

RESEARCH HIGHLIGHTS

The hallmark of sound research is adoption of new scientific techniques and approaches tailor-made for the changing needs and situations. Several such novel needs were utilized this year both in the basic as well as applied research fields. Use of positive deviance approach in community nutrition, integrated feeding and care interventions, Integrated Behavioural and Biological Assessment to study HIV-related aspects and commercialization of fortification (of salt) technology are some such examples. The Food and Drug Toxicology Research Centre (FDTRC) also carried out a series of community based and laboratory - based investigations to study endemic kidney damage in some segments of population, surveys to assess community's perception towards practices related to food and drug use, pre-clinical toxicity of JE vaccine are some research investigations done at the division. This year also witnessed the accelerated growth of the Centre for Advanced Research for Pre-clinical Toxicity established in the recent past.

1. COMMUNITY STUDIES

1.1 Impact Evaluation of Positive Deviance Programme in West Bengal

The 'Positive Deviance' (PD) is an asset based approach, built on the belief that in every community there exists few mothers with special efforts or better child care practices, which enable them to prevent undernutrition among their children, compared to their counterparts, who live in similar socio - economic background and resources, and are exposed to the same risks from the existing environment". The Government of West Bengal, with the assistance of UNICEF, initiated PD programme in 4 districts, utilizing the existing ICDS infrastructure, to accelerate the process of reduction and prevention of undernutrition among children in the age group of 0-3 years in a short time.

At the request of UNICEF, the National Institute of Nutrition, carried out the impact evaluation of the programme.

The study revealed that a high proportion of women opt for institutional deliveries and a majority of the home deliveries are conducted by Trained Birth Attendants (TBAs) in the PD areas as compared to control areas. Recording of birth weights of newborns was significantly higher in PD areas, as compared to control areas. Similarly, a significantly higher proportion of mothers fed colostrum, and initiated breast-feeding within 3 hours of delivery. Initiation of complementary feeding at 6 months of age among children currently aged 12-23 months was significantly higher in PD areas. A significantly higher proportion of children were completely immunized by one year of age; they were covered for massive dose vitamin A supplementation and were subjected to regular growth monitoring in PD area. The overall prevalence of stunting was significantly lower in PD area as compared to control area.

1.2 Assessment of Nutritional Status of < 5 year children in the select districts in the country

In response to UNICEF's request, NIN undertook a study entitled "Assessment of Nutritional Status of < 5 year children in the select districts in the country" to generate baseline data, where UNICEF interventions are in operation.

A total of 12,879 children were covered from 9,576 households in 480 villages of 16 districts representing 13 States. About 57-98% women reportedly fed colostrum to their newborns and about 40-79% of the mothers initiated breast-feeding within 3 hours of delivery, in various districts surveyed. About 20-58% of mothers reportedly gave pre-lacteal feeds to the newborn. About 30-87% of the mothers of 6-11 months children initiated complementary feeding by the age of 6 months. The proportion of completely immunized among 12-23 months children ranged from a low 52% to a high 98%. About 33-64% of pre-school children were of underweight, while prevalence of stunting and wasting was in the ranges of 31 - 54% and 15-25% respectively in different districts.

2. CLINICAL STUDIES

2.1. Assessment of prevalence of osteoporosis in adult population in India—An ICMR Task Force study

A major ICMR Task Force study on the prevalence of osteoporosis in adult population in India has been completed. In addition, the assessment of the peak bone mass in population who have no constraints to growth has also been carried out.

Preliminary results suggest that the bone densities of population from the high, middle and low socio-economic groups are significantly different. The bone densities at all the 3 sites (hip, spine and forearm) improve and the prevalence of osteoporosis decreases as one goes up the class scale in men and women.

Over 43% of women (ages 30+) from the low income group are osteoporotic at the spine but after the age of 60+ years the prevalence of osteoporosis is over 90% when compared to women from the high income group (16% overall osteoporosis at the spine after the age of 30+ years and 45% after the age of 60+ years).

The age at peak bone mass of populations who have no constraints to growth appears to be between 25 to 30 years at all the 3 sites and it is not very different from the BMD values reported from the west. This study will provide an important data base for future studies on bone health in India.

