998).

The prevalence of severe underweight (weight for age<median-3SD) tended to increase significantly with age from 11% among infants to 36% in 1-3 years children, which, thereafter, decreased to 12.6% in 3-5 year age group. The overall prevalence of stunting (height for age<median-2SD), an indicator of long duration undernutrition was to the extent of 68%, while wasting (weight for height < Median-2SD), an indicator of short duration of undernutrition was seen in 13%.

The prevalence of underweight, though statistically not significant was relatively higher among boys than girls (27.6% Vs 16.4%). However, a significantly higher (P<0.05) proportion of boys were stunted compared to girls (42.3% Vs 24.6%).

The overall prevalence of chronic energy deficiency (CED) (BMI<18.5) among adults was 56%, which was higher than the figures reported during the drought (40%) as well as that in Rajasthan (45%) (DWCD, 1998). The prevalence of CED was relatively higher among males (60%) as compared to females (53%).

Breast feeding and complementary feeding practices

Majority of the women (85%) reported that they initiated breast feeding on the third day of delivery. The newborns were fed with Jaggery water (80%) or goat milk (20%) during the first two days. Eighty five percent of the mothers stated that they discarded colosotrum, the proportion of which was much higher than that reported for the State (53%) by DWCD. They reportedly did so because of the belief that colostrum is not good for health of the new borns (36.4%), as a traditional practice (33.3%) or because of elders' advice (30.3%). Among those who were currently giving complementary feeding 68% initiated the same during 13-18 months, while only about 26% started it during 7-12 months of age.

Particulars of mortality

A total of 27 deaths in different age/sex groups were reported during the previous six months in the villages covered, with an estimated crude death rate of 8.1 as against 8.9 per thousand population reported for the State by SRS (1997). Of these, seven were neonates, two were post neonates, two were preschoolers, one was a school age child, two were adolescents and 13 were adults. The major cause of death among neonates was prematurity, while in the case of others, death was due to infectious diseases like malaria, pulmonary tuberculosis or respiratory tract infections. None of the deaths could be attributed to starvation.

Comments

The Saharia is a primitive tribal group with poor socio-economic status and low literacy level. The major household occupation was either agricultural or other labour. The intake of various foods, barring cereals & millets was very poor, compared to levels suggested in balanced diets. The intake of protective foods such as green leafy vegetables and milk was very low which was reflected in higher prevalence of micronutrient deficiencies such as conjunctival xerosis and Bitot spots, indicating vitamin A deficiency among children. The prevalence of CED among adults was relatively higher.

About 90% of the HHs surveyed were availing the benefit of the Special Component Programme, being implemented by State Government for Saharia Community. In addition, the HHs were also participating in supplementary feeding programme under ICDS and MDM. These programmes could have averted severe forms of undernutrition to a certain extent.

No deaths attributable to starvation were reported in the villages surveyed. Premature delivery and infectious diseases contributed to a majority of the deaths among young children. Higher rate of illiteracy, ignorance, inappropriate infant and child feeding practices and lack of early treatment in case of morbidities seemed to have aggravated the situation. These observations highlight the need for strengthening health and nutrition programmes such as RCH, ICDS and MDM in conjunction with health and nutrition education.

2. STUDY ON THE CURRENT STATUS OF FLUOROSIS AND STEPS TO CONTROL HEALTH RISKS OF FLUOROSIS IN THE NORTH-WESTERN DISTRICTS OF TAMIL NADU

Fluorosis is one of the major public health problems in India affecting 62 million population, including 6 million children in 18 States and Union territories in the country. Drinking water is the main source of fluoride, though certain foods/beverages like tea also contribute to a significant amount of fluoride intake. Ingestion of low content of fluoride leads to dental caries, while excess intake over a period of time leads to dental and skeletal fluorosis.

Studies conducted earlier in Tamil Nadu State have shown that about 8 out of 29 districts were declared endemic for dental fluorosis and the districts such as Vellore, Krishnagiri, Dharmapuri, Salem and Erode were reported to be having relatively higher fluoride content in drinking water sources. No systematic studies were conducted in the area to assess the magnitude of fluorosis and its association with fluoride levels in drinking water. The present study was therefore, undertaken at the request of Ministry of Health and Family Welfare, Government of India in the five North-Western districts of Tamil Nadu viz., Vellore, Dharmapuri, Krishnagiri, Salem and Erode.

Objectives

- To estimate the fluoride content of drinking water sources,
- To assess the clinical prevalence of fluorosis,
- To estimate the population "at risk" of dental fluorosis at district level and
- To recommend suitable remedial measures.

Methodology

A cross sectional survey adopting stratified sampling procedure was carried out. The data generated by the Tamil Nadu Water supply and Drainage Board (TWADB) formed the sampling frame for selecting the villages. The water sources in each of the districts were stratified into three categories on the basis of fluoride levels in drinking water viz., < 2, 2-4, and 4-6 ppm. One water source was selected randomly from each of these categories in the selected districts and the corresponding village was identified for the survey. About 250 households (HHs) with approximately 1000 population, residing around the selected water source were covered for carrying out various investigations.

Investigations

- Household socio-economic and demographic particulars such as community, family size, occupa tion and source of drinking water.
- Examination of all the available individuals for presence of clinical signs of dental and skeletal fluorosis in the households selected.

- Information regarding the type, depth, and age of the water sources of the HHs covered for clinical examination and collection of sample from drinking water source for estimation of fluoride levels and
- Assessment of intake of different foods associated with fluorosis in a sub sample of 25 HHs of the total HHs covered for clinical examination in each village by semi quantitative diet survey using food frequency questionnaire.

Estimation of population "at risk":

The prevalence of dental mottling observed in the current survey at a particular fluoride level was extrapolated on the proportion of population catered by water sources with that level of fluoride to obtain the population 'at risk'.

RESULTS

Coverage

A total of 8700 individuals, including 1745 children of 5-14 years of age from 2800 HHs from 13 villages in the five selected districts were examined for presence of clinical signs of fluorosis. In addition, water samples from 126 drinking water sources were collected for estimation of fluoride content, while semi quantitative diet survey was carried out in a sub-sample of 254 HHs.

Fluoride levels in drinking water

Bore wells fitted either with hand pumps (36-89%) or electric pump and connected to over-head tank (OHT) (11-100%) formed the major source of drinking water among the villages surveyed, while in two villages of Salem district and one village of Erode district, drinking water was also supplied from Cauvery river. Of the 13 water sources selected for the current study, 6 were found dry at the time of survey. The fluoride levels in remaining 7 sources ranged from a low of 1.0 ppm to a high 6.0 ppm. It was observed that the fluoride levels assessed during the current survey were comparable to those reported by TWAD board within intra class correlation coefficient of 0.96, $p<0.01$. The fluoride content of the water sources collected from various sources in these villages ranged from 0.47 ppm to 6.6 ppm. At village level, the mean fluoride levels ranged from a low of 0.6 to a high of 4.6 ppm. In 7 out of the 13 villages, the mean fluoride was more than the WHO cut off level of 1.5 ppm. However, considering $\leq$1 ppm of fluoride in drinking water as safe level for tropical climates, all the villages except for Cinniam Palayam village of Erode district had unacceptable levels of fluoride. The proportion of water sources having fluoride level of >1 ppm was found to be in the order of 62% in Krishnagiri, 60% in Vellore, 56% in Salem, 53% in Dharmapuri and 26% in Erode districts.

The mean fluoride level as assessed in current survey in different villages was found to be comparable with the mean values reported by TWAD board, with an intra class correlation of 0.7 ($p<0.05$), indicating that fluoride levels estimated by TWAD board are still valid and can be used for making reliable estimates of fluorosis.

Prevalence of Clinical signs of Fluorosis

The clinical prevalence of fluorosis was mostly in the form of dental mottling while that of skeletal fluorosis was found to be negligible.

Dental fluorosis

The overall prevalence of dental mottling among the total population ranged from a low 17% in the district of Vellore to a high of 36% in the district of Dharmapuri. A wide variation in the prevalence was observed in the three categories of villages surveyed within each district.

The age group wise prevalence of dental mottling pooled for all the districts was least (21%) among 5-9 years children, which increased to a maximum of 56% among 10-14 years children. Thereafter, the prevalence tended to decrease with increase in age to a minimum of 10% among 50-59 year individuals. Similar trends were observed in all the districts. The prevalence of dental mottling among the children of 10-14 years was maximum in Krishnagiri (67%), followed by Salem (58%), Dharmapuri (57%), Vellore (48%) and Erode (30%).

The prevalence of severe dental mottling (Grade III) was observed to be maximum in the districts of Salem and Dharmapuri (3%), followed by Krishnagiri (1.9%), Vellore (1.5%) and Erode (0.4%). A higher prevalence of dental mottling with very low prevalence of skeletal deformities among the children of 5-14 years in all the districts surveyed, indicate that the disease is of recent origin. The prevalence of dental mottling was observed in significant proportion of population above the age of 15 years in the districts of Dharmapuri and Salem as compared to other Districts which may be attributed to the presence of Granite cutting and polishing units which release silica dust into the atmosphere and exposure to silica in fluorotic area is known to aggravate the manifestations of fluorosis.

Skeletal Fluorosis

The overall prevalence of skeletal fluorosis in all the age groups was found to be less than 1%. The prevalence was relatively higher in the villages of Dharmapuri district (1.3%) and was least in the villages of Vellore district (0.5%). Only in the districts of Salem and Erode, skeletal fluorosis was observed to be prevalent in the age group of 5-14 years.

Frequency of consumption of specific foods

Ragi, a rich source of calcium and known to reduce the risk of fluorosis was consumed by a majority of the HHs, either daily or twice a week, in all the districts surveyed, except in the district of Erode. Their proportion ranged from a high 82% in the district of Krishnagiri to a low 42% in Dharmapuri District. Consumption of milk & milk products, yet another source of calcium, was also observed to be regular in a majority of the HHs (62-84%) in all the districts surveyed.

The association between different foods consumed and the dental mottling, was however, not observed in the current study.

Estimated population "at risk"

It is estimated that about 30 lakhs population are "at risk" of fluorosis in all the five districts surveyed, of which 5-14 year children constitute about 9 lakhs. The Salem district is having highest number of cases of about 10.2 lakhs dental mottling of all age groups of which 2.8 lakhs are 14 year children, with an estimated prevalence rate of about 34% and 48% respectively. The estimated prevalence among total population and children of 5-14 years in the rest of districts were 30% & 45% in Dharmapuri, 24% & 30% in Erode, 23% & 25% in Krishnagiri and 17% & 34% in Vellore district respectively.

Recommendations

- Creating awareness among the community about fluorosis through health education and to discourage them consuming water from high fluoride sources.
- Sensitizing the concerned administrators regarding the need for identifying water sources with permissible levels of fluoride to provide safe drinking water to the community.
- Supplementation of the affected population, especially children and adolescents, with therapeutic doses of micrantrients such as calcium, vitamin D and vitamin C to decrease the consequences of fluorosis.
- Propagation and supply of domestic de-fluoridators which is the most economic and practicable choice of supply of fluoride free water.
- Avoiding ingestion of fluoride rich foods such as tea, tobacco, and use of fluoride rich toothpastes and simultaneously encouraging the community to consume foods rich in calcium, vitamin C and protein.
- Plugging of bore wells identified to be having high fluoride levels in water and making concerted efforts to dig bore wells in low fluoride zones.
- Wherever feasible, surface water either from the sources of harvested rainwater or from rivers to be supplied as a permanent solution to alleviate the problem. In the context of districts surveyed in the State of Tamil Nadu, the river Cauvery, with the districts of Dharmapuri and Salem on one side and Erode on the other side can form the right choice for supply of drinking water in these districts. In the current study, it has also been observed that most of the villages were already having community based over-head tanks and pipeline system for the supply of drinking water. This facility can be utilized for the supply of safer drinking water from the river Cauvery.

3. SMOOTHING CENTILE CURVES OF BMI FOR RURAL INDIAN CHILDREN BY USING LMS METHOD

Anthropometric data are used world wide to assess the growth status of individuals of different age/sex groups. Body Mass Index (BMI) is being used for the past 25 years, as a simple summary measure of nutritional status of adults, especially, for assessing the chronic energy deficiency or over-weight/obesity. Changes in the BMI in adults with the advancement of age is fairly slow and hence common cutoff levels are being used in assessing the nutritional status, which are independent of age. The BMI, however, changes substantially with age among children below 18 years, rising during infancy, falling during the preschool age, and then again rising through adolescence to adulthood. Therefore, for assessing the nutritional status of children using BMI, age/sex specific centile curves have to be developed and used.

Objective

To construct age/sex specific BMI centile curves by applying the Lamda Mu Sigma (LMS) method for rural Indian children.

Materials & Methods

Anthropometric data on 21,070 children aged 1 to 17 years collected by the National Nutrition Monitoring Bureau (NNMB) in 9 States during 2000-01 surveys was utilized. T.J. Cole et al developed software to obtain normalized growth centiles, by using 'LMS' method which simplifies assessment of growth status of children using BMI. This software is obtained from Dr.T.J. Cole and the normalized mean (M), the Coefficient of variation (S) and the Box-Cox power (L) curves were obtained as smoothed functions of age/sex by using the above data base. These age/sex specific BMI centiles can be converted into SD scores.

Results

Curves were derived using Cole's LMS method, which adjusts the BMI distribution for skewness. LMS values served to generate sets of seven or nine centiles, from the 3rd to the 97th for boys and girls separately. The 50th centile values were slightly higher in boys up to the age of 9 years, while in later ages girls had higher values. LMS curves, Centile values and SD scores of boys and girls are provided in Tables 1 & 2 and Figure 1.

Table 1. LMS and Centile BMI values of rural Indian boys

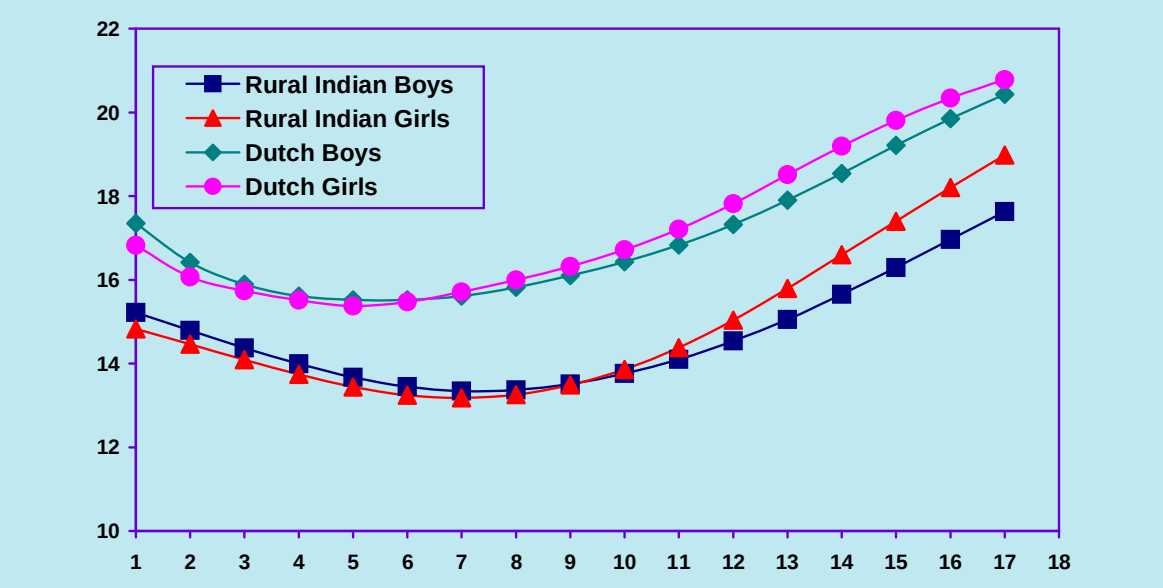
Age	L	M	S	-2.0001	-1.3334	-0.6667	0	0.6667	1.3334	2.0001
1	0.44	14.83	0.11	11.77	12.74	13.77	14.83	15.94	17.10	18.31
2	0.40	14.46	0.11	11.57	12.49	13.45	14.46	15.51	16.60	17.74
3	0.36	14.09	0.10	11.37	12.23	13.14	14.09	15.08	16.12	17.20
4	0.31	13.74	0.10	11.17	11.99	12.84	13.74	14.68	15.66	16.69
5	0.27	13.44	0.10	11.00	11.77	12.58	13.44	14.33	15.27	16.25
6	0.23	13.24	0.10	10.88	11.63	12.41	13.24	14.10	15.01	15.97
7	0.19	13.18	0.10	10.86	11.59	12.36	13.18	14.03	14.93	15.88
8	0.15	13.26	0.10	10.92	11.66	12.44	13.26	14.13	15.05	16.01
9	0.11	13.49	0.10	11.07	11.83	12.63	13.49	14.39	15.34	16.35
10	0.07	13.86	0.10	11.32	12.12	12.96	13.86	14.81	15.83	16.91
11	0.03	14.38	0.10	11.68	12.52	13.42	14.38	15.41	16.51	17.68
12	-0.01	15.04	0.11	12.13	13.03	13.99	15.04	16.16	17.36	18.65
13	-0.05	15.79	0.11	12.65	13.62	14.66	15.79	17.02	18.34	19.77
14	-0.09	16.60	0.12	13.20	14.24	15.37	16.60	17.93	19.38	20.96
15	-0.13	17.40	0.12	13.76	14.87	16.08	17.40	18.85	20.44	22.18
16	-0.17	18.20	0.12	14.31	15.48	16.78	18.20	19.76	21.49	23.39
17	-0.21	18.98	0.13	14.84	16.08	17.46	18.98	20.66	22.53	24.61

The L values show the skewness of the BMI distribution, a value of 1 indicate normality and smaller values represent progressively greater skewness. The degree of skewness is reflected in the spacing of the BMI centiles. The S values which define the coefficient of variation of BMI of boys and girls are given in Tables 1 & 2 respectively, which on multiplication with 100, provide percentages. The extent of variability in S values was about 11% in younger age groups (1-4 years) which tended to decrease to 9% in 5-10 year age group and then again rise with age to reach a peak value of 12-13% at 17 years. This rise occurred two years earlier in girls, reflecting the timing of the onset of adolescent growth spurt.

Table 2. LMS and Centile BMI values of rural Indian girls

Age	L	M	S	Centiles						
				3rd	10th	25th	50th	75th	90th	97th
				(-2.0001)	(-1.3334)	(-0.6667)	(0)	(0.6667)	(1.3334)	(2.0001)
1	0.64	15.22	0.11	12.08	13.09	14.14	15.22	16.33	17.46	18.63
2	0.54	14.79	0.10	11.87	12.81	13.78	14.79	15.83	16.89	18.00
3	0.45	14.37	0.10	11.66	12.53	13.43	14.37	15.35	16.36	17.40
4	0.36	13.99	0.10	11.46	12.27	13.11	13.99	14.91	15.87	16.86
5	0.26	13.67	0.09	11.28	12.04	12.84	13.67	14.55	15.46	16.42
6	0.17	13.45	0.09	11.16	11.88	12.64	13.45	14.29	15.18	16.12
7	0.07	13.34	0.09	11.11	11.81	12.55	13.34	14.18	15.06	15.99
8	-0.02	13.37	0.09	11.14	11.84	12.58	13.37	14.21	15.10	16.05
9	-0.11	13.51	0.09	11.26	11.96	12.71	13.51	14.37	15.29	16.28
10	-0.21	13.76	0.09	11.44	12.16	12.93	13.76	14.66	15.62	16.67
11	-0.30	14.10	0.10	11.69	12.43	13.23	14.10	15.05	16.09	17.21
12	-0.40	14.54	0.10	12.00	12.77	13.61	14.54	15.55	16.67	17.90
13	-0.49	15.05	0.10	12.37	13.18	14.07	15.05	16.15	17.36	18.72
14	-0.58	15.65	0.11	12.79	13.65	14.59	15.65	16.83	18.16	19.66
15	-0.68	16.29	0.11	13.26	14.16	15.16	16.29	17.57	19.02	20.69
16	-0.77	16.96	0.11	13.75	14.69	15.75	16.96	18.34	19.93	21.78
17	-0.87	17.63	0.12	14.24	15.23	16.35	17.63	19.12	20.86	22.91

Figure 1. Median BMI curves of rural Indian boys and girls aged 1-17 years compared with Dutch boys and girls



Age in years

4. NUTRITION PROFILE OF COMMUNITY IN UTTAR PRADESH - A DISTRICT LEVEL SURVEY

Government of India in its National Plan of Action on Nutrition (1995), as a part of the National Nutrition Policy (1993), envisages preparation of plan of action on nutrition at district levels. Earlier, the results of nutrition profile of the States of Punjab, Haryana, Himachal Pradesh, Assam, Orissa and West Bengal based on district level surveys were presented (Ann. Rep. 1995-96, 1996-2000, 2001-2002). At the request of Department of Women and Child Development (DWCD), Government of India, the study was extended to the state of Uttar Pradesh to assess the food and nutrient intakes of rural communities and to assess the nutritional status of the representative segments of population in terms of anthropometry and clinical status at district level during the year 2001-2002.

Methodology

Keeping in view the manpower and the time frame, a total of 400 HHs were covered in each of the 54 districts for demographic, socio-economic particulars and anthropometry and clinical examination of the individuals. Every alternate household of these 400 HHs was covered for the assessment of food and nutrient intakes by 24 hour recall method of diet survey. From these households, mothers having at least one preschool child were interviewed to assess their knowledge and practices (K & P) on breast feeding, child-rearing and socio-cultural aspects with reference to food consumption.

The results can be summarized as below:

1. A total of 21,739 households from 1080 randomly selected villages from 54 districts were covered for the assessment of nutritional status of all the available individuals. Family diet survey was carried out by 24-hour recall method in a sub- sample of 10,291 households by selecting every alternate household covered for nutrition assessment; individual dietary intakes were also assessed on 22,416 individuals of different age and sex groups from every alternate HH covered for diet survey. Assessment of knowledge and practices (K&P) on breast feeding, child rearing practices and socio cultural aspects of food consumption was carried out on 7,963 women, having at least one preschool child.

2. A majority of the HHs surveyed (44%) belonged to backward castes, followed by Scheduled Caste (35%), other castes (19.4%) and Scheduled Tribe (1.2%). About 46% houses were kutcha in nature and about 31% of HHs were landless. The major occupation of the head of the HHs was cultivation (36%) or non-agricultural labour (31%).

3. The average intake of cereals & millets (533 g) was above the RDI (460g), while that of pulses and legumes (37g) was comparable to the RDI (40g). The average intake of protective foods such as GLV is very much below (8g) as compared to RDA. Consumption of income elastic foods such as milk (111 ml) and sugar and jaggery (16 g) were below the recommended levels.

4. The mean intakes of energy (2445 Kcal), protein (73g) and calcium (517 mg) either comparable or more than the recommended levels, while that of other micronutrients like Iron (25mg), Vitamin A (214 µg) and riboflavin (1 mg) were below the RDI.

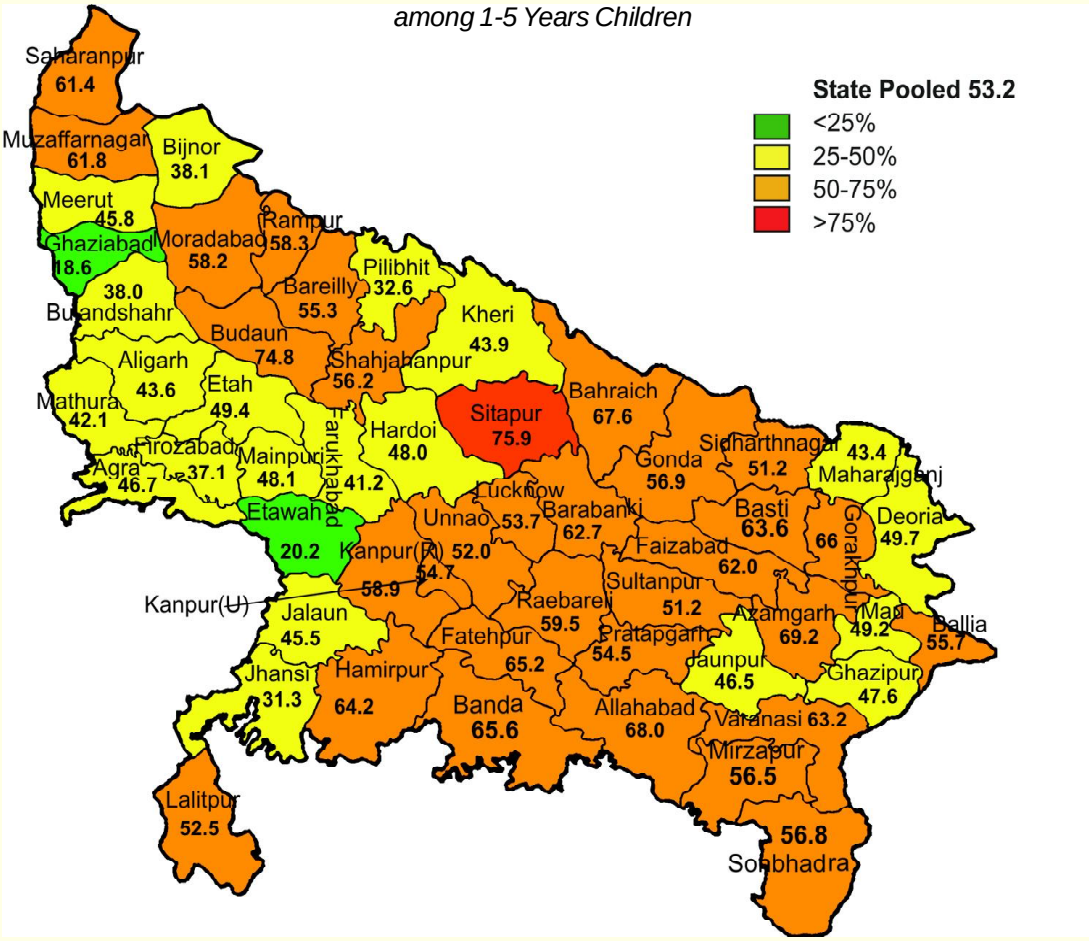
5. Distribution of households according to protein calorie adequacy status revealed that in about 64% of the HHs, the intake of both the nutrients was adequate, while in 28% of HHs, the intake of dietary energy was inadequate while that of protein was adequate. In 8 % of HHs, the intake of both the protein and energy were inadequate.

6. At the individual level, in general the intake of roots & tubers was above the recommended levels among preschool children and school age children. The deficit was more with regard to intake of income elastic foods such as milk & milk products, fats & oils and sugar & jaggery. In case of children of other age groups and adults, the intakes of cereals & millets, roots & tubers and other vegetables were above the RDI. The intake of all the nutrients except for protein, thiamin and niacin was below the recommended levels in children of all the age groups. In case of adults, the mean consumption of protein, energy, calcium, thiamin, niacin and vitamin C was above the RDI. The diets in general were grossly deficient with regard to iron, vitamin A and riboflavin.

7. The prevalence of severe underweight among preschool children, as judged by weight for age (<60% of standard), was observed to be 9%. The proportion of severe grade undernutrition was higher in 1-3 year children (11.4%) as compared to 3-5 year group (6.7%).

8. Stunting (height for age < Median -2SD) was observed in about 72% of preschool children, while wasting (weight for height < Median -2SD) was noticed in about 13%, indicating that chronic undernutrition was more common. Underweight (< Median -2SD) was observed in 53% of children (Figure 2).

Figure. 2. Prevalence (%) of Underweight (Weight for Age <Median - 2SD) among 1-5 Years Children



9. Prevalence of chronic energy deficiency (CED) among adults, as assessed by BMI (<18.5), was about 38%; with the prevalence being relatively higher among males (42.4%) compared to females (34.4%).

10. Among the mothers who were currently giving complementary food to their children, a majority (50.2%) of them initiated the same to their children during 7-12 months of age. The most commonly used complementary foods were rice/roti (45.3%) and milk (35%). Majority of the mothers, in general, were aware of nutritional deficiency disorders such as night blindness (87.3%), anaemia (76.5%) and protein energy malnutrition (80%). Major causes attributed for the same were dietary inadequacy and vitamin deficiency.

11. A majority of the mothers interviewed preferred to consume foods such as milk and fruits during pregnancy, while during lactation, milk was preferred. Also, mothers reportedly avoided certain foods such as papaya fruit, flesh foods and colocasia during pregnancy and green leafy vegetables, fermented rice and spicy foods during lactation.

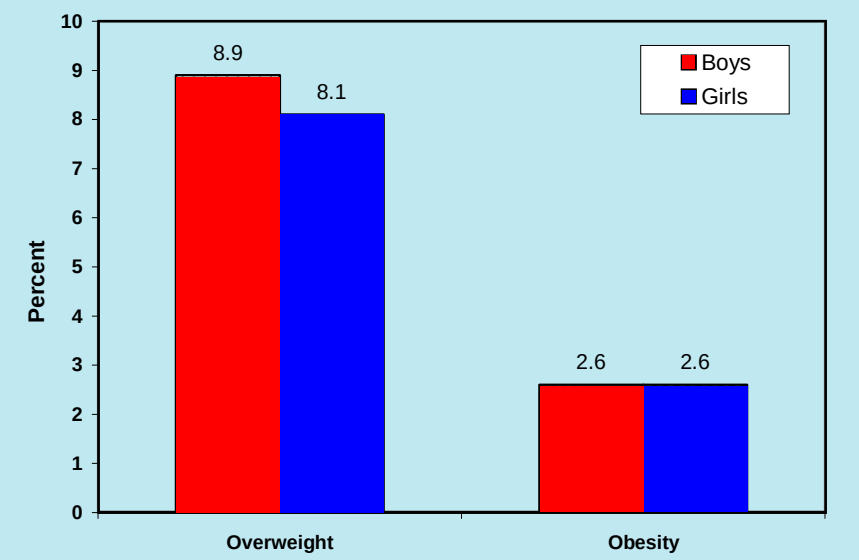
12. Study of dietary preferences during illness indicated that majority of the mothers preferred green gram pulse, milk, fruits and rice during diarrhoea and measles while flesh foods and spicy / oily foods were avoided. In general, the majority of mothers opined that the foods such as cereals & millets, pulses, meat & poultry, GLV and milk & milk products were useful for providing energy, maintaining healthy eyes, better growth and to improve blood.

5. PREVALENCE OF OVERWEIGHT AND OBESITY AND ITS PREDICTORS AMONG URBAN ADOLESCENT SCHOOL CHILDREN (12-17 YEARS) OF RANGAREDDY DISTRICT, ANDHRA PRADESH

Rising prevalence of overweight and obesity and its health consequences has prompted the World Health Organization (WHO) to identify it as one of today's most important public health problem posing global epidemic ramifications. The problem is not only confined to adults but also exists among children and adolescents. Adolescence is an important period in the life cycle of human beings, which is characterized by rapid rate of growth. The prevalence of overweight and obesity among children and adolescents has been reportedly increasing significantly in both developed and developing countries during the past two decades. The most significant long-term consequence of childhood and adolescent obesity is its persistence even during adulthood, with all the associated health risks. Estimation of prevalence of overweight and obesity and its correlates is, therefore, of paramount importance for the formulation of strategies to avert overweight and obesity. Therefore, a study was undertaken in continuation of an earlier study carriedout in the twin cities of Hyderabad and Secunderabad, with an objective to estimate the prevalence of overweight & obesity and its correlates among urban adolescent school children (12-17 years) in the urban agglomeration of Rangareddy district.

Fourteen schools catering to low (3), middle (4) and upper middle-income (7) groups were selected by multistage stratified random sampling procedure. Anthropometric measurements, viz., height (cms) and weight (kgs) were taken on 1825 adolescent school children (Boys: 1048; Girls: 777) using standard procedures. Information on socioeconomic and demographic particulars, their perceptions and practices on diet, life style patterns, and physical activities was assessed using pre-tested questionnaires. Obesity (95 percentiles) and overweight (85th - <95 Percentiles) were defined using BMI for age and sex specific percentiles (NHANES). Chi-square test was applied to identify the association between prevalence of overweight & obesity. The results revealed that in general, the prevalence of overweight and obesity was 11%, (Figure 3), and was comparable among the girls (11.5%) and boys (10.7%). The prevalence was significantly ($p < 0.001$) higher among children studying in private and private aided institutions (12.7% and 17.2%) as compared to those studying in government institutions (3%); among those belonging to high socioeconomic status (19.3%) as compared to the low (4.5%) and low middle (9%) socio-economic status (Figure 3)

Figure. 3 Distribution (%) of adolescents (12-17 Years) according to overweight and Obesity and Gender



BMI ≥ 85-< 95 centiles BMI ≥ 95 centiles

It was marginally lower among the children who were reportedly participating in the household activities for 3hrs/day. On the other hand, it was marginally higher among children (11.5%) who are watching TV for more than one hour/day as compared to the children, who are watching TV for less than an hour (6.5%). Thus, the study revealed that prevalence of overweight and obesity among urban adolescent school children in the Rangareddy District of Andhra Pradesh was higher (11%) than in their rural counterparts (0.6%: NNMB 2001). The prevalence was more among the children of upper middle and high socioeconomic status groups compared to the children of low and lower middle socioeconomic status. The prevalence was relatively less among children participating in games & sports or engaged in physical exercises and higher among the children with no physical exercise or who were watching TV for long hours. There is a need to initiate programmes on health and nutrition education for the school children incorporating benefits of physical activity in the form of games and sports and sticking to healthy food habits and good life styles.

II. WOMEN AND CHILD HEALTH

1. BODY COMPOSITION MEASUREMENT BY DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA) IN WOMEN FROM AN URBAN SLUM

Overweight and obesity are rapidly escalating problems in developed as well as developing countries. Excess body fat, in particular abdominal fat, is a harbinger of several adverse metabolic consequences, including impaired glucose tolerance, hyperlipidaemia and other features of metabolic syndrome. Overweight and obesity are commonly defined by the measurement of body mass index (BMI). However, this is an imperfect measure, because it cannot differentiate between fat and fat-free mass (bone, muscle, viscera and body water). A more accurate definition of overweight and obesity should be based on the total amount of body fat. The upper limits of body fat for defining obesity have been set as 25% for males and 30% for females *(Hortobagyi et al. Eur J Clin Nutr 1994; 48:769-75)*. In Caucasian men and women a BMI of 30 corresponds to 25% and 30% body fat in males and females respectively.

