

# वार्षिक प्रतिवेदन ANNUAL REPORT 2006 - 2007



राष्ट्रीय पोषण संस्थान  
National Institute of Nutrition

(भारतीय आयुर्विज्ञान अनुसंधान परिषद)  
(Indian Council of Medical Research)

हैदराबाद - 500 604  
Hyderabad - 500 604

# **ANNUAL REPORT**

## ***2006-2007***



**NATIONAL INSTITUTE OF NUTRITION**  
(Indian Council of Medical Research)  
Hyderabad-500 604, INDIA  
[www.ninindia.org](http://www.ninindia.org)



आचार्य एन. के. गांगुली

महानिर्देशक

**Prof. N.K. Ganguly**

MD.FRC Path. (London), FAMS, FNA, FASc, FNASc  
Director General

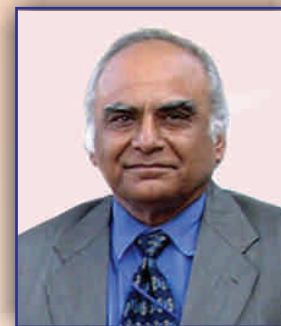


भारतीय आयुर्विज्ञान अनुसंधान परिषद

अंसारी नगर, पोस्ट बॉक्स 4911, नई दिल्ली -110 029

**Indian Council of Medical Research**

Ansari Nagar, Post Box 4911, New Delhi-110029



## MESSAGE

The interdisciplinary nature of research activity has always been the main feature of NIN's scientific endeavours. A good blend of basic, clinical and community research and right emphasis on appropriate extension programmes and training methodologies have helped in the strengthening of institute's research component this year.

I am happy to note that the research work done this year has paved way for the transfer of technology relating to double fortification of salt with iron and iodine. Commercialization of this technology is now expected to draw the attention of both small and large scale industries and make this value added salt available to the common man. The interest evinced by the scientists this year in the areas of HIV/AIDS, pre-clinical toxicity studies on JE vaccine, use of innovative approaches like positive deviance in community health and capacity building in the realm of food and drug safety is indeed commendable.

I take this opportunity to inform you all of the establishment of a new Research Centre in the honour of Dr.Yellapragada SubbaRow, the eminent medical mind of yesteryears. This Centre located on the Institute's campus will act as a storehouse for all the materials of archival importance – films, monographs, audio-visual material, research papers, personal diaries and other priceless documents pertaining to this inimitable genius. I hope that this Centre, which is expected to start functioning this year, would attract the attention of the younger generation towards the works of this remarkable scientist.

I extend my best wishes to all the staff of this institute and hope that their research endeavours address the hitherto unmet needs, especially of the marginalized sections of our society.

Prof. N.K. Ganguly



# CONTENTS

	Page No.
RESEARCH STAFF	i
RESEARCH HIGHLIGHTS	iii
I. COMMUNITY STUDIES	
1. Evaluation of positive deviance programme in West Bengal	1
2. Assessment of nutritional status of under 5 year rural children in select districts of India: Govt of India –UNICEF programme of co-operation: 2003-2007	3
II. CLINICAL STUDIES	
1. Double blind, randomized, controlled, clinical trial of Zinc supplementation to full term infants	16
2. Assessment of prevalence of osteoporosis in adult population in India - Indian Council of Medical Research (ICMR) Task force study	17
III. MICROBIOLOGY AND IMMUNOLOGY	
1. Role of zinc – In the triad of truncal adiposity, insulin sensitivity and proinflammatory cytokines: Baseline data	21
IV. BASIC STUDIES	
1. Validation of iron bioavailability using the caco-2 cell model	23
2. Commercialization of Double Fortified Salt	25
3. Developmental origins of adiposity and insulin resistance: Role of maternal and postnatal Magnesium status	26
4. Anticataractogenic effect of <i>Embllica officinalis</i> and its tannoids against streptozotocin induced diabetic cataract in rats	31
5. Molecular chaperone function of $\alpha$ -crystallin under hyperglycemic conditions: Modulation by dietary factors – Modulation of chaperone activity of $\alpha$ -crystallin under hyperglycemic conditions by antiglycating agent MYB2	34
6. Screening and testing of aldose reductase inhibitors and antiglycating agents from dietary sources – Specificity of dietary aldose reductase inhibitors	37
7. Importance of $\alpha$ - crystallin heteropolymer in the eye lens: Chaperone-like activity and physicochemical properties	39
8. Acute effect of AOA rich legume sprout salads on oxidative stress and antioxidant status of adult human volunteers	42
9. Health beneficial effects of foods	44
i. Antioxidant activity and phenolic content of cereals and millets	
ii. Antioxidant activity and phenolic content of spices commonly consumed in India	

	Page No.
10. Pro12 ala polymorphism in PPAR $\gamma$ and its association with metabolic syndrome in a cohort from South India	48
11. Understanding the mechanism of action of PPAR $\gamma$ in regulation of glucose metabolism	50
<b>V. PATHOLOGY</b>	
1. EDAX – ray analysis of oil seeds; sesame (TIL), mustard, groundnut, castor, cotton and coconut	53
<b>VI. EXTENSION &amp; TRAINING</b>	
Service Activities	54
<b>VII. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE</b>	
<b>A. FOOD SAFETY</b>	
1. Exploratory study on kidney disease cases in Uchapally village, Nellore district, Andhra Pradesh	57
2. Multicentric study on analysis of pesticide residues in sugar samples	60
3. Capacity Building Project on Food and Drug Safety	61
<b>B. CANCER AND XENOBIOTICS</b>	
1. Effect of diallyl sulphide on atherogenic properties in subjects with diabetes mellitus	64
<b>VIII. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES (NCLAS)</b>	
Service Activities	66
<b>IX. PRE-CLINICAL TOXICOLOGICAL STUDIES</b>	
1. Non-clinical toxicity studies of Japanese encephalitis vaccine (Live Attenuated SA-14-14-2)	70
2. Pre-clinical toxicity evaluation of proprietary peptide molecule GENOPEP 1 (ISSAR 1)	71
INSTRUMENTATION SERVICES	73
LIBRARY AND DOCUMENTATION SERVICES	76
Ph.D PROGRAMMES	78
AWARDS/ HONOURS CONFERRED ON SCIENTISTS	80
PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS/ WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING	82
WORKSHOP/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN	83
SERVICES RENDERED TOWARDS INCOME GENERATION	84
SCIENTIFIC PUBLICATIONS	85
SCIENTIFIC ADVISORY COMMITTEE	90