2.2 Double blind Randomized controlled trial of zinc supplementation to full term infants

Zinc is an important nutrient for growth and other functions. Results of a randomized controlled trial of over 470 subjects where half of them received zinc (5mg/day) and the other half received placebo for a period of 14 months, showed that there were no significant differences in weights or heights between supplemented and control children. There was a small but significant increase in body fat percentage in supplemented children at 12 months of age (20.2 ± 5.34 Vs 19.0 ± 4.4 , $p=0.02$). Similarly, mean number of episodes of diarrhoea was not significantly different between supplemented and control groups (1.46 Vs 1.62) and mean diarrheal duration was significantly low in

supplemented children, per 100 days followup (6.8 ± 5.6 Vs 8.1 ± 6.53 , $p=0.048$).

3. NUTRITION AND INFECTION

Insulin resistance and its association with zinc and adipokines in young adults

It has been shown that there is several fold increase of coronary heart disease from 1960 to 1990 in the urban India. A major proportion of these patients are relatively young and 35-40% show no major risk factors. Higher triglyceride, lower HDL and increased visceral fat and insulin resistance have been proposed as reasons for the higher risk of CHD and type 2 diabetes. Role of sub clinical micronutrient deficiency in this phenotype of central obesity and increased insulin resistance and its association with adipokines and CHD risk factors in young adults (20-30) was studied.

3.5% of the subjects under study had high serum glucose values (≥ 110 mg/dl), a significant proportion (43.8%) of them had poor insulin sensitivity ($\text{HOMA} \geq 3.16$). A proportion of 36.3% had $\text{BMI} \geq 23.0$. Adiponectin was inversely associated with BMI while resistin was comparable. When the subjects were stratified based on HOMA, subjects with $\text{HOMA} \geq 3.16$ had significantly lower Zinc and adiponectin compared to subjects with $\text{HOMA} < 3.16$ group.

A significant proportion of young adults have high BMI and insulin resistance as indicated by HOMA. Similar to other reports, adiponectin, an adipokine was inversely associated with insulin sensitivity. Zinc might have a beneficial role in insulin sensitivity. The present study also indicates a male predisposition to develop insulin resistance and higher BMI associated with low zinc status.

4. BASIC STUDIES

4.1 Micronutrients

Developing strategies for the prevention and control of micronutrient deficiencies has been the major focus of the institute. The institute has developed operationalized strategies like DFS with iron and iodine and whole wheat flour fortified with iron, folic acid and vitamin A. Biofortification of staple crops is an emerging area in the field of nutrition science. It involves

screening, selecting and conventional breeding of germplasm for high micronutrient density. Department of Biotechnology is funding a networking project on crop biofortification and NIN is coordinating all the nutritional studies under this project and has become a networking unit for nutritional studies planned under the DBT's Crop Biofortification Networking project.

Caco-2 cell line (cell line derived from human intestinal enterocytes) is an alternative to the laborious animal experimentation and is relatively simple to use it as a screening method for assessing bioavailability of nutrients. NIN has established a coupled *in vitro* digestion/Caco-2 cell iron bioavailability method for screening for iron bioavailability in staple foods. This method has been developed using ferritin content of Caco-2 cell as a surrogate marker of iron bioavailability, measured using an indigenously developed ELISA for human serum ferritin. Currently, the target of iron crop biofortification is phytoferritin and iron bioavailability study in Caco-2 cell with phytoferritin from pea seed has shown that the pea ferritin is susceptible for gastric and intestinal digestion and the iron bioavailability is similar to that of the chemical source of iron, ferrous sulphate.

4.2 ISOTOPE DIVISION

4.2.1. Commercialization of double fortified salt (DFS)

The Ministry of Health & Family Welfare, Government of India constituted a Technical Committee of Experts under the Chairmanship of Dr. M. K. Bhan, Secretary of the Department of Biotechnology, Government of India on "Formulation of guidelines for use of iodine & iron double fortified salt (DFS) as a measure to reduce prevalence of anaemia" vide letter No. Z 28020/16/2005-CH/PH dated 14th July 2005. Dr. Bhan Committee, after detailed deliberations, recommended for the commercial production of DFS according to NIN's formula and to introduce it in nutritional programmes for vulnerable groups (Recommendations of Dr. Bhan Committee dated 16th November 2006).

As per the recommendations of the ICMR Expert Committee on commercialization of DFS, an advertisement was released during December 2006 in newspapers [The Times of India: All editions, Nav Bharat Times (Hindi: Mumbai + Delhi), Dinamalar (Tamil), Eenadu

(Telugu), Divya Bhaskar (Gujarat) and Dainik Bhaskar (Rajasthan)] with regard to technology transfer of NIN-DFS. In response to the advertisement, 12 applications from salt manufacturers were received. Furthermore, letters have been written to the Secretaries of Women Development and Child Welfare Department of all the States & UTs in the country (total: 35) advocating the introduction of NIN-DFS in nutritional programmes of the States & UTs. The details of DFS according to NIN formula and its large-scale production in factories have been published in peer reviewed scientific journals. Therefore, IPR issues are not involved in the technology transfer. The modalities of the transfer of technology are being worked out.