Numerous methods are available to assess body composition, all with their own advantages and limitations. Since the development of Dual Energy Xray Absorptiometry (DXA) in early 1990s, it has emerged as one of the most widely accepted methods of measuring body composition. It is a safe, convenient and non-invasive method. It can measure % fat with great precision and it correlates well with other methods of measuring body composition. Asian Indians have a characteristic phenotype, consisting of excess body fat, abdominal adiposity and less lean tissue. Excess body fat and less lean tissue complement each other in volume and weight so that the value of BMI does not increase. Further, the risk for co-morbidities such as diabetes, dyslipidaemia and hypertension in Chinese residing in Hong Kong started to increase from a BMI of 22 kg/m^2 onwards. *(Ko et al., Int J Obesity 1999; 23:1136-42).*

Taking these into consideration, the WHO working group has redefined the criteria of obesity in Asian population acknowledging the need for 'different standards that are culturally specific'. The proposed reclassification of overweight for adult Asian is >23 kg/m^2 and for obesity it is >25 kg/m^2. Obesity is an emerging problem in all the socio-economic groups in India, and even the urban slum-dwellers. Many of them are migrants from rural areas and have changed their life-styles during adulthood. This study was therefore carried out to measure the body composition by DXA in women from the low socio-economic group residing in an urban slum.

Hypothesis

Indian women have a characteristic body composition with higher levels of body fat per cent at lower BMI levels than other ethnic groups.

Objectives

1. To assess body composition by DXA in non-pregnant,non-lactating women between the ages of 30-60 years from low socio-eonomic group.
2. To relate body composition parameters such as lean mass, fat mass and % fat to the anthropometric parameters.
3. To explore the relationship of BMI to the body fat per cent.

Methodology

Sample size: All the women were a part of earlier study on bone status of women from low income group. Two hundred and seventy eight perimenopausal women from a large urban slum (Addagutta) in Hyderabad between the ages of 30 to 60 years were recruited for this study. They belonged to a poor socio-economic group and were involved in various occupations in the non-formal sector. Background information including reproductive history such as age at menarche, number of children, duration of breast feeding, menopausal status and age at menopause was collected. Anthropometric indices including height, weight, arm circumference and skin fold thickness at triceps, biceps, sub-scapular and suprailliac regions were measured using standard procedures.

Body composition measurements were carried out using DXA (Hologic 4500W).

Results

1. Characteristics of the study group (Mean $\pm$ SD)

Age	-	41.0 $\pm$ 8.60 years
Height	-	149 $\pm$ 5.49 cm
Weight	-	49.2 $\pm$ 9.85 kg
BMI	-	22.1 $\pm$ 3.99
Parity	-	3.3 $\pm$ 1.38
Number of postmenopausal women	-	120 (41%)
Number of premenopausal women	-	170 (59%)
Age at menopause	-	40.8 $\pm$ 5.86 yrs
WB-Fat mass	-	16.5 $\pm$ 6.24 kg
WB-Lean mass	-	30.3 $\pm$ 4.10 kg
WB-Lean +bone mass	-	31.8 $\pm$ 4.27 kg
WB- %fat	-	33.0 $\pm$ 6.38

2. Women stratified by weight

When the subjects were divided into 5 weight groups (<40, 40-45, 45-50, 50-55 and $\geq$ 55 kg), all the components i.e. WB-Fat mass, Lean mass and % fat increased significantly with increasing weight ($P<0.001$).

3. Women stratified by BMI groups

When the women were divided in 3 BMI groups ie. < 18.5, 18.5-22.9 and $\geq$ 23, it was observed that the BMI increased whole body (WB) fat mass increased disproportionately to the lean mass, thereby increasing the body fat per cent. Even the women with a desirable BMI i.e. 18.5 to 23 had a high level of body fat per cent (32%).

4. Women stratified by height groups

Women were divided into four height groups i.e. <145, 145 - 150, 150-155 and >155cm. As expected, the mean body weight and WB-fat increased with increasing height. But interestingly whole body lean mass also increased significantly with increasing height in all the height groups, thus increase in height was not associated with increase in body fat per cent.

5. Body composition parameters such as WB-Fat mass, WB-Lean mass as well as % body fat were not related to the age or the menopausal status in this group of women.

6. Relationship of BMI and body fat per cent - When body fat per cent was plotted against BMI (Figure.4) it showed a curvilinear relationship as shown by many of the previous studies (*Flegal KM. Et al. Obes.Res.1997; 5:93S).*

To create the prediction model for body fat per cent, inverse of BMI (1/BMI) was used as the mean predictor variable as done by Gallagher et al. *(Am J Clin Nutr 2000; 72:694-701).* This approach improved the linearity of the association between body fat per cent and BMI (Figure 5). It also increased the per cent explained variance and reduced the Standard Error of the Estimate (SEE) thereby improving the accuracy of the prediction.

The relationship between body fat per ent as dependant variable and BMI, age and menopause as independent variables was analysed and a prediction formula for calculating body fat per cent was derived as follows:

Body fat % = 65.468-671.044/BMI
(SEE = 3.32%)

Figure 4. Correlation of BMI & % fat (DXA)

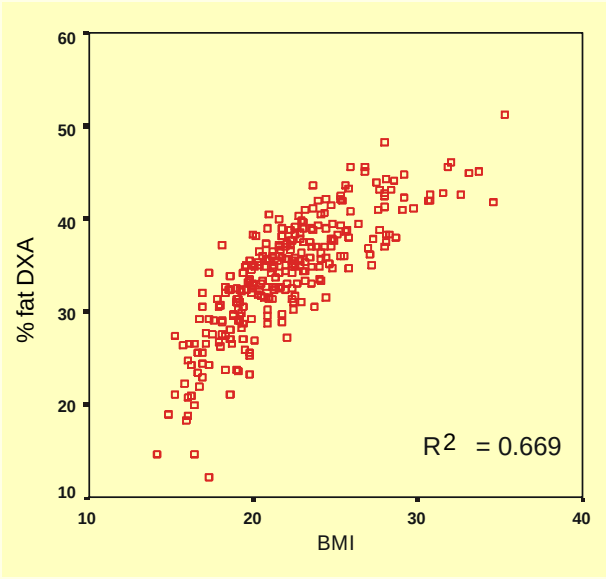
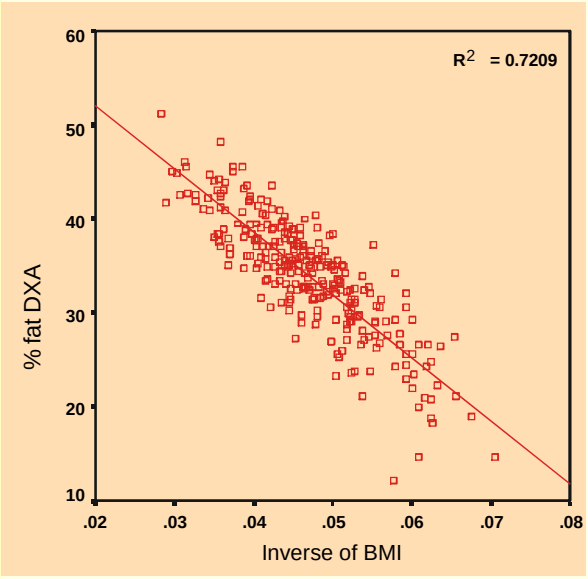


Figure 5. Correlation of inverse of BMI & % fat (DXA)



Conclusions

This estimation of body fat per cent from BMI is less dependant on intra and inter observer errors than the routinely used skinfold method. This study thus confirms that Indian women have high levels of body fat per cent at comparatively lower BMI levels than the values reported for the other ethnic groups. Increasing weight and BMI is associated with increase in body fat per cent levels. Whereas increase in height is associated with increase in lean mass but not body fat per cent. Increase in height during growth phase appears to be an important way to improve the muscle mass without increasing body fat per cent at population level. Role of childhood nutrition is important.

2. PREGNANCY INDUCED HYPERTENSION AND ANTIOXIDANT NUTRITION

Pregnancy induced hypertension is seen in approximately 10-20% of all pregnant women in India. It is associated with increased maternal morbidity and mortality related to intrauterine growth retardation, premature delivery and perinatal asphyxia. Pregnant women with pre-eclampsia are at increased risk for abruptio placenta, intra-cerebral hemorrhage and hepatic and renal failure *(Cunningham FG, 1992).*

Pre-eclampsia is associated with oxidative stress, Imbalance between pro-oxidants and anti-oxidants leads to potential cell or tissue damage *(Hubel CA, 1989).* Vascular endothelial damage is known to play a key role in the pathophysiological mechanism of pre-eclampsia *(Robert JM, 1989).* Free radical mediated lipid peroxidation may be involved in the endothelial damage in pre-eclampsia. Available data suggests increase in lipid peroxidation products and decrease in antioxidant activity in Pre-eclampsia compared with normal pregnancy *(Wang Y, 1992*). Excess free radical disturbances are associated with increased utilization of antioxidants resulting in decrease in their concentration *(Mikhail MS, 1994).* Reduced ascorbic acid is the biological labile form of ascorbic acid. It is a water-soluble antioxidant, which acts as the first antioxidant defense against free radicals present primarily in plasma. Plasma ascorbic acid levels were decreased with mild and severe pre-eclampsia. α -tocoferol and ß carotene are lipid soluble antioxidants, which have capacity to quench free oxygen radicals primarily present in the lipid membrane. α Tocoferol and β carotene were decreased only in severe form of Pre-eclampsia *(Mikhail MS, 1994).* This study was carried out to assess the antioxidant nutritional status in pregnant women with pre-eclampsia belonging to low socio-economic group attending a local government hospital.

Hypothesis

Decreased antioxidant nutrient status in pre-eclampsia may predispose to adverse outcome (course of the disease and outcome of pregnancy).

Objectives

To assess the plasma levels of antioxidants (reduced ascorbic acid, α tocoferol and ßcarotene) in pregnant women with different grades of pre-eclampsia and its relation to pregnancy outcome.

Sample size

Taking 20% as prevalence of pre-eclampsia with 80% power at 5% significance and 15% variation in proportions - 50 pregnant women with pre-eclampsia with matched controls were recruited.

Methods and Material

Subject recruitment

Fifty pregnant women with different grades of pre-eclampsia with matched control were enrolled.

Criteria

Demographic, socioeconomic, nutritional and obstetric data was recorded using a precoded proforma. A detailed clinical nutritional anthropometric and obstetric examination was carried out. Fasting

blood samples were collected to estimate antioxidant nutrients by standard techniques. Pregnant women were followed up till delivery to record pregnancy outcome and birth weight of infants.

Results

Of fifty pregnant women with pre-eclampsia recruited, twenty two had severe toxaemia while 28 had mild to moderate toxemia. The mean antinatal weight and post natal BMI were found significantly (P< 0.05) higher among cases compared to controls (Table 4). Antioxidants (vitamin C, vitamin E and carotene) were found significantly lower in pregnant women with pre-eclamptic toxaemia compared to control group (Table 5). Vitamin C was found significantly (p<0.05) lower in women with severe pre-eclampsia compared to mild to moderate group. The mean gestational age (weeks) at delivery and birth weight (kg) were lower in women with pre-eclampsia compared to control group. The premature delivery and intrauterine growth retardation were higher in women with pre-eclampsia (Table 6).

Table 4. Obstetric profile

	PET (50)	CONTROL (50)
Age (yr)	20.9±2.65	20.4±2.06
ANW (kg)	56.8±8.75	52.9±5.81*
P Natal BMI	22.1±3.41	20.7±2.58*

** P<0.05*

Table 5. Pre-eclampsia and antioxidants

Antioxidants	Pre-eclampsia	Control	Significance
Vit C µmol/L	31.9 ± 18.26	43.1 ± 22.15	P<0.05
Vit E µg/ml	4.3 ± 2.90	6.2 ± 3.88	P<0.02
β-carotene ng/ml	45.8 ± 31.86	68.1 ± 36.88	P<0.01

Table 6. Delivery outcome

	PET (50)	CONTROL (50)
G Age at Del [wk]	36.4±3.01	38.9±1.14*
BW kg	2.38±0.52	2.68±0.31*
PMD	46 %	8 %
Term IUGR	20 %	12 %
Hb g/dl	8.6 ±1.80	9.5±1.76

Conclusions

- Significant differences were observed between pre-eclampsia and controls with respect to obstetric profile and antioxidant levels.
- Serum vitamin C levels showed significant differences between mild to moderate group and severe degree of pre-eclampsia.
- Effect of antioxidant supplementation in primigravida on prevalence of pre-eclampsia has to be planned.

3. VITAMIN A MODULATES IMMUNE RESPONSE IN ACUTE RESPIRATORY INFECTIONS

Micronutrients can influence and modulate immune response and alter the course and outcome of most infectious illnesses. Poor nutritional status, or deficiency of specific nutrients like zinc and vitamin A, has been shown to suppress several facets of immune response. Among the various nutrients, vitamin A, iron and zinc have a significant role in immune response. Animal studies have shown that in addition to generalized effects on immune function, zinc and vitamin A can influence Th1 and Th2 cytokine responses and thus have a profound impact on the outcome of infectious illnesses. Studies have documented low serum retinol and zinc levels during acute infection. Based on these findings clinical trials were conducted on the assumption that infectious disease outcome might improve with zinc or vitamin A supplements.

However, though zinc supplementation studies proved beneficial; vitamin A supplementation studies on children with respiratory infection have been disappointing. Some researchers suggested that vitamin A (VA) should not be used therapeutically in patients with pneumonia unless there is clinical evidence of vitamin A deficiency or concurrent measles infection. Considering the above views, It is hypothesized that vitamin A may modulate the Th1 andTh2 bias and alter the course of respiratory infection. Thus in this study the micronutrient status of children during acute respiratory infection (ARI) and their association with local cytokine (Th1, Th2) response was determined. In addition; to study the impact of large dose vitamin A on Th1 andTh2 modulation, cytokine response was studied after oral administration of 2 lacs IU of vitamin A in normal children.

Materials and Methods

Study design

Children aged 10 months to 3 years, suffering from ARI (pneumonia, bronchiolitis and upper respiratory tract infection), with a history of illness for not more than 5 days, were recruited from Niloufer Hospital, Hyderabad. Clinical diagnosis of pneumonia was confirmed by X-ray chest; and bronchiolitis was diagnosed in infants based on the classical clinical signs of wheezy cough, dyspnoea and irritability, with or without x-ray evidence of hyperinflation of lungs. Children with cough; fever and rhinitis were grouped as upper respiratory tract infection (URTI). Children with congenital heart disease, chronic lung disease or family history of asthma were excluded from the study. Sample size estimate was based on mean and SD of IL2 cytokine in vitamin A deficient ARI children with 90% power and 5% significance. This yielded a sample size of 20 ARI children with low vitamin A (20 μg/dl). Thus, 72 children with ARI were recruited, of whom there were 38 children with vitamin A deficiency for comparison of cytokine response. Thirty apparently normal children of similar age group and socioeconomic status were taken as control group. The control group was taken to compare micronutrient status in the ARI children.

Nutritional status

Anthropometric measurements were taken to assess their weight for age using Gomez classification. After obtaining an informed consent from parents, 2 ml of blood sample was collected from children, and their hemoglobin (Hb) status, serum zinc and vitamin A levels were determined. Serum retinol was measured by HPLC. Serum zinc was measured using atomic absorption spectrophotometry (AAS) after diluting (1 in 5) serum in deionised water. Heamoglobin was determined by cyanmethemoglobin method.

Systemic and local, Th1/Th2 response in children with ARI

Nasopharyngeal aspirates (NPA) were collected aseptically by passing size 5 feeding tube into the nasopharynx and applying gentle suction with a syringe. NPA secretions were rinsed into collecting vials containing 1ml phosphate buffer. After centrifugation of nasopharyngeal secretions to precipitate cells, the supernatant was frozen at -70 C till analyzed for cytokines by ELISA. An aliquote of serum was also preserved at -70 C to analyze cytokines at a later date.ELISA (Diaclone research) was used to determine Cytokines (IL2, IL4 and IFN-γ) from NPA and serum. Recombinant cytokines of known concentrations were used to produce the standard curves. The lower limit of detection for IL2, IL4 and IFN-γ were 5.6, 1.1 and 12.5 pg/ml respectively, with intra and inter assay variability of less than 10% and 5% respectively. The total protein from NPA was determined by modified Lowry's method.

Th1/Th2 response after 2 lacs IU of oral vitamin A in normal children

In ten apparently normal children of same age group and weight for age, blood sample was collected initially and 15 days after 2 lacs IU of vitamin A orally. The blood samples were processed for isolation of peripheral blood mononuclear cells (PBMC). PBMC was isolated on Ficoll hypaque and stimulated with PHA for 18 hours at 37°C and 5% CO_2. . After 18 hours culture, the supernatant was harvested and IL2, IL4 and IFN-γ were analyzed by ELISA. Cytokine production by cultures without PHA was below the limit of detection of ELISA.

Statistical Analysis

Statistical analysis was done with SPSS PC software. A 'P' value of 0.05 was used to determine significance. Student's t test was used to compare nutritional variables (WFA, Hb, zinc and vitamin A) between ARI and control groups. Relationship of cytokine response and vitamin A concentration in ARI children was done using the nonparametric Mann-Whitney test, as the cytokine values were not distributed normally. Correlation coefficient was done after log transforming the cytokine data. Paired t test was used to compare cytokine response from PBMC of normal children before and after 2 lacs IU of vitamin A.

Results

The mean age in months and weight for age (WFA) was comparable between ARI and the control group. Mean serum zinc, vitamin A and Hb were significantly lower in the ARI children compared to the control group (Table 7). Vitamin A was <20µg/dl in 38 of 72 ARI children and 30 children had hemoglobin less than 9 g/dl. Low zinc (<70µg/dl) level was seen in 32 ARI children. Thus, more than 50% of the ARI children had low vitamin A.

Table 7. Correlation coefficient for nutritional status and NPA IL2 response in children with acute respiratory infections and in children with pneumonia alone.

	r value (ARI, n= 50)	r value (Pneumonia, n=14)
WFA	0.069	0.337
Hb	0.175	0.134
VA	-0.324*	-0.563*
Zinc	0.142	0.108

** P<0.05* *WFA: weight for age; Hb: hemoglobin; VA: serum vitamin A*

1. Local cytokine response

NPA IL2 was detectable in 53 (73.6%) of 72 ARI children and ranged from nondetectable to 472.4 pg/mg protein. The mean CI was103 (64.1, 142.9) pg /mg total protein.

NPA IL4 was detectable in 84.7% of ARI children and ranged from nondetectable to 103.4 pg/mg protein. The total mean and CI was 9.3 (-5.2, 23.9) pg/mg protein. On the other hand, IFN-γ was detectable in only 7of 72 ARI children.

2. Serum cytokine response

Of the 72 ARI children, only 8 and 21 showed serum IL2 and IL4 in the detectable range, with mean (CI) of 8.3 (-6.7, 23.5) and 2.3 (0.2,4.4) respectively. Serum IL2 was not correlated with NPA IL2 levels, while IL4 showed a negative correlation between NPA and serum. Serum IFN-γ was below detectable level in all the ARI children.

3. Nutritional status and cytokine response in children with ARI

When the mean values of NPA IL2 was compared between children with Hb of 9 g/dl and <9 g/dl, there was no significant difference. Similarly a cut off value of zinc at 70ug/dl showed no difference in IL2 response, while WFA and vitamin A were inversely related with NPA IL2 concentration.

However, the inverse association of IL2 with WFA disappeared when the data was controlled for vitamin A, while the association with vitamin A ($P<0.05$) remained strong when controlled for other nutritional parameters.

Correlation coefficient on log-transformed data showed a significant ($P<0.05$) inverse association of serum vitamin A and NPA IL2 in children with ARI (Table 7). A similar association was seen in children with pneumonia; that is after excluding cases of URTI and bronchiolitis. Other nutritional parameters (WFA, Hb, zinc) showed no correlation with NPA IL2.

NPA IL4 was not related either with WFA, Hb, and vitamin A or zinc levels. Correlation of IFN-γ with nutritional status was not attempted as very few children had detectable levels. Serum cytokines were not associated with nutritional parameters.

4. Effect of oral vitamin A on IL2, IL4 and IFN-γ response in normal children

To examine the direct effect of vitamin A supplementation and since vitamin A cannot be supplemented to children with ARI the effect of large dose vitamin A was studied in normal children.

The baseline vitamin A status of these children was adequate, however, serum vitamin A increased 15 days after vitamin A (2 lakhs IU) supplementation, while the IL2 concentration in the PBMC culture supernatant reduced significantly ($P<0.05$; paired t test) from the baseline concentration.

The concentration of IL4 and IFN-γ were comparable to the baseline response (Figure 6). Furthermore, increasing concentration of vitamin A added to PHA stimulated cultures showed a progressively decreasing secretion of IL12, in addition to lower IL12 secretion in the presence of vitamin A sufficient serum compared to deficient serum, indicating that vitamin A might regulate Th1 (IL2) response by modulating IL12, which is a Th1 regulatory cytokine.

Figure 6 Effect of Vitamin A on IL2 response from PBMC

Conclusions

1. Vitamin A suppresses Th1 response (IL2), which may be mediated by down regulation of IL12; that is vitamin A could be anti-inflammatory.
2. Vitamin A might alter the course of immune response in acute respiratory infection (URTI, pneumonia and bronchiolitis) and thus influence the outcome of respiratory infection.
3. Weight for age (WFA), hemoglobin (Hb) and zinc did not show any association with Th1 or Th2 cytokines.

Though the present study does not show the effects of vitamin A on respiratory morbidity, it does signify that vitamin A modulates Th1 response and thus might alter the course and outcome of infectious diseases. In depth studies are needed to delineate the role of vitamin A on Th1 and Th2 response and it's effect on IL2 receptors in acute respiratory infection in children.

III. MICRONUTRIENTS AND TRACE ELEMENTS

1. NATIONAL FACILITY FOR DRIED BLOOD SPOT (DBS) TECHNOLOGY FOR VITAMIN A ESTIMATION

Vitamin A deficiency is the leading cause of blindness in children. Therefore assessment of sub - clinical form of vitamin A deficiency forms an integral part of the efforts of introducing and monitoring programs aimed at reducing vitamin A deficiency from the population. In this context a 'National facility for Dried Blood Spot (DBS) Technology for vitamin A Estimation' has been established at the Institute with the financial support of MI and MOST, India during the year 2003-2004. During the year technical services for analysis of vitamin A and validation of stability of DBS with time have been undertaken.

Methodology

Technical services for analysis of vitamin A

Recently NNMB carried out the survey in eight states on the prevalence of micronutrient deficiencies in the country. To assess the sub clinical form of vitamin A deficiency DBS samples were collected from a sub-sample of 576 preschool children per State who were covered for clinical examination (N=9508 preschool children per State). Storage and analysis of the DBS samples were done at the DBS facility.

A typical set of a days analysis consisted of a blank, standard retinol, tocol for internal recovery, and about 50-60 samples.

Stability of DBS retinol

Overnight fasting blood samples (10 ml) were collected from 10 adult male volunteers for the study. Blood collected into heparinized vacutainers were used for preparing 50 strips of 4 spots each and stored at -20°C. Matching plasma samples were aliquoted (100µl) and stored at -20°C and analysed periodically.

Result

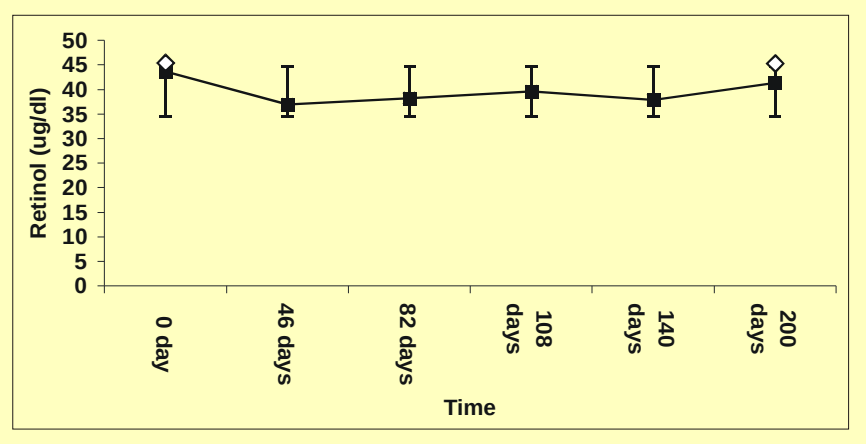
A total of 6000 samples were analyzed during the year. The period of collection and analysis varied between 90 days to 170 days. The data is being tabulated for assessing the extent of sub clinical deficiency of vitamin A by DBS method.

Stability of retinol in DBS at various time points is given in figure 7. The retinol in DBS was found to be stable and comparable to plasma retinol concentration over a period of 200 days.

Conclusion

The facility is operational and handled about 6000 samples during the last 2 years with an estimated cost of Rs 600/- per sample, which could make the facility sustainable.

Figure7. Stability of retinol in dried blood spot



2. A COUNTRY INVESTMENT PLAN FOR FOOD FORTIFICATION IN INDIA

Considering the long-term implications of micronutrient deficiencies in the population, there is an urgent need to devise technologies aimed at controlling them. A draft proposal entitled 'Country Investment Plan (CIP)' for the provision of micronutrient fortification of food supplements to 0- 6 year children through Integrated Child Development Scheme (ICDS) in the States of Andhra Pradesh and Rajasthan was prepared and submitted to the DWCD, Government of India. The CIP was presented at the Inter-Ministerial meeting and suggested to revise the CIP.

Following are the suggestions

- To include three districts from Orissa and Rajasthan
- To include 6-14 years children, adolescent girls, pregnant women and lactating mothers
- To include fortified wheat flour and DFS
- NIN to carryout zinc estimation in serum of the children
- NIN to carry out monitoring at the time of fortification
- NIN to carry out baseline diet and nutrition survey and evaluate the program at periodic intervals

Materials and methods

The draft CIP was revised based on the suggestions of the Inter-Ministerial Committee except for inclusion of adolescent girls in CIP as they are benificaries of ICDS and MDM. Instead of Andhra Pradesh and Rajasthan the plan envisages implementation of CIP in the states of Orissa and Rajasthan for introduction of micro-nutrients-iron, iodine, vitamin A, zinc, thiamin, riboflavin, folic acid and ascorbic acid at RDA level to the beneficaries.

Results

A draft proposal on 'Country investment plan (CIP) for micronutrient fortification of food supplements of ICDS and mid day meal in the States of Orissa and Rajasthan' was prepared and submitted to

DWCD. The estimated investment for the proposal is US$ 9.13 million (Rs 43 crores and 80 lakhs) for 3 years.

Conclusions

Opportunities are available for investment in micronutrient fortification of food supplements of ICDS and mid day meal, which will reduce the prevalence of hidden hunger.

3. STABILITY OF IODINE IN DOUBLE FORTIFIED SALT (DFS): A SHORT-TERM STUDY

Iodine deficiency disorders and iron deficiency anaemia are widely prevalent and often coexist in the country. As a part of the dietary diversification, fortification of food with iodine and iron is recommended as one of the strategies to prevent and control these two deficiency disorders. The National Institute of Nutrition (NIN) developed a suitable technology for dual fortification of common salt (DFS) with iodine and iron [Food and Nutrition Bulletin, 1994; 15(1): 32-39]. The large-scale production in the factory [Food and Nutrition Bulletin, 1996; 17(1): 73-78], its bio-safety [Nutrition Research 1998; 18(1): 121-129], its ultra structure [SCANNING, The Journal of scanning Microscopies, 1998; 20(3): 271-273], and its efficacy in community [British Journal of Nutrition 2001; 85: Suppl. 2, S167-S173] have been evaluated. As per the recommendations of the ICMR Committee of Experts on DFS, the stability of DFS was evaluated under programmatic conditions in six different places in India (RMRC-Bhubaneswar, TRC-Chennai, MRC-Delhi, RMRC-Dibrugarh, NIRRH-Mumbai and GMC-Surat) under the project entitled "Operational Evaluation of the Stability of Iodine in Double fortified Salt: A Multicentric Study" during 2001-02.

The results of the study revealed that,

a) Iodized refined salt had satisfactory stability of iodine and the distribution (%) of samples with iodine content of >15 ppm was 99% at the end of 6 months.

b) Iodized ordinary salt had satisfactory stability of iodine and the distribution (%) of samples with iodine content of >15 ppm was 95% at the end of 6 months.

c) Double fortified ordinary salt had poor stability of iodine, as the iodine content was less than 7 ppm, right from the first month.

d) Double fortified refined salt had iodine content lower than expected, particularly those stored in coastal areas and the distribution (%) of samples with iodine content of >15 ppm was 60% at the end of 6 months.

e) The substantial time gap between the dates of sampling and analysis contributed to considerable variations in DFS and hence the results were not conclusive.

To resolve these, a rapid survey was undertaken under the supervision of Prof. M. G. Karmarkar, Senior Advisor, ICCIDD, New Delhi. Salt samples were randomly selected from 1 kg pouches of double fortified refined salt (DFS) from the available bags at each center for simultaneous time-controlled analysis, at ICCIDD Laboratory and NIN. These results indicated that the stability of iodine was quite satisfactory in DFS, with 76% of the samples had >15 ppm of iodine even after 22 months of fortification, which was much higher than that was reported at the end of 6 months (Table 8).

Table 8. Distribution (%) of DFS samples having >15 ppm of iodine

Center	Percent*	
	6 Months	22 Months
Bhubaneswar	40	75
Chennai	66	73
Delhi	77	74
Dibrugarh	53	67
Mumbai	72	91
Surat	56	77
Average	*60*	*76*
** Mean values of duplicates, n = 40/center.*		

These results were discussed in the Scientific Advisory Committee of NIN in August 2003 and the committee recommended a short-term study to assess iodine stability under normal room conditions at NIN and the ICCIDD Laboratory by ensuring that no gap existed between sampling and analysis. The present study therefore, was carried out during 2004-05.

Objectives

a. To study the stability of iodine in DFS prepared as per NIN formula and stored under room conditions over a period of six months, and

b. To study the weekly loss of iodine, if any, in DFS kept in plastic pouches and sampled frequently, simulating the domestic conditions.

Methodology

As per the project protocol, 500 kg of double fortified refined salt (DFS), 500 kg of iodized ordinary common salt (IOS) and 500 kg of iodized refined salt (IRS) were produced by dry mixing process prescribed for DFS as well as iodized salt in the salt factory of M/s Prince International at Bhubaneswar during 29-31 March 2004 under the technical supervision of the investigators. All the raw materials were tested for their quality before the use and were found acceptable. After confirming the satisfactory iodine levels in samples (n = 20) drawn randomly from each type of salt, independently by NIN and the ICIDD laboratory, the Managing Director of the salt factory gave the colour codes to the three salts before transportation to ensure blinding. The key to the codes was handed over in a sealed envelope to the Director of NIN for safe custody, which was decoded at the end of the study.

The three types of fortified salts were packed in colour coded 0.5 kg LDPE pouches (orange/white/yellow) at the salt factory. Twenty-five pouches of each type of salt were further packed in double lined HDPE bags of respective colour. Seventeen bags of each coloured bags were transported by road during the first week of April 2004 from Bhubaneswar to NIN, Hyderabad as well as ICCIDD Laboratory, New Delhi. The consignments reached the laboratories safely during the second week of April 2004. The remaining salt bags (6 of each colour) were transported to the Orissa Unit of the National Nutrition Monitoring Bureau (NNMB) at the Regional Medical Research Centre (RMRC), Bhubaneswar. The salt bags were stored inside a room under normal local conditions at NIN, ICCIDD Laboratory and RMRC. The storage of salt bags at Bhubaneswar was intended to assess the stability of iodine under coastal environment. Sampling of salts and estimation of iodine were done simultaneously at NIN and ICCIDD Laboratory on the same pre-determined dates, to ensure uniform conditions of storage, duration and analysis. Every month one pouch was randomly picked up from each bag and the iodine content was estimated in duplicate. Loss of iodine, if any, under conditions simulating household environment was tested in a sub-sample of each type of salt during the first three months. For this purpose, 6 pouches of each salt were drawn randomly. After estimating the initial iodine content, the pouches were closed with rubber bands and stored for subsequent analysis on 7th, 14th, and 21st days of every month in the

first 3 months. When the iodine content of DFS was estimated by the conventional iodometric titration using sulphuric acid, a wide variation in the iodine content of duplicate samples was observed (a) on the same day and (b) at different time points. This led to a lot of confusion in the interpretation of the results with regard to the stability of iodine in DFS. Therefore, a modified method using orthophosphoric acid was employed after a thorough validation. Both the laboratories employed the same method for iodine estimation in all the three types of fortified salts throughout the study period, to ensure uniformity.