# RESEARCH STAFF

**DIRECTOR**  
B.Sesikeran, MD

## CLINICAL DIVISION

### MATERNAL AND CHILD HEALTH

Veena Shatrugna, MD  
(Deputy Director)  
B.A. Ramalakshmi, MBBS, DGO  
K. V. Radhakrishna, MBBS, DCH  
G. Jagjeevan Babu, MBBS  
Bharati Kulkarni, MBBS, DCH  
G. Amarendra Reddy, MA, MPhil  
Prabhavati Paranjape, BSc

### PATHOLOGY AND MICROBIOLOGY

L. Singotamu, MSc, PhD  
(Deputy Director-Sr-Gr)  
P. Uday Kumar, MD  
R. Hemalatha, MD  
M. Shiva Prakash, MSc, PhD  
SSYH. Qadri, MVSc  
L.A. Ramaraju, MSc

## BIOCHEMISTRY DIVISION

A. Vajreswari, MSc, PhD  
(Deputy Director)  
V.Vijayalakshmi, MSc, PhD  
Ahmed Ibrahim, MSc, PhD  
C. Suresh, MSc, PhD  
Sanjay Basak, MSc, PhD  
S.Hemalatha, MSc, PhD  
S. M.Jeyakumar, MSc, Mphil, PhD  
A.Prashanth, MSc  
G.Sashikiran, MSc  
Sheril Alex, MSc  
SSV.Prasad, MSc  
J.Shashidhar, MSc PhD

### MOLECULAR BIOLOGY

Nasreen Zafar Ehtesham, MSc, PhD  
(Deputy Director)  
Sudip Ghosh, MSc, PhD  
N. Vijaya Banu, MSc, PhD  
Abdul Haseeb, MSc  
B. Aruna, MSc  
Mohd. Nasiruddin, MSc  
M.Srivani, MSc  
D.Munni Kumar, MSc

## BIOPHYSICS DIVISION

K. Madhavan Nair, MSc, PhD  
Y. Venkataramana, MSc, PhD  
S. Ranganathan, MSc, PhD  
P. Raghu, MSc, PhD  
P. Ravinder, MSc, PhD  
B. Satyanarayana, MSc  
K. Sreenivasulu, MSc  
Vasuprada Iyengar, MSc  
Gitanjali, MSc

## FOOD CHEMISTRY DIVISION

T. Longvah, MSc  
(Deputy Director)  
P. Amrutha Rao, MBBS, DPH  
K. Bhaskarachary, MSc, PhD  
J.Sreenivasa Rao, MSc  
S.Devendra, MSc, Mphil, PhD  
P. Sujata, MSc, PhD, PGDN & DM  
P. Ramulu, MSc, PhD

## ENDOCRINOLOGY & METABOLISM DIVISION

M. Raghunath, MSc, PhD  
(Deputy Director)  
G. Bhanu Prakash Reddy, MSc, PhD  
Rita Saxena, MSc, PhD  
P. Suryanarayana, MSc, PhD  
K.Rajender Rao, MSc, PhD  
C. Vijayakumar Reddy, MSc, PhD  
S. Chennaiah, MSc, PhD  
D. Sreeramulu, MSc, PhD  
P. Anil Kumar, MSc  
Megha Saraswat, MSc  
T. Mrudula, MSc  
PNBS Srinivas, MSc  
Y. Durgakishore, MSc  
IJN. Padmavathi, MSc  
Manisha Ganeshan, MSc  
P.B.Sainath, MSc  
K.Anand Kumar, MSc  
P.Muthenna, MSc



### FIELD DIVISION

G.N.V. Brahmam, MBBS, DPH  
(Deputy Director-Sr-Gr)  
Shahnaz Vazir, MA, PhD  
A. Laxmaiah, MBBS, MPH  
R. Harikumar, MBBS, DPH  
N. Arlappa, MBBS  
Ch. Gal Reddy, MA, MPhil  
K. Mallikharjuna Rao, MSc, PhD  
I.I.Meshram, MBBS, MD(PSM)  
M.S. Radhika, MSc, PhD  
Sharad Kumar, MA, MPhil  
M. Ravindranath, MA  
Little Flower Augustine, MSc

### STATISTICS DIVISION

K. Venkaiah, Msc  
(Deputy Director)  
T. Prasanna Krishna, MSc, PhD  
M. Vishnuvardhan Rao, MSc, PhD  
N. Balakrishna, MSc, PhD  
Grace Maria Antony, MSc, PGDCA

### EXTENSION & TRAINING DIVISION

Krishnakumari Menon, MSc  
D. Raghunatha Rao, MSc, PhD  
T. Vijaya Pushpam, MA, Mphil  
M.Maheshwar, MCom, MA, MCJ, LLB  
G. M. Subba Rao, MA, PGDJ, PGDT  
K. Damayanthi, MSc, PhD  
Anilkumar Dube, MA, MCJ, DPM  
R. Nageswara Rao, MSc, BJ

### FOOD & DRUG TOXICOLOGY RESEARCH CENTRE (FDTRC)

Director  
B.Sesikeran, MD  
Kalpagam Polasa, MSc, PhD, MBA  
(Deputy Director)