4.2.2. Commercialization of iodine & iron Field Kits

The demand for the Field Kits for testing iodine in iodized salt as well as double fortified salt and iron in double fortified salt is increasing day by day. Therefore, the large-scale production of these kits was taken up. The colour gradation card of the kit is replaced by a colour gradation sticker with instructions for use and is pasted on the plastic dropper bottle of the test solution. The cost of each of iodine kit is Rs. 7/- and the cost of iron kits is Rs. 10/- and one kit can be used to test 200-250 salt samples.

5. DEGENERATIVE DISEASES

5.1. RESEARCH ON CATARACT AND RETINAL DEGENERATION

5.1.1 Crystallins in health & disease and modulation by dietary factors

(I) Modulation of chaperone activity of α -crystallin under diabetic cataract by cumin:

Impaired chaperone function of α -crystallin could be involved in the formation of diabetic cataract. We have demonstrated that in diabetic cataract, α -crystallin chaperone activity is diminished. Interestingly, dietary cumin delayed progression and maturation of diabetic cataract by modulating the chaperone activity of α -crystallin. The antiglycating effect of cumin appears to be the predominant mechanism for the modulatory effect on α -crystallin chaperone activity in diabetes and thereby delaying cataract in rats.

(ii) Regulation of Expression of α -crystallins under diabetic conditions

Earlier, for the first time, elevated expression of small heat shock protein, α -crystallin, in various diabetic tissues and the significance of which is being studied was reported. Heat shock protein (Hsp) expression is regulated transcriptionally by heat shock factors (Hsf) and there are three Hsf (Hsf 1, Hsf 2 & Hsf 4) in vertebrates. Among the three Hsf, Hsf1 appears to control the expression of α -crystallins under diabetic conditions.

5.1.2. Studies on role of functional foods and nutraceuticals in degenerative conditions

(i) Emblica Officinalis and its isolated tannoids delayed diabetic cataract in rats

Preliminary screening studies and *ex vivo* lens organ culture studies suggest that tannoids of amla have significant aldose reductase inhibitory potential. Results from animal studies indicate that Emblica and its tannoids are effective against development of diabetic cataract in rats mainly by countering the polyol pathway induced osmotic and oxidative stress. Moreover, these results indicate that Emblica and its tannoids may act downstream to glucose-mediated changes. Further, these results have implications in terms of exploring the ingredients of dietary sources for the treatment of diabetic complications other than diabetic cataract.

(ii) Specificity of dietary aldose reductase inhibitors

Although many aldose reductase inhibitors (ARI) have been extensively studied with promising results, none have demonstrated sufficient efficacy in human clinical trials. The likely cause of these side effects is a lack of selectivity towards related enzymes involved in the detoxification of reactive aldehydes, particularly aldehyde reductase (ALR1). Many ARI are shown to equally interact with aldehyde reductase. During the last three years, many dietary sources for their potential to inhibit aldose reductase were screened and found significant inhibition with some dietary sources. Then the specificity of these dietary ARI against ALR1 was investigated. Specific inhibition of aldose reductase by the dietary sources in the study has given a direction to explore the potential of

dietary sources against secondary complications of diabetes as a food based therapeutic approach.

5.2. STUDIES ON PHYSIOLOGICALLY ACTIVE NON-NUTRIENTS IN FOODS

5.2.1. Foetal programming for adult diseases

Effect of maternal dietary trace element (Mg, Mn, Cr and Zn) restriction on the body adiposity and insulin resistance in the offspring.

In earlier reports WNIN rats that maternal chronic magnesium restriction irreversibly increased the body fat % and decreased lean body mass (LBM), fat free mass (FFM), insulin response to glucose challenge in the offspring up to 18 months of age. Insulin resistance (HOMA IR) which was significantly higher at 6 months of age continued to be high till 18 months albeit not significant. In addition to lowered muscle mass (decreased LBM & FFM) basal glucose uptake was also significantly and irreversibly reduced in magnesium restricted (MgR) offspring at 18 months of age. The increased adiposity was associated with significant hypoleptinemia and increase in TNF levels while adiponectin levels were unchanged. In line with these observations, expression of fatty acid synthase (FAS) and fatty acid transporter protein 1 (FATP1) was increased in liver and adipose tissue. However, preliminary observations seem to suggest that the observed alterations in the expression of the proteins mentioned above may not be due to altered levels of corresponding mRNAs.