Procedure adopted for the estimation of iodine in the three fortified salts

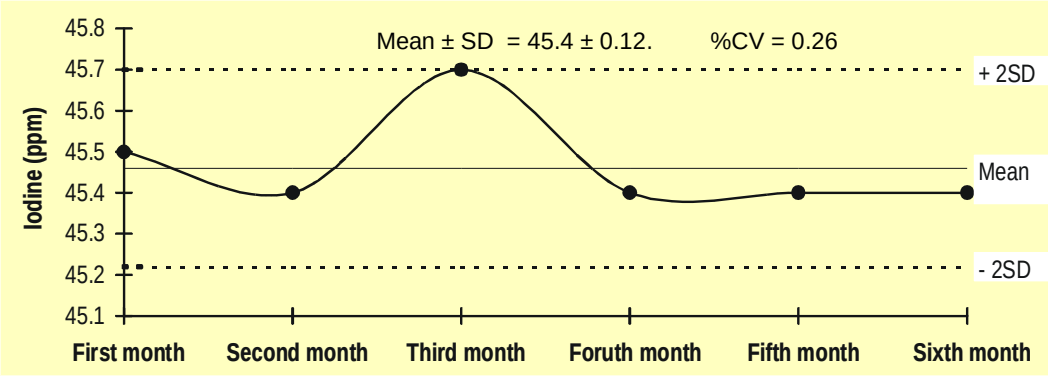
Acid used	Procedure
H_3PO_4	10 g DFS/IS + 0.25 ml of 1% KI + 50 ml distilled water +1 ml of 4N H_3PO_4 Keep in dark for 10 minutes and titration with 0.005M $Na_2S_2O_3$

In order to ensure the reliability of the results, NIN and ICCIDD Laboratory strictly adhered to the internal as well as external quality control measures. For the internal quality control, multiple analyses (20 times) for iodine content of a known reference salt and standard KIO3 (in 10g of plain non-iodized salt) were performed. The 95% confidence interval of mean iodine values were calculated along with the operating control range (Mean ± 2 SD) for preparing the Quality Control Charts. Reference salt and standard KIO3 were also analysed, whenever the iodine content of the three fortified salt samples were estimated on the pre-determined dates. For the external quality control, 10 samples each of the three fortified salts were drawn randomly at NIN and sent to the ICCIDD Laboratory. There the samples were analysed in duplicate, simultaneously by the investigators from both the centers using the same reagents. Analysis of variance was performed considering the laboratories, duration and duplicates as independent variables and iodine values as dependent variable. Percent frequency distribution according to iodine content of the three fortified salts estimated at the two laboratories was also made.

Results

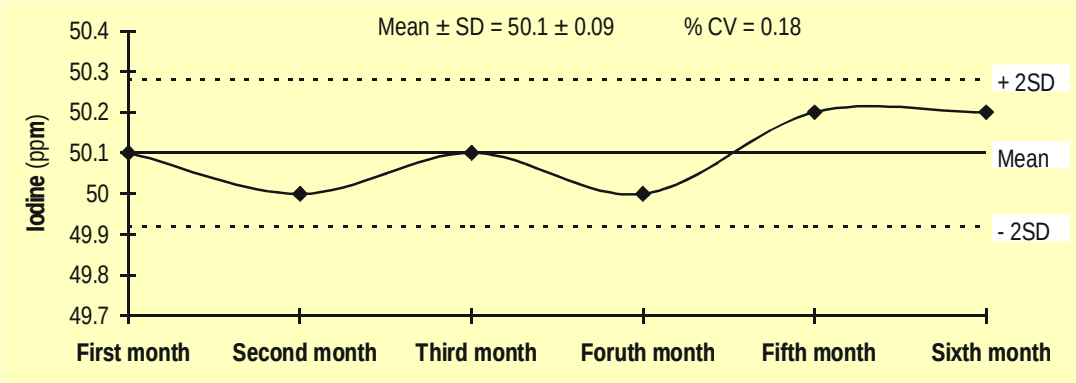
Estimation of initial iodine by the titration method using orthophosphoric acid at NIN and ICCIDD Laboratory showed that the mean iodine content was 30.0 ± 2.0 ppm in iodized ordinary salt (IOS), 42.7 ± 3.5 ppm in iodized refined salt (IRS) and 40.3 ± 3.8 ppm in DFS. The results of the internal quality control (IQC) carried out at NIN showed that the operating range of iodine content for the reference salt was 45.2 to 45.6 ppm (Figure 8) and that of the standard KIO3 was 49.9 to 50.3 ppm (Figure 9).

*Figure 8. Reference salt iodine IQC Chart at NIN**



** Similar results were obtained at the ICCIDD Laboratory*

*Figure 9. Sandard KIO_3 iodine IQC Chart at NIN**



** Similar results were obtained at the ICCIDD Laboratory*

The results of the external quality control (EQC) revealed good agreement between duplicate values of each laboratory as well as between the laboratories, irrespective of the type of fortified salt (Table 9). The intra-class correlation was very close to the unity (ρ= 0.97).

*Table 9. External Quality Control at ICCIDD laboratory**

Laboratory	DFS		IOS		IRS	
	Iodine (ppm)	CV	Iodine (ppm)	CV	Iodine (ppm)	CV
ICCIDD	42.5 ± 3.0	7.0 %	35.9 ± 2.8	7.8 %	46.9 ± 3.1	6.6 %
NIN	41.5 ± 2.9	7.0 %	35.1 ± 2.9	8.2 %	46.1 ± 2.8	6.1 %

Mean value of duplicates, n = 10/salt/Lab.
DFS: Double fortified salt; IOS: Iodized ordinary salt; IRS: Iodized refined salt.
*10 Samples of each salt were analysed simultaneously in duplicate by the investigators of the two laboratories, using the same reagents, at ICCIDD Laboratory, Delhi.

As per the project protocol, a draft report of the results at the end of six months was prepared and sent to the then members of the ICMR Expert Committee on DFS. The Committee concluded that there was no loss of iodine in any of the three types of fortified salts and recommended to decode and prepare the final report, as suggested by the Director-General of ICMR. Decoding of the three fortified salts revealed that orange pouches contained DFS, white pouches contained IOS and the yellow pouches contained IRS.

Regular monthly analysis of the salt samples carried out simultaneously at the two laboratories showed that the mean iodine content in DFS as well as IRS was about 40 ppm, while it was 30 ppm in IOS (Table 10). The iodine content of all the three salts estimated every month at NIN and ICCIDD Laboratory during the study period of six months was essentially similar.

Table 10. Mean iodine content of fortified salts stored at Hyderabad and Delhi by duration

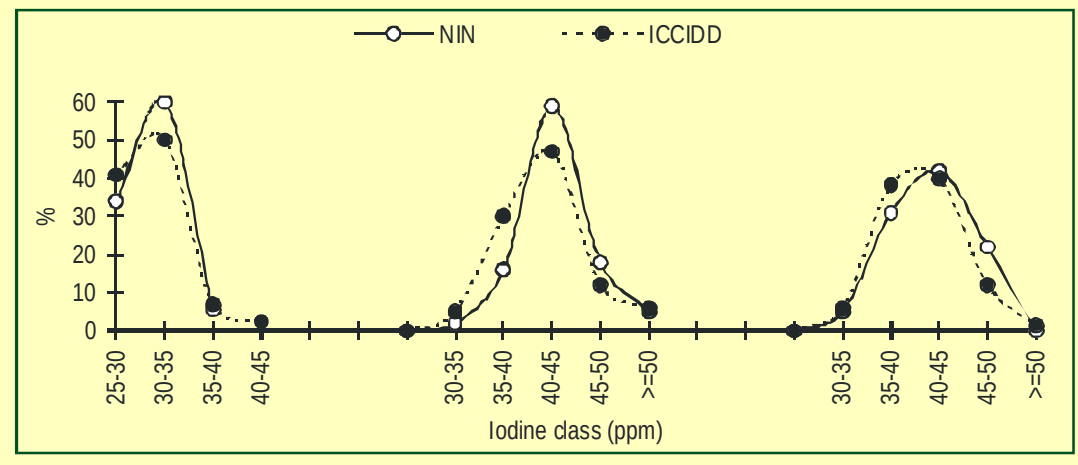
Time	Iodine content (ppm)					
	DFS		IOS		IRS	
	NIN	ICCIDD	NIN	ICCIDD	NIN	ICCIDD
Initial	40.1 ± 3.9	40.4 ± 3.6	30.7 ± 1.3	29.7 ± 2.1	42.3 ± 3.7	43.1 ± 3.9
Month 1	40.1 ± 4.3	40.1 ± 4.3	30.7 ± 2.7	33.6 ± 4.2	44.3 ± 4.0	44.5 ± 6.0
Month 2	42.5 ± 4.7	42.5 ± 4.7	32.4 ± 1.9	33.9 ± 2.1	43.8 ± 4.9	45.5 ± 4.0
Month 3	42.0 ± 4.3	42.0 ± 4.2	31.9 ± 1.2	31.2 ± 1.5	43.1 ± 3.3	43.0 ± 3.3
Month 4	42.0 ± 3.6	39.3 ± 1.8	32.4 ± 3.0	30.8 ± 1.8*	42.9 ± 2.5	40.3 ± 2.1
Month 5	41.4 ± 3.3	40.4 ± 1.6	*30.2 ± 0.9*	*28.8 ± 1.6**	*41.4 ± 3.0*	*38.2 ± 1.8**
Month 6	40.2 ± 2.1	39.6 ± 1.8	*30.0 ± 1.2*	*28.2 ± 1.4**	*41.6 ± 2.3*	*37.6 ± 2.4**

Mean value of duplicates, n = 17/salt/lab/month.
DFS: Double fortified salt; IOS: Iodized ordinary salt; IRS: Iodized refined salt.
*Between laboratories significant at $p < 0.05$.

Analysis of variance revealed that there was no significant difference between duplicate samples. Post-hoc analysis indicated minor differences in iodine content between the laboratories at some points mainly in IOS and IS. Since the external quality control showed good agreement between the laboratories and the frequency distributions of the iodine values were identical, the minor differences noted between laboratories have no practical relevance (Figure 10).

Even these small differences could be attributed to the inherent nature of the raw salts or distribution of iodine. However, the percentage of salt samples having >15 ppm of iodine was 100% in all the three salts over the study period of 6 months.

Figure 10. Frequency (%) distribution of fortified salts according to iodine content during the study period of 6 months



IOS: Iodized ordinary salt; IRS: Iodized refined salt; DFS: Double fortified salt.

Table 11 shows the mean iodine content of DFS, IOS and IRS stored at Bhubaneswar and tested at NIN and ICCIDD Laboratory. The mean iodine content of DFS and IS was about 40 ppm while that of IOS was 30 ppm, throughout the study period. No differences of practical relevance were observed in the iodine content.

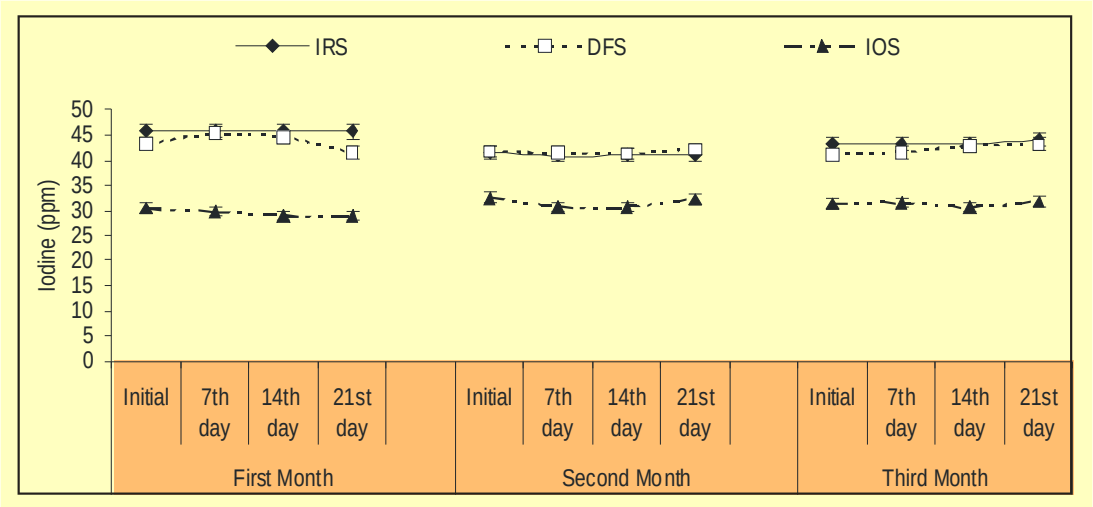
Table 11. Mean iodine content of fortified salts stored at Bhubaneswar by duration

Time	Iodine content (ppm)					
	DFS		IOS		IRS	
	NIN	ICCIDD	NIN	ICCIDD	NIN	ICCIDD
Initial	40.1 ± 3.9	40.4 ± 3.6	30.7 ± 1.3	29.7 ± 2.1	42.3 ± 3.7	43.1 ± 3.9
Month 1	40.2 ± 1.4	43.5 ± 3.0	33.6 ± 1.0	34.0 ± 0.8	41.2 ± 1.1	42.3 ± 1.5
Month 2	40.9 ± 1.3	41.6 ± 1.0	32.8 ± 1.4	31.9 ± 1.4	41.4 ± 2.1	41.3 ± 3.4
Month 3	44.4 ± 3.3	40.2 ± 0.7	30.8 ± 1.0	30.3 ± 0.2	42.5 ± 5.6	38.5 ± 2.2
Month 4	*44.4 ± 3.3*	*41.3 ± 1.1**	32.0 ± 0.3	31.4 ± 0.8	*43.2 ± 5.0*	*40.2 ± 1.0**
Month 5	40.7 ± 0.6	39.8 ± 0.9	*28.7 ± 2.6*	*30.7 ± 0.9**	*44.0 ± 0.6*	*40.0 ± 1.6**
Month 6	40.7 ± 0.9	40.4 ± 0.8	29.8 ± 0.8	29.5 ± 0.8	40.5 ± 1.1	40.0 ± 0.8

Mean value of duplicates, n = 6/salt/lab/time point.
DFS: Double fortified salt; IOS: Iodized ordinary salt; IS: Iodized refined salt.
*Between laboratories significant at $p < 0.05$.

Weekly analysis of sub-samples of the three fortified salts stored under household storage conditions, during the first 3 months of the study period revealed no significant change in the iodine content (Figure 11).

Figure 11. Weekly variation of iodine content in fortified salts during the first 3 months (Pooled for centers)

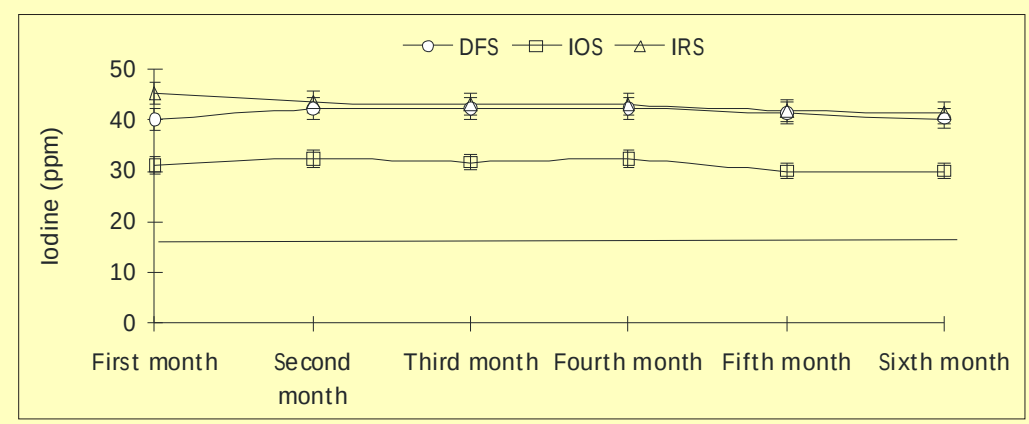


Mean value of duplicates, n = 6/salt/Lab/Time point.
IRS: Iodized refined salt; DFS: Double fortified salt; IOS: Iodized ordinary salt.

The present study has reconfirmed that large-scale production of DFS and iodized salts by dry mixing, packing and long distance transportation is feasible. There was a difference in the initial iodine content of different salts though the amount of KIO3 used was same. This could be due to nature of the raw salts used. The modified method was found to be suitable for iodine estimation in all the types of salts. The IOS, IRS and DFS stored at New Delhi, Hyderabad and Bhubaneswar showed excellent iodine stability by retaining more or less the initial iodine content (30-40 ppm) at all the time points, throughout the study period. All the three fortified salts retained >15 ppm of iodine throughout the period of 6 months. The coastal environmental conditions at Bhubaneswar did not affect the stability of iodine in all the three fortified salts, indicating that the poor stability noted at Bhubaneswar in the earlier multicentric stability study on DFS appears to be due to the inadequacies in the method of estimation of iodine. The salt pouches stored and handled under simulated household conditions did not show any loss of iodine in all the three fortified salts. The conventional iodine estimation using H2SO4 is not suitable as it yields inconsistent levels of iodine in DFS. Monitoring the iodine content by the modified method using H3PO4 demonstrated good stability of iodine in DFS.

The study, thus, revealed that over a period of 6 months, the stability of iodine (Figure 12) was consistently good in DFS, IOS and IRS.

Fig 12. Mean iodine content (ppm) of fortified salts by duration (Pooled for centers)



DFS: Double fortified salt; IOS: Iodized ordinary salt; IRS: Iodized refined salt

Conclusions

1. The results conclusively proved that iodine in double fortified refined salt prepared according to NIN formula has very good stability with very little loss of iodine in six months.
2. This study also demonstrated that a modified method is essential for consistent and accurate iodine estimation in DFS.

These results are in consonance with those of the positive and beneficial effects of DFS observed earlier.

IV. STUDIES ON MOLECULAR BIOLOGY

1. ROLE OF DIETARY **n-**3 PUFA OR TRANS FATTY ACIDS IN FOETAL PROGRAMMING OF INSULIN RESISTANCE IN RATS: BIOCHEMICAL AND MOLECULAR MECHANISMS

During development of fetus there are critical and restricted periods which are often coincident with periods of rapid cell division during which individual tissues/organs differentiate and mature. According to theory of 'fetal origins' of chronic adult disease, nutritional deprivations/imbalances *in utero* alters physiology and metabolism of developing tissues/organs postnatally and increases the risk of chronic adult diseases. Long chain polyunsaturated fatty acids (LC PUFA) are integral components of cell membrane and are important determinants of fetal growth and development. Docosahexaenoic acid is one of the abundant fatty acid present in brain, other neural tissues and the retina.

In India, low birth weight has been a major problem for quite a long period and in recent years there has been a steep increase in insulin resistance and its sequale leading to diet-related chronic diseases. The major target tissues of insulin action are skeletal muscle and adipose tissue. Recent evidence indicates that although adipocyte glucose uptake is small, it plays a significant role in insulin resistance. Adipocytes release free fatty acids, leptin and adipocytokines. LCPUFA content of skeletal muscle and adipocyte plasma membrane have been correlated with insulin sensitivity. Further, PUFA regulate the expression of genes involved in carbohydrate and lipid metabolism by binding to nuclear receptors known as PPARs. n-6 and n-3 PUFA have distinct biological effects and therefore both absolute levels and n-6/n-3 ratio are important for various physiological functions. Our recent studies have shown that keeping total PUFA in the diet constant and increasing α -linolenic acid (18:3n-3, vegetable oil) or LCn-3 PUFA (fish oil) increased insulin sensitivity in target tissues in sucrose induced insulin resistant rats. On the other hand Trans fatty acids (TFA) from Indian vanaspati increased insulin resistance. The present study is designed to evaluate whether dietary fatty acids (TFA or 18:3n-3 or LCn-3 PUFA) affect foetal programming of insulin resistance.

Hypothesis

Low maternal n-3 PUFA nutritional status or high TFA intake may predispose the pups to insulin resistance in adult life.

Aims and Objectives

To investigate the effects of increasing dietary n-3 PUFA (decreasing n-6/n-3 ratio) or TFA before conception, during pregnancy and lactation predisposes to insulin resistance in adult rats.

Methodology

Study design: WNIN female weanling rats (n=32) were divided into 4 groups and fed cereal pulse based diets containing 10% fat. The total dietary PUFA was ~ 10en% but the n-3 PUFA levels varied and were as follows:

Group I : ~ 0.2en% 18:3n-3 (n-6/n-3 ratio ~30)
Group II : 2.5 en% 18:3 n-3 (n-6/n-3 ratio 2)
Group III : 0.2 en% 18:3n-3 + 0.5 en% LC n-3 PUFA (n-6/n-3 ratio 10)
Group IV : Vanaspathi, which furnished 1en% TFA and 10en% total PUFA

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

The above diets were fed for 90 days. After 90 days of feeding, blood was collected from rat in-groups I to IV. After 5 days of bleeding (95 days feeding) rats in groups I to IV were mated with males fed stock colony diet (2 females + 1 male). The pregnant rats were continued on the respective diets throughout the period of pregnancy and lactation. The pups were weaned at 21 days. Half of the pups in each group were continued on the respective diets for 90 days whereas the other half were switched to diets providing n-6/n-3 ratio = 30. At the end of 45 and 90 days, blood was collected after 18hr fasting. Plasma glucose, insulin, total and HDL cholesterol and triglycerides were estimated. At the end of 90 days following blood collection, animals were sacrificed; liver, pancreas, epididymal and retroperitonial fat pads and skeletal muscle (diaphragm) were dissected out. Adipocytes were isolated from epididymal fat pads and the following parameters were estimated.

1. Plasma free fatty acids
2. Adipocyte glucose transport
3. Adipocyte lipolysis and antilipolytic effect of insulin
4. Liver TBARS
5. Liver antioxidant enzyme activities (Catalase, GSH-px and SOD)

Results

1. The fasting plasma free fatty acid levels were comparable between the groups.
2. Adipocyte lipolysis, insulin mediated antilipolysis and glucose transport were similar among different groups.
3. Compared with offspring on the control diet (n-6/n-3 ratio of 30), offspring on n-3 PUFA diet which provides n-6/n-3 ratio of 2 (vegetable oil) or 10 (fish oil) had significantly higher activities of liver catalase and glutathione peroxidase. There were no significant differences in liver TBARS among the groups.

2. IS RESISTIN A PRO-INFLAMMATORY MOLECULE ?

Resistin, an adipocytokine, is found to be elevated in genetic and diet-induced mouse models of obesity. This protein is expressed exclusively in adipocytes in rodents. However, in humans it is secreted mainly by macrophages. Reduced insulin-stimulated glucose uptake in mice that were administered recombinant resistin and reversal of the same by anti-resistin IgG indicates a role for this molecule in the development of insulin resistance. Plasma resistin levels are elevated in individuals with type 2 diabetes mellitus. Diabetics with insulin resistance and reduced glucose uptake suffer from cytokine-induced acute-phase inflammation.

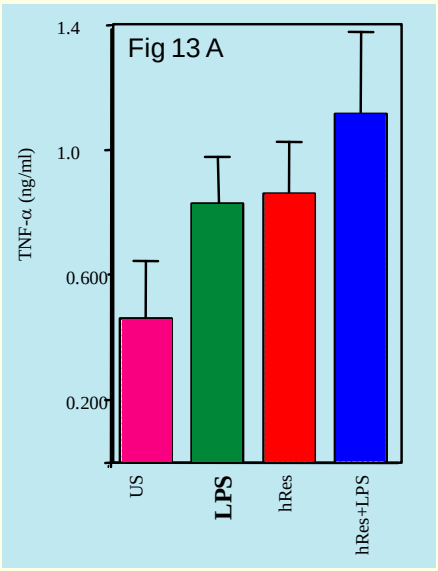
Inflammation in relation to obesity and insulin resistance has often been correlated with the over-production of the pro-inflammatory cytokine TNF-α.TNF-α is one of the major inflammatory mediators secreted by macrophages upon stimulation with pro-inflammatory molecules. TNF-α is expressed

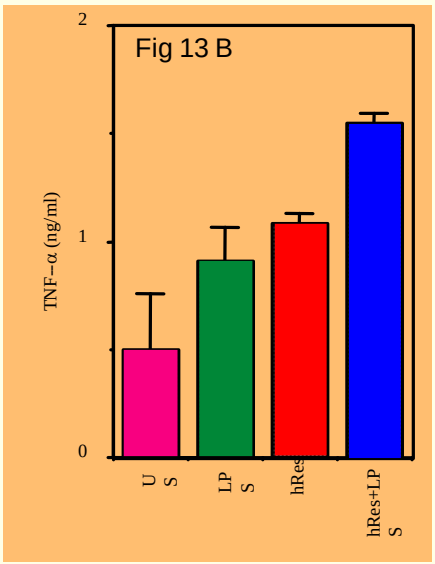
constitutively at a low level in monocytic cells. This basal level expression has been shown to be altered by the inflammatory millieu leading to either its upregulation or downregulation. In monocytes, the nuclear factor NF-kappa B has been established as an important transcription factor in the expression of cytokine genes including TNF-α. It is interesting to elucidate the immunomodulatory functions of human resistin. The effect of resistin on the production of pro-inflammatory cytokines in macrophages was demonstrated. The results showed that human resistin acts as a pro-inflammatory molecule, stimulating the synthesis and secretion of TNF-α and IL-12 and this involves the activation of NF-κB transcription factor. Given a direct positive correlation between TNF-α (and/or) inflammation and resistin expression we investigated whether resistin itself directly activates macrophages for the production of TNF-α. These studies were done in *in vitro* maintained U937 cell line as a source of human monocyte/ macrophage lineage. Cells were seeded in 35 mm dish and were differentiated in the presence of 25 nM PMA for 24 h. These differentiated macrophages were then stimulated by the addition of hResistin (30 g/ml) for 48 h. As a positive control, macrophages were also stimulated with bacterial LPS. TNF-α level was assayed by EIA in these cells. Incubation of cells with hResistin protein results in induction of TNF-α (Figure 13A &13B). As compared to unstimulated U937 macrophages, the stimulation of TNF-α by hResistin was about two fold. Similar experiments were performed using mouse RAW 264.7 macrophages. These results, therefore, conclusively demonstrate that addition of hResistin to macrophages induces TNF-α secretion in both human as well as in mouse macrophages.

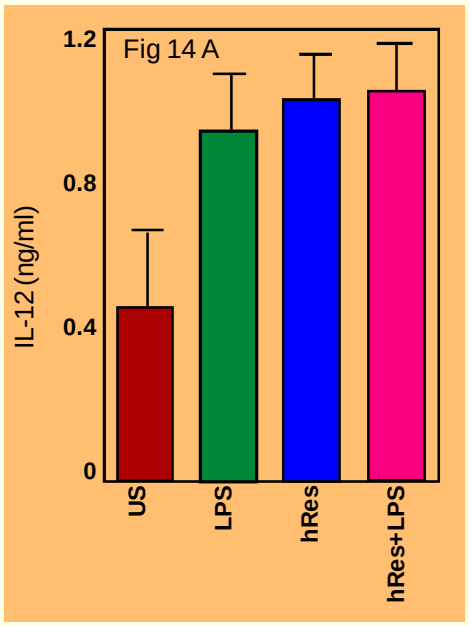
It is known that macrophage activation during inflammation also leads to increased production of other cytokines such as IL-12. The level of IL-12 in the culture supernatant was measured by EIA at 48 h post-stimulation (Figure 14A & 14B). These results categorically demonstrate the unique property of hResistin to act as an inducer of pro-inflammatory cytokines in both murine and human macrophages. The stimulation of TNF-α was also analysed at the transcriptional level. RAW 264.7 cells were stimulated with human resistin and the mRNA levels of TNF-α were measured by quantitative RT-PCR. Stimulation of mouse macrophages with recombinant resistin lead to the increased transcription of TNF-α gene. These results demonstrate that treatment of RAW 264.7 cells by hResistin also leads to an increase in *de novo* synthesis of TNF-α mRNA. Since human resistin is a secretory protein, there is always a possibility that recombinant resistin purified from a bacterial expression system under denaturing conditions might not have refolded into its native form. To further address the issue of protein folding, we cloned human resistin gene into mammalian expression vector pCDNA 3.1. The recombinant plasmid pCDNA-AShRes carrying resistin gene under the transcriptional control of CMV promoter was used for transient transfection (Figure 15A). The expression of human resistin in transfected RAW 264.7 cells was confirmed by RT-PCR. TNF-α mRNA expression was analysed as a function of time in the transiently transfected cells by RT-PCR (Figure 15B).

In order to evaluate the mechanism of action of resistin in eliciting a pro-inflammatory response in macrophages, the role of NF-κB family of transcription factors, which are known to be involved in regulating inflammatory responses, was investigated. This was carried out initially by assaying for nuclear translocation of NF-κB using electrophoretic mobility shift assay in macrophages exposed to resistin. U937 cells were stimulated with increasing concentrations of purified recombinant human resistin for four hours. It can be clearly seen that nuclear extract derived from U937 cells treated with hResistin shows increased DNA-Protein complex corresponding to the p50 and p65 heterodimer (NF-κB) (Figure 16). Furthermore, this complex is generated in a dose dependent manner. These results provide the first line of evidence that exogenous resistin stimulates the p50/p65 heterocomplex formation and its localization into the nucleus. The induction of pro-inflammatory cytokines in macrophages by the addition of resistin therefore appears to be mediated through the NF- κB transcription factor. The NF-κB activity in macrophages was blocked by using a dominant negative I κBα plasmid. U937 cells were transfected with dominant negative Iκ Bα plasmid lacking $Ser^{32/36}$ residue. In the absence of the serine residue, phosphorylation of Iκ Bα is inhibited and consequently, the translocation of NF- κB into

the nucleus is blocked. U937 cells were then allowed to stabilize for 12 hours followed by stimulation with purified human resistin. TNF-α levels were checked by ELISA after 24 hours and compared with macrophages transfected with control vector (pBSK from Stratagene). Cells transfected with dominant negative Iκ Bα plasmid secrete significantly lower levels of TNF-α in response to human resistin as compared to cells transfected with pBSK plasmid alone (Figure 17A). This therefore clearly suggests that in the absence of functional NF- κB the pro-inflammatory action of resistin is greatly reduced. To provide additional evidence on the role of NF- κB, PDTC, an inhibitor of I κBα , was used. PDTC blocks the dissociation of NF- κB/ Iκ Bα complex thereby inhibiting the translocation of NF- κB (Figure 17B). Although, resistin is believed to be a link between type 2 diabetes and obesity, it is tempting to suggest that the overproduction of resistin during obesity actually influences type 2 diabetes through activation of TNF-α. TNF-α is not only a major candidate for induction of inflammation, as observed frequently in diabetic patients, it has also been associated with increased insulin resistance in obesity.







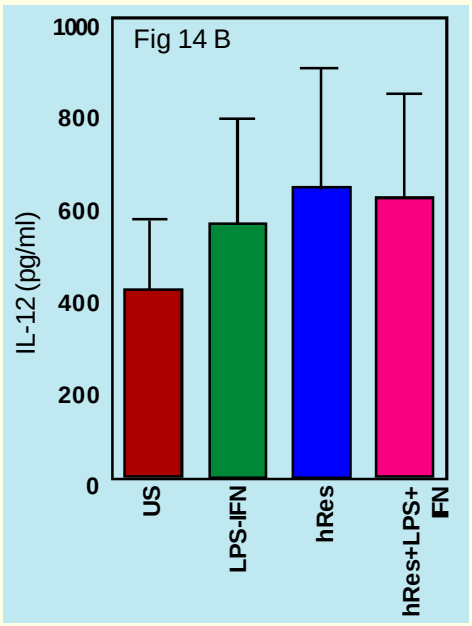


Fig. 15 A

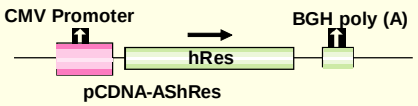


Fig. 15B

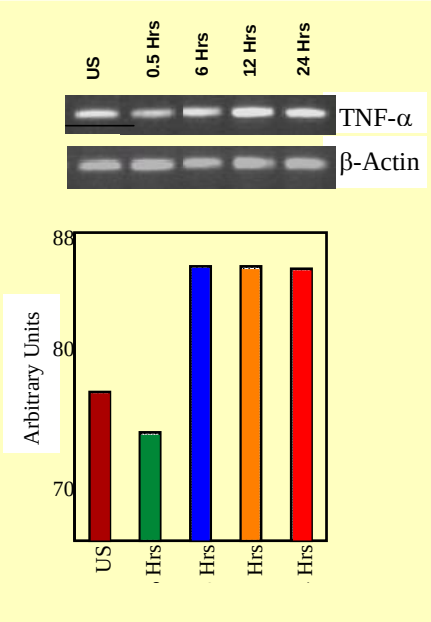


Fig. 16

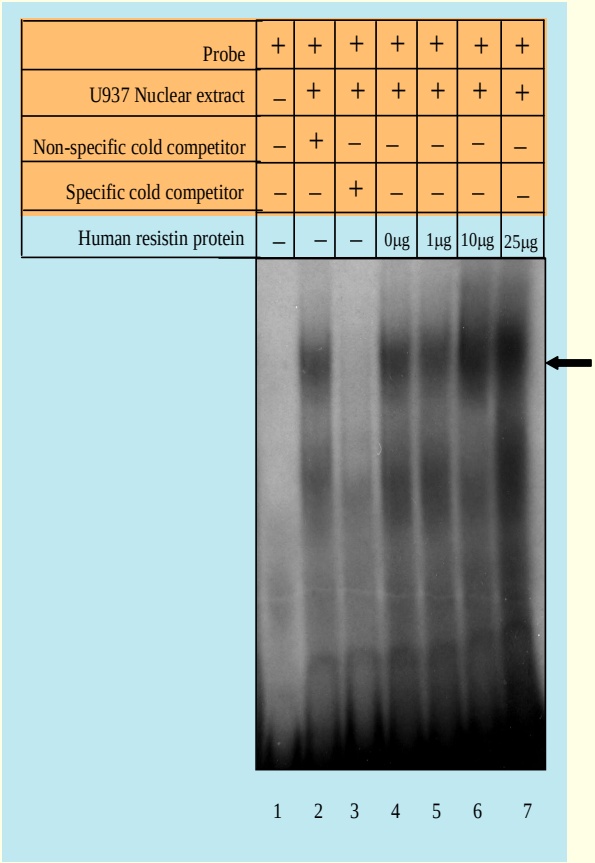


Fig. 17 A

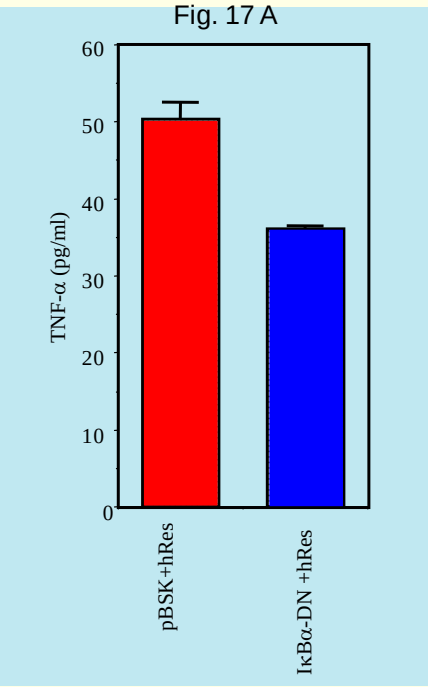
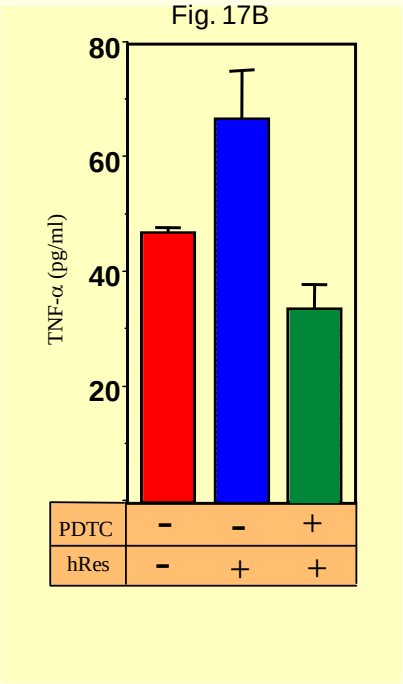


Fig. 17B



V. STUDIES ON CATARACT

1. CURCUMIN AND TURMERIC DELAY STREPTOZOTOCIN - INDUCED DIABETIC CATARACT IN RATS

Chronic hyperglycemia is a major determinant in the development of secondary complications of diabetes, including diabetic cataract. Studies indicate that diabetes increase the risk of development of cataract. Though the etiology of cataract is not fully understood, oxidative damage to the constituents of the eye lens is considered to be a major mechanism in the initiation and progression of various types of cataracts, including diabetic cataract. Diabetes causes increased oxidative stress in various tissues including lens.