#### FOOD TOXICOLOGY

Arjun L Khandare, MSc, PhD  
J. Padmaja, MSc, PhD  
V. Sudershan Rao, MSc, PhD  
S. Vasanthi, MSc, PhD  
Priyanka Shankar, MSc

#### DRUG TOXICOLOGY

M.P. Rajendra Prasad, MBBS, MSc (AN), PhD  
B. Dinesh Kumar, MSc, PhD

### NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES (NCLAS)

Director

B.Sesikeran, MD

N.V. Giridharan, MSc, PhD  
(Deputy Director)

S. Kalyanasundaram, MSc

P. Suresh Babu, MVSc

N. Hari Shanker, MSc, PhD

B. Kiran Kumar, MSc

B.Pratibha, Msc

### PRE-CLINICAL TOXICOLOGY

Director

B.Sesikeran, MD

Kalpagam Polasa, MSc, PhD, MBA  
(Deputy Director)

N.V. Giridharan, MSc, PhD

(Deputy Director)

T. Prasanna Krishna, MSc, PhD

S. Kalyanasundaram, MSc

P. Uday Kumar, MD

P. Suresh Babu, MVSc

R. Hemalatha, MD

B. Dinesh Kumar, MSc, PhD

SSYH. Qadri, MVSc

N. Hari Shanker, MSc, PhD

### LIBRARY

M. Devidas, MA, MLISc

### INSTRUMENTATION

M. Raghunath, MSc, PhD

(Deputy Director)

Ramachander Chowgule,

Dip. in Electronics

### ADMINISTRATION

G. Krishna Reddy, B.Com

Alexander Verghese

M.J. Radha Bai

A. Subba Reddy, BA

Mohd.Shamsuddin, B.Com

M. Ashok Raj, B.Com

### MAINTENANCE

P. Rajamohan Rao, LCE, PGDCPEMM

# 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 ( $20.2 \pm 5.34$  Vs  $19.0 \pm 4.4$ ,  $p=0.02$ ). 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 ( $6.8 \pm 5.6$  Vs  $8.1 \pm 6.53$ ,  $p=0.048$ ).

## 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 ( $\geq 110$  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 14<sup>th</sup> 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 16<sup>th</sup> 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 $\alpha$ -crystallin under diabetic cataract by cumin:

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

*(ii) Regulation of Expression of  $\alpha$ -crystallins under diabetic conditions*

Earlier, for the first time, elevated expression of small heat shock protein,  $\alpha$ -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  $\alpha$ -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.



# I. COMMUNITY STUDIES

## 1. EVALUATION OF POSITIVE DEVIANCE (PD) PROGRAMME IN WEST BENGAL

Integrated Child Development Services (ICDS), which was launched in India on 2<sup>nd</sup> October 1975, constitutes one of the important direct policy instruments of the National Nutrition Policy (NNP). Even after two decades, the national nutrition goals set were not achieved to the desired levels. The Government of West Bengal under the aegis of UNICEF-West Bengal has been implementing special interventional programmes to prevent and control the problem of undernutrition since more than a decade.

Introduction of 'PD' programme was a step to accelerate the process of reduction and prevention of undernutrition among under 3 year children, in a short time, by enabling the communities to adopt 'best local practices of childcare', on a sustained basis. The 'PD' is defined as "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 with similar socioeconomic background and resources, and are exposed to the same risks from the existing environment".

Therefore, it is assumed that the PD programme acts as a quality improvement tool for ICDS, to improve its process as well as outcome variables. It emphasizes on 'community investment' and 'participation by change in behaviors' through practice. In the year 2001, the PD Programme was initiated in 32 villages in two Districts, namely South-24 Paraganas and Murshidabad. Subsequently, it was extended to other areas in a phased manner and is currently in operation in 10 blocks of Murshidabad, 2 blocks of South-24 Paraganas, 4 blocks in Purulia and 8 blocks of Dakshin Dinajpur Districts.

At the request of UNICEF, West Bengal, the National Institute of Nutrition (NIN) undertook a study to assess the impact of PD programme on well-being of <3-year children, before the programme is extended to other districts.

### OBJECTIVES

1. To assess various aspects related to the process of implementation of the Positive Deviance programme in terms of training, resource mapping, methods of nutrition counseling and child care services, and
2. To assess the impact of the programme in terms of weighing efficiency among the workers, prevalence of undernutrition, extent of relapse in undernutrition, recording of birth weights, incidence of morbidities viz., diarrhoea, and ARI, extent of exclusive breastfeeding, complementary feeding, immunization of the children, in the areas where the programme is in operation for one year or more and control areas.

### METHODOLOGY

#### STUDY DESIGN

The study was a cross sectional one, by adopting systematic random sampling procedure, with the Anganwadi Centres (AWCs) as 1<sup>st</sup> stage sampling units, and households (HHs) with <3 year child as 2<sup>nd</sup> stage sampling units.

#### SAMPLE SIZE COMPUTATION

Sample size calculations were made on the basis of estimation of the extent of reduction in moderate to severe grade undernutrition among <3-year children. Assuming reduction in the prevalence of moderate to severe grade undernutrition from 30% at base line to 15% (a change by about 50%) after implementation of the programme, with 95% confidence interval and 90% power, the minimum size required for each of the control and intervention areas was worked out as 174 (or say 200). However, to enable sub-set analysis 1000 children of 0-3 years age group were covered from 40 AWCs



(@25 children/AWC) from PD areas and an equal number from control areas.

### **SAMPLING FRAME**

All the AWC villages with the PD programme in operation for one year or more, from the districts of South 24 paraganas, Murshidabad, Dakshin Dinajpur and Purulia formed the sample frame. From these, forty AWC villages were selected for the purpose of study, by systematic random sampling, adopting probability proportion to the size of AWCs in the Districts.