5.2.2. Generation of database on the phenolic content and health enhancing effects of plant foods commonly consumed in India

As part of efforts to generate a database on the phenolic content of plant foods commonly consumed in India and on their health enhancing effects including antioxidant activity (AOA), the total phenolic content and AOA (by three different methods : FRAP, DPPH – radical scavenging activity and reducing power) of some commonly consumed cereals, millets, legumes, pulses green leafy vegetables, fruits (including dry fruits) and spices were determined. In general, phenolic content and antioxidant activities determined by the three different methods showed a wide range of distribution in

all the types of plant foods studied. Among the foods tested the AOA showed the following order : spices > vegetables and fruits > legumes and pulses > cereals and millets. Correlation analysis indicated that phenolics were correlated variably with different types of AOA in different classes of foods tested. For example, they were correlated maximally to DPPH radical scavenging activity and FRAP in dry fruits, with only FRAP in cereals and millets whereas they showed no significant correlation with any type of AOA in legumes and pulses. In spices, PC was correlated maximally with DPPH radical scavenging activity followed by inhibition of auto oxidation of carotene in carotene- linoleic acid mixture.

6. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

6.1 Food Toxicology

6.1.1. Exploratory study on kidney disease cases in Uchapally village, Nellore District, Andhra Pradesh

A community-based cross-sectional study was carried out during October 2006 in Uchapally village of Nellore district, Andhra Pradesh, India. A total of 52 subjects including 32 subjects suffering from kidney related problems (Group A), (identified by District Health Officials) and 20 apparently normal subjects, randomly selected from the health camp (Group B) for comparison was studied. Community based cross-sectional study was carried out in Uchapally village of Andhra Pradesh to know the cause of endemic kidney damage.

Chronic kidney disease staging was done according to MDRD formula (Internet version). As per the formula all the subjects in Group A were in different stages of kidney disease. Of them, about 81% were in stage 4 and 13% were in stage 5 of chronic kidney disease. Similarly, about 80% of the subjects in Group B were also in different stages of kidney disease, with 40% in stage 2 followed by 20% in stage 3 and 10% in stage 4 of chronic kidney disease.

The higher proportion of osteosclerosis, secondary to kidney damage may be due to high levels of strontium and silica in ground water, which is the only drinking water source in the village.

6.1.2. Multicentric study on analysis of pesticide residues in sugar samples

The presence of pesticides in foods and beverages is a matter of concern. Some commonly consumed carbonated drinks were analysed for pesticide residues and the report has been submitted to the concerned authorities.

6.1.3. Capacity Building Project on Food and Drug Safety

In order to create awareness on food safety and strengthen food safety monitoring system in the country, Government of India has initiated several projects under overall World Bank assisted Capacity Building Project. One of the important components of the project is to conduct surveys which cover household and street foods. The household survey was carried out by FDTRC, NIN with the assistance of four regional centres with the objective of understanding community perceptions and practices related to food and drugs. A grant of Rs. 1.27 crore was given to conduct the survey. The survey was conducted in 28 states. NIN was nodal centre and regional centre for south.

The results of the survey indicated that about 64% Indians are non-vegetarians of whom 63% store cooked non-vegetarian food at room temperature. Only 29% consumed stored food after reheating. Majority of households reported that they buy packed foods, out of whom, only 21% look for symbols on the label. About 54% of respondents did not know or associate diarrhoea as a symptom of foodborne disease, while about 56% to 70% did not associate abdominal pain, nausea and vomiting as symptoms of foodborne diseases. The reported prevalence of foodborne diseases was 13.2% at household level and 3% at the community level.

With reference to drug quality, the study revealed that most of the respondents (84%) claim to consult qualified doctor and 80% stated that their pharmacists insist on doctors' prescription. About 66% of the respondents buy drugs in packed condition and 46% check the expiry date. About 63% on an average are aware of disposable syringes and out of them atleast 75% insist on use of disposable syringes and needles. Based on the findings of the survey, the Ministry of Health and Family Welfare is developing intervention strategies to ensure total food and drug safety in the country.