On the other hand, a number of studies suggest that intake of antioxidant rich foods may slow the progression of cataract. Curcumin, the active principle of turmeric, has been shown to have significant antioxidant activity. Earlier, the effect of curcumin against galactose-induced cataract model using two levels of curcumin, 0.002 and 0.01% in the diet were studied. Interestingly, though curcumin delayed the onset of cataract at both the levels, maturation was delayed by 0.002% curcumin, but not by 0.01% (*Mol Vis* 9: 223-230, 2003). Since galactose-induced cataract does not mimic typical diabetic cataract of humans, the effects of curcumin was investigated in another model of diabetic cataract, streptozotocin (STZ) induced diabetic cataract model.

Methodology

WNIN rats were selected and diabetes was induced by streptozotocin (35 mg/kg body weight; IP) and divided into 4 groups (Group II-V). The control (Group I) rats received only vehicle. While Group I and Group II animals received AIN-93 diet, rats in Group III, Group IV and Group V received 0.002%, 0.01% curcumin and 0.5% turmeric in AIN-93 diet respectively, for a period of 8 weeks. Cataract progression due to hyperglycemia was monitored by slit lamp biomicroscope and classified into 4 stages. At the end of 8 weeks, the animals were sacrificed and the biochemical pathways involved in the pathogenesis of cataract such as oxidative stress, polyol pathway, alterations in protein content and crystallin profile in the lens were investigated to understand the possible mechanism of action of curcumin and turmeric. Blood glucose and insulin were also determined.

Results

1. Despite the increased food intake, the body weight of Group II animals was decreased (194g), when compared to the controls (385g). However, the decrease in body weight due to hyperglycemia was not ameliorated either by treatment with curcumin or turmeric.
2. Both curcumin and turmeric did not prevent the streptozotocin-induced hyperglycemia, as assessed by blood glucose and insulin levels indicating that curcumin and turmeric treatment had no effect in the state of STZ-induced hyperglycemia (Figure 18).

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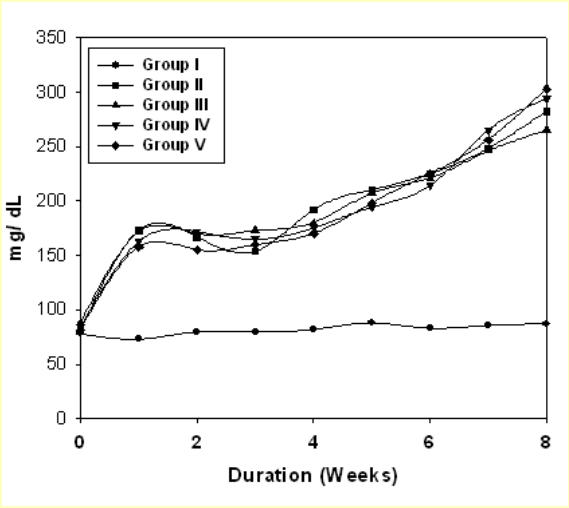
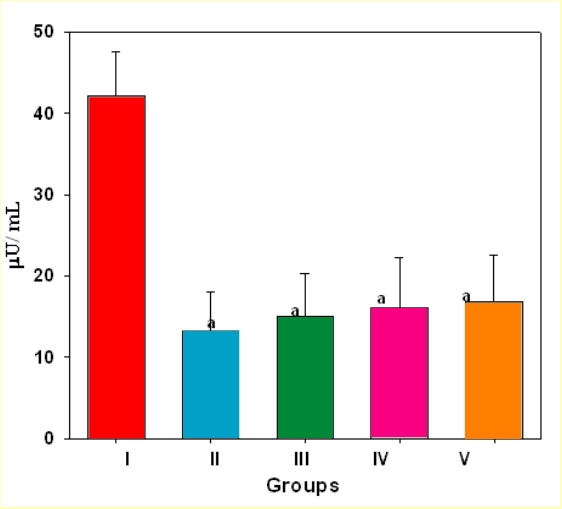
Figure18: Effect of curcumin and turmeric on blood glucose (A) and insulin (B) levels in streptozotocin-induced diabetic rats





3. Interestingly, curcumin delayed the progression and maturation of STZ-induced diabetic cataract in a dose dependent manner. The effect was even more pronounced with turmeric treatment (Figure 19,20).

Figure19: Effect of curcumin and turmeric on average stage of streptozotocin-induced cataract as a function of time

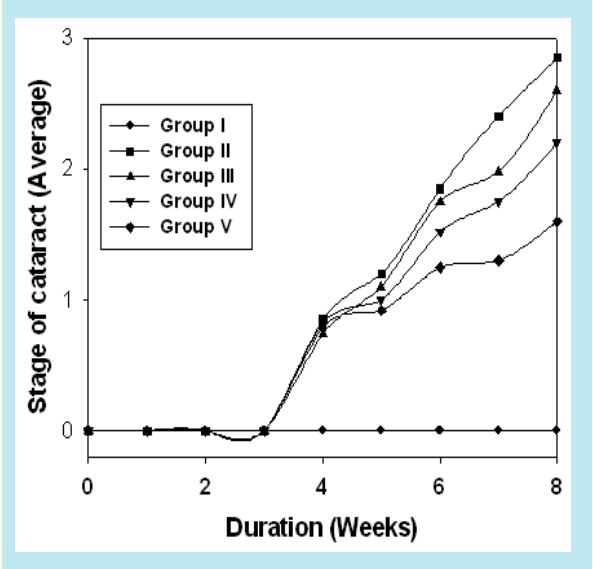
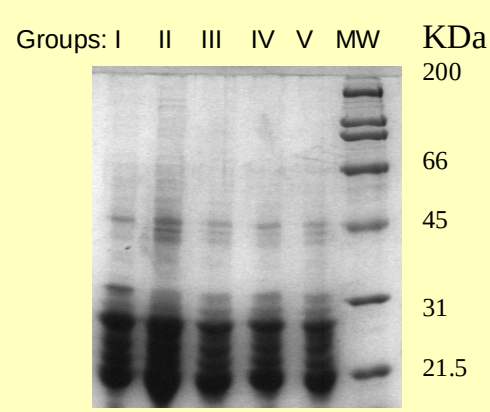


Figure 20: Effect of curcumin and turmeric on protein cross-linking in soluble fraction of lens. Soluble protein was loaded onto a polyacrylamide gel. Molecular weight standards in kDa are indicated alongside of the gel



4. Curcumin and turmeric treatment appear to have countered the hyperglycemia-induced oxidative stress, since there was a reversal of changes with respect to lipid peroxidation, reduced glutathione, protein carbonyl content and activities of antioxidant enzymes in a significant manner (Tables 12 & 13).

Table-12. Effect of curcumin/turmeric on lipid peroxidation, protein carbonyls and glutathione in rat lens

Parameter	(Group I)	(Group II)	(Group III)	(Group IV)	(Group V)
TBARS (n mol/g lens)	6.68±0.78	12.35±0.92[a]	9.52±5.25	7.32±2.36[b]	8.48±2.7[b]
Carbonyls (μ mol/mg protein)	2.00±0.45	3.20±0.34[a]	2.69±0.72	2.34±0.88	2.27±0.74
GSH (μg/g lens)	434±42.46	174±5.99[a]	198±12.71[a]	209±6.84[a]	224±8.9[ab]

The data presented above are the mean ±SD (n=4). The superscript 'a' denotes that the data are significantly different from G I and the superscript 'b' denotes that the data are significantly different from G II ($p<0.05$).

Table 13. Effect of curcumin/ turmeric on activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glucose-6-phosphate dehydrogenase (G6PD) in rat lens

Enzyme	Group I	Group II	Group III	Group IV	Group V
SOD	39.6±3.54	40.8±5.74	39.7±5.62	38.4±8.53	40.3±9.08
GPx	17.0±0.83	23.4±1.62[a]	21.1±1.31[b]	19.9±2.21[b]	20.8±1.01[b]
G6PD	5.2±0.36	4.2±0.55[a]	4.2±0.40	4.5±0.18	5.0±0.52[b]

The data are the mean ± SD (n=4). The superscript 'a' denotes that the data are significantly different from G I and the superscript 'b' denotes that the data are significantly different from G II ($p<0.05$). While SOD activity was expressed as units/min/100 mg protein, activity of GPx and G6PD was expressed as mol of NADPH oxidized/h/100 mg protein and mol of NADP reduced /h/100 mg protein respectively.

5. Also treatment with turmeric or curcumin appears to have minimized osmotic stress as assessed by polyol pathway enzymes (Table 14).

Table 14. Effect of curcumin/ turmeric on activities of polyol pathway enzymes, aldose reductase (AR) and sorbitol dehydrogenase (SDH), in rat lens

Enzyme	Group I	Group II	Group III	Group IV	Group V
AR	22.6±0.70	29.2±3.23[a]	26.8±0.86[b]	22.4±1.81[b]	23.1±0.64[b]
SDH	3.6±0.53	4.0±1.24	3.9±0.01	3.5±1.01	3.1±0.94

The data are the mean ±SD (n=4). The superscript 'a' denotes that the data are significantly different from G I and the superscript 'b' denotes that the data are significantly different from G II. AR activity was expressed as moles NADPH oxidised/h/100 mg protein and SDH activity as moles NADH oxidised/h/100 mg protein.

6. Interestingly, feeding of curcumin and turmeric improved the altered total and soluble protein levels in diabetic lens (Table 15). The ability of curcumin/ turmeric to prevent the loss of soluble proteins of lens in STZ-treated rat was remarkable (Table 15).

Table 15: Effect of curcumin/ turmeric on lens protein content in rat lens

Parameter	Group I	Group II	Group III	Group IV	Group V
Total protein (mg/g lens)	495±37.3	385±27.8[a]	398±53.1	472±48.3[b]	468±17.5[b]
Soluble protein (mg/g lens)	359±33.8	180±40.5[a]	237±50.3	297±39.1[b]	305±20.4[b]
% Soluble protein	72.5	46.9	59.7	62.9	65.1

The data are the mean ± SD (n=4). The superscript 'a' denotes that the data are significantly different from G I and the superscript 'b' denotes that the data are significantly different from G II.

7. Most importantly, curcumin and turmeric treatment not only prevented the decrease in protein content but also aggregation and insolubilization of lens proteins due to hyperglycemia as assessed by HPLC (Table 16) and SDS-PAGE (Figure 19).

Table 16. Distribution of crystallins in soluble protein fraction. Data are average of three HPLC runs for area under the curve

Peak	Group I	Group II	Group III	Group IV	Group V
HMW Peak	8631	24655	13853	10493	11172
α-Crystallin	215461	201370	202881	206158	202936
β-Crystallin	351479	277659	298147	308971	317596
γ-Crystallin	347580	294613	299900	318304	340523

Data are arbitrary units for absorbance at 280 nm.

Conclusions

The results indicate that turmeric and curcumin are effective against development of diabetic cataract in rats. Moreover, these results thus provide a clue, for the first time, that turmeric or curcumin may act downstream to glucose-mediated changes. Further, these results imply that the ingredients in our dietary sources, such as turmeric, may provide a viable food based, as well as pharmacological approach in the treatment of diabetic complications.

2. CHAPERONE ACTIVITY OF α-CRYSTALLIN UNDER DIABETIC CONDITIONS: MODULATION BY CURCUMIN

The α-Crystallin contributes to 30% of total lens protein and consists of two different subunits of each 20 kDa. These subunits self associate to form heterogeneous polydisperse complex of molecular mass of 800 kDa. α-Crystallin, being a member of small heat shock protein family acts as molecular chaperone and prevents the aggregation of other lenticular proteins/enzymes denatured by heat and other stress conditions. It has been established that chaperone function of α-crystallin is critical in maintenance of transparency of the lens *vis-à-vis* cataract formation. Diabetes is one of the major risk factors of cataract formation. In view of the prevailing and predicted epidemic of diabetes in developing countries like India, diabetic cataract may become the major leading cause of blindness along with senile cataract.

Chaperone function of α -crystallin in hyperglycemic conditions is of great concern with respect to lens transparency. It has been shown that oxidative stress can deteriorate the α-crystallin chaperone activity. Studies implicate that impaired chaperone function of α -crystallin could be involved in the formation of diabetic cataract. Therefore, it is essential to understand the role of chaperone function in diabetic conditions and the ways and means by which we can maintain and/ or modulate the chaperone potential of α-crystallin under diabetic conditions. The aim of the study was to investigate the effect of diabetes on chaperone activity of α-crystallin and to investigate modulatory effect, if any, of curcumin on chaperone activity of α-crystallin.

Methodology

Three month old male WNIN rats (b.w. 225 g) received 0.1 M citrate buffer pH 4.5 as vehicle (Group I; n=8), where as the experimental rats received a single intraperitioneal injection of streptozotocin (STZ; 35 mg/kg) in the same buffer. After 72 h, fasting blood glucose levels were monitored and animals having blood glucose levels less than 250 mg/dl were excluded from the experiment and rest were distributed into three groups. Experimental animals received either only AIN-93 diet (Group II; n=13) or received AIN-93 diet containing 0.002% (Group III; n=9) and 0.01% curcumin (Group IV; n=9). Animal care and protocols were in accordance with and approved by the Institutional Animal Ethics Committee. After 8 weeks of STZ injection rats from all the four groups were sacrificed by CO_2 asphyxiation and eye balls were enucleated. Lenses from three rats in each group were pooled for the studies. Water soluble proteins were analyzed on a Sephacryl S-300 gel filtration column and fractions corresponding to αH and αL-crystallins were pooled separately. Chaperone activity of α-crystallin (both αH and αL) was assessed by aggregation and enzyme inactivation assays. Far- and near-UV CD spectra of αH- and αL-crystallins were recorded. Intrinsic tryptophan fluorescence and fluorescence of 8-anilino-1-naphthalene-sulfonic acid (ANS) bound to α-crystallin was also measured.

Results

There was a marked difference in the relative distribution of crystallins between the groups. αH-crystallin peak has been elevated in diabetic rat lens (Group II) compared to control rat lens (Group I). Further there was a decrease in both ß- and γ-crystallin fractions also in Group II (Figure 21).

- Feeding of curcumin (Group III and IV) reverted the altered crystallin profile in a dose dependent manner (Figure 21).
- αL-Crystallin from Group II rat lens showed 50% decrease in chaperone activity in suppressing the heat-induced aggregation of ßL-crystallin when compared to the activity of αL-crystallin from Group I (Figure 22). Similar results were observed with αH-crystallin.
- Interestingly, the chaperone-like activity of αL from Group III and IV was improved than Group II rat lenses. Strikingly, aggregation kinetics of ßL-crystallin displayed longer lag time in the presence of αL from Group III and IV compared to αL from Group II (Figure 22). Curcumin feeding also improved chaperone activity of αH-crystallin isolated from diabetic lenses.
- Similar to aggregation assays, the ability of αL-crystallin from Group II to prevent heat-induced inactivation of G6PD was declined as compared to Group I (Figure 23).
- Furthermore, in contrast to the marginal protection in aggregation assays, αL-from Group III and IV (curcumin treated) rat lens exhibited a remarkable protection against G6PD inactivation(Figure 23).
- αL from Group II showed lesser ANS binding when compared with αL from Group I (Figure 21), which correlated well with the decreased chaperone activity of αL-crystallin from Group II (Figure 22).

- Furthermore, improved chaperone activity of αL-crystallin from Group III and IV is also reflected in increased ANS binding when compared to that of αL-crystallin from Group I (Figure 24).
- Far-UV CD signal for α-crystallin isolated from diabetic rat lens is decreased compared to control rat lens, indicating altered secondary structure. Altered tryptophan fluorescence (Figure 25) and changes in near-UV CD spectra indicated altered tertiary structure of α-crystallin due to hyperglycemia.
- Although, curcumin treatment has not affected the altered secondary structure due to hyperglycemia in a significant manner, curcumin-mediated modulation of altered tertiary structural changes are quite noticeable (Figure 25).

Figure21: Separation profile of total soluble lens protein on gel filtration. Protein (80 mg) from Group I, Group II, Group III and Group IV was loaded onto a Sephacryl S-300 HR column

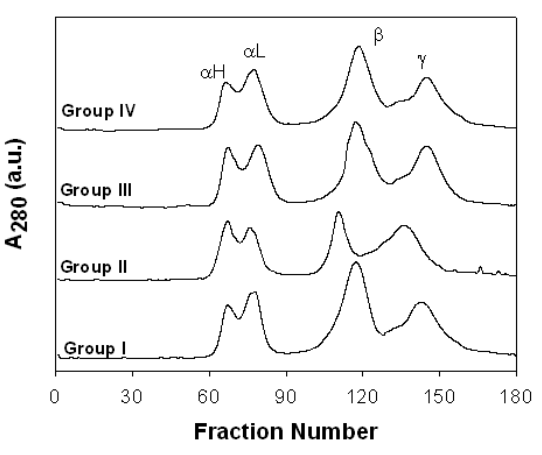


Figure 23: Chaperone activity of αL-crystallin in enzyme inactivation assays. Protection of heat-induced inactivation of glucose-6-phosphate dehydrogenase at 42°C by αL-crystallin Bar 1-G6PD alone, Bars 2, 3, 4 and 5, are G6PD along with αL-crystallin from Group I, Group II, Group III and Group IV, respectively. Data were average of three chaperone assays

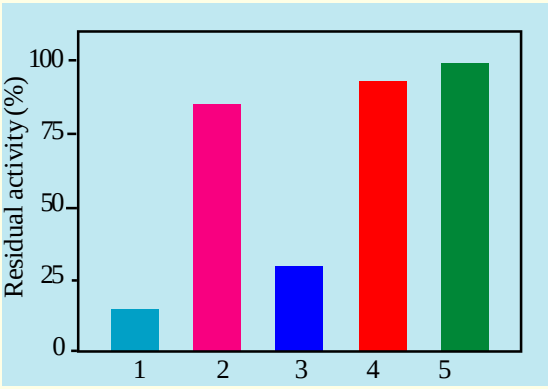


Figure 22: Chaperone activity of αL-crystallin as assessed by the suppression of heat-induced aggregation of ß$_L$-crystallin. ß$_L$-Crystallin was incubated at 60°C in the absence (trace 1) or in the presence of αL-crystallin (0.025 mg/ml) from Group I (trace 2), Group II (trace 3), Group III (trace 4) and Group IV (trace 5). Data were average of three chaperone assays

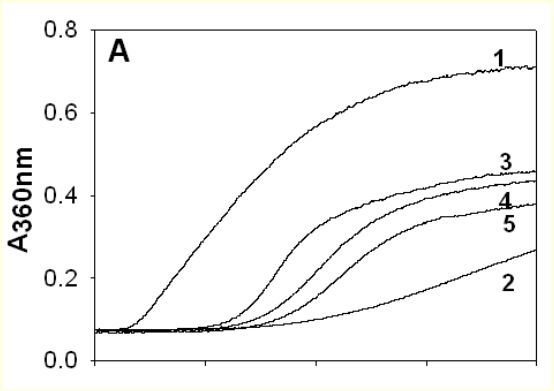


Figure 24: Hydrophobicity of αL-crystallin as assessed by ANS fluorescence. Traces 1-4 correspond to αL-crystallin from Group I, Group II, Group III and Group IV, respectively. Data were average of three assays.

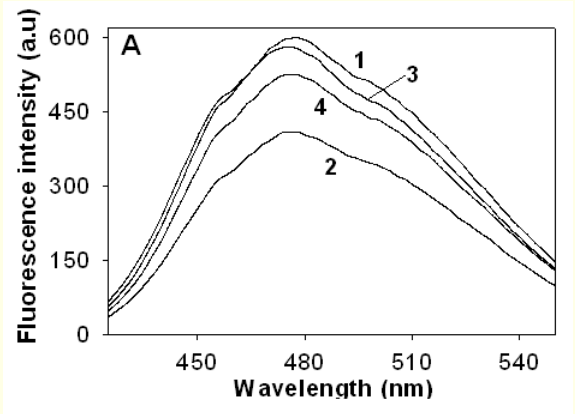
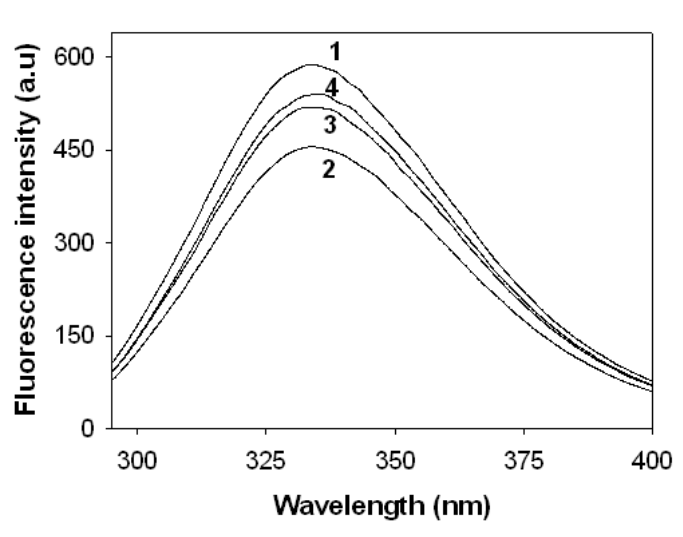


Figure 25: Tryptophan fluorescence spectra of αL-crystallin. Traces 1-4 correspond to αL-crystallin from Group I, Group II, Group III and Group IV, respectively. Data were average of three experiments



Conclusions

Present study demonstrated that in STZ-induced diabetic cataract α-crystallin exhibited diminished chaperone activity, which was positively modulated by dietary curcumin and delayed progression and maturation of cataract. Though, multiple actions of curcumin could be involved in delaying STZ-induced cataract in rats, we observed that antioxidant effect of curcumin was the predominant mechanism (Invest Ophthalmol Vis Sci, 2005). Thus, one of the possible explanations for the modulatory effect of curcumin on α-crystallin chaperone activity in diabetes could be decreased oxidative stress by curcumin in hyperglycemia.

3. INHIBITION OF PROTEIN GLYCATION BY DIETARY AGENTS

Diabetic complications is a major cause of morbidity and mortality in both the developed and developing nations, which are manifested in several organ systems in the form of vascular complications such as retinopathy, nephropathy, neuropathy and non-vascular complications such as cataract and glaucoma. Hyperglycemia is the primary factor that initiates and promotes the complications. Multiple molecular mechanisms have been proposed to explain the pathogenesis of long term complications of diabetes, one of them which is considered to be a prominent upstream phenomenon down the line that leads to various deleterious consequences is non enzymatic glycation. The high glucose levels in diabetes may cause tissue damage by the nonenzymatic glycation of proteins. Excess reducing sugars react non-enzymatically with the amino groups of proteins to form Schiff's base intermediates, which rearrange to more stable Amadori products via the Maillard reaction. These Amadori products further undergo chemical modification to form advanced glycation end products (AGE). It has been shown that formation of AGE in vivo contributes to several pathophysiologies associated with ageing and diabetes mellitus, such as cataract.

Hence role of antiglycating agents delaying the onset or progression of diabetic complications has gained the considerable importance. Aminoguanidine and few other chemical compounds have been shown to be effective against protein glycation, but none of them are clinically successful. Therefore, the aim of the study was to investigate antiglycating potential of dietary agents for the prevention of secondary complications of diabetes.

Methodology

Briefly, total soluble lens proteins (TSP) from bovine eyes were used for in vitro glycation. Each 1ml incubation mixture contained 10 mg of protein, 0.2 M PBS, glycating sugar (100 mM fructose), 50 µg of penicillin & streptomycin and 0.01% sodium azide. Various concentrations of aqueous extracts of dietary sources were added to the above mixture when used. Tubes were then sealed and incubated in dark at 37oC for 3 weeks. After incubation solutions were dialyzed extensively against buffer to remove unbound sugars and the extent of glycation was assessed by various complimentary methods;

(i) Determination of protein cross linking on SDS-PAGE,
(ii) Advanced glycation related fluorescence (ex: 370 nm and em: between 400-500 nm) and
(iii) Protein carbonyls and tryptophan fluorescence as function of protein oxidation and conformational changes.

Results

Incubation of bovine lens total soluble protein (TSP) with fructose resulted in cross-linking of polypeptides in a dose dependent manner and these cross-links are similar to the cross-links observed in many types of cataractous lenses. Similarly, upon glycation there was an increase in non-tryptophan fluorescence due to formation of AGE. Glycation of lens proteins also leads to oxidative damage and altered conformational changes as shown by increased carbonyl content and decreased tryptophan fluorescence respectively. Since there is very little information on antiglycating agents, particularly from dietary sources, the dietary agents were coded that have shown promising antiglycating potential, to protect intellectual property rights and the code names are given in Table 17.

On the basis of the results obtained after analyzing the extent of inhibition of glycation, the extracts that are found to be promising are MB1, MB2, MAB1, MSB1, MYB1, MYB2, MYB3, MYB4, MYB5 and MYB6 (Table 17). They inhibited protein glycation in a dose dependent manner. Inhibition of glycation in comparison to control was assessed by different parameters. Order of effectiveness against glycation is given based on concentration of extracts required for inhibiting glycation by various methods

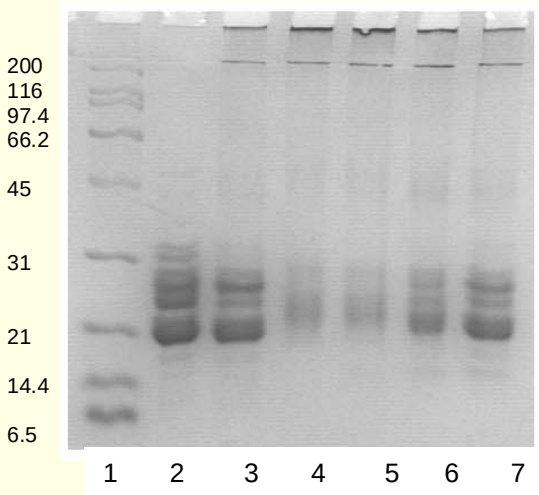
- The following agents in that order were effective in preventing the formation of cross-links of lens protein profile based on SDS-PAGE; MYB1 > MYB5 > MYB2 > MAB1 > MB1 > MSB1 > MB2 (Figure 26)
- Inhibition of AGE fluorescence was found to be in the following order; MYB1 > MB1 > MYB2 > MYB5> MAB1 > MB2 > MSB1 (Figure 27)
- Reduction in the carbonyl content of the glycated protein by dietary extracts was observed in the following order; MYB1 > MB2 > MYB2 > MAB1 > MYB5 > MB1 > MSB1 (Figure 28)
- Recovery brought about by the extracts in the tryptophan fluorescence of protein found in the following order; MYB5 > MB2 (Figure 29)

Table 17: Code names of the dietary extracts screened for the inhibition of protein glycation.

S.No.	Code Name	Ranking*	S.No.	Code Name	Ranking*
1	MB1	**3**	6	MYB1	**1**
2	MB2	5	7	MYB2	**2**
3	MAB1	6	8	MYB3	**4**
4	MSB1	8	9	MYB4	9
5	MSB2	10	10	MYB5	7

** Ranking was assigned by averaging the potential of the extract in preventing protein glycation by all the three methods, cross-linking, AGE fluorescence and protein carbonyls methods. Results were reproduced 3 times for the agents with ranking 1-4.*

Fig. 26: SDS-PAGE of lens protein upon in vitro glycation in the absence and presence of MYB1



Lane 1: MW standards in kDa
Lane 2: Control protein
Lane 3: Protein + MYB1 (0.1 mg/ml)
Lane 4: Protein + Fructose
Lane 5: Protein + Fructose + MYB1 (0.01 mg/ml)
Lane 6: Protein + Fructose + MYB1 (0.05 mg/ml)
Lane 7: Protein + Fructose + MYB1 (0.1 mg/ml)

Fig. 27. AGE fluorescence on in vitro incubated protein with fructose in the absence and presence of MYB1

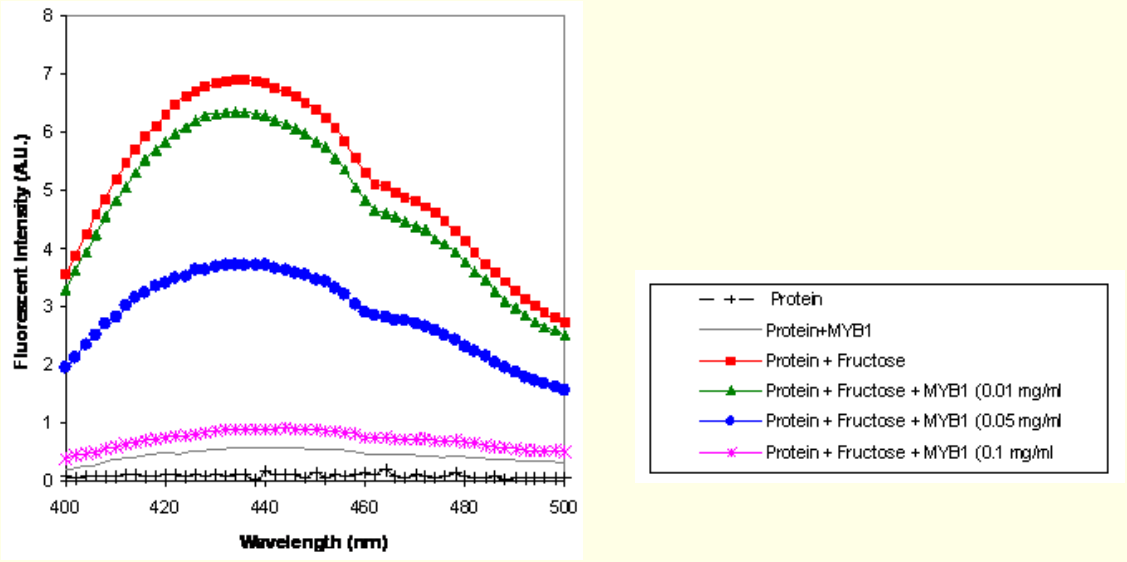
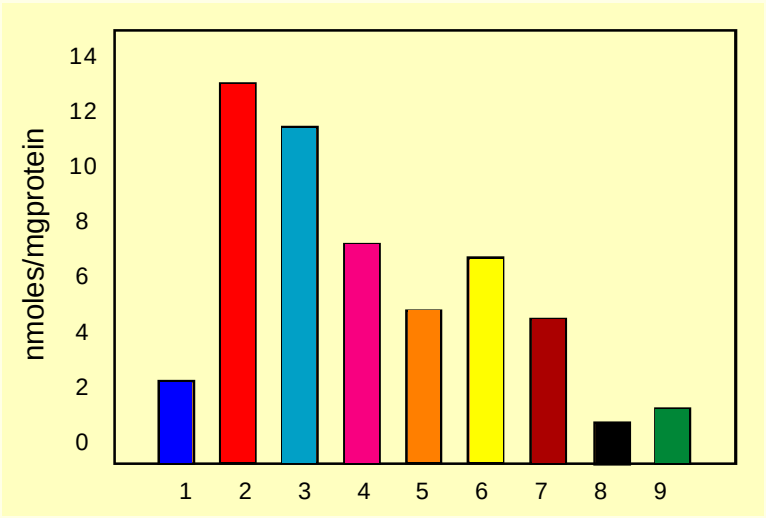
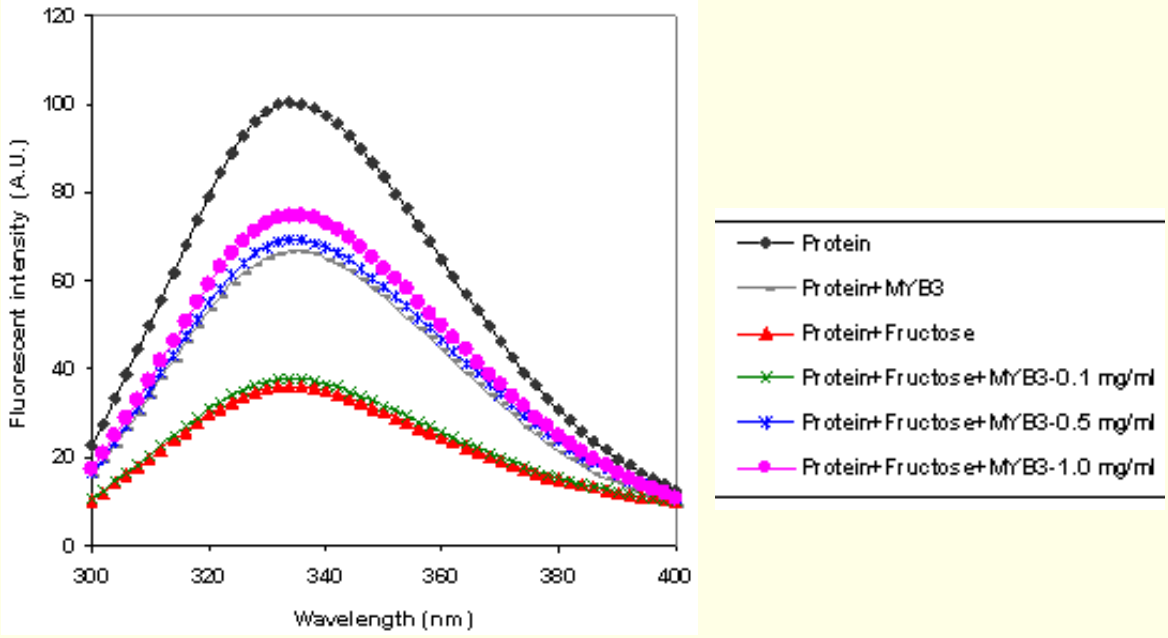


Fig. 28: Carbonyl content of lens soluble protein upon in vitro glycation in the presence and absence of MB2



1 - Protein
2 - Protein + Fructose
3 - Protein + Fructose + MB2 (0.1 mg/ml)
4 - Protein + Fructose + MB2 (0.5 mg/ml)
5 - Protein + Fructose + MB2 (1.0 mg/ml)
6 - Protein + Fructose + MB2 (2.5 mg/ml)
7 - Protein + Fructose + MB2 (5.0 mg/ml)
8 - Protein + Fructose + MB2 (7.5 mg/ml)
9 - Protein + Fructose + MB2 (10 mg/ml)

Fig. 29. Tryptophan fluorescence of TSP upon in vitro glycation in the presence and absence of of MYB3



Conclusions

MYB1, MYB2, MB1 and MYB3 were found to be the effective inhibitors of protein glycation in vitro, MYB1 being the most potent. Hence, these agents may be exploited for their potential in the management of secondary complications of diabetes. Studies are underway to investigate the mechanism of antiglycation by the effective agents and their significance in the pathophysiology.