For the purpose of control, AWC villages from the same districts or neighbouring district without PD programme, and matched for total population and female literacy rate at the village level. Care was taken to ensure that the villages selected as control had no influence of PD from the neighbouring intervention areas.

### **STUDY POPULATION**

In each of the selected AWC villages, twenty-five 0-3 year children were selected for the study, by adopting simple random procedure. In addition, a sub-sample of mothers, members of Village Health Committee (VHC)/ Self Help Groups (SHG)/ Village Education Committee (VEC) and programme functionaries were also covered.

### **INVESTIGATIONS**

#### **PROCESS VARIABLES**

Focus Group Discussions (FGDs) were held with the stakeholders (NGO, Govt. Officials, Members of Panchayati Raj Institutions (PRI) about their role and cooperation in the programme and with AWW, Supervisors, SHGs on programme implementation.

#### **OUT-COME VARIABLES**

The out-come variables assessed included, efficiency of weighing of 0-3 year children, proportion of children receiving complementary food at 6 months, the type, frequency and quantity of complementary food given to the beneficiary, proportion of 12-24 months children covered for each of the vaccinations and those completely immunized by one year of age, proportion of 9-36 months children who received first dose of massive vitamin A along with measles

immunization, extent of reduction in the prevalence of undernutrition.

### **ETHICAL ISSUES**

Necessary ethical clearance was obtained from the Institutional Review Board (IRB) before initiation of the study. Informed consent was obtained from the respondent mothers and the village leaders.

### **RESULTS**

The salient features of the study are as follows:

- ♦ A total of 1900 under 3-year young children (950 each from PD and control areas) from 80 AWCs (40 each from PD and control areas) were covered for the study.
- ♦ A significantly higher proportion of HHs (64%) in PD area lived in semi pucca houses, while in contrast 66% of HHs lived in Kutcha houses in control area.
- ♦ A significantly higher proportion of the mothers (64%) in PD area were literate compared to 47% in the control area.
- ♦ Significantly higher proportion of HHs in the PD area followed healthy practices in handling of drinking water, environmental sanitation and waste disposal compared to control area.
- ♦ A significantly higher proportion (37%) deliveries in PD area took place in a health facility compared to control area (29%) mostly assisted by a medical doctor.
- ♦ A significantly higher proportion of home deliveries conducted with the assistance of Trained Birth Attendant (TBA) in the PD area (25%), as compared to 8% in the control area.
- ♦ Extent of recording of birth weight was significantly ( $p < 0.01$ ) higher in the PD area. Also, the mean birth weight was significantly higher ( $p < 0.05$ ) in the PD area (2730 g) compared to control area (2560 g).
- ♦ A significantly higher proportion of mothers in PD area initiated breast feeding within 3 hours of delivery (76%) and did not give pre-lacteal feeds (78%) compared to 44% and 35% respectively in the control areas.



- ♦ The extent of feeding of colostrum (90%) and exclusive breastfeeding up to 4-5 months (70%) were significantly higher in PD area compared to control area (82% and 61% respectively).
- ♦ Among children aged <12 months, 12-23 months and 24-35 months about 19%, 44% and 45% respectively started receiving complementary foods at 6 months of age compared to 13%, 29% and 23% respectively in the control area.
- ♦ A significantly higher proportion of children ( $p<0.01$ ) in the PD area received were fed complementary food with a spoon compared to control area.
- ♦ A significantly ( $p<0.01$ ) higher proportion of children (86%) in PD area were completely immunized, compared to 68% in control area.
- ♦ Supplementation of massive dose vitamin A, a significantly ( $p<0.01$ ) higher proportion (84% in PD area) received one or two doses as compared to 74% in control area.
- ♦ The proportion of children weighed 9 times a year (satisfactory growth monitoring) was significantly ( $p<0.01$ ) higher in PD area (50%), compared to control area (13%).
- ♦ A significantly higher proportion ( $p<0.01$ ) of mothers in PD area (85%) compared to control areas (34%) reported that the AWW discussed the nutritional status of their children with them.
- ♦ A significantly ( $P<0.01$ ) higher proportion of children in PD areas received treatment for diarrhoea (26%) and ARI (48%) from health functionaries such as AWW/ANM/MO compared to 13% and 18% respectively in the control area.
- ♦ The proportion of referrals by the AWW to a government medical / private doctor was significantly ( $p<0.01$ ) high in PD area (37%) compared to 18% in control area.
- ♦ A significantly ( $p<0.01$ ) higher proportion of mothers in PD area (69%) reportedly received Health and Nutrition Education (H & NE) compared to 27% in control areas.
- ♦ A higher ( $p<0.01$ ) proportion of mother's committees, Mahila Mandals, village health

committees and women working groups were operating in PD areas compared to control area.

- ♦ The overall prevalence of undernutrition, according to IAP classification, was similar between the PD and control groups. However, among 12-17 months children the prevalence was significantly ( $p<0.05$ ) lower in PD (55%) compared to control (64%) area.
- ♦ The overall prevalence of underweight and wasting according to SD classification was similar between the two groups while, that of stunting was significantly lower ( $p<0.01$ ) in PD area (26.5%) compared to control area (32%).
- ♦ The prevalence of underweight and stunting (45.6% and 25.2% respectively) was significantly ( $p<0.01$ ) lower in the PD area compared to control area (63.2% and 37.4% respectively) in children aged 12-17 months.
- ♦ No gender differentials were observed in the case of underweight or wasting between the two groups.

The study revealed that the positive deviance programme improved the infant and young child feeding, hygiene practices, improved the coverage for ICDS & health services and facilitated better inter-sectoral coordination and convergence in PD area, in addition to reducing the prevalence of underweight and stunting in the critical age group of 12-17 months.