6.2 CANCER AND XENOBIOTICS

6.2.1. Effect of diallyl sulphide on atherogenic properties in subjects with diabetes mellitus

Plant foods are rich in non-nutrients, phytochemicals and some of them are extensively used in traditional medicine to treat some common ailments. Incidence of non-communicable diseases is on the rise and require management of diseased state alongside treatment. Spices / condiments used in Indian diet can help in preventing some of the complications associated with long duration of diseased state. Allium vegetables like garlic and onions are known to possess many medicinal properties. Diallyl sulphide (DAS), one of the major chemical constituent is known to contribute to antioxidant property of garlic. In this study, antiatherogenic potential of diallyl sulphide was assessed by measuring the ADP induced platelet aggregation in the presence and absence of DAS using blood samples obtained from diabetic subjects. There was significant reduction in platelet aggregation by DAS. The results suggest that regular intake of garlic through diet may be helpful in reducing one of the secondary complications in diabetes mellitus.

7. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

The Centre carries out both service as well as basic research activities. It also extends research support to the Institute in terms of supply of animals and technical help.

Breeding and supply of animals, supply of stock and experimental feed, supply of blood/sera from animals are some of the major service activities of the centre. The supply of animals increased by 20% this year and correspondingly there was an increase of 20% income compared to the previous year. It was also true for the supply of stock animal feed, which showed an increase of 36%. There was lot of enquiries for experimental animal feeds and the centre supplied 458.25 kg of need based diets. The centre continued to supply blood/sera from various laboratory animals to several institutes.

Routine microbiological health monitoring was carried out in randomly selected animals as well as in accessory items entering the animal

cages like bedding, feed and water. There was an increase in the incidence of air-borne organisms, which is expected to be reduced once the renovation of the facilities is taken up.

The renovation of the animal facilities has started and it is expected to be completed in 9-10 months. Efforts to establish a 'National Animal Resource Facility' at the Genome Valley, Turkapally, Hyderabad, gathered momentum with the registration of 102 acres of land gifted by the state government in favour of ICMR. The proposal is with the planning commission and the budgetary requirement is projected to be Rs.244.34 crores for five years.

Research support

The centre supplied animals and extended technical help for conducting 8 IAEC approved regular animal experiments of the Institute. It also extended help to Pre-clinical Toxicology Centre, and evaluation of proprietary peptide molecule Genopep I and II were completed in mice, rabbits and the report was submitted to sponsor. Studies in tetravalent vaccine supplied by Indian immunologicals have been initiated this year.

With the registration of primate facilities during the period, the requests for conducting experiments in monkey from other Institutions (Private and Government), especially for pharmacokinetics studies can now be entertained.

8. PRE-CLINICAL TOXICOLOGY (PCT)

8.1 Centre for Advanced Research for Pre-clinical Toxicology

In the recent past, with the development of biotech products, DNA vaccines, plant products, drugs, chemicals, genetically modified foods etc. using indigenous technology, the clinical use of such products requires methodology development specially for their safety evaluation both at pre-clinical and clinical levels. In view of this, ICMR has established a Centre for Advanced Research for Pre-clinical Toxicology at NIN in the year 2000 and with financial support for a period of 5 years to conduct safety studies (pre-clinical toxicology data) especially for biotech products and other pharmaceuticals.

The centre has not only got the recognition at national level, but has generated scientific data

as well as financial turnover to the tune of Rs.1.5 crore. Among the various important programmes undertaken, Non-clinical toxicity studies of Japanese Encephalitis Vaccine (LIVE ATTENUATED SA-14-14-2), Safety evaluation of Proprietary peptide molecules as anti cancer agents, Pre-Clinical Toxicity Evaluation of Ayurvedic Bhasmas (herbo-mineral preparations) have been reported in 2005 - 06. The center also has conducted brain storming sessions and became a centre for developing human resources whose services are needed for various industrial sectors. In appreciation of the efforts, the centre has received grant of Rs.2.5 crore from DST to upgrade the facility on par with International standards. In the current year, efforts are being made to get the GLP accreditation.

8.2 Non-clinical toxicity studies of Japanese encephalitis vaccine (Live Attenuated SA-14-14-2); Pre-clinical toxicity evaluation of proprietary peptide molecule GENOPEP 1 (ISSAR 1)

The pre-clinical toxicological testing of Japanese Encephalitis Vaccine (JE) and Genoep 1 which was developed for use as anticancer drug has been completed. The neurovirulence was negative with administration of JE vaccine by intrathecal suggesting its safety under conditions of acute exposure. Sub-chronic toxicity test indicated no abnormalities. The results were submitted to Ministry of Health, so that phase I and phase II clinical trials can be taken up. The Genoep 1 showed no abnormal toxicity except for mild genotoxicity in rats.