4. EFFECT OF METHYLGLYOXAL ON DEGRADATION AND STABILITY OF α- CRYSTALLIN

Several diabetic complications including cataract are thought to be result of accumulation of advanced glycation end products (AGE) generated from modification of proteins by different glycating agents. Methylglyoxal (MGO), a major dicarbonyl compound, is present in high concentrations in lens compared to plasma or any other tissue and its levels increase several folds during diabetes. Compared to other potential glycating agents, MGO has very high affinity for proteins and is known to react with Arg, Lys, His and Cys residues forming AGE. α -Crystallin, molecular chaperone of eye lens plays an important role in maintaining the transparency of eye lens by preventing the inactivation or aggregation of several enzymes/proteins and also as a key structural element. Being a long-lived protein and rich in basic amino acids, α -crystallin may be more susceptible to non-enzymatic browning by MGO. Earlier it was reported that MGO-modified α -crystallin exhibited enhanced chaperone-like activity in aggregation assays but it was less effective in preventing enzyme inactivation (Biochem J, 379, 1-10, 2004).

Further, modified α -crystallin has showed decreased hydrophobicity, altered secondary/ tertiary structure and increased oligomeric size (*Biochem J*, 379, 1-10, 2004). Although altered/damaged proteins are known to be more susceptible to degradation, effect of non-enzymatic glycation of protein on its susceptibility to degradation was not well studied. Eye lens contains high levels (3-5 mM) of ATP and its binding has been shown to protect α B crystallin from proteolytic digestion. Therefore, we have investigated the effect of MGO-modification on α-crystallin degradation and stability and the role of ATP mediated protection. Finally, physiological significance of MGO effects on α -crystallin *vis a vis* lens transparency was studied using lens organ culture system.

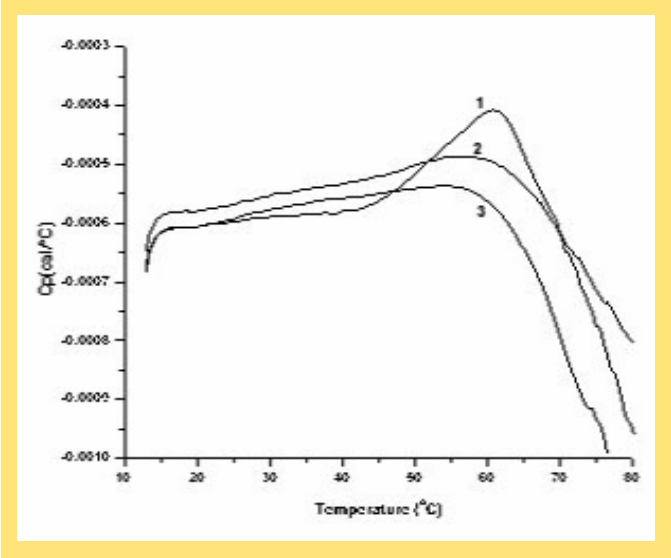
Methodology

The α-Crystallin was isolated from bovine lenses by gel filtration (Sephacryl S-300HR) and incubated with various concentration of methylglyoxal (MGO) for different time periods at 37oC under sterile conditions. Unbound MGO was removed by extensive dialysis. Extent of protein damage was analyzed by assessing formation of protein carbonyls by 2, 4-DNPH method. Effect of MGO-modification on stability was studied by differential scanning calorimetry (DSC) and denaturant induced unfolding. Susceptibility of MGO-modified α -crystallin to proteolytic digestion by trypsin and chymotrypsin was analyzed by SDS-PAGE. ATP binding to native and MGO-modified α -crystallin was studied using tryptophan fluorescence quenching. Bovine lenses were cultured in modified TC-199 medium with antibiotics at 37°C under 95% air and 5% CO_2. Lenses were incubated with and without 1 mM MGO for different time periods. After incubation, lenses were homogenized and soluble and insoluble fractions were prepared by centrifugation.

Results

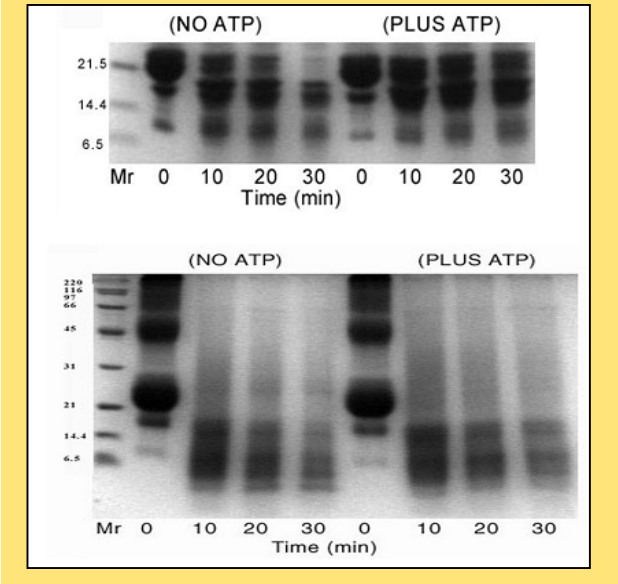
- MGO modification leads to severe oxidative damage as shown by increased protein carbonyl formation.
- Methylglyoxal modification of α -crystallin, reduced its stability in a concentration dependent manner as studied by differential scanning calorimetry, Tp was shifted to 56 and 53 from 65°C respectively for 10 and 100 mM MGO-modified α-crystallin (Figure 30).
- Denaturant induced unfolding studies show that MGO-modified α-crystallin unfolds at lower concentrations of denaturant compared to native α-crystallin. These results further support DSC data and suggest that methylglyoxal modification decreases the stability of α-crystallin.

Fig. 30. Tracings of typical differential scanning calorimeter thermograms (heat capacity versus temperature) of native and modified α-crystallin. Trace 1- native α-crystallin, trace 2 & 3 - 10 and 100 mM MGO modified α-crystallin.



- Proteolytic digestion studies of native and MGO-modified α-crystallin suggest that methylglyoxal modification increases the susceptibility to degradation by proteases such as trypsin and chymotrypsin.
- Binding of ATP could protect native α-crystallin against proteolytic digestion by trypsin but not MGO-modified α-crystallin (Figure 31)

Fig. 31. Trypsin digestion of native (A) and MGO-modified (B) α-crystallin in the absence & presence of ATP at 37oC as analyzed by SDS-PAGE. α -Crystallin was modified by incubating with 5 mM MGO for 24 h. The time course for the proteolysis was indicated at the bottom of the gel. Molecular weight standards in kDa are marked alongside.



- Stem-Volmer plot for the ATP binding to native and MGO-modified α-crystallin indicates decreased affinity for the binding of ATP to MGO-modified α-crystallin. Scatchard analysis indicates decreased binding sites for the ATP upon MGO-modification of α-crystallin (Table 18).

Table 18. Binding of ATP to native and MGO modified α-crystallin.

Group	Binding constant (K_d, nM)	Binding sites (N)
Native α-crystallin	3.130	7.9
Modified α-crystallin		
1 mM	1.611	4.5
5 mM	1.27	0.14
10 mM	2.296	0.10

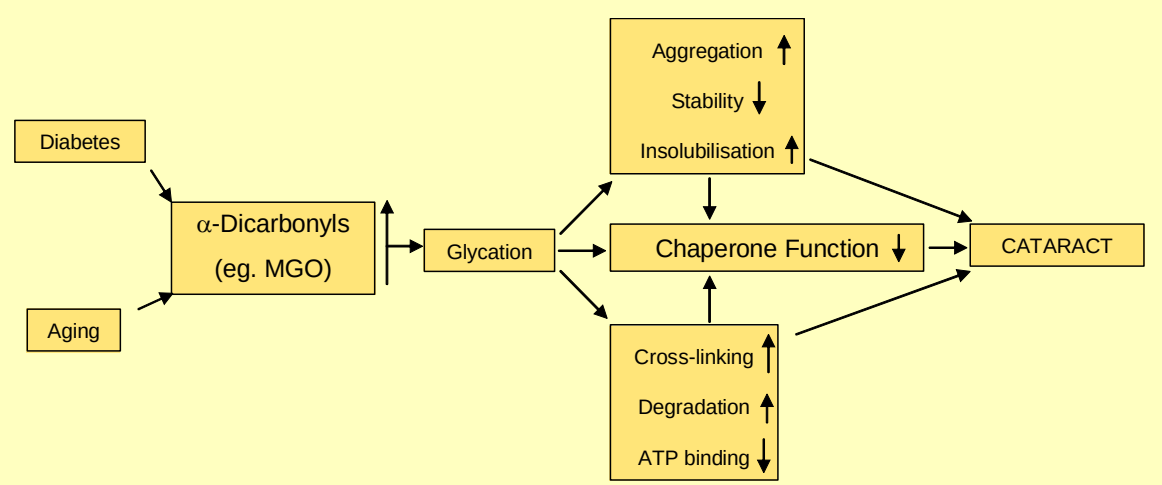
Values are average of three experiments

- Lens organ culture studies indicate presence of MGO in the culture medium leads to the opacification of the calf lenses (Figure 32).
- Total soluble protein analyzed by gel filtration indicates MGO treated lenses having high amounts of high molecular weight α-crystallin (α_H) compared to control lenses.
- Furthermore, α-crystallin fractions isolated from MGO-treated lenses showed diminished chaperone activities.
- Western blot analysis of the soluble and insoluble fractions of MGO-treated and control lenses indicates that MGO-modication of α-crystallin leads to AGE formation and insolubilisation causing the scattering of light.

Fig 32. Methylglyoxal-induced opacification of lens in organ culture: Calf lenses were cultured in modified TC-199 in the presence of 1 mM MGO for '0' days (A), 2 days (B) and 4 days (C). Transparency of the lens that was cultured for 4 days in the absence of MGO was similar to '0' days lens

A **B** **C**

Fig. 33. Schematic representation of possible molecular effects of methylglyoxal on α-crystallin Vis a vis lens opacification



Conclusions

The studies show that nonenzymatic browning of α-crystallin by MGO leads to decrease in its stability and unfolding that in turn leads to the exposure of buried proteolytic sites causing enhanced proteolytic degradation. The ATP could not protect the glycated α-crystallin from proteolytic degradation as was observed with native α-crystallin. As depicted in figure 33, unfolding and conformational changes due to MGO modification increase susceptibility to degradation and subsequent light scattering due to cross-linking/ insolubilisation. Hence, it is quite possible that posttranslational modification imposed by dicarbonyls may have unfavorable effects on the ability of α-crystallin to inhibit protein aggregation/ enzyme inactivation in vivo. Results of the present study provide the basis for the role of non-enzymatic glycation on α-crystallin chaperone activity in age-related brunescent and diabetic cataracts.

5. INSIGHTS INTO THE HYDROPHOBICITY AND CHAPERONE FUNCTION OF α-CRYSTALLIN: ISOTHERMAL TITRATION CALORIMETRY STUDY

The α-Crystallin, a member of the sHSP group constitutes a major portion of the eye lens cytoplasm. Lenticular α-crystallin is a hetero-oligomer with two subunits, αA and αB, mostly present in a stoichiometry of 3:1. αA and αB are 20 kDa each and share ~60% sequence identity. Both homo and heteropolymers of α-crystallin exhibit chaperone-like activity by suppressing protein aggregation. It is clear that, in addition to providing refractive properties to the eye lens, α-crystallin is instrumental in maintaining transparency of the eye lens with its chaperone-like activity. Despite their high sequence homology, the relative importance of αA- and αB-crystallins is not completely understood. Although the mechanism of chaperone function is not understood completely, numerous studies implicate surface-exposed hydrophobic sites on α-crystallin and other sHSP in binding to partially unfolded proteins.

The finding that increased exposure of hydrophobic surfaces on structurally perturbed α-crystallin is associated with increased chaperone like function, substantiates the role of hydrophobicity in chaperone function of sHSP. However, the enhanced chaperone-like activity with increase in temperature was not

similarly associated with increased hydrophobicity of αA- and αB-crystallins. αA- and αB-Crystallins differ not only in their hydrophobic character with temperature but also in their secondary and tertiary structure, molecular size and other physicochemical properties. This makes an exact correlation between hydrophobicity and chaperone activity difficult to establish. In the present study isothermal titration calorimetry (ITC) was employed to determine the number of binding sites and the thermodynamics of 8-anilino-1-napthalene sulfonic acid (ANS) binding to αA- and αB-crystallin. Hydrophobicity and chaperone activity of αA- and αB-crystallins were correlated at different temperatures to get greater insight into the role of hydrophobicity in the chaperone-like function of α-crystallin.

Methodology

Recombinant αA- and αB-crystallins were purified according to previously reported methods (*FEBS Lett,* 2002, 522, 59-64). The chaperone activity of αA-and αB-crystallin was studied by assessing their ability to suppress the aggregation of insulin B chain at 15 and 30°C (induced by DTT). Hydrophobicity was measured with ITC using ANS as a hydrophobic ligand. In brief, 4-8 µl aliquots of ANS stock solution were added to αA or αB crystallin solution and heat changes accompanying these additions were recorded. The data so obtained were fitted using non-linear least squares minimization method for determining the binding stoichiometry (N), binding constant (K_b), and change in enthalpy (ΔH^b), using Origin software (Microcal Inc) and two site model provided the best fit for the data. The change in entropy (ΔS) was calculated from $\Delta G = \Delta H - T\Delta S$. For chaperone assays performed in the presence of ANS, αA and αB-crystallins were preincubated with saturating amounts of ANS at specified condition as described in figure legends and excess ANS was removed by dialysis.

Results

1. The quantification of the hydrophobic surfaces in αA- and αB-crystallins by ITC indicate presence of two binding sites, one with high affinity, low capacity and another with low affinity, higher capacity. At 30°C, αB showed higher number of ANS binding sites than αA (Table 19 & Figure 34).

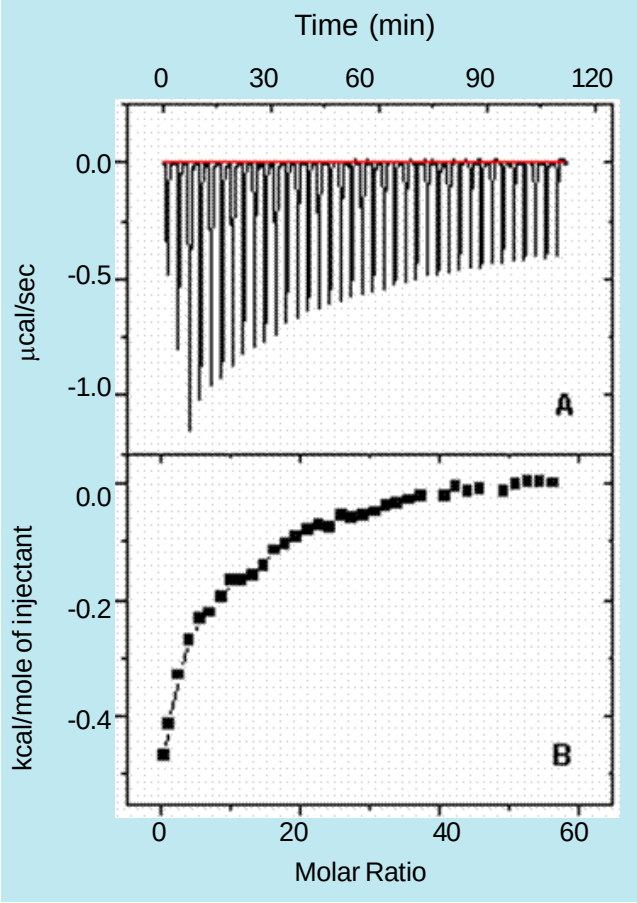
Table 19. Calorimetric data of ANS binding to αA- and αB-crystallins at 15°C and 30°C

Parameters	15°C		30°C	
	αA	αB	αA	αB
N1	3.5 ± 0.47	3.1 ± 0.32	0.98 ± 0.06	5.9 ± 0.26
K1 (M^{-1}) X10^5	2.4 ± 0.16	4.6 ± 0.42	7.8 ± 0.66	2.1 ± 0.42
ΔG1 (kcal/mol)	–7.0 ± 0.67	–7.4 ± 0.61	–6.4 ± 0.56	–7.0 ± 0.89
ΔH1 (kcal/mol)	–368 ± 22.4	–612 ± 29.1	–1075 ± 93.3	–1262 ± 196
ΔS1 (cal/mol/K)	23.4 ± 2.61	23.8 ± 1.62	18.8 ± 2.31	20.2 ± 3.21
N2	12.2 ± 1.40	11.9 ± 1.57	18.0 ± 2.32	27.1 ± 3.05
K2 (M^{-1}) X10^5	1.9 ± 0.09	0.42 ± 0.037	0.73 ± 0.079	4.1 ± 0.60
ΔG2 (kcal/mol)	–5.6 ± 0.63	–4.7 ± 0.62	–5.0 ± 0.65	–10.6 ± 1.20
ΔH2 (kcal/mol)	–127 ± 10.6	–506 ± 39.2	–243 ± 16.1	–56.9 ± 5.70
ΔS2 (cal/mol/K)	19.2 ± 2.35	14.8 ± 1.25	16.8 ± 1.05	20.9 ± 2.5

(N= Number of binding sites; K= binding constant; ΔG= Change in free energy; ΔH= Change in enthalpy; ΔS= Change in entropy; The numbers 1 and 2 indicate thermodynamic parameters associated with site 1 and site 2)
In each case the errors associated have been mentioned.

2. In agreement with higher number of hydrophobic sites, αB-crystallin, demonstrated higher chaperone activity than A at 30°C.
3. Thermodynamic analysis of ANS binding to αA- and αB-crystallins indicate that high affinity binding is driven by both enthalpy and entropy changes with entropy dominating the low affinity binding (Table 19).

Fig. 34. Calorimetric titration profile of the binding of ANS to native αA-crystallin at 30°C. Panel A: Exothermic heats associated with the injection of ANS into the sample cell containing αA-crystallin. Panel B: Binding isotherm corresponding to the data in panel A.

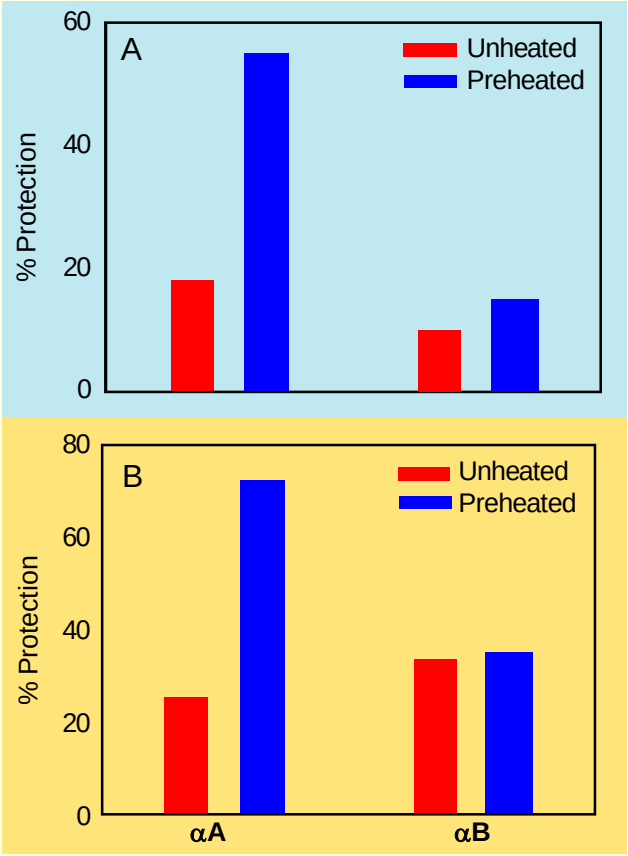


4. Interestingly, although ANS binding sites were similar for αA and αB, at 15°C, A was more potent than αB in preventing aggregation of insulin B-chain.
5. While, there was no change in high affinity sites of αA and αB for ANS upon preheating, there was an increase in low affinity sites for αA and αB.
6. However, preheated αA, in contrast to αB, exhibited remarkably enhanced chaperone activity indicating no quantitative correlation to its surface hydrophobicity (Figure 35).
7. Chaperone activity of αA and αB-crystallins studied both in the absence and presence of ANS suggests that there is no direct quantitative relation existing between hydrophobicity and chaperone like activity (Table 20).

Table 20. Percentage loss of chaperone activity of αA and αB crystallin (native and preheated) in the presence of ANS at 15 and 300C. Data are average of three chaperone assays. The chaperone activity in the absence of ANS was considered as 100%

	αA		αB	
	15°C	30°C	15°C	30°C
Native	42	44	62	65
Preheated	60	54	55	63

Fig. 35. Chaperone activity of native and preheated αA and αB-crystallins studied at 15 (Panel A) and 30oC (Panel B). Aggregation of insulin with DTT in the absence of α-crystallin was considered as 100 % to calculate the % protection observed in the presence of chaperone.



Conclusions

ITC data along with the data on chaperone activity suggest that although, hydrophobicity appears to be a factor in chaperone-like activity of α-crystallins, it does not correlate quantitatively to its chaperone function. Factors other than hydrophobicity could be involved in the chaperone activity of α-crystallin, which needs to be investigated. Further, this approach of studying the surface hydrophobicity using ITC may be employed to establish the role of hydrophobicity in chaperone activity of other sHSP.

VI. PATHOLOGY

EFFECT OF MATERNAL MALNUTRITION ON FETAL PANCREAS

Animal studies have revealed that maternal malnutrition and hyperglycemia during pregnancy, affect the development of fetal pancreas-endocrine in particular. It is also known that fetal undernutrition in long term may lead to impaired glucose tolerance & insulin resistance while the structure & function of the islets particularly the β -cells is also affected.

Objective

To look into the status of pancreatic islets with respect to morphological changes in aborted fetuses obtained by MTP, from undernourished mothers & compare them to those obtained from adequately nourished mothers.

Methodology

All fetuses of gestation age between 16-20 weeks were collected over a 28 month period. Only samples from uncomplicated MTPs (medically terminated pregnancies) were included for the study.

Obstetric history of the mothers was obtained through a questionnaire and mothers were classified as undernourished and adequately nourished, based on BMI of 18 and 20.0 or above respectively. Three ml of blood was collected for fasting glucose and Hemoglobin (Hb) estimations from each of the subjects. A total of 15 fetal samples were obtained, of which 6 were from mothers < 18 BMI and other 9 belonged to the group with BMI >20.

Fetuses obtained were immediately injected with Bouins fluid intraabdominally and put into 10% neutral buffered formalin overnight. On the following day, the crown rump length (CRL) of the fetus was measured and subsequently dissected to retrieve the pancreas, which was then segregated into head, body and tail regions. Each part was fixed in Bouins fluid further and processed separately. From each region, an average of 150 paraffin sections of 4 thickness were obtained and thus a total of 450 sections were obtained from each pancreatic specimen. After standardization of various islet parameters in each section, it was found that evaluation of every fifth section would be adequate without any compromise on the quality of data obtained. Thus, at the end, an average of 30 paraffin sections were studied for each region of an individual pancreas (90/ pancreatic sample). Masson trichrome stained sections (for better delineation of the islets) of each region were studied and morphometric estimates were done at 10X magnification using an ocular grid. First, the pancreatic volume was estimated by selecting the cross-section with the maximum dimensions (length, breadth etc). Each islet was marked in all the 30 sections and followed through. Thus, the length, maximum width and total thickness were obtained and the unit area and volume were calculated. Immunohistochemical study of cells with anti-insulin monoclonal antibodies was also carried out for their morphological assessment, which would also reflect their functional status.

Results

The various maternal and fetal parameters studied including those of the pancreas were as follows:

Table 25. Pancreatic characteristics according to maternal nutritional status in uncomplicated MTP

Sl.No	Parameter	BMI <18.0 mean ±S.E (N)	*BMI >20* mean ± S.E (N)
1.	*MATERNAL* : BMI **	16.78 ± 1.295 (6)	22.72 ± 1.706 (9)
2.	Weight (kgs) **	37.85 ± 2.887 (6)	52.69 ± 5.153 (9)
3.	Height (cms)	150.27 ± 5.357 (6)	152.17 ± 4.134 (9)
4.	Gestational age (weeks)	17.00 ± 1.265 (6)	18.78 ± 1.986 (9)
5.	Hb (g%)	9.36 ± 1.421 (4)	10.53 ± 2.469 (8)
6.	FBS (mg%)	61.00 ± 21.494 (4)	88.00 ± 28.914 (5)
7.	FOETAL CRL (cms)	14.83 ± 1.60 (6)	14.56 ± 2.33 (9)
8.	Total Pancreatic volume (mm^3)	26.76 ± 15.03 (6)	40.88 ± 19.66 (9)
9.	Islet count / pancreas	335.50 ± 180.75 (6)	534.33 ± 461.66 (9)
10.	Maximum length of islet (µ)	300.00 ± 113.13 (6)	318.89 ± 98.54 (9)
11.	Minimum length of islet (µ)	25.00 ± 7.74 (6)	24.44 ± 6.82 (9)
12.	Maximum width of islet (µ)	221.67 ± 53.82 (6)	231.11 ± 59.46 (9)
13.	Minimum width of islet (µ)	22.50 ± 6.12 (6)	24.44 ± 6.82 (9)
14.	Mean islet area (mm^2) / Pancreas	0.0079 ± 0.0010 (6)	0.0090 ± 0.0022 (9)
15.	Mean islet volume (mm^3) / Pancreas	0.0001 ± 0.00 (6)	0.0002 ± 0.00 (9)
16.	Islet density (Islets / cu.mm of pancreas)	19.12 ± 16.27 (6)	13.36 ± 8.95 (9)
17.	Fully formed islets in section studied	35.06 ± 21.44 (5)	29.49 ± 15.01 (8)
18.	Number of β-cells / unit area	55.05 ± 19.16 (5)	57.05 ± 12.88 (8)
19.	Number of acinar cells / unit area	853.34 ± 271.16 (5)	936.19 ± 198.92 (8)
20.	Ratio of beta : Acinar cells	15.87 ± 2.50 (5)	17.25 ± 5.27 (8)

*** Significant*

Conclusion

It was observed that there were no significant differences in the number, size or density of the islets as well as the beta and acinar cell counts between the pancreas of aborted fetuses (aged 16 to 20 weeks) belonging to undernourished and adequately nourished mothers (Table 25).

VII. EXTENSION AND TRAINING

A. SERVICE ACTIVITIES

1. Publications

The quarterly periodicals, namely, Nutrition (English), Poshan (Hindi), Poshana (Telugu) and a semi-technical bulletin Nutrition News, covering popular articles of public interest and scientific information on nutrition are being published. During the year, Urdu version of Dietary Guidelines for Indians - A Booklet was brought out.

Publications reprinted during 2004-2005

1. Some Theraputic Diets
2. Diet and Heart Diseases
3. Fruits
4. Count What You Eat
5. Recommended Dietary Allowance
6. Dietary Guidelines for Mother and Child (Telugu)
7. Mother and Child
8. Diet and Diabetes
9. Dietary Tips for the Elderly
10. Nutritive Value of Indian Foods

2. Training Programmes

This year, a total of twenty-six candidates have attended the regular training programmes of the Institute viz. (i) Post-Graduate Certificate Course in Nutrition (10 participants) (ii) Annual Training Course in Endocrinological Techniques (8 participants) and (iii) Techniques for Assessment of Nutritional Anaemias (8 participants). In addition, ad-hoc training programmes were conducted for 7 WHO Fellows, of whom four were from Bangladesh, two from Nepal and one from Orissa. In the training courses, care has been taken to expose the participants to the latest information both in theoretical as well as practical aspects in the field of nutritional sciences through lectures and demonstrations using multimedia educational approaches by the Institutes as well as expert guest faculty. Besides, visits to the Nutrition wards in the hospitals and community programmes were organized to expose the participants to prevailing nutritional problems in the population and IEC techniques in educating the community to prevent malnutrition.

3. Extension Activities

3.1. Exhibitions

1. Portable exhibition was displayed at New Model High School, Doodhbowli, Hyderabad in connection with National Technology day Celebrations on 11th May 2004. Teachers, students and other staff members of the school (200) were explained about the importance of nutrition and health.

2. To commemorate 50th year celebration of the Gandhi Medical College, an exhibition stall with portable exhibition system was put-up in the Social and Preventive Medicine Unit of the medical college during 6th-17th December 2004. There was an overwhelming response from the general public and as well as the students (3000) of the medical college.
3. As part of 92nd session of Indian National Science Congress, a nutrition education stall was put up in an exhibition organized by ICMR, New Delhi at Ahemdabad during January 3rd - 7th 2005 at Nirma University, Ahmedabad. About 2000 people from different walks of life visited the stall during the exhibition.
4. As part of 2nd Nutraceutical summit an exhibition stall was organized on Nutrition & Health in the International Seminar & Expo during February 3rd - 5th 2005 at New Delhi. About 600 visitors visited the stall.
5. To commemorate Consumer Week Celebrations, a portable exhibition was put up on 15th March 2005 depicting the dietary guidelines for all the age groups. About 100 visitors, who participated in the consumer day celebrations, witnessed the portable exhibition.

Sri. Narendra Modi, Chief Minister of Gujarat visiting the NIN stall in the 92nd session of Indian National Science Congress, Organized by ICMR, Ahmedabad, New Delhi

Consumer Week Celebrations on 15th March 2005

3.2. Popular Talks

1. Organized Nutrition awareness programmes (6) to the school children living in slums and youth volunteers of Confederation of Voluntary Associations (COVA) in the old city of Hyderabad in the summer camps organized by COVA between 25th April 2004 and 30th May 2004. About 700 youth volunteers including school children participated in the awareness programme.
2. A popular lecture on "Food, Nutrition and Health" and a cooking demonstration in association with Food and Nutrition Board, Hyderabad were organized to the employees of Andhra Pradesh State Transport Corporation (APSRTC), Karimnagar Depot on 25th May 2004.About 300 employees and their families participated in the programme.
3. A popular lecture on "Balanced diet, nutritive values of foods, health and nutrition" on the occasion of Telugu Academy Foundation day on 6th August 2004 was given to the employees (60) of the academy.
4. Delivered a lecture on "Nutrition and Health" with special reference to general Nutrition, low cost nutritious recipes, good cooking practices and Food and Personal hygiene to the cooks (150) working in Social Welfare Department hostels on 21st May 2004 in a training programme organized by the Department of Social Welfare, Ranga Reddy District.
5. Delivered a popular lecture on "Nutrition and Health" to the employees of a workshop South-Central Railway, Lalaguda, Secunderabad on 15th June 2004. About 180 employees including the senior officials and technical staff participated in the programme.
6. An orientation programme on "Nutrition and Health" including personal hygiene and cooking demonstration on low-cost nutritious recipes were organized for the volunteers (50) of an NGO i.e., Nrityanjali Academy, Secunderabad working with HIV patients on 16th June 2004.
7. A popular talk on "Nutrition and Health" with special reference to the Nutritional requirements for school children was given to the delegates (70) in the 3rd International workshop on quality education for all on 7th July 2005, organized by ALPAKS Kids world, Hyderabad.
8. A nutrition awareness programme on 20th July 2004 for community health workers (60) (CHWs) in Mylaram Village, Bommala Ramaram Mandal, Nalgonda District was organized in association with Rural Organization For Social Education (ROSE), an NGO working in that area.
9. Demonstration of intervention material on Nutrition in the study settings of projects on Adolescent Girls and FMFH Programme for schoolchildren at MJM Girls High School, Shali Banda and Neo School Aizza, New Malakpet respectively, during the visit of Dr. Rukhsana Haider, Regional Adviser (Nutrition), WHO SEARO, New Delhi on 18th August, 2005.
10. A lecture on "Nutrition for adolescent girls" in Masjid-E-Jafferi Shariff was given to the adolescent girls (60) on 26th October 2004 in a programme organized by COVA in Yakutpura, Hyderabad.
11. A talk on "Nutrition and Health" with special reference to the nutritional requirements for HIV patients was given to women peer educators (50) and out reach workers in a training programme on "Nutrition and HIV/AIDS" organized by Research in Environment, Education and Development society (REEDS) on 29th October 2004 at Kodangal, Mahabubnagar District.
12. A popular lecture on "HIV and Nutrition" to the health educators (40) of an NGO i.e., Development Action for Rural Environment (DARE), Hyderabad on 28th December 2004.