## **2. ASSESSMENT OF NUTRITIONAL STATUS OF UNDER 5 YEAR RURAL CHILDREN IN 16 SELECT DISTRICTS OF INDIA: GOVT OF INDIA – UNICEF PROGRAMME OF COOPERATION : 2003-2007**

Undernutrition continues to be a major public health problem in the developing world, including India. The most vulnerable groups being women of reproductive age group and young children. Maternal undernutrition, faulty infant and young child feeding practices, lack of personal hygiene and environmental sanitation,



coupled with recurrent infections and infestations and a host of socio-economic and demographic factors largely contribute to high prevalence of undernutrition in the community.

Children, who are subjected to socio-economic and dietary constraints during their critical years of growth and development, end up as adults with small body size. Such adults may be apparently healthy, but there is evidence that their productivity and earning capacity might be impaired. Repeat surveys by National Nutrition Monitoring Bureau (NNMB, 1999) in eight States revealed that, despite of very little or no change in the dietary intakes of rural population over a period of time, there was a decrease in the prevalence of severe forms of undernutrition among young children with concomitant increase in normal grade. However, the proportion of children with mild to moderate undernutrition remained similar. Recent survey carried out by NNMB (2006) in the rural areas of nine states revealed that about 55% preschool children are of underweight, 52% are stunted and 15% are wasted.

The Government of India (Gol) and the respective State Governments have been implementing several health, nutrition intervention and developmental programmes for the overall improvement of health and nutrition status of the community. UNICEF-India has initiated 'Gol-UNICEF programme of co-operation during 2003-2007' in 42 'focal districts' spread over 14 States of the country, for the improvement of health and well-being of women and children. In order to facilitate assessment of impact of the programme at a later stage of implementation, a baseline survey was carried out by National Sample Survey

Organization (NSSO), during 2005-06 in all the 42 districts, to assess the, (i) Health of children and mothers (ii) Schooling and educational attainment of children, especially of girls, (iii) Sanitation, hygiene and water supply, (iv) Child protection and (v) HIV-AIDS.

In order to develop data base on the nutritional status of under five-year children, at the request of UNICEF-India, NIN carried out survey in a sub sample of 16 districts from 13 States, by covering 50% of the villages surveyed earlier by NSSO (Table 1).

### OBJECTIVES

1. To assess the nutritional status of <5 year children in terms of anthropometric measurements such as heights & weights.
2. To assess the breast feeding and complementary feeding practices among children aged 0-23 months, and
3. To estimate iodine levels in the salt samples used for cooking in the households (HHs), using spot testing kit.

### METHODOLOGY

#### STUDY DESIGN

It was a cross sectional, community survey, adopted random sampling procedures.

#### SAMPLING PROCEDURE

#### SAMPLE SIZE

Assuming an overall prevalence of underweight (weight for age < median-2SD of NCHS standards) of 50% among <5 year children, with 5% relative precision and 95% CI, a sample size of 383 (or 400) children per district was arrived at.

**Table 1. States and Districts covered for the study**

Sl. No.	District	State	Sl. No.	District	State
1.	Medak	Andhra Pradesh	9.	Chandrapur	Maharashtra
2.	Dibrugarh	Assam	10.	Latur	Maharashtra
3.	Vaishali	Bihar	11.	Nandurbar	Maharashtra
4.	Valsad	Gujarat	12.	Koraput	Orissa
5.	East Singbhum	Jharkhand	13.	Tonk	Rajasthan
6.	Raichur	Karnataka	14.	Krishnagiri	Tamil Nadu
7.	Guna	Madhya Pradesh	15.	Lalitpur	Uttar Pradesh
8.	Shivpuri	Madhya Pradesh	16.	Purulia	West Bengal



## SELECTION OF VILLAGES

For the purpose of survey, NSSO divided each district into 30 strata and covered two villages from each stratum. For the purpose of present study, one village was randomly selected from each stratum, thus covering a total of 30 villages per district.

## SELECTION OF HOUSEHOLDS

In each of the selected villages, a total of 20 HHs having at least one index child of <5 years of age were covered by adopting cluster sampling procedure. For this purpose, the main village and its hamlets, if any, were divided into 5 geographical areas, based on natural groups of household/ streets/ mohallas/ areas etc. HHs belonging to Scheduled Caste and Scheduled Tribe communities formed one group. From each of these groups, four consecutive HHs were surveyed by starting from the Northeast corner of the area. In each of the selected HH, all the children of <5 year were covered for the survey. Thus, a total of about 600 children were covered from 30 villages in a district.

A total of 12,879 children of below five years of age from 9,576 HHs, in 480 villages of 16 districts, in 13 states were covered for anthropometry. Mothers of 2,962 children of 0-12 months & 2,880 children of 12-23 months were interviewed for child feeding practices. In addition, salt samples from all the HHs were tested for iodine content using spot testing kits.

*District-wise salient observations are as follows:*

### 1. MEDAK DISTRICT, ANDHRA PRADESH

- ◆ About two thirds of the HHs belonged to Other Backward Communities, while a fourth of HHs belonged to Scheduled Castes and only 3% to Scheduled Tribes. About 39% each were joint and nuclear families. About 58% of the fathers and 36% mothers of index children were literates. The average family size was 6.0. The major occupation of the head of HH was labour (49%), followed by agriculture (25%), business (9%) and service (7%).
- ◆ Eighty one percent of the HHs were semi-pucca and 9% each were kutcha and pucca. The major sources of drinking water were tap (83%), followed by tube well (17%).