3.3. Radio talks

All India Radio broadcast following popular talks in local language "Telugu" on different occasions:
1. Importance of Carbohydrates on 12th August 2004

2. Importance of proteins in the diet on 17th August 2004, and
3. Role of fats in the diet on 19th August 2004. AIR repeatedly broadcast these talks during the year.

4. Special Events

4.1. National Technology Day (11th May 2004)

A nutrition awareness camp was organized for school children, teachers and other staff members (200) of New Model High school, Dhoodhbowli in the old city of Hyderabad in association with COVA on 11th May 2004.

4.2. National Nutrition week (Sep 1 -7 .2004)

A Nutrition awareness programme for farmers and cooking demonstration were organized to the farmers and their family members (40) on 3rd September, 2004 in association with Central Research Institute for Dry land Agriculture (ICAR), Hyderabad at Kodangal village, Ranga Reddy District.

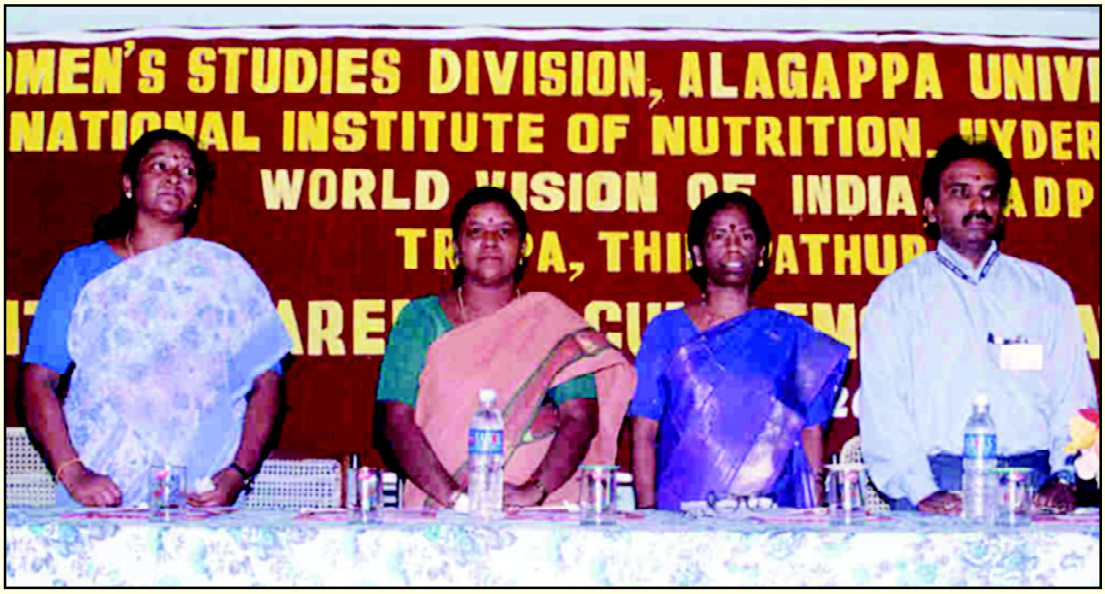
The Urdu version of 'dietary Guidelines for Indians - A Booklet' was released at a modest function organised in 'Urdu Ghar' in the old city of Hyderabad. Media covered the event.

Inter-school competitions in elocution, essay writing and painting were organised on this year's theme - 'Malnutrition - A silent emergency' over 170 students took part in various competitions. These contests were organised in association with the School Students' Nutrition Club and COVA

4.3. World Food Day

Nutrition awareness programmes (3) were organized in Tamilnadu State. One camp was organized at Madurai on 15th October 2004 in association with Food Nutrition Board (FNB), Madurai for adolescent girls, pregnant and lactating women and anganwadi workers (50). The other two camps were conducted

for the students (250) from different faculties, women self help groups (350) from nearby villages in association with FNB, Madurai on 15th and 16th October 2004 at Allagappa University, Karaikudi in association with Department of Women studies of the University.



4.4. Year of Scientific Awareness 2004

An interactive session on "Nutrition and Health" in association with Confederation of voluntary Associations (COVA) was organized to the girl students (80) of Fatima's Girls High School, Hyderabad on 15th September 2004.

5. Public and Media Relations

The Nutrition Museum continues to attract students from school and colleges, health workers, nurses and NGO groups from all parts of the country. Lecture-cum-video programmes on various nutrition themes were conducted for these visitors in batches. A total number of 86 groups consisting of 2580 students from ten States (Andhra Pradesh, Gujarat, Maharashttra, Kerala, Tamilnadu, West Bengal, Madhya Pradesh, Uttar Pradesh, New Delhi and Punjab) visited the institute during the year. Technical information was provided to general public on nutrition and health-related aspects and dietary counselling was offered to the needy general public.

Reporters from several newspapers interacted with the scientists of the Institute working on different aspects published research highlights. In addition, articles from the Institute's periodicals were also picked up by various newspapers in different Indian languages. All the extension activities of the institute were covered well by the local media in English, Telugu and Urdu.

The staff of the Extension and Training Division took active part in ensuring media coverage for the entire special events organized by the institute. Curtain raisers, press releases and follow-up reports were coordinated by the staff. Staff was also actively interacting with media and was successful in improving visibility of the institute as well as ICMR. Major events like the visit of Dr. Anbumani Ramadoss, Honourable Union Minister for Health and Family Welfare and Mrs. Panabaka Lakshmi, Honourable Minister of State for Health and Family Welfare were also covered widely in the news.

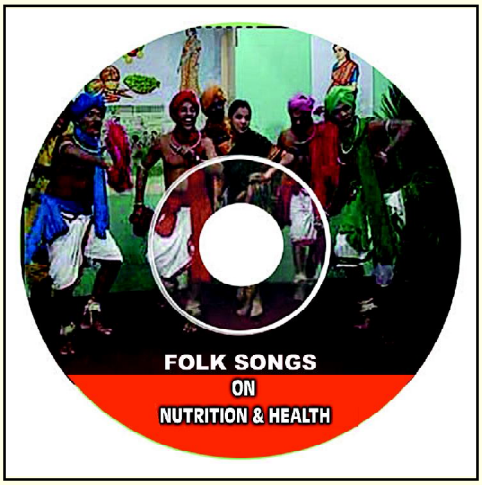
Dr. Anbumani Ramadoss, Honourable Union Minister for Health and Family Welfare addressing the media

Mrs. Panabaka Lakshmi, Honourable Minister of State for Health and Family Welfare interacting with the media personnel.

Expertise of NIN's scientists was also used by the Educational Media Research Centre (EMRC), Hyderabad in making educational films on nutrition and food security.

The staff of ET Division assisted in the designing, development and editing of brochures for National Workshop on Pesticide Residues organized by the Institute in January 2005. The staff of the Division also involved in designing, editing and printing of a Report on Prevalence of Fluorosis in the North-western Districts of Tamilnadu.

An educational film using the folk media was developed by the staff of the division covering various aspects of nutrition for educating the NSS volunteers was developed as part of "Development of communication strategies to improve nutrition and health related knowledge of NSS volunteers" project.



6. NIN's Website

The institute's website, www.ninindia.org, recorded more than 22,400 hits since its launch in March 2004. The division has been updating the website. Employment opportunities are being regularly put up on the website.

B. RESEARCH ACTIVITIES

IMPLEMENTATION OF FAO'S FEEDING MINDS, FIGHTING HUNGER (FMFH) PROGRAMME - AN EXPLORATORY STUDY

Despite the Green Revolution and great economic and scientific strides, nearly 800 million people are chronically undernourished and over 200 million children under the age of five suffer from acute malnutrition. In this respect, Governmental, Non-governmental and international organisations have launched various programmes. The FAO is one of them. Besides promoting food production and food security, one of the aims of FAO is to create a world, in which all children can grow, learn and flourish, developing into healthy, active and caring members of society. In tandem with this objective, FAO, along with some like-minded organisations, has launched the Feeding Minds, Fighting Hunger (FMFH) Programme.

The FMFH Programme, through a set of lesson plans, aims to introduce the topics of 'hunger and malnutrition in the world' to the schoolchildren through their teachers. Three lesson plans have been provided for each of the three broad school levels - Primary, Intermediate and Secondary. Each lesson contains background information for the teacher and outlines the main objectives, concepts and content areas to be covered. Guidelines for a variety of classroom activities, teaching aids and discussion points are provided. Given the wide variety of problems, cultures and environments around the world, the lessons have been prepared as a framework for teachers. The programme envisages that the teachers, after understanding the lesson plans should educate the children in their respective classrooms. The scope, language, discussions and activities provided for each lesson, have to be tailored to fit the students and local conditions.

In order to popularise this initiative in the South-east Asian region, FAO and UNESCO organized an inter-country workshop from 27th to 29th August, 2002, at the National Institute of Nutrition (NIN), Hyderabad. As many as 52 delegates from Southeast Asian countries viz., Bangladesh, India, Indonesia, Nepal, Philippines and Sri Lanka along with representatives of FAO and UNESCO participated in the workshop. The delegates deliberated over the ways to effectively implement the FMFH lesson plans and unanimously felt that the success of FMFH programme is dependent on the involvement of school students and teachers to fight against hunger and malnutrition (FMFH Workshop Report, 2002).

Following this workshop an exploratory study was carried out to assess the efficacy FMFH lesson plans in improving the knowledge levels of the school children. The study was conducted with the following objectives:

1. To asses the current knowledge levels of the middle-level school children and their science teachers on topics related to FMFH lessons
2. To educate the teachers about the concepts of FMFH, by adapting intermediate-level lesson plans and to educate middle-level school children through them
3. To evaluate the impact of school-based teaching of FMFH lessons on the improvement of knowledge levels of school children on these topics.

Materials and Methods

Study setting

The study was conducted in the schools of old-city of Hyderabad in association with a Non-Governmental Organisation i.e., Confederation of Voluntary Associations (COVA). A total number of 49

member schools of COVA were considered for the study. Informed consent was obtained from the heads of the participating schools and pupils.

Sample size

In the pilot study, a pre-tested questionnaire consisting of 20 multiple-choice questions was administered to 75 school children selected randomly from five schools. The 20 questions were related to hunger, food insecurity, nutrition, nutrition deficiency disorders, FAO and two additional questions related to their preferences in methods of learning were also included. For the main study, cluster randomisation procedure was used to determine the number of schools.

The sample size was worked out with an expected improvement of 4 points (on a scale of 20) in the mean scores after intervention, at 95% level of significance and 80% power. From the total number of schools available, 10 schools were selected randomly and each school was again randomly allocated to either control or experimental group. Thus, five schools were treated as control group and the remaining five as experimental group. All the children from VIII and IX grades and their biological science teachers of these classes were included in the study.

FMFH lesson plans

Three different lesson plans of FMFH are suggested for Primary, Intermediate and Secondary school levels. Each lesson contains background information for the teacher, objectives, concepts and contents to be covered in the classroom during implementation. The three common lesson plans for all school levels deal with three different topics namely 1. What are hunger and malnutrition and who are hungry? 2. Why are people hungry and malnourished? 3. What can we do to help end hunger?

Apart from these guidelines, a variety of classroom activities including teaching aids and discussion points are also provided. For the study purpose, only the intermediate level lesson plan was used to educate the students in the experimental schools.

Assessment of knowledge levels of school children

Baseline data of schoolchildren and teachers of control group (n=358) and experimental group (n=312) were collected by administering a pre-tested knowledge assessment questionnaire (KAQ).

Statistical analyses

Data from KAQ were analysed using SPSS package (version 11.5).

Significant improvement in the knowledge-levels of schoolchildren was observed even in the control group in an earlier study conducted in schools of Hyderabad by Vijaya Pushpam et al. Therefore, in this study effect size was used to assess extent of effect of intervention in improving the knowledge levels in the experimental schools as compared to control group schools.

Intervention

Teachers Training Workshops

A Teachers' Training Workshop was organized in March 2003 for the biological science teachers of VIII and IX grades of the experimental schools. The teachers were trained in the concepts of FMFH and different strategies of communication in order to modify the FMFH lesson plans to suit the local needs of their school children. A follow-up workshop was conducted in July 2003 to reinforce the

knowledge acquired in the previous workshop before they implemented FMFH lesson plans in their respective schools.

Development of Communication Material

The following communication materials were developed based on the preferences indicated by the students in the pilot study, in consultation with the teachers during the Teachers Training Workshop. Posters - One poster on functions of foods and three posters on micronutrient deficiency disorders viz., Anaemia, Vitamin A and Iodine Deficiency Disorders (IDD) were identified from the existing posters of NIN and modified. In addition, posters related to Hunger Map of Asia, Hunger Map of world, vulnerable groups, food systems chart and what can children do to help end hunger and malnutrition were adapted from the FMFH lesson plans. These topics were identified with the help of teachers during the first Teachers Training Workshop. Each school was given a complete set of 10 posters.

Skit - A skit covering all the concepts mentioned in FMFH lesson plans was developed and performed using children's theatre group of COVA to reinforce classroom education.

Implementation of FMFH Lesson plans

In the experimental schools, teachers implemented FMFH lesson plans between August and October 2003 using the communication material along with various classroom activities.

Post-intervention Knowledge assessment

The questionnaire that was administered at baseline was used on 254 students in the control and 216 students in the experimental schools after intervention and the children were instructed not to discuss among themselves while answering the questionnaire. One school opted out of the study and some children were not present at the time of administration of post-intervention questionnaire(s). However, the drop out did not affect the over all outcome of the study. Further, retention of the concepts of FMFH lesson plans was also studied by administering the same questionnaire after a gap of two months for the experimental group. For the purpose of analysis, each right answer was assigned one mark and the wrong answer was given a zero.

Results

Pilot Study

The mean of scores of 75 children of all five schools was 8.36 + 3.13 (SD). About 80% of the schoolchildren preferred to learn through classroom lectures, followed by teaching aids (like charts and posters) and the play way etc.

Main Study

The baseline data on the concepts of FMFH lesson plans of biological science teachers in the control schools were 14.20 + 1.48 (SD) and in the experimental group the mean score was 13.00 + 2.58. However, after intervention there was drop in the mean scores of control schools i.e., 13.60 + 1.82 as compared to a significant increase in the knowledge levels among the biological teachers of the experimental schools (17.50 + 1.29). Baseline data of schoolchildren in control and experimental schools showed that there was no significant difference ($p>0.05$) between the groups, indicating homogeneity in the groups (Table 25).

Table 21. Knowledge levels of the school children before and after intervention

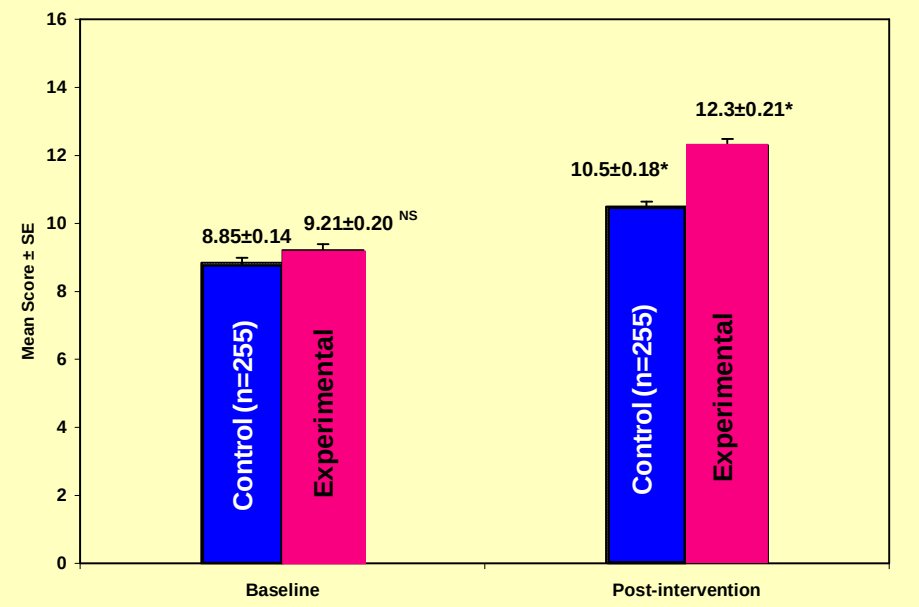
Group	n	Mean ± SD	Significance
BASELINE			
Control	358	8.66 ± 2.28	p>0.05 NS
Experimental	312	8.95 ± 2.96	
INTERVENTION			
Experimental Group			
Post-intervention –1	213	12.31 ± 3.14	p>0.05 NS
Post-intervention - 2	213	12.54 ± 2.89	

Post intervention results indicated a significant improvement in the knowledge levels in the experimental group (Figure 36). Significant improvement was also observed in the knowledge levels of the control group (Figure 36). However, when comparisons were made between the mean improvement in the knowledge-levels of control (1.65 + 0.21 (SE)) and experimental groups (3.09 + 0.19 (SE)), it was found that there was a significant increment in the experimental group as compared to the control group indicating the efficacy of the intervention (Figure 37). As regards the retention of knowledge gained during intervention, post-intervention-1 and post-intervention-2 mean scores of school children in the experimental group were compared and no significant difference was observed (Table 21), indicating that there was retention of knowledge.

Effect size

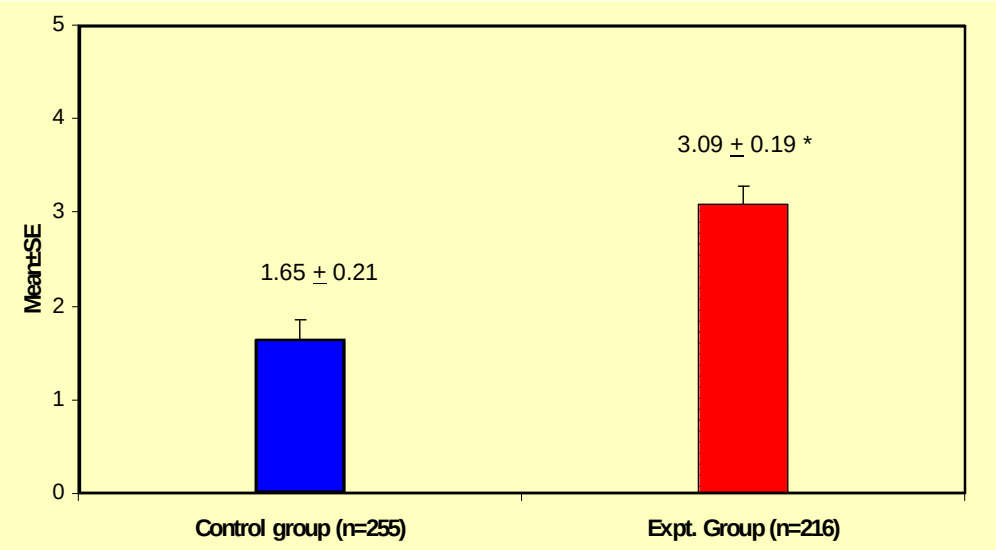
The effect size of the difference in improvement in the nutrition knowledge after intervention between experimental and control groups was d = 0.40, indicating that the effect of intervention was medium as per Cohen's standard.

Fig 36. Comparision of mean scores of control and experimental groups at baseline and after intervention



NS- Not significant ** Significant*

Fig 37. Improvement in the mean scores of the school children after intervention



** Significant*

Conclusions and scope for further study

The significant improvement in nutrition knowledge of experimental group through classroom education using FMFH lesson plans over control group was medium as shown by effect size (Cohen's d). According to Slavin (2003), the impact of the educational programmes can be successful where the effect size is 0.2 or greater. In the present study, the effect size was medium (d = 0.40) indicating that the FMFH programme is effective, efficacious and can be implemented in classroom setting. It is clear that school-based FMFH lesson plans, adapted to local circumstances have significantly improved the knowledge levels of the pupils. However, their efficacy in making the children as the change-agents can be explored through a follow-up study. Similar studies can be initiated to assess the efficacy of the programme in other schools in different set-ups.

VIII. FOOD & DRUG TOXICOLOGY RESEARCH CENTRE

A. FOOD SAFETY

1. OCCURRENCE OF TRANSGENIC MATERIAL IN DIFFERENT VARIETIES OF BT COTTON

The genetically modified BT cotton that is currently being cultivated and marketed in India carries Cry 1AC gene which expresses insect resistance protein. BT cotton may enter the food chain as feed for cattle or as edible oil. This study was initiated to detect presence of transgenic DNA and its protein in cotton seed and oil.

Aims and objectives

Detection of transgenic DNA and protein (Cry1Ac) in cotton seed samples from farmers.

Work done during the year

1. Collection of samples

A total of 10 BT cottonseed samples, 4 cottonseed extractions and 4 cottonseed oil samples were collected from farmers and oil extraction plants in Warangal and Guntur districts. Authentic BT cottonseed samples namely, MECH 12, 162 and 184 were procured from MAHYCO Company for comparison as standard.

2. Analysis of BT cottonseed for Cry1Ac delta endotoxin

BT cottonseed samples (10) along with the standard BT cottonseed samples were analyzed for Cry 1Ac using commercial kit manufactured by Envirologix Inc. USA. The kit method is based on ELISA (Sandwich format) with primary antibody coated wells to which a clarified protein extract of sample is applied.

After reaction with Cry1Ac enzyme conjugate, the absorbance is read at 450nm. Cry1Ac level is calculated on the basis of OD values of standard /calibrators (provided with the kit) and samples. The results indicated that Cry1Ac levels ranged from 0.35 to 1.92 ppm in samples collected from farmers and 1.46-1.77 ppm in the MAHYCO BT samples MECH 12, 162, 184.

Conclusions

Presence of Cry 1AC protein was detected in samples collected from the farmers. The levels were comparable to that of standard seed samples obtained from MAHYCO.

2. EFFECT OF DIFFERENT TYPES OF DOMESTIC PROCESSING ON PHENOLIC CONTENT AND AOA OF SELECTED FOODS

Plant foods are good sources of antioxidants. Several studies have reported antioxidant content of various foods, mostly in their raw form. However, during processing, interactions among nutrients and/or antioxidants and/or oxidants, may modify the antioxidant activity of foods. Therefore, information on antioxidant activity of foods, in the form they are consumed is useful. Hence, studies have been undertaken to determine antioxidant activity of foods, both in the raw and processed forms. This study is an attempt to determine the effect of processing on the antioxidant activity (AOA) of foods, and to use the data thus generated, for the development of suitable recipes with higher antioxidant activity.

Aims and Objectives

To determine the effect of different types of domestic processing on phenolic content (PC) and AOA of selected foods of different categories.

Work done during 2004-2005

Keeping in view that most plant foods are usually consumed after some kind of processing or the other, plant foods belonging to various categories, which were found to have higher AOA in raw form (Annual Report 2003-2004) were selected to study the effect of different types of domestic processing on their AOA and PC. The foods selected to study the effect of domestic processing were: wheat (cereals), red gram dhal and black gram dhal (dehusked legumes), green gram, Bengal gram and moth beans (whole legumes), groundnut and sesame (oil seeds), spinach (green leafy vegetables), tomato (other vegetables) and onion (roots and tubers). As mentioned earlier (Annual Report 2003-2004), three samples of each of these foods were purchased from each of the three selected local markets. They were cleaned thoroughly and subjected to commonly used domestic processes such as boiling in water, pressure-cooking, shallow frying, deep-frying, sprouting, malting and microwave cooking appropriately. The raw and processed food samples were extracted with 70% methanol, the AOA and phenolic content were determined in these extracts as mentioned earlier.

Results

The results are given in Tables 22 and 23 and the salient findings of the study are mentioned below.

Antioxidant Activity

- During sprouting and malting there was an increase in AOA of wheat albeit, the increase was not significant. In general, other processing methods studied had no significant effect on the AOA of wheat.
- AOA decreased significantly during deep-frying of soaked black gram dhal ($P < 0.05$). Other types of processing had no significant effect on the AOA of dehusked legumes.
- Sprouting had no significant effect on the AOA of whole legumes.
- There was no significant difference in the AOA of raw and processed vegetables (GLVs, other vegetables, roots and tubers) and oil seeds.

Keeping in view our earlier finding that PC could account for the AOA in only some types of foods but not others, we determined the effect of processing on the PC of foods and evaluated the relationship between the AOA and PC in the processed food. The salient findings on the PC of processed foods are as given in table 22.

Table 22. Effect of domestic processing of Cereals and Legumes on AOA & PC

FOOD STUFF		Process	AOA*	PC**
Cereals	Wheat	RAW	5.8 ±2.7	24.0±5.5
		BOIL	5.3 ±2.8	18.1±4.6
		PR.COOK	4.4 ±0.6	13.5±2.6
		PR.C.SEP	4.7± 1.5	15.5±4.2
		R+ OIL	4.9 ±1.4	26.0±7.1
		SH.FRY	4.6 ±1.3	24.5±6.7
		PURI /sf	5.6 ±2.1	25.8±3.0
		ROTI	4.7 ±2.1	31.0±9.6
		DR	5.4 ±1.8	30.6±2.6
		SPROUT	3.8 ±0.5	56.6±17.0
		MALT	3.8 ±2.0	101.2±20.8
Dehusked legumes	Black gram dal	RAW	1.2± 0.2	26.1±0.7
		PR.COOK	1.8 ±0.1	22.6± 2.0
		PURI /sf	2.6 ±0.8	26.2± 2.8
		DR	1.8 ±0.1	36.3± 3.6
	Red gram dal	RAW	1.5 ±0.3	24.9± 7.9
		BOIL	1.3 ±0.5	27.2± 5.2
		PR.COOK	1.9 ±0.5	23.4± 5.8
		PR.C.SEP	1.5 ±0.4	28.7± 9.4
		R+ OIL	0.8 ±0.2	24.4± 1.1
		SH.FRY	2.5 ±1.4	32.5± 8.8
		DR	2.0 ±0.9	34.9± 5.6
Whole legumes	Bengal gram whole	RAW	0.8 ±0.3	36.0± 11.8
		SPROUT	1.0 ±0.1	40.6± 5.5
	Green gram whole	RAW	0.8 ±0.1	69.4± 3.7
		SPROUT	0.9 ±0.2	100.9± 6.1
	Moth	RAW	1.0 ±0.1	34.8± 2.9
		SPROUT	0.8 ±0.1	186.6± 2.9

*AOA expressed in mg of raw food required for 50% inhibition of auto-oxidation of β carotene - linoleic acid emulsion under the conditions of the assay.
** PC expressed in mg of gallic acid equivalent in 100g of raw food stuff

Phenolic content

- During sprouting and malting there was a significant increase in phenolic content of wheat. Other processing methods had no significant effect on PC of wheat.
- Phenolic content of dehusked legumes increased significantly only during dry roasting but no change was observed on other types of processing.
- Sprouting in general increased the PC of whole legumes but the effect varied with different legumes.
- PC of spinach was significantly increased during boiling ($P<0.05$) and pressure-cooking ($P<0.05$). Other processing methods also increased the PC of spinach but the increase was not significant. No significant changes were observed in PC of tomato and onion during any type of processing studied.
- Groundnut and sesame had the highest amount of PC among all the foods studied. However there was no significant effect of any of the processing methods tested on their PC.

*Table 23. Effect of domestic processing of vegetables and oilseeds on AOA & PC**

FOOD STUFF		Process	AOA*	PC**
Vegetables	Spinach	RAW	3.2 ±1.5	33.8± 7.3
		BOIL	2.9 ±1.5	53.6± 10.2
		PR.COOK	3.4 ±1.7	51.8± 9.6
		PR.C.SEP	2.5 ±1.7	42.7± 8.0
		R+ OIL	4.3 ±2.2	30.4± 5.0
		SH.FRY	3.4 ±1.5	48.2± 10.6
		Micro	3.2 ±1.7	47.3± 9.2
	Tomato	RAW	3.6 ±1.6	35.9± 3.4
		BOIL	4.3 ±1.8	36.8± 2.6
		PR.COOK	3.5 ±0.8	35.7± 4.1
		PR.C.SEP	3.3 ±1.0	35.8± 2.7
		R+ OIL	3.1 ±1.1	32.7± 6.9
		SH.FRY	4.1 ±1.2	39.1± 1.9
		Micro	4.0 ±1.2	36.9± 5.0
	Onion	RAW	4.2 ±1.2	39.2± 6.1
		BOIL	5.6 ±1.7	32.7± 2.2
		PR.COOK	5.0± 2.0	31.3± 4.1
		PR.C.SEP	4.8±1.7	32.0± 3.2
		R+ OIL	3.8±1.4	43.9± 3.7
		SH.FRY	4.9±1.3	45.2± 11.8
		Micro	4.8±1.9	36.0± 5.8
Oil seeds	Groundnut	RAW	2.3±0.9	95.8± 15.6
		BOIL	2.6±1.9	99.7± 47.6
		PR.COOK	2.4±1.6	105.7± 25.2
		PR.C.SEP	2.0±1.2	127.9± 31.4
		R+ OIL	1.8±1.5	125.8± 21.0
		SH.FRY	2.6±1.4	116.0± 20.6
		DR	3.0±1.2	94.9± 19.5
	Sesame	RAW	3.7±0.3	89.1± 14.4
		DR	3.4±0.2	89.2± 17.1

*AOA expressed in mg of raw food required for 50% inhibition of auto-oxidation of βcarotene - linoleic acid emulsion under the conditions of the assay.
** PC expressed in mg of gallic acid equivalent in 100g of raw food stuff

In line with the observation we made earlier in raw foods, the AOA and PC of processed foods also do not seem to go hand in hand always and in all foods. Also, the effect of processing could be different on the AOA and PC of not only foods of different groups but also among foods of same groups as well. In general, all the processing methods tested, either had very marginal or no effect on their AOA. Among different types of domestic methods of processing studied PC seemed to increase during sprouting but the increase varied among different legumes. Sprouted legumes had the highest AOA amongst all the processed foods tested.

B. CANCER AND XENOBIOTICS

1. ANTIGENOTOXIC POTENTIAL OF GINGER

Some frequently consumed spices in India and other countries have been claimed to exhibit antimutagenic/anticarcinogenic potentials. The antimutagenic property of ginger was reported in previous report (Ann Rep, NIN 2003-2004). One of the suggested mechanisms of antimutagenicity is through scavenging of free radicals that are generated during xenobiotic metabolism. The antioxidant enzymes in the tissues effectively counteract the endogenously formed free radicals. Nutrients and non-nutrients in diet are known to possess antioxidant property and also stimulate antioxidant enzymes in tissues. Therefore an experiment was conducted to investigate, whether ginger fed through diet can improve the antioxidant status in experimental animals.

Rats were fed ad lib with ginger through diet at various levels namely 0.5%, 1%, 5% for a period of one month. The animals were sacrificed and organs of interest namely liver and kidney tissues were dissected out and processed for antioxidant enzymes.

Results

In the previous Ann Rep (2003-2004) results on TBARS and protein carbonyls were reported. It was found that there was significant reduction in the levels of TBARS in liver and kidney. The level of protein carbonyls in liver was decreased in 5% ginger fed groups. Hepatic superoxide dismutase, catalase and glutathione peroxidase activities were measured. The results indicated significantly higher activity of liver SOD, catalase and GSHPx at all the levels of ginger feeding ($P<0.001$) compared to control (Table 24).

Table 24. Hepatic antioxidant enzymes in ginger fed rats

Group	SOD U/mg. prot.	Catalase U/mg prot.	GSHPx	
			Cytosol oxidized/ mg.prot per min	RBC U/gm Hb
Control	2.9 ± 0.48 [a]	40.4 ± 3.47 [a]	332.9 ± 38.64[a]	267.0 ± 35.1[a]
0.5% ginger	5.1 ± 1.21 [b]	55.4 ± 10.06 [b]	370.1 ± 29.90[a]	312.0 ± 29.2[b]
1.0% ginger	6.1 ± 0.89 [b]	66.7 ± 7.87 [c]	401.3 ± 27.62[b]	327.7 ± 6.78 [b]
5% ginger	7.0 ± 0.89 [bc]	78.4 ± 2.72 [d]	433.3 ± 23.86 [c]	379.5 ± 25.8[c]

Values are Mean ± SD of 6 rats per group
Different superscripts are significant at $p<0.05$ by Duncan's multiple range test

Conclusion

Ginger ingestion can result in improved antioxidant status and this could be one of the bases for its antimutagenic property.

2. ETHNOPHARMACOLOGICAL VALIDATION OF BIODYNAMIC COMPOUNDS IN TRADITIONAL MEDICINE

Natural and synthetic antioxidants play a vital role in protecting cells and tissues against oxidative damage caused by free radicals. Previous studies (Annual Report-2002) indicated that plant extracts coded 4308,4212,3107,3223 & 5322 have potential antioxidant activity as evaluated by battery of *in vitro* tests. Further, the therapeutic potential of plant extracts 4212, 3223 was evaluated in patients suffering with rheumatoid arthritis and found co-relation between the therapeutic efficacy and the anti-oxidant potential (Annual Report-2003).

Explant culture system, an ex-vivo testing tool, a classic model for studying pharmacological, toxicological and drug metabolizing studies was used to confirm antioxidant potential of 4212 and 4308 extracts.

Hypothesis

To assess the antioxidant potential of aqueous (4212) and aqueous alcoholic (4308) extract of "Rasna panchaka" a combination medicine for the treatment of rheumatoid arthritis using a novel serum free explant culture system.

Methodology

Mice liver was excised aseptically, cut explanted to 1-2mm3 (1-2 mg each) cubes and cultured in Serum Free Medium 199 (Sigma) at pH 7.4 under aseptic conditions. In each petri plate, a fixed number (12-15) of explants were maintained in 5ml of medium 199 in a humidified CO2 incubator at 370C. Extracts varying from 1-10µg/ml were added to the culture plates.

The antioxidant property was assessed by determining the primary defense oxidative enzymes viz. SOD, Catalase, GSH and MDA in the cultured mice liver explants.

Results

1) Cultured tissue harvested at various time points clearly indicated that the cellular architecture of the tissue was well conserved in the first 6hrs with gradual display of specific changes in the next 24hrs (Figure 38).

2) The extracts showed significant protection against oxidative stress at the dose of 2 µg/ml (Figure 39).

3) Lipid peroxidation measured using malonaldialdehyde (MDA) as a marker was reduced by 50%. This effect was accompanied with an increase in the first defense enzymes superoxide dismutase (50%) and catalase (18%) with no change in reduced glutathione levels (Figure 40).

Fig. 38. Photomicrographs of percent normal cells at various time points

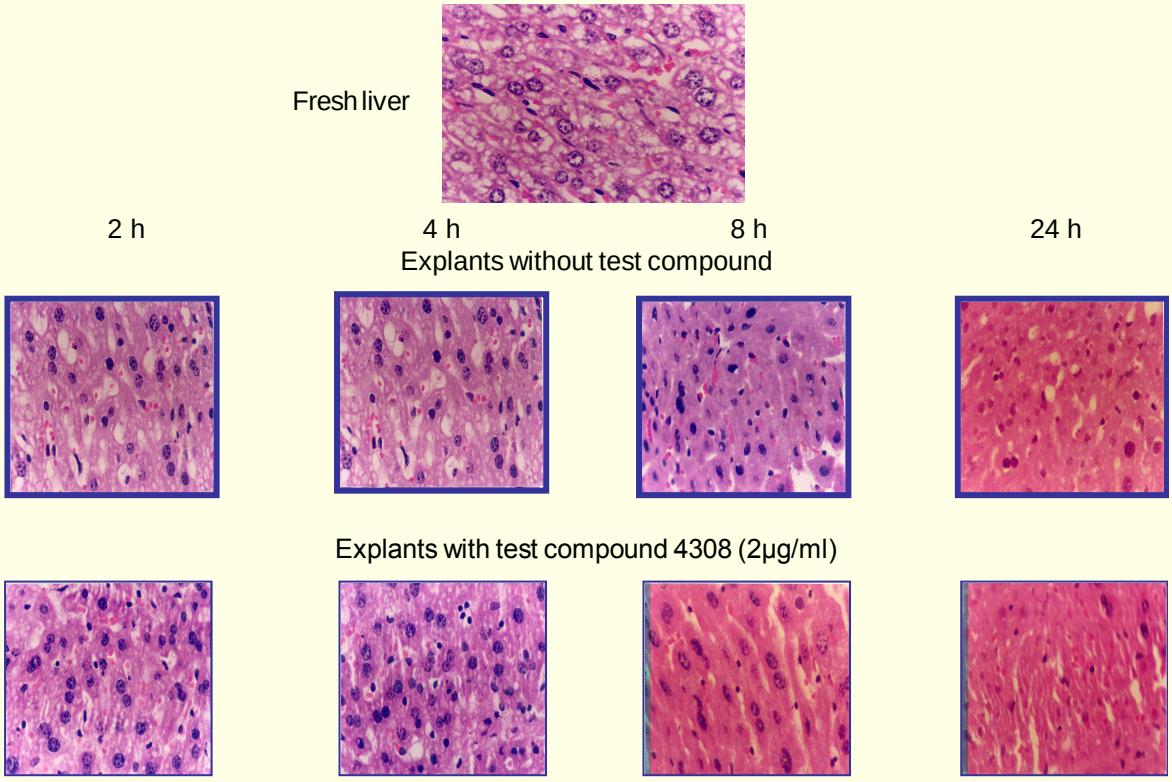


Fig. 39. Percentage of normal cells with reference to time and dose

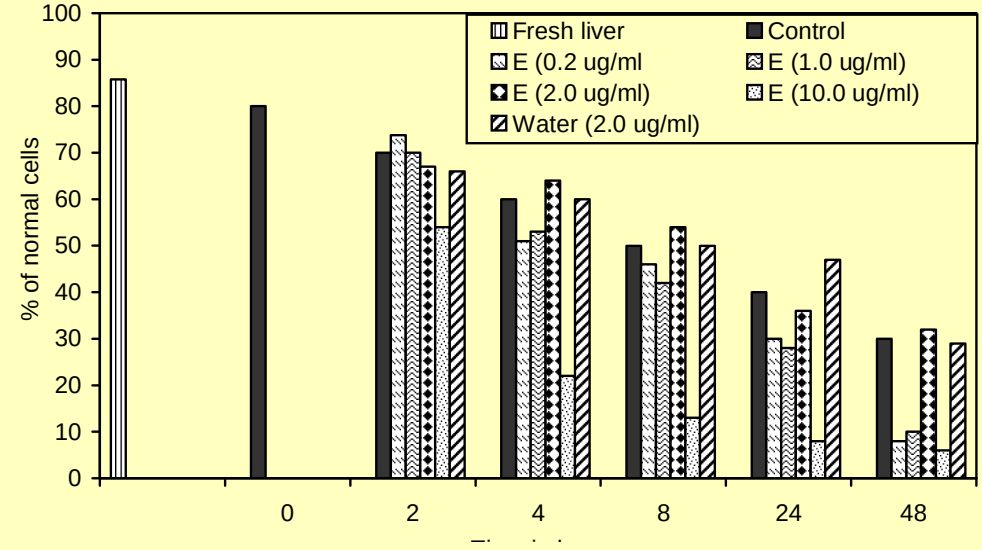
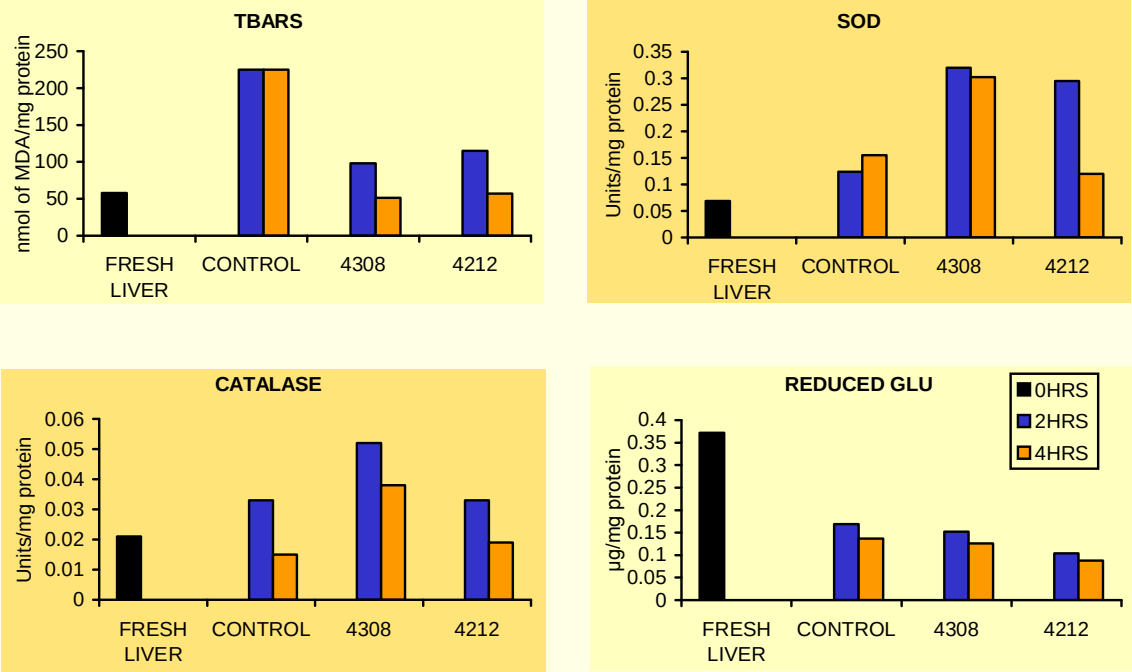


Fig. 40. Antioxidant status of explants with and without test compounds



Conclusions

a) The study results related to antioxidant activity supported our earlier in vitro and in vivo observations validating the use of explant culture system to detect antioxidant potential of plant products.

b) The results suggest that water plus methanol extract possess superior antioxidant property compared to the traditionally used water extracts.

c) The results demonstrated that "Rasna panchaka" an indigenous drug used in the treatment of rheumatoid arthritis has a potential anti-oxidant property and can counteract the oxidative damage associated in the pathogenesis of disease.

3. IMPACT OF INTERVENTION PROGRAMME ON RATIONAL USE OF DRUGS (RUD). (PHASE - III)

In recent years, the use of drugs has considerably increased in almost all countries. Use of irrelevant and unnecessary drugs not only escalates the cost of health services but it is also harmful to the consumer under certain circumstances. Earlier studies conducted in various parts of Andhra Pradesh (Annual Report 2001/02) have indicated irrelevant use of drugs specially injectables, antibiotics, nutritional products etc. In a developing country like India with average literacy/socio-economic status it is imperative to initiate intervention studies to educate the public on the rational use of drugs. In view of this an attempt was made to develop intervention tools, which include film titled "Haridas - Tale of medicines", a brochure for creating awareness on concepts of rational drug use in community. A pilot interventional trial has shown a significant impact (Annual Report 2003-04).

The present study has been undertaken with the following objectives:

To create awareness on the concepts of RUD with special emphasis on harmful effects of unnecessary use of injectables, antibiotics, nutritional product etc.

To evaluate the impact of advocacy programme on RUD practices on target group.

Methodology

The study has been undertaken in a selected area in Karimnagar district of Andhra Pradesh. The baseline and post intervention data (after 2-3 weeks of advocacy programme) on drug consumption profile has been collected from 11 public health centres (7PHC, 3CHC & DH) which were selected based on the distinct socio-economic factors and from all directions in a pre-tested schedule. The intervention programme consists of a film show "Haridas - A tale of medicines" of 36 min followed by distribution of brochures at least twice in five days at health centers and public places. The data has been analyzed using various indicators viz. Prescribing, Patient care, Facility and Communication indicators as per WHO standard guidelines using SPSS package version 10.0.

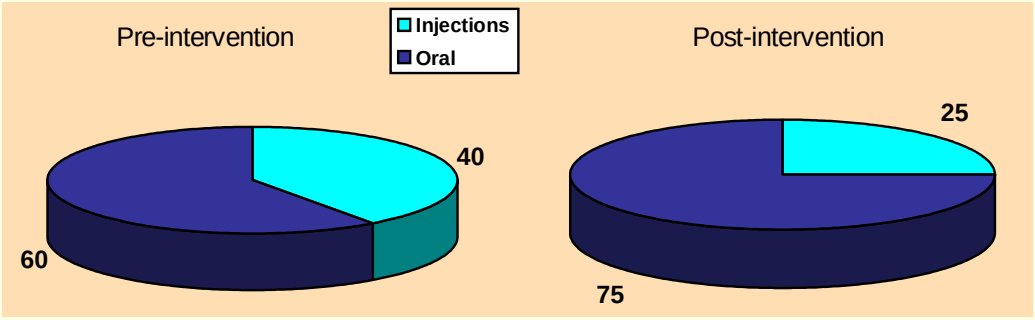
Results

1. Approximately 50-100 patients of different age groups at health centers, about 150 community members during the show at public places/ schools/ panchayat office etc. have received the benefit of this intervention programme (Figure 41).

Fig. 41. Community education programme on rational use of drugs

2. The various categories of the drugs prescribed and dispensed included analgesics (17%), vitamins (18%), antibiotics (10%) and other categories of drugs like anti-allergics, vaccines, topical applications etc (Figure 42).

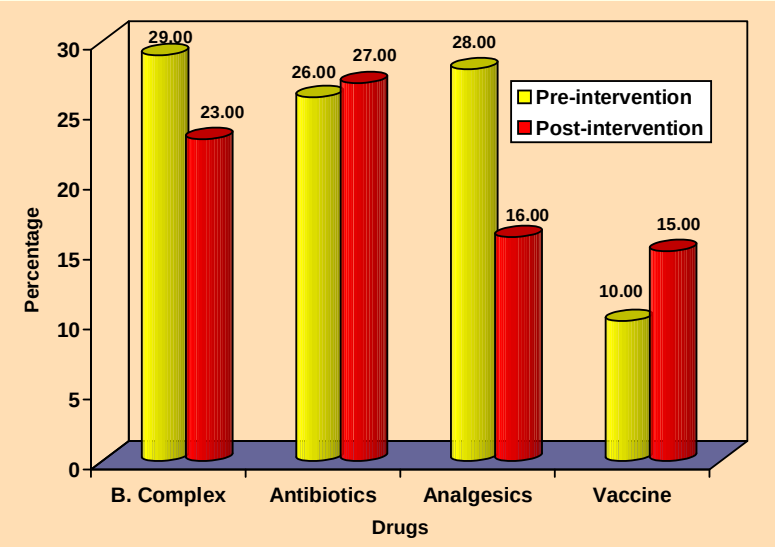
Fig. 42. Episodes of injections



3. The use of injections was to the extent of 40%, for vitamins (22%), antibiotics (27%), analgesics (28%) etc.

4. The post intervention programme has shown a reduction in injection rate from 40 to 25%. (Figure 43).

Fig. 43. Categories of Parenteral Formulations Dispensed



5. A marginal group of population has clearly understood the importance of antibiotic dosage schedule.

6. The media coverage and encouraging support from medical and other officials has made a positive impact.

Conclusions

1. The study revealed excess use of injections (especially vitamins/ antibiotic formulations).

2. The interventional programme had a significant positive impact on target audience.

3. Good media coverage, active participation of community leaders and other officials especially from police and health departments have popularized the intervention programme in the community. There was a great demand for such programmes in other areas too.

The well-devised IEC programme can effectively promote RUD concept especially among under privileged sections of population.

IX. PRE-CLINICAL TOXICOLOGY

SAFETY/TOXICITY STUDIES ON AYURVEDIC FORMULATIONS (a,b,c,d,e) (WHO Biennium Programme)

The traditional use of Ayurvedic formulations is one of the widely accepted therapies especially in chronic diseases viz. arthritis, asthma, and inducing fertility, rejuvenation etc. The data on safety of the Ayurvedic formulations has become important for wider global acceptance. The coded Ayurvedic formulations developed by CCRAS, MoH & FW are reported to have potential therapeutic activity in chronic diseases and are considered essential for pre-clinical toxicity screening as per WHO guidelines. The present investigations are therefore undertaken to evaluate the safety of five Ayurvedic formulations "a, b ,c ,d & e' by acute/ sub-acute toxicity tests in mice/rats as desired by sponsor.

Objectives

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

Methodology

The animals were selected, conditioned, and exposed to the test compounds (Ayurvedic Formulation-[a, b, c, d & e]) through oral route at various dose levels (Table 26). The animals were observed for lethality for 14 days in acute toxicity study after single exposure at 10x of therapeutic dose (table 27). In sub-acute toxicity study (28 days) the following observations were recorded before and after exposure to test compound (Table 27).

Table 26. Dosage details

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

** Route of administration Oral*

Table 27. Test Details (species/doses/duration)

Test details	Test species	Dosage	Duration	Study duration
Acute	Swiss Albino Mice 5 M +5F	10x	Once	14 days*
Sub acute	Rats (Wistar rat) 20 M+20F@	1x Therapeutic 5x Average Dose 10x High Dose VC Vehicle control	Daily 28 days	30 days*

@ 10 (5M+5F) in each group (1x, 5x, 10x & VC) 1x = (therapeutic dose), 5x = (5 times of the therapeutic dose), 10x = (10 times of the therapeutic dose), Additional 5 days for conditioning animals.

Observations: Food intake, body weight, routine physical, physiological examinations have been recorded at frequent intervals. Hematology, clinical chemistry in blood samples and gross necropsy, histopathology of liver, kidney, lungs and brain has been investigated at the end of experiment.

Data is compiled and analyzed for significant difference between treatment groups and vehicle control group by appropriate tests.

Results

The experimental work was initiated in august 2004 with formulation 'a' and the work was completed by October 2004. The results of formulation 'a' are provided here with. However, studies are in progress with formulation 'b' 'c"d' & 'e'.

1) Acute:

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

2) Sub Acute

There were pre-terminal death's in animals receiving therapeutic dose(10%), average dose(30%), high dose(70%) for formulation-(a).

No significant treatment related effect on food intake, body weight, clinical signs and behavioral activity etc were observed in the animals found alive during the experiment.

No significant changes were observed in hematological parameters in the surviving animals. There were no significant changes in clinical chemistry parameters in the surviving animals.

No specific test compound induced pathological changes were observed in various organs collected during euthanization of the animals.

Conclusion

1. No abnormal findings were recorded after administering single dose of 10x dose.
2. Pre-terminal deaths ranging between 30-70% in animals receiving 5 and 10 times of the recommended therapeutic dose were observed for formulation-(a).

Current status: Data compilation and report writing is in progress for formulations b,c,d & e.

X. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

A. SERVICE ACTIVITIES

1. Breeding and Supply of Animals

During the nine months period 18195 animals were bred and 16,307 animals were supplied to various institutions including the parental organisation. However, the number of animals supplied to NIN including the animals for health monitoring is only 528 which is 2.1% of the total number of animals available. Rest of the animals were supplied to various research institutions including pharmaceutical R & D centres. The percentage of animals died or disposed were 5.95 or 4.91 respectively including mutant colonies like nude mice, Ob/Ob and GR/Ob rats. When the mutant colonies were excluded, the percentage of animals died and culled were 5 and 3.75 which is within the normal range of large colonies. The details of individual species and strains supplied are shown in Tables 29 & 30. The income generated from this activity was Rs.16.94 lakhs.

2. Supply of animal feed

a. Stock animal feed

In the nine months period 13163 kg of rat/mice feed and 570 kg of rabbit/g.pig feed were supplied to Pharmaceutical R & D Centres and 3219 kg of rat/mice feed and 1136 kg of G.pig/Rabbit feed were supplied to Govt. institutions. The income generated from this activity was 11.97 lakhs (Table 28).

b.Experimental animal feed

There has been demand for making some of the experimental animal feeds as the facility has got the expertise as well as ingredients required for making experimental feed.

Table 28. During the year the following experimental animal feeds were prepared and supplied:

No.	Type of Diet	Quantity	Institution
1	A high fructose diet	14 kg	TN Medical College & Byl Nair Ch.Hospital, MUMBAI
2	Galactose diet	27.2 kg	Osmania University, Hyd.
3	Safflower oil based high fat diet	2.5kgx 8 times	Lupin Limited (Research Park) Pune
4	Tryptophan deficient diet	13 kg Exptl. and 4 kg ontrol	Osmania University, Hyderabad
5	Iron deficient diet	20 kg	National Brain Research Centre, Nainwal More, MANESAR
6	Protein Deficient diet	20 kg	
7	High protein diet	40 kg	
8	High Fat diet	10 kg x 10 times	National Centre for Cell Sciences, (NCCS) Pune
9	High Fat diet	60%10 kg 40%10 kg 4% 10 kg	Suven Life Sciences, Hyderabad.

Apart from this, herbal powder incorporated pelleted stock diet of 320 kg was supplied to M/s.Leila Impex, Vijayawada and a hypocholesterimic oil based guinea pig feed was supplied to M/s.Shantha Biotech Ltd., Hyderabad.

3. Supply of Blood & Blood products

During this period a total of 445 ml of blood, sera and plasma were supplied to 8 different institutions on 32 occasions. A sum of Rs.18,740/- was realized. Apart from that 20 ml of blood was supplied to the institute.

4. Health Monitoring

A total of 657 samples from conventional and barrier maintained colonies were taken for microbiological monitoring during this period. This included samples from all the animal colonies maintained in the facilities. Apart from that, feed, water, bedding and equipments were also screened for microbiological screening. Organisms like Corynebacterium spp. E.coli, Klebsiella spp., Proteus spp., Listeria monocytogenes, Streptococcus spp., Staphylococcus spp., Bacillus spp., Micrococcus spp., and Acinitobacter calco.var anitrat were identified from all rat strains from both the colonies. Liver cysts were occasionally found in WNIN and SD rats from both colonies. Most of them were identified in older animals; the production and supply stock were unaffected. Once the facilities are modernized - especially increased exchange of air in the rooms - many of these organisms can be eliminated. Endoparasite, Syphacia obveleta, one of the common nematode was also found in most rats, especially WNIN, SD, and Fischer-344. Giardia spp, was frequently found in hamsters. The other common symptoms found in a wide spectrum of strains in the facility (especially in SD and WNIN rats) due to non-specific etiology were allopacia, exfoliate dermatitis, and mammary tumors. Apart from this, specific dermatitis due to ectoparasites, Mycoptes musculinus and Myobia spp. were also seen in the two strains of mice BALB/c and Swiss in the facility and appropriate control measures were taken to contain them.

Sentinel Monitoring

As explained last year, animals were identified from different age groups and placed in different areas within the colony with a specific labeling. They are being monitored for bacterial and parasitical examinations. The initial data show that most of the bacteria observed as on routine random checking (as reported above) were present in sentinels as well. Sick animals from different colonies: A total 114 animals have been reported sick during this period and they were as follows: rats 92, guinea pigs 12, rabbits 7, and Swiss mice 3., They were found to have subcutaneous tumors, hair loss, middle ear infection and severe dermatitis. These animals were physically examined and samples were collected for microbiological and histopathological examinations.

5. Human Resource Development

37th Annual Laboratory Animal Technicians' Course was held between 14th June and 30th July and was attended by 12 candidates.

25th Laboratory Animal Supervisor's Training Course was conducted from 1st Sept. to 30th November and 10 participants successfully completed the course.

A five day orientation programme with practical demonstration was given to 31 students of the DMLT course from Medwin Hospitals.

A total number of 11 persons were given adhoc training this year. This consists of one week orientation training in laboratory animal science (6 persons from private and government organization) as well as 3 months training in biotechnology in the molecular biology and animal physiology laboratories of NCLAS.

Table 29. Details of breeding and supply of different species and strains of laboratory animals (barrier maintained colony) during the period from 01.4.2003 to 31.03.2004.

Sl. No.	Species	Strain or Breed	Stock As on 1.4.2004	Total Number of animals							Balance as on 31.12.04
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied	Died	Disp	
1	Mouse	BALB/c An. N (inbred)	1170	2242	3412	216	2764	2980	1	226	205
		C57BL/6J (inbred)	631	1903	2534	-	2071	2071	104	-	359
		N:NIH(S) Nude (athymic) (inbred)	234	295	529	32	217	249	92	78	1'0
2	Rat	Wistar/NIN (inbred)	564	2424	2988	15	2163	2178	54	152	604
		SD (Sprague Dawley) (Outbred)	434	489	923	-	524	524	88	-	3'1
		Fischer 344 N (inbred)	49	97	146	10	32	42	23	12	69
3	G. Pig	N:HART (Hartley)	73	412	620		263	263	63	1	293
		Dunkin (Hartley)	135	-	-		-	-	-	-	-
		N:NIH (Coloured)	142	206	348	-	212	212	34	3	99
4	Rabbit	New zealand white	47	39	86	8	34	42	6	-	38
TOTAL			3479	8107	11586	281	8280	8561	465	472	2088

Percentage of animals supplied to other Institutions : 71.5 %
NIN : 2.42 %
( ) Values are percentage of number of animals available in each species.

Table 30. Details of breeding and supply of different species and strains of laboratory animals (conventional colony) during the period from 1.4.2004 to 31.12.2004

Sl. No	Species	Strain or Breed	Stock As on 01.04.04	Total Number of animals								Balance as on 31.12.04
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disposed		
										Old age	Sick	
1	Mouse	Swiss (inbred)	659	3250	3909	76	2692	2768	402	70		669
2	Rat	WNIN (inbred)	932	5102	6034	162	4530	4692	139	218		985
		WNIN/Ob-Ob inbred)	381	430	811	-	-	-	148	88		572
		WNIN/GR-Ob	486	425	911	9	-	9	100	206		596
		Wkyoto (inbred)	133	184	317		12	12	40	64		201
		CFY/NIN (inbred)	72	145	217		-	-	8	13		196
		Holtzman (inbred)	222	12	234		-	-	100	86		48
		Wild White	44	3	47		-	-	21	-		26
3	Hamster	Golden (inbred)	148	530	678		265	265	48			365
4	Monkey	Macaca mulatta (Rhesus)	24	-	24		-	-	-			24
5	Sheep		1		1							1
6	Rabbit		6	7	13	-	-	-	-	-	-	13
TOTAL			3108	10088	13196	247	7499	7746	1009	745	-	3696

Percentage of animals supplied to other Institutions :

NIN :

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

B. RESEARCH ACTIVITIES

1. PCR BASED DNA FINGERPRINTING OF WNIN STRAIN AND ITS OBESE MUTANTS

Two mutant obese rat strains, WNIN/Ob and WNIN/GR-Ob were developed from the existing WNIN rat colony, which is being maintained at NCLAS in an inbred status for the past 84 years. Both the mutants are obese, but WNIN/GR-Ob has impaired glucose tolerance additionally. The present project was undertaken to establish genetic identity for these two obese mutant rat strains. It was decided to make a DNA fingerprint profile, employing the RAPD (Randomly Amplified Polymorphic DNA) approach using random primers to establish the genetic identity. Three standard strains WNIN, WKY and Fischer-344 were used as controls. The two phenotypes of the mutants lean (+/+) and carrier (+/-) were also included for comparison.

Methodology

1. Genomic DNA was isolated from the blood samples of WNIN (parental Strain), WKY (related strain), Fischer-344 (unrelated strain), WNIN/Ob, WNIN/GR-Ob and their phenotypes lean (+/+) and carrier (+/-) , six replicates in each.
2. PCR was carried out using random primers (over 60) from kit A, kit B and kit E (Operon Technologies) by standard protocols. The PCR products were then run on 8% polyacrylamide gels.
3. PCR products unique to WNIN/GR-Ob were cloned into the vector PCR-2.1 TOPO and the inserts were sequenced using M13 forward and reverse primers.
4. Southern blotting and hybridization of the PCR products amplified by the primerOPB10 was carried out using 32P labelled clone containing 390bp insert as probe as per standard protocol.

Results

1. The primer OPE7 generated initially a DNA profile unique to WNIN/Ob, but in subsequent generations, it was found to be untenable.
2. The M13 repetitive sequence, when used as a primer, also could not generate a profile unique to WNIN/Ob.
3. The (GATA)n primers and microsatellite markers linked to obesity loci also could not establish a profile unique to WNIN/Ob.
4. The random primer OPB10 generated a 'DNA fingerprint' profile for WNIN/GR-Ob which differentiated the mutant from the control strains and it's two phenotypes lean and carrier and also from the other mutant WNIN/Ob.
5. The PCR products unique to WNIN/GR-Ob were of the sizes- 360bp, 390bp, 400bp and 600bp.
6. The above sequences were successfully cloned in vector pCR 2.1- TOPO and the inserts could be sequenced using M13 forward and reverse primers.
7. BLAST Search revealed that the cloned PCR products 360bp, 390bp, 400bp have homology to the sequences on rat chromosome no.3 and chromosome no.8.The 600 bp PCR product has partial homology to chromosome X (only upto150 bp).
8. Southern blotting and hybridization of the PCR products with the clone containing 390bp insert as probe showed hybridization to the WNIN/GR-Ob indicating that the cloned regions are part of the rat genome.

Conclusions

The efforts to generate a DNA fingerprint unique to WNIN/Ob were not successful. However, a 'molecular signature' specific to the WNIN/GR-Ob mutant could be generated and the unique PCR products thus generated have homology with chromosome no.3 and chromosome no.8 and partially to chromosome X.

2. GENETIC TYPING OF WNIN/Ob AND WNIN/GR-Ob STRAINS USING MICRO-SATELLITE MARKERS

The two obese mutant rats developed from WNIN rat colony at NCLAS, WNIN/Ob and WNIN/GR-Ob need to be genetically typed to establish a DNA profile unique to them. Both the mutants are obese, but WNIN/GR-Ob has impaired glucose tolerance additionally. Microsatellite markers are abundant, randomly distributed throughout the genome, polyallelic and are genetically more informative. Therefore, approximately 100 microsatellite markers were selected, spanning all the 20 chromosomes and the X chromosome taking three to four markers per chromosome to type the obese mutant strains. Three standard strains, WNIN, WKY and Fischer-344 were used as the controls and the two phenotypes of the mutants, lean (+/+) and carrier (+/-) were also included in the study.

Methodology

1. Genomic DNA was isolated from the blood samples of WNIN (parental strain), WKY (related strain), Fischer-344 (unrelated strain), WNIN/Ob and WNIN/GR-Ob and their phenotypes lean and carrier, six replicate in each group.
2. PCR was carried out using microsatellite markers by the method by Serikawa et al (1992). The PCR products were then run on 12% polyacrylamide gels.
3. Hierarchical cluster analysis using centroid method was performed on the data generated using SPSS package version 11.5.

Results

1. A genomic scan of the standards and the mutant strains was performed using 96 microsatellite makers and out of which, 62 primers yielded good genetic profiles.
2. Out of these 62 primers, 9 microsatellite markers were found to be useful for rat strain identification.
3. Cluster analysis of 62 microsatellite primers based data, indicated the formation of two clusters; the first one contained the parental strain WNIN and the mutants, WNIN/Ob and WNIN/GR-Ob, the second cluster contained the standard strains -WKY and Fischer-344.

Conclusions

Nine primers were identified which can be used for genetic monitoring of rat strains. Amongst the nine, the microsatellite, primer leukosianin has great promise as it could detect length polymorphism between the three standard rat strains and also differentiate the mutant obese strain from the parental strain WNIN.

INSTRUMENTATION SERVICES

The activities of the department can be catogorised into 5 aspects:

I. Procurement and installation of new equipment.

II. Maintenance of existing equipment.

III. Training Programmes.

IV. Institution Building Activities.

V. Participation in research activities.

I. Procurement and installation of new equipment.

Procurement of equipment involves the following steps:

a. Invite suggestions from scientists.

b. Tabulation of the same and discuss at the Equipment Committee, with HOD, Institute and the Convener.

c. Prepare document for placing at the SAC and defend.

d. Prepare Tech, Specifications and get them approved by the Technical Committee.

e. Stores to float tender in two bids form (Tech & Commercial).

f. Scrutiny of Technical Bids and make recommendations and obtain approval of Technical Committee.

g. Open Commercial Bids and make recommendations.

h. The list is submitted to Council for funds.

i. After the funds are released, LC will be opened. And order released.

j. Once the order is placed, pre-requisites for installation of equipment are obtained.

k. Note put up to Director, on arrival of equipment, mentioning the names of indenters with location for installation suggested.

l. After the approval is obtained, action will be taken to install the equipment completing the formalities of consignment verification and reporting for short shipment, damage etc. if any.

m. After the instruments are installed, tests will be performed to ensure that the specifications are matched and reports will be issued. Simultaneously, arrangements for training on operation and service are also carried out (Table 31).

Table 31. List of New Instruments Installed:

S. No	Name of the Equipment	Make	Model	Location
1	Digital Photocopier	Toshiba	E-studio 200	Room No.104 Field Unit.
2	Digital Photocopier	Toshiba	E-studio 200	Library
3	Digital Platform Balance	OSAW	600 Kg.	Primate Facility
4	Digital Platform Balance	OSAW	300 Kg.	NCLAS
5	Binocular Microscope	Nikon	E-200	R.No.38 Bio.chem.
6	Trinocular System Microscope	Olympus	BX51	Room No. 32 Clinical Immu.
7.	Fluorimeter with PC and Printer (hp 3535)	Jasco	P.C.6500	Endocrinology Division
8.	C.D. Polarimeter with PC & Printer (hp3745)	J 810 (150-l)	Jasco	Endocrinology Division
9.	Micro plate Reader spectrophotometer	RPR WI HT WAVE	Biotek	Endocrinology division
10.	Hp Desk Jet Printer	HP	3845	Attached to Varian GC 3800
11.	Hp Desk Jet Printer	HP	3650	Attached to Spectramax Plate Reader cum Spectrophotometer
12.	Table Top Ref. Centrifuge	HERAEUS	Multifuge 3S-R	Clinical R.No11
13.	Whole Blood Aggregometer	Chronolog	500VA	R.No215
14.	Electrophoresis	Bio-Rad	Power Pack	R.No 55
15.	Microwave Oven-2nos.	LG	30LT	1) Bio-Chem.
16.	Digital Multimeters	Yokogawa	73401	Instrumentation
17.	Digital pH Meters- 8nos.	Thermo Orion	420A+	Biophysics – 2Nos. Molecular Biology–1No. Bio-chemistry – 2Nos. Clinical Division – 1 No. Endocrinology – 1 No. Food Toxicology – 1 No.
18.	RO System	Labconco	90750-02	Endocrinology
19.	RO System	Labconco	90750-02	Food Toxicology
20.	Millipore Water Purification System	Milli-Q	ELIX – 10	Biophysics
21.	Gel Dryer	Hydro Tech	583	Molecular Biology
22.	0.5KVA UPS –3nos.	Wipro	Wipra e-merge.	1&2-Libr. 3-Personnel div
23.	Off-line UPS 0.5 KVA		BE 5005	C-Bills Section
24.	1 KVA UPS	VIVCON		Clinical Division
25.	1 KVA UPS	VIVCON		Drug Toxicology
26.	1 KVA UPS	VIVCON		Library
27.	Off-line UPS 0.5 KVA–2 No	APC	ES500	Library
28.	Window Air Conditioner	Voltas	2000DV Vectra S.No.RAC-502500007	Room No.307
29.	Deep Freezer(Vertical D.D)	Bluestar	BFS –345 S.No. 20041305578	Room No.68
30	Deep Freezer		HF500 S.No.31031001795	Room No.68

S. No	Name of the Equipment	Make	Model	Location
31.	Water Cooler- 40 Lit.	Sriram	S.No. SRA 200BF8/2020K30347. Compressor No.CCK21883	
32.	High Pressure Water Jet:	OAH	TW13/170. 3804672	
33.	Air Cooler:	Kenstar	DXPO 118H	Stores Dept.
34.	Refrigerators(D/D, Frost Free) 260Lit	Godrej	GF28	FDTRC R.No.310
35.	Refrigerator(D.D	LG	GLT322GP. S.No.J.B CG4081I L0066491N	R.No.51 Analytical Carridor
36.	Refrigerator D.D	LG	S.No: J44G3091 L004577N	Dr.Vijayalaxmi Biochemistry
37.	Refrigerator D.D	LG	S.No.: 50045961 N0045931	Dr.Veena Shatrugna's Lab
38.	Refrigerator	LG	S.No.: 50045961 N0045931	Dr.Vajreswari's Lab-Biochemistry
39.	Refrigerator (D/D) 310Lit	LG	S.No.J43G309I L004261N	Dr.Singotam's Lab
40.	Refrigerator170lit.	Godrej	Champion	Crutch
41.	Refrigerator	Kelvinator	Electrolux S.No.: 40401188	R.No.40
42.	Refrigerator D.D, F.F -310 Lit	LG	322GD S.No.: 40401188	Dr.Vajreswari Dr.Madhavan Nair Mr.T.Longvah
43.	Warranty Replacement of Compressors (Sealed)		2000Q. S.No.: 20MJ1739	Molecular Biology Lab
44.	D.F Condensing Unit	Kirloskar Copeland	KCH-410	R.No.214
45.	Cold Room Condensing Unit			Gopalan Block II Floor
46.	Glass Distilled Water Set	Shanti Scientific		Ocular Biochemistry
47.	Bacteriological Incubator	Osworld	JRI'C-9	Molecular Biology
48.	Floor Scrubbing Machine	Unitec	43-CT	Primate Facility
49.	Vacuum Cleaner	Eureka Forbes	Euroclean	Primate Facility
50	Vortex Mixers-20nos.	Scientific Industries	G360E	Biophysics
51.	Seed Germinator	Remi	SG 65	NCLAS
52.	Water Bath	Cintex	CIC12	Food Chemistry
53.	Colour Television	Thomson	21 F TSV	NCLAS
54.	Wet Grinder	Maharaja	--	Biophysics
55.	Multimedia Projector-3nos.	Epson	EMP 74 EMP 75 EMP 735	Director's Office Assembly Hall Conference Hall

II Maintenance of Existing Equipment

Table 32. Total no. of complaints registered

Name of the Divisions	Complaints Received	Completed	Pending
Electronics	210	198	12
Electromechanical & Winding	160 50	154 48	6 2
Refrigeration & Air-Conditioning	241	240	1
Electrical	723	714	9
Total	13 8 4	1354	30

III Training Programmes and other activities

Participation of staff in Training Programmes:

In plant Training in Biomedical Engineering was imparted to six candidates from the Advanced Training Institute for Electronics and Process Instrumentation, Ramanthapur; under the aegis of Ministry of Labour, Government of India, for a period of six weeks from 12-07-2004 to 20-8-2004. Mr.R.Chaugule, Mr.AKV.Rajamouli, Mr.V.Satish Babu, Mr.Prasanna Kumar, Mr.K.Srinivasa Rao and Mrs Durga explained the theoretical and practical aspects of various scientific and test equipments used in the Biomedical research. The staff of the Division demonstrated Isotope Counters to the participants of the Thirty Second Annual Training Course on Endocrinological Techniques and their Applications on August 20th 2004 (Mr.R.Chaugule & Mr.A.K.V.Rajamouli).