- ◆ About 84% women reportedly fed colostrum to the newborn. About 61% of the women initiated breast feeding within three hours of delivery. About 44% of the women reportedly gave pre-lacteal feeds, the most common ones being honey water (23%) and cow/goat milk (7%).
- ◆ About 57% of 6-11 months children started receiving complementary foods before 6 months of age. About 14% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 87% were receiving complementary feeding in addition to breast feeding, while the rest were solely breastfed. About 64% of the children were receiving complementary feeding  $\geq 3$  times a day, while 24% were receiving 4-5 times a day.
- ◆ About 89% of 12-23 months children were completely immunized. About 98% and 91% received BCG and Measles vaccines respectively.
- ◆ About 39% of the HHs were consuming adequately iodized ( $\geq 15$  ppm) salt.
- ◆ The prevalence of Bitot spots was about 1.9%. Fever (37%), ARI (9.4%) and diarrhoea (6.2%) were the common morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 51% of children were underweight (weight for age < median-2SD), 35% were stunted (height for age < median-2SD), and 16% were wasted (weight for height < median-2SD).
- ◆ Significant ( $P < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was relatively higher among children of SC and ST communities, HHs engaged in labour, having illiterate parents, living in semi-pucca or kutcha houses, using firewood as cooking fuel and those not having electricity and sanitary latrine.

### 2. DIBRUGARH DISTRICT, ASSAM

- ◆ A fourth (25%) of the HHs covered belonged to ST, while 11% belonged to SC. OBC and OC



accounted for 41% and 24% of HHs respectively. A majority of the HHs were nuclear (54%), followed by joint (25%) and extended nuclear families (21%). About 73% fathers and 57% mothers of index children were literates. The average family size was 6.0. The major occupation of the head of the HH in a majority of the HHs was labour (55%), followed by agriculture (15%), business and service (11% each).

- ◆ Fifty four percent of the HHs were living in kutcha houses, 38% in semi pucca and 8% in pucca houses. Tube well formed the major source of drinking water in a majority (95%) of the HHs.
- ◆ About 83% of women reportedly fed colostrum to the newborns. About 62% of the women initiated breastfeeding within three hours of delivery. About 21% reportedly gave pre-lacteals, the most common ones being, cow/buffalo/goat milk (20%), honey and plain water (4% each).
- ◆ Of the 6-11 months children, about a half (50%) started receiving complementary foods before the age of 6 months, while about 21% were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 90% were receiving complementary foods in addition to breast milk, while the rest were solely breastfed. The frequency of complementary feeding was 3 times a day in about 67% of the children, while about 30% were receiving the same 4-5 times a day.
- ◆ About 65% of 12-23 months children were completely immunized. About 90% and 67% received measles and BCG vaccines respectively.
- ◆ About three fourths (73%) of the HHs surveyed were consuming adequately iodized (15 ppm) salt.
- ◆ The prevalence of Vitamin A Deficiency (VAD) signs such as Bitot spots was about 0.4%. Prevalence of dental caries was 11%. About 51% and 28% of children reportedly had fever and ARI respectively during the preceding 15 days of survey.

- ◆ About 45% of the children were underweight (weight for age < Median -2SD), and 32% were stunted (height for age < Median -2SD) indicating chronic undernutrition. Wasting (weight for height < Median -2SD) was observed in 15% of the children, indicating current undernutrition.
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. Undernutrition (underweight and stunting) was observed to be significantly higher among children of SC and ST communities, those from HHs engaged in labour, having illiterate fathers, those living in kutcha or semi-pucca houses and in HHs using firewood as cooking fuel. The prevalence of underweight, stunting and wasting was significantly high among children of cultivators, illiterate mothers, in HHs not having electricity and where a sanitary latrine was not available/not used. The prevalence of underweight and wasting was also significantly high among children with history of morbidity in the preceding 15 days of survey.

### 3. VAISHALI DISTRICT, BIHAR

- ◆ Of the HHs covered, about 45% belonged to SC, 21% to OBC, and 32% belonged to Other Communities. About 45% were joint families and 33% were nuclear families. About 60% fathers and 34% mothers of index children were literates. The average family size was 8.0. The major occupation of the head of the HH was labour (42%), followed by agriculture (15%), business (14%) and service (9%).
- ◆ Thirty nine percent of the houses were kutcha, 31% were semi-pucca and 30% were pucca in nature. The major source of drinking water in a majority of the HHs was tube well (92%).
- ◆ About 87% women reportedly fed colostrum to the newborns. Forty two percent of women initiated breastfeeding within three hours of delivery and more than half (58%) of the women reportedly gave pre-lacteals, the most common ones being, sugar water (27%) and cow/buffalo/goat milk (17%).



- ◆ About 14% of 6-11 months children started receiving complementary foods before 6 months of age while large proportion (62%) was solely breastfed even after 11 months of age. Of the 12-23 months children covered, 87% were receiving complementary foods in addition to breast milk, while the rest were solely breast-fed. Fifty five percent of the children were receiving complementary feeding 3 times, while 39% were receiving 4-5 times a day.
- ◆ Only about half (52%) of 12-23 months children were completely immunized. About 77% and 60% received measles and BCG vaccines respectively.
- ◆ About two third (63%) of the HHs were consuming adequately iodized (15 ppm) salt.
- ◆ The prevalence of Bitot Spots and Conjunctival Xerosis was 3.1% and 2.3% respectively, indicating a problem of public health significance. Prevalence of Dental Caries was 8%. High prevalence of morbidities such as fever (35%), diarrhoea (36%) and ARI (28%) were reported in children during the preceding 15 days of survey.
- ◆ About 47% of the children were underweight (weight for age < Median -2SD), and 45% were stunted (height for age < Median - 2SD) indicating chronic undernutrition. Wasting (weight for height < Median -2SD) was observed in 17% of the children, indicating current undernutrition.
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. All the three forms of undernutrition (underweight, stunting and wasting) was observed to be significantly higher among children of SC and ST communities, landless labourers, those living in kutcha and semi-pucca houses and HHs where sanitary latrine was not available / not used. The prevalence of underweight and stunting was significantly high among children of illiterate parents, while a significantly high proportion of children in HHs using firewood as cooking fuel were underweight. Wasting was observed in a significantly high proportion

of children in HHs not having electricity. The prevalence was also significantly high among children with history of morbidity in the preceding 15 days of survey.

#### 4. VALSAD DISTRICT, GUJARAT

- ◆ A majority of the HHs (73%) belonged to ST, followed by Other Communities (14%) and OBC (10%). About 48% were nuclear and 32% were joint families. About 78% of the fathers and 71% mothers of index children were literates. The average family size was 5.0. The major occupation of the head of HH was labour in a majority of the HHs (44%), followed by agriculture (32%), service (17%) and business (6%).
- ◆ Fifty seven percent of the houses were semi-pucca, 42% were kutcha and 11% were pucca in nature. The major source of drinking water in a majority of HHs was tube well (76%), followed by open well (16%) and tap (8%).
- ◆ Majority (79%) of women reportedly fed colostrum to the newborn and about 79% of the women initiated breastfeeding within three hours of delivery. Only 12% of the women reportedly gave pre-lactal feeds, the most common ones being sugar water (5%) and milk (4%).
- ◆ About 32% of 6-11 months children started receiving complementary foods by 6 months of age. About 27% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 75% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 43% of the children were receiving complementary feeding  $\leq 3$  times a day, while 37% were receiving 4-5 times a day.
- ◆ About 87% of 12-23 months children were completely immunized. About 96% and 88% received BCG and measles vaccines respectively.
- ◆ About 44% of the HHs were consuming adequately iodized (15 ppm) salt.
- ◆ None of the children examined had Bitot spots. Fever (27%), ARI (11%) and diarrhoea (3%) were the common morbidities experienced by



the children during previous fortnight of the survey.

- ◆ About 55% of children were underweight (weight for age <median-2SD), 37% were stunted (height for age <median-2SD) and 22% were wasted (weight for height <median-2SD).
- ◆ Significant ( $P<0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was significantly higher in children of SC and ST communities, labourers, illiterate parents, those living in semi pucca or kutcha houses, HHs using firewood as cooking fuel, those not having electricity and sanitary latrine.

#### 5. EAST SINGHBHUM DISTRICT, JHARKHAND

- ◆ About 44% of the HHs belonged to ST, while 23.4% belonged to SC. OBC and OC accounted for 16.1% each. Of the HHs covered, 44% were nuclear families, while joint and extended nuclear families accounted for 41.3% and 14.7% respectively. About 54% of the fathers and 37.5% mothers of index children were literates. The average family size was 6.0. The major occupation of the head of HH was labour (52.1%), followed by agriculture (32.6%), business (7.2%) and artisans (2.9%).
- ◆ About 45% each of the houses were either kutcha or semi-pucca in nature and 9.9% were pucca. The major sources of drinking water in a majority of HHs was tube well (69.6%), followed by open well (26.4%).
- ◆ About 80% women reportedly fed colostrum to the newborn. Majority (62.4%) of the women initiated breast feeding within three hours of delivery. About 24% of the women reportedly gave pre-lacteal feeds, the most common ones being honey water (10.6%), plain water (4.5%), cow/buff/goat milk (4.5%) and sugar water (2.9%).
- ◆ Forty five percent of 6-11 months children started receiving complementary foods before 6 months of age. Thirty eight percent of children were solely breastfed even after 11 months of age. Of the 12-23 months children

covered, 95% were receiving complementary feeding in addition to breast feeding, while the rest were solely breastfed. About 53% of the children were receiving complementary feeding 4-5 times a day, while 34% were receiving 3 times a day.

- ◆ About 75% of 12-23 months children were completely immunized. About 95% and 76.9% received BCG and Measles vaccines respectively.
- ◆ Majority (97.5%) of the HHs were consuming adequately iodized (15 ppm) salt.
- ◆ The prevalence of conjunctival xerosis was 4.6%. ARI (45.1%), fever (28.6%) and diarrhoea (8.3%) were the common morbidities experienced by the children during previous fortnight of survey.
- ◆ About 57% of children were underweight (weight for age < median-2SD), 36.3% were stunted (height for age < median-2SD) and 23.9% were wasted (weight for height <median-2SD).
- ◆ Significant ( $P<0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of underweight and stunting was significantly high among children born to illiterate parents, fathers involved in labour activities and staying in the HHs, where electricity was not available. The prevalence of stunting was also significantly higher among children with history of morbidity during preceding 15 days of survey, from the HHs, where sanitary latrine was not available or not using the same and among landless Hhs.

#### 6. RAICHUR DISTRICT, KARNATAKA

- ◆ About a fifth of the HHs each (21%) belonged to ST, and SC, while 33% belonged to OBC. About 40% each were joint & nuclear families. About 43% fathers and 24% mothers of index children were literates. The average family size was 7.0. The major occupation of the head of HH was labour (54%), followed by agriculture (27%), business (6%) & service (5%).
- ◆ Fifty one percent of the houses were semi pucca, 27% were kutcha and 22% were Pucca



in nature. The major sources of drinking water were tube well (57%), followed by tap (27%).

- ◆ About 57% women reportedly fed colostrum to the newborn. Only about 33% of the women initiated breast feeding within three hours of delivery. About 62% of the women reportedly gave pre-lacteal feeds, the most common ones being, sugar water (20%) and honey water (18%).
- ◆ About 33% of 6-11 months children started receiving complementary foods before 6 months of age. About 17% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 85% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 82% of the children were receiving complementary feeding  $\leq 3$  times a day, while only 3% were receiving 4-5 times a day.
- ◆ About 72% of 12-23 months children were completely immunized. About 91% and 74% received BCG and Measles vaccines respectively.
- ◆ Only about 10% of the HHs were consuming adequately iodized ( $\geq 15$  ppm) salt.
- ◆ The prevalence of Bitot spots was about 1.1%. Fever (21%), diarrhoea (3.5%) and ARI (3%) were the common morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 59% of children were underweight (weight for age  $<$  median-2SD), 47% were stunted (height for age  $<$  median-2SD), and 18% were wasted (weight for height  $<$  median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of the children and the different socioeconomic variables. The prevalence of undernutrition was significantly higher in children of SC and ST communities, landless labourers, illiterate parents, those living in semi-pucca or kutcha houses, HHs using firewood as cooking fuel, not having electricity and where sanitary latrine was not available / not used.