Training

Attended Training on HVAC Systems organized by GMP Compliance India at Hyderabad, (Mr.B.Ramulu).

Refrigeration Staff attended Inplant Training by BPL Engineer on Refrigerators at the Institute.

Seminar attended:

"Innovation in Chromatograph" conducted by M\s Perkin Elmer India on29-09-2004 (Mr.V.Satish Babu and Mr.K.Sreenivasa Rao).

IV. Audio-Visual Arrangements were made for various scientific and cultural functions.

Institution Building Activities:

1. Equipment Committee (Technical and Purchase).
2. ICMR and DGHS Technical Committee- Capacity Building Project.

V. Research Activities: The following papers have been published

1. A study of the structural characterization and cyclohexanol dehydrogenation activity of Cu/-Al2O3 Catalysts.
   Anita Rachel a, V.Durga Kumari*a, R.Subramanianb, K.V.R.Charya & P.Kanta Raoa
   Indian Journal of Chemistry vol.43A, June 2004,pP.1172-1180
2. A Protective role for Zinc on intestinal peroxidative damage during oral iron reletion.
   B.Sreedhara, R.Subramanian b and K. Madhavan Nair a
   Biochemical and Biophysical Research Communications 318 (2004) 992-997.
3. Helped the scientists in the simulation of ESR specific using appropriate software and interpretation of data.

LIBRARY AND DOCUMENTATION SERVICES

Library continued to cater to the documentation and information needs of the Institute and other research organizations, home science and medical colleges. The Library has played a key role in reference activities by offering information services like MEDLINE Searches. ProQuest Searches and other on-line retrieval activities using the LAN network. Library continued to participate in exchange of data and information usin the URL< http://Groups.yahoo.com/Goup/ICMRLibrarians>.

Automation activity (Cataloguing) using the ISIS and LIBRIS softwares has been continued during the year.

The Library has continued to provide an excellent Photostat support to the scientists, technical as well as to the administrative staff. Resource Sharing and User-Education Programmes etc are continuously being undertaken by the Library. Institute's scientific papers going in for publication in Journals etc., are being routed through the Library and a data-base of the published papers is also made accessible through on-line services.(www.ninindia.org).

During the period a Total of 2861 ProQuest ML Full Text database Searches Jan-Dec'04 were made and there is overall 491% (Jan-Dec'04) increase in its usage.

British library Institutional membership is renewed for 2004 and Corporate Membership for "Universities Federation for Animal Welfare" UK for the year 2004, has also been taken out during the year under report.

The following journals have been added to the existing subscriptions list of 2004

1. Perspectives in Education

The following Library services were expanded as detailed below

1. New Additions

Books		. . . . .	207
Reports		. . . . .	501
Thesis / Dissertations		. . . . .	17
Microforms		. . . . .	27
CDROMS -MEDLINE	32		
ProQuest ML CD's	53	. . . . .	137
Misc. CD's	52		

2. Other activities

Journals Bound	 221
Visitors using the Library	 8,378
Circulation of Books/Journals etc.	 1,928
MEDLINE Abstracts provided	 2,945
No. of E-mails sent outside	 199
No. of E-mails received	 759

Photocopying (No. of pages)	. . . . .	4,09,450
Number of Annual Reports mailed	. . . . .	510
No. of Books/Journals received on Inter Library Loan	. . . . .	90
No. of Duplicate Journals sent out	. . . . .	25
No. of INTERNET Searches provided	. . . . .	54
No. of Reprints sent	. . . . .	75
ProQuest Full Text Database Searches provided	. . . . .	8
No. of Bibliographies	. . . . .	7

3. Total Library Collections

Books	. . . . .	15,336
Journals (Bound Volumes)	. . . . .	26,729
Journals subscribed for 2003	. . . . .	238
Journals received (Gratis/Exchange)	. . . . .	285
Microforms (Microfiche)	. . . . .	1,017
Reports	. . . . .	10,874
Reprints	. . . . .	3, 07,550
Theses & Dissertations	. . . . .	351
MEDLINE CDROM Discs	. . . . .	160
Current Contents on Diskettes with Abstracts.	.. . .	664
ProQuest (Ful Text E-Journals) on CDROMS	. . . . .	387
Misc. CD's	. . . . . .	193

Ph.D PROGRAMMES

Ph.D Awardees

Research Scholar/Staff	University	Year	Title of thesis
1. Vijayalakshmi A.	Osmania	2004	Long-term dietary manipulation of intestinal epithelial cell apoptosis in rats

Research scholars

Research Scholar/Staff	Title of the project	Guide
1. Rajendraprasad M.P. (1997)	Nitrosamines and its relevance to cancer in India	Dr.Kamala Krishnaswamy
2. Nirmala K. (1999)	Antigenotoxic potential of ginger	Dr. Kalpagam Polasa
3. Pratima Rao (1999)	Multicentric study on intake of food colours	Dr.Ramesh V Bhat
4. 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
5. Jeyakumar S.M. (2000)	Studies on food intake regulation and obesity in WNIN/Ob and WNIN/GR-Ob rats	Dr. Vajreswari, A.
6. Rita Saxena (2000)	Role of food processing on antioxidant activity and development of recipes with high antioxidant activity	Dr. M.Raghunath
7. Sreedhar B (2000)	Iron and Zinc interactions at the site of absorption	Dr.Madhavan Nair K
8. Venu L. (2001)	Foetal metabolic programming for insulin resistance: Role of maternal dietary micronutrients	Dr. Raghunath, M.

Research Scholar/ Staff	Title of the project	Guide
9. Satish Kumar M (2001)	Molecular chaperone function of alpha crystallin	Dr. Bhanuprakash Reddy G.
10. Krishna Kumari Menon (2001)	Positive Deviance in child nutrition	Dr.Vijayaraghavan, K.
11. Manjula T. (2001)	Ethno-pharmacological validation of biodynamic compounds in traditional medicine	Dr. Dinesh Kumar, B.
12. Aruna B. (2002)	Biophysical characterization of resistin	Dr.Nasreen Z. Ehtesham
13. Haseeb A. (2002)	Understanding the mechanism of action of PPARγ as a link molecule between obesity, type 2 diabetes and CHDs	Dr.Nasreen Z.Ehtesham
14. Uma Devi A. (2002)	Study of energy metabolism in WNIN obese rat mutants	Dr.Giridharan N.V.
15. Kiran Kumar B. (2002)	Genetic typing of WNIN/Ob and WNIN/GR-Ob strains using microsatellite markers	Dr.Giridharan N.V.
16. Anil Kumar (2002)	Molecular chaperone function of alpha crystallin under hyperglycemic conditions: Modulation by dietary factors	Dr.Bhanuprakash Reddy G.
17. Megha Saraswat (2003)	Screening of aldose reductase inhibitors and antiglycating agents from dietary sources and assessing their anticarcinogenic potential	Dr.Bhanuprakash Reddy G.
18. Mrudula T. (2003)	Charecterisation and significance of a novel fatty acid elongase of the eye lens	Dr.Bhanuprakash Reddy G.
19. Mr. Prasahanth A. (2003)	Role of scavenger receptors class B-1 (SR-B1) in reticulocyte differentiation, absorption of fat and fat soluble vitamins (vitamin A) and female infertility using WNIN/Ob rat model	Dr.Vajreshwari A.

Research Scholar/ Staff	Title of the project	Guide
20. Durga Kishore Y. (2004)	Role of maternal and postnatal zinc status in the development of insulin resistance in adult life	Dr.Raghunath M.
21. Vijay Kumar V. (2004)	The role of specific nutrients on the pancreatic progenitors/ stem cell specific to ductal epithelial cell	Dr.Vijayalakshmi V.
22. Subba Rao G.M (2004)	Approaches to Nutrition communication: A comparative study of effectiveness	Prof. Vinod Pavarala (Univ. of Hyderabad)
23. Md.Naseeruddin (2004)	Understanding the role of resistin in inflammatory process leading to type 2 diabetes	Dr.Sudeep Gosh
24. Padmavathi I.J.N. (2004)	Role of maternal chromium status in the development of insulin resistance in the offspring	Dr. Raghunath M
25. Satyanarayana B. (2004)	Biological significance of phytoferritins	Dr. Madhavan Nair K.
26. Sreenivasulu K. (2004)	Caco-2 cell as a model to study bioavailability, mechanism of absorption and cytoprotective effects of zinc.	Dr.Madhavan Nair K.
27. Vasuprada I. (2005)	Studies on the response and interaction of iron and zinc in Caco-2 cells	Dr. Madhavan Nair K.

AWARDS/HONOURS CONFERRED ON SCIENTISTS

Name of the Scientist	Award/Honour
Mr.M. Satish Kumar, CSIR-SRF	Annual Research Award – 2004 as a 2nd runner, for his research work on "Effect of dicarbonyls on chaperone-like function α-crystallin : Implications in cataract".
Dr. K. V. Rameshwar Sarma	Expert of the IDF Standing Committee on Nutrition and Health of the Indian National Committee of the International Dairy Federation.
Mr.G.M. Subba Rao	Junior Young Scientist Award in Community Nutrition for his paper titled "Impact of FAO's global school-based nutrition education initiative – Feeding Minds, Fighting Hunger (FMFH) on school-children", at the XXXVI Annual Meeting of the Nutrition Society of India.
Dr. L Singotamu	CFTRI Award for best poster presentation on "Scanning Electron Microscope and EDAX-Ray Studies on processed milk samples" at the XXXVI Annual Meeting of the Nutrition Society of India.
Dr.N. Hari Shanker	CFTRI Award for best poster presentation on "Determination of body composition and activity by non-invasive procedures in three commonly used rat strains", at the XXXVI Annual Meeting of the Nutrition Society of India.
Dr.D. Raghunatha Rao	Certificate of Merit for his excellent Paper presentation on "Nutrition Knowledge and Dietary Habits of School Going Adolescent Girls in Hyderabad" in oral session at the UGC sponsored National Conference on "Human Health and Nutrition : A Biotechnological Approach",Organised by VPM's B.N. Bandodkar College of Science, Mumbai
Dr.P. Suryanarayana	Young Scientist Award for his paper entitled "Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats", presented at SERI-ARVO Meeting on Research in Vision and Opthalmology held at Singapore from 16-20th February 2005.

PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS

Date	Scientist	Conference/Meeting/Workshop/Seminar
2004		
April 25-29	Mr.M. Satish Kumar	Annual Meeting of Association for Research in Vision and Opthalmology, Florida, USA. Presented a poster entitled "Enhanced degradation and altered AFP binding of MGO-modified α-crystallin".
April 27-29	Dr. L. Singotamu	The Fifteenth Annual International Scientific Meeting on Scanning Microscopies, sponsored by the Foundation for Advances in Medicine and Science and Scanning, at Washington DC, USA. Presented a paper on "Scanning Electron Microscope Studies on Small Millets".
Aug. 10-13	Dr. Ghafoorunissa	Meeting of the International Nutrition Advisory Council and World Congress of Clinical Nutrition-2004, at Brisbane, Australia.
Nov. 1-5	Dr.B. Sivakumar	Twenty sixth Session of Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU), at Bonn, Germany.
Dec. 7-9	Dr.B. Sivakumar	Bi-Regional Meeting for Development of an Integrated Strategy on Optimal Foetal Growth and Development, held at Bangkok, Thailand.
2005		
Feb. 16-20	Dr.P.Suryanarayana	Second SERI-ARVO meeting on Research in Vision and Ophthalmology, at Singapore.

WORKSHOPS/CONFERENCES/ SEMINARS/TRAINING PROGRAMMES HELD AT NIN

1. Training for field investigators of RCH projects in the techniques of blood sample collection for estimation of haemoglobin, at NIN, Hyderabad; Regional Occupational Health Centre, Kolkatta and Directorate of Public Health and Preventive Medicine, Chennai (Conducted in three batches with 20 participants in each batch belonging to various States) (April 6-8, 12-14, 21-23).
2. Dietitians Academic Meet, organized by Indian Dietitic Association. Dr.Shyam Sunder, Intensivist, Yashoda Hospital delivered a lecture on "Introduction to Parenteral Nutrition" (May 14).
3. Pre Clinical Toxicology -Scientific Advisory Committee meeting (July 20).
4. Meeting of the NNMB Steering Committee (July 23).
5. Meeting of the Pre-SAC and Scientific Advisory Committee of NIN/FDTRC/NCLAS (Aug. 5-7).
6. XXXIII Annual Training Course on Endocrinological Techniques and their Applications (Aug.17-Sept.30).
7. Year of Scientific Awareness Workshop, organized by Department of Science and Technology in association with Cerana Foundation (Sept. 11-12).
8. Meeting of the Academic Committee on training courses conducted by the institute (Oct.1).
9. Training of Trainers: Programme on Nutrition and Health Education for ICDS functionaries, organized in association with Food and Nutrition Board, Hyderabad (Oct. 11-15).
10. An ad-hoc training programme for four WHO participants from Bangladesh in the field of Community Nutrition (Nov.29-Dec.3).
11. Nutrition Orientation Programme for Middle-Level Health Functionaries of A.P. State Government. Twenty three participants from all over the State have participated in the training programme (Dec. 6-10).
12. A two day National Seminar on "Pesticide Residues and their Risk Assessment" (Jan. 20-21, 2005).
13. XXXXII Post-Graduate Certificate Course in Nutrition. Ten candidates from different States of the country participated (Jan.3 - March 15, 2005).
14. An ad-hoc training programme for two WHO Fellows from Nepal in the field of Community based Nutrition (Feb.7-March 4).
15. Training Course on "Techniques for Assessment of Nutritional Anaemias". Ten candidates from different States of the country participated in the Course (March 21-31).
16. Orientation Programme in Nutrition for Youth Volunteers from North-East, organized by the Institute, at Rajiv Gandhi National Institute for Youth Development, Sriperumbudur, Tamil Nadu (March.18-22).

SERVICES RENDERED TOWARDS INCOME GENERATION

1. Pathology services

During the year, a total income of Rs.3, 00,000/- was generated from various projects of institute's preclinical toxicology and surgical pathology and cytology samples.

SCIENTIFIC PUBLICATIONS - 2004

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Anita Rachel, Durga Kumari V, Subramanian R, Chary KVR, Kanta Rao P : A Study of the structural characterization and cyclohexanol dehydrogenation activity of Cu/Y-Al2O3 catalysts. Indian J Chem. 43A : 1172-1180, 2004.
2. Bamji MS, Murthy PVVS, Vishnuvardhan Rao M, Dangoria D : Impact of women health and nutrition entrepreneurs and mobilizers on health and nutrition of rural children and mothers' knowledge and health-related practices. Regional Health Forum. 8(1): 92 - 103, 2004.
3. Bhanuprakash Reddy G: Fluoride toxicity and oxidative stress. Fluoride. 37 (1): 43-44, 2004.
4. Bhargavi V, Khandare AL, Venkaiah K, Sarojini G: Mineral content of water and food in fluorotic villages and prevalence of dental fluorosis. Biol.Trace Element Res. 100 (3) : 195-203, 2004.
5. Chennaiah, S, Qadri SSYH, Rama Rao SV, Shyamsunder G, Raghuramulu N : Cestrum diurnum leaf as a source of 1,25 (OH)2 Vitamin D3 improves egg shell thickness. J. Steroid. Biochem. Mol. Biol. 89-90: 589-594, 2004.
6. Ghafoorunissa, Hemalatha S, Vishnuvardhana Rao M: Sesame lignans enhance antioxidant activity of vitamin E in lipid peroxidation systems. Mol. Cell Biochem. 262 (1-2) : 195 - 202, 2004.
7. Goud IK, Hasan Q, Balakrishna N, Prabhakar Rao K, Ahuja YR : Genotoxicity evaluation of individuals working with photocopying machines. Mutat Res. 563: 151 - 158, 2004.
8. Hemalatha S, Ghafoorunissa: Lignans and tocopherols in Indian sesame cultivars. JAOCS. 81 (5): 467 - 470, 2004.
9. Hemalatha S, Raghunath M, Ghafoorunissa: Dietary sesame (Sesamum indicum cultivar linn) oil inhibits iron-induced oxidative stress in rats. Brit J Nutr. 92: 581- 587, 2004.
10. Khandare AL, Uday Kumar P, Shanker Rao G, Venkaiah K, Lakshmaiah N: Additional Beneficial effect of tamarind ingestion over defluoridated water supply to adolescent Boys in a fluorotic area. Nutrition. 20: 433-436, 2004.
11. Kondapi AK, Neelima M, Mandraju RK, Sasikaran B, Subba Rao K : Analysis of age dependent changes of Topoisomerase II and in rat brain. Int J Devl Neuroscience. 22 : 19-30, 2004.
12. Nair KM, Bhaskaram P, Balakrishna N, Ravinder P, Sesikeran B : Response of hemoglobin, serum ferritin, and serum transferrin receptor during iron supplementation in pregnancy : A prospective study. Nutrition. 20 (10): 896-899, 2004.
13. Padmaja RJ, Pratima Rao, Bhat RV, Nadamuni Naidu A : Type, extent and use of colours in ready-to-eat (RTE) foods prepared in the non-industrial sector - A case study from Hyderabad, India. Int J Food Sci Technol. 39: 125-131, 2004.
14. Polasa K, Naidu AN, Ravindranath I, Krishnaswamy K: Inhibition of B(a) P induced strand breaks in presence of curcumin. Mutat Res. 557 : 203-213, 2004.
15. Pratima Rao, Bhat RV, Sudershan RV, Krishna TP, Naidu N: Exposure assessment to synthetic food colours of a selected population in Hyderabad, India. Food Addit. Contam. 21 (5) : 415 - 421, 2004.
16. Raghu P, Ghosh S, Soundarya K, Haseeb A, Aruna B, and Nasreen Z. Ehtesham: Dimerization of human recombinant resistin involves covalent and noncovalent interactions. Biochem Biophys Res Commun. 313 (3): 642-646, 2004.

17. Raghu P, Sivakumar B: Interactions amongst plasma retinol-binding protein, transthyretin and their ligands: implications in vitamin A homeostasis and transthyretin amyloidosis. Biochim Biophys Acta. Vol.1703:1-9, 2004.

18. Ramulu P, Udayasekhara Rao P: Effect of maturity and processing on total, insoluble and soluble dietary fiber contents of Indian green leafy vegetables. Int J Food Sci. Nutr. 55(7) : 561-567, 2004.

19. Satish Kumar M, Mrudula T, Mitra N, Bhanuprakash Reddy G: Enhanced degradation and decreased stability of eye lens -crystallin upon methyglyoxal modification. Exp Eye Res. 79(4): 577-583, 2004.

20. Satish Kumar M, Yadagiri Reddy P, Anil Kumar P, Surolia I, Bhanuprakash Reddy G: Effect of dicarbonyl-induced browning on α - crystallin chaperone - like activity : physiological significance and caveats of *in vitro* aggregation assays. Biochem J. 379: 273- 282, 2004.

21. Sreedhar B, Nair KM: Iron dependence and zinc inhibition of duodenal cytosolic aconitase of rat. Indian J Biochem Biophys. 41 (5): 250-253, 2004.

22. Sreedhar B, Subramanian R, Madhavan Nair K: A protective role for zinc on intestinal peroxidative damage during oral iron repletion. Biochem Biophys Res Commun. 318: 992 - 997, 2004.

23. Sreeramulu D, Ramalakshmi BA, Balakrishna N, Raghuramulu N : Serum dehydroepiandrosterone and lipid peroxides in human volunteers of different age groups. Indian J Clin Biochem. 19(1): 79 - 82, 2004.

24. Suryanarayana P, Anil Kumar P, Megha Saraswat, Mark Petrash J , Bhanuprakash Reddy G : Inhibition of aldose reductase by tannoid principles of Emblica officinalis : Implications for the prevention of sugar cataract. Molecular Vision. 10: 148 - 154, 2004.

25. Vasanthi S, Sesikeran B, and Bhat RV: Starlink genetically modified corn and allergenicity in an individual. J Allergy Clin Immunol. 113(5): 1003 - 1004, 2004.

26. Venkata Ramana Y, Surya Kumari MVL, Sudhakar Rao S, Balakrishna N: Comparison of training loads and physiological responses in athletes: consideration of body weight implications. J Exercise Physiologyonline. 7 (3): 134 - 139, 2004.

27. Venkata Ramana Y, Surya Kumari MVL, Sudhakar Rao S, Balakrishna N: Variations in basal metabolic rate with incremental training load in athletes. J Exercise Physiologyonline. 7 (1) : 26 - 33, 2004.

28. Venkata Ramana Y, Surya Kumari MVL, Sudhakar Rao S, Balakrishna N: Effect of changes in body composition profile on VO_2 max and maximal work performance in athletes. J Exercise Physiologyonline. 7 (1): 34 - 39, 2004.

29. Venu L, Harishankar N, Prasanna Krishna T, Raghunath M: Does maternal dietary mineral restriction per se predispose the offspring to insulin resistance? Eur J Endocrinol. 151: 287 - 294, 2004.

30. Venu L, Harishankar N, Prasanna Krishna T and Raghunath M: Maternal dietary vitamin restriction increases body fat content but not insulin resistance in WNIN rat offspring up to 6 months of age. Diabetologia. 47: 1493 - 1501, 2004.

B. PAPERS PUBLISHED IN PROCEEDINGS OF WORKSHOPS / CONFERENCES

1. Ghafoorunissa: National facility for systematic analysis of foodstuffs. In “Towards National Nutrition Security”, Nutrition Foundation of India, Silver Jubilee Symposium, 29th November -1st December 2004, New Delhi, NFI, 2004, p. 129-133.

2 Khandare AL, Hari kumar R, Venkaiah K, Gal Reddy Ch, Sivakumar B: Exploratory study on fluorosis in five districts (Vellore, Krishnagri, Dharampuri, Salem and Erode). In National workshop on nutritional effects on fluorosis, held on 19th & 20th Nov 2004, at Tamilnadu Water and Drainage Board, 87-100pp, 2004.

3. Narayana AS, Khandare AL, Krishnamurthi MVRS: Mitigation of fluorosis in Nalgonda district villages. In " 4th International Workshop on fluorosis prevention and defluoridation of water", ISFR, EnDeco, ICOH, Colombo, Sri Lanka held on 2nd -6th March 04

4. Raghunatha Rao D, Vijayapushpam T, Grace Maria Antony, Subba Rao GM, Rameshwar Sarma KV : Nutrition knowledge and dietary habits of school going adolescent girls in Hyderabad. Proceedings of National Conference on human health and nutrition : A Biotechnological approach, December 12-13, 2004 : 84-89, 2004.

5. Rameshwar Sarma KV: Nutrition scenario in India - challenges and solutions. Proceedings of National Conference on human health and nutrition: A Biotechnological approach, December 12-13, 2004: 13-26, 2004.

6. Sivakumar B: Dual fortification of common salt-Technological hurdles and way ahead. In towards National Nutrition Security", Nutrition Foundation of India, Silver Jubilee Symposium, 29th November -1st December 2004, New Delhi, NFI, 2004, p. 148-150

7. Subba Rao GM, Raghunatha Rao D, Venkaiah K, Dube AK, Rameshwar Sarma KV: Nutrition education for school children using FAO's global school-based programme - feeding minds, fighting hunger (FMFH). In Proceedings of UGC sponsored National Conference on human health and nutrition: A Biotechnological approach, Dec' 12-13, 2004, Botany Department, B.N.Bandodkar College of Science, Thane, Maharashtra, pp.94-100.

8. Veena Shatrugna, Gita Ramaswamy, Srividya Natarajan: Taking charge of our bodies: A health handbook for women, edited by K. Lalitha et.al. New Delhi, Penguin Books: PP 1-427, 2004.

9. Venkaiah K, Harikumar L, Khandare A, Gal Reddy Ch, Brahmam GNV, Sivakumar B: A Stastical model for estimation of population at risk of dental fluorosis using data from a rapid survey. In "National workshop on nutritional effects on fluorosis", 19th & 20th November 2004, held at Tamil Nadu Water Supply & Drainage Board, p.5, 2004.

C. POPULAR ARTICLES

I. NUTRITION NEWS

1. Arlappa N, Balakrishna N, Sharad Kumar, Brahmam GNV, Vijayaraghavan K: Nutritional status of the aged in rural India. Nutrition News. 25 (1&2) : 1-6, Jan/Apr' 2004.

2. Padmaja RJ, Pratima Rao, and Bhat RV: Food colours in ready-to-eat foods in unorganized sector - A Case study. Nutrition News. 25 (3&4): 1-6, July/Oct'2004.

II. OTHERS

1. Dube AK: Mr. Chimney's paradox: A health communication perspective. Health Action.17 (7): 14 - 17, 2004.

2. Subba Rao GM, Rameshwar Sarma KV: Ideal breakfast. Indian Express (Health page), dt. 22-6-2004.

SCIENTIFIC ADVISORY COMMITTEE

NIN/FDTRC

1. Dr.M.K.Bhan .... Chairperson
   Secretary, Government of India
   Department of Biotechnology
   NEW DELHI - 110 003

2. Dr.Lalji Singh
   Director
   Centre for Cellular & Molecular Biology
   HYDERABAD - 500 009

3. Dr.Satish Kumar Gupta
   Staff Scientist-VII and Chief
   Gamete Antigen Laboratory
   National Institute of Immunology
   NEW DELHI - 110 067

4. Dr.S.Majumdar
   Professor & Head
   Dept. of Experimental Medicine &
   Biotechnology
   P.G.I.M.E.R, CHANDIGARH - 160 012

5. Dr.Prema Ramachandran
   Director
   The Nutrition Foundation of India
   NEW DELHI - 110 016

6. Dr.Jayaprakash Muliyil
   Professor
   Community Health Department
   Christian Medical College,
   VELLORE - 632 002

7. Dr.Rajesh Kumar
   Professor & Head
   Department of Community Medicine,
   P.G.I.M.E.R, CHANDIGARH - 160 012

8. Dr.Indu Gupta
   Professor (Perinatology)
   Dept. of Obstetrics & Gynaecology
   P.G.I.M.E.R, CHANDIGARH - 160 012

9. Dr.D.Balasubramanian
   Director - Research
   L.V.Prasad Eye Institute
   HYDERABAD - 500 034

10. Dr.Shahid Jameel
    Group Leader, Virology
    ICGEB, NEW DELHI - 110 067

11. Prof.K.Ramachandran
    8, G-2, Visveswarapuram
    Mylapore, CHENNAI - 600 004

12. Dr.Indira Chakravarty
    Director, Professor & Head
    All India Institute of Hygiene &
    Public Health, KOLKATA - 700 073

13. Dr.Arvind Pandey
    Director
    Institute for Research in Medical
    Statistics, NEW DELHI - 110 029

14. Dr. Vatsla Dadhwal
    Associate Professor
    Department of Obstetrics & Gynaecology
    All India Institute of Medical Sciences
    NEW DELHI - 110 029

15. Dr.B.S.Kodkany
    Chief, HRRC
    Department of Obstetrics& Gynaecology
    JLN Medical College,
    BANGALORE - 590 010

16. Dr.H.P.S.Sachdev
    EE-6/12, Vasant Vihar
    NEW DELHI - 110 057

17. Dr.C.S.Yajnik
    Director, Diabetes Unit
    KEM Hospital, PUNE - 411 011

NIN/FDTRC (Contd..)

18. Dr.Anoop Misra
Professor
Department of Medicine
All India Institute of Medical Sciences
NEW DELHI - 110 029

19. Dr.S.V.Madhu
University College of Medical Sciences
(UCMS) & GTB Hospital
Shahdara, DELHI - 110 095

20. Dr.M.G.Karmarkar
Senior Adviser, ICCIDD
Centre for Community Medicine
All India Institute of Medical Sciences
Ansari Nagar, NEW DELHI - 110 029

21. Dr.Shally Awasthi
Professor
Department of Paediatrics
K.G.Medical College, LUCKNOW

22. Dr.Nita Bhandari
Department of Paediatrics
Scientist II
B-10, Soaminagar, NEW DELHI

23. Dr.Satyajit Rath
Scientist-7
National Institute of Immunology
NEW DELHI

24. Prof. N.K.Ganguly
Director General
Indian Council of Medical Research
NEW DELHI - 110 029

25. Mr.P.D.Seth
Financial Adviser
Indian Council of Medical Research
NEW DELHI - 110 029

26. Dr.G.S.Toteja
Dy. Director General
Indian Council of Medical Research
NEW DELHI - 110 029

27. Dr.Malabika Roy
Dy.Director General
Indian Council of Medical Research
NEW DELHI - 110 029

28. Dr.B.Sivakumar
(Member Secretary)
Director
National Institute of Nutrition, Hyderabad

SPECIAL INVITEES

29. Dr.B.S.Narasinga Rao
102, Brigade Hill View Apartments
Gavipuram
BANGALORE - 560 019

30. Dr.Mahtab S.Bamji
Former Director Gr. Scientist, NIN
211, Sri Dattasai Appts
Golconda X Roads
HYDERABAD - 500 029

31. Dr.Kamala Krishnaswamy
Former Director, NIN &
Emeritus Medical Scientist, ICMR
National Institute of Nutrition
HYDERABAD - 500 007

32. Prof.K.S.Ratnakar
Anand Sai
H.No.1-216/159, Karthikeyanagar
Nacharam, HYDERABAD

33. Prof.Asis Datta
Director
National Centre for Plant Genome
Research
JNU Campus, NEW DELHI - 110 067

NCLAS

1. Dr.Sandip K.Basu …. Chairperson
Director
National Institute of Immunology
NEW DELHI 110 057

2. Dr.Chander P. Puri
Director
National Institute for Research in Reproductive Health, MUMBAI- 400 012

3. Dr.Krishna Ashrit
Director of Animal Husbandry (Retd.)
H.No.4-150/4/1, Road No.1
Srivenkateswara Colony, Saroor Nagar
HYDERABAD - 500 035

4. Dr.P.S.Chauhan
Emeritus Medical Scientist (ICMR)
Plot No. 99, Sector 18 A
Neruli West, Navi Mumbai - 400 076

5. Dr.R.V.Thampan
Director
Rajiv Gandhi Centre for Biotechnology
THIRUVANANTHAPURAM - 695 014
KERALA

6. Dr.O.P.Asthana
Head, Clinical and Experimental Medicine
Central Drug Research Institute
LUCKNOW - 226 001

7. Dr.Syamal Roy
Deputy Director
Indian Institute of Chemical Biology
Jadavpur, KOLKATA

8. Prof.P.B.Seshagiri
Associate Professor
Department of Molecular Reproduction Development & Genetics
Indian Institute of Science, BANGALORE

9. Dr.R.R.Bhonde
National Centre for Cell Science, NCCS Complex, Ganeshkhind, PUNE - 411 007

10. Dr.B.S.Das
Adviser
Department of Biotechnology
Ministry of Science & Technology
NEW DELHI - 110 003

SPECIAL INVITEE

11. Prof.P.Kondaiah
Associate Professor
Molecular Reproduction Development & Genetics, Indian Institute of Science,
BANGALORE - 560 012