## 7. GUNA DISTRICT, MADHYA PRADESH

- ◆ About 44% of the HHs covered belonged to OBC, while 15% belonged to OC. SC and ST accounted for 26% and 15% respectively. About 56% of fathers and only 18% of mothers of index children were literates. The average family size was 5.0. The major occupation of the head of the HH in a majority of HHs was labour (45%), followed by cultivation (42%).
- ◆ Fifty eight percent of the houses were semi-pucca, 38% were kutcha and only 4% were pucca in nature. The major source of drinking water was tube well (81.5%), followed by open well (15.8%).
- ◆ About 84% women reportedly fed colostrum to the newborns. Sixty six percent of women initiated breastfeeding within three hours of delivery. Only 11% of women reportedly gave pre-lacteal feeds, the most common ones being cow/goat milk (5.4%) and sugar water (3.2%).
- ◆ Only about 6% of 6-11 months children started receiving complementary foods before 6 months of age. About 73% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 63% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 28% of children were receiving complementary feeding 3 times a day, and 27% were receiving 4-5 times a day.
- ◆ About 74% of 12-23 months children were completely immunized. About 75% and 91% received measles and BCG vaccines respectively.
- ◆ Thirty five percent of the HHs was consuming iodized salt containing adequate amount of iodine (15 ppm).
- ◆ The prevalence of Bitot Spots was 1.7%, which is indicative of public health significance of the problem. Fever (9%), ARI (9.1%) and diarrhoea (3.7%) were the common morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 58% of the children had underweight (weight for age  $<$  Median-2SD), 46% were



stunted (height for age <Median-2SD) indicating chronic undernutrition and 24% were wasted (weight for height <Median-2SD) indicating acute undernutrition.

- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables, including occupation of HH, literacy status of mother, presence of electricity and sanitary latrine.

## 8. SHIVAPURI DISTRICT, MADHYA PRADESH

- ◆ About 26% and 15% of the HHs surveyed belonged to SC and ST respectively, while 44% belonged to OBC. About 69% of the families were nuclear and 28% were joint families. About 56% of the fathers and 18% of mothers of index children were literates. The average family size was 5.0. The major occupation of the head of the HH in a majority of HHs was labour (47%), followed by agriculture (42%), business (8%) and service (2%).
- ◆ About 59% of the houses were semi-pucca, 26% were kutcha & 15% were pucca in nature. The major sources of drinking water were tube well (61%), followed by open well (37%).
- ◆ About 67% women reportedly fed colostrum to the newborns. Only about 65% of the women initiated breastfeeding within three hours of delivery. About 7% of the women reportedly gave pre-lacteal feeds; the most common ones were cow/goat milk (5.3%) and sugar water (1%).
- ◆ Only about 8% of 6-11 months children started receiving complementary foods before 6 months of age. About 58% of children were solely breastfed even after 11 months of age. Of the 12-23 months children covered, 72% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 39% of the children were receiving complementary feeding 3 times a day, while 27% were receiving 4-5 times a day.
- ◆ About 82% of 12-23 months children were completely immunized. About 92% and 83% received BCG and measles vaccines respectively.

- ◆ Only about 6% of the HHs were found to be consuming adequately iodized (15 ppm) salt.
- ◆ The prevalence of VAD signs such as conjunctival xerosis and Bitot spots were 1.3% and 0.6% respectively. Fever (11%), diarrhoea (2%) and ARI (8%) were the common morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 58% of children were underweight (weight for age < Median -2SD), 54% were stunted (height for age < Median -2SD), and 16% were wasted (weight for height < Median -2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was significantly higher among children of SC and ST communities, landless labourers, illiterate parents, and those living in semi pucca or kutcha houses, HHs not having electricity and where sanitary latrine was not available / not used.

## 9. CHANDRAPUR DISTRICT, MAHARASHTRA

- ◆ About a fifth of the HHs belonged to ST (18.9%), and 14.7% belonged to SC, while more than half of the houses (53.3%) belonged to other BC. More than half (54.4%) of the HHs were nuclear and about a fourth (27.5%) were joint families. About 84% of the fathers and 82% mothers of index children were literates. The average family size was 5.0. The major occupation of the head of HH was labour (52.3%), followed by agriculture (28.6%), business (9%) and service (7%).
- ◆ Sixty two percent of the houses were semi-pucca, 28% were kutcha & 10% were Pucca. The major sources of drinking water in majority of HHs were tube well (47.6%), followed by open well and tap (about 26% each).
- ◆ About 83% women reportedly fed colostrum to the newborn. About three fourths (73.5%) of the women initiated breastfeeding within three hours of delivery. Only about 18% of the women reportedly gave pre-lacteal feeds, the most common ones being sugar water (8.8%) and honey water (2.9%).



- ◆ About 45% of 6-11 months children started receiving complementary foods before 6 months of age. About 21% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 90% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 62% of the children were receiving complementary feeding  $\leq 3$  times a day, while about a fourth (27.1%) were receiving 4-5 times a day.
- ◆ About 96% of 12-23 months children were completely immunized. About 99% and 96% received BCG and Measles vaccines respectively.
- ◆ About 59% of the HHs were consuming adequately iodized ( $\geq 15$  ppm) salt.
- ◆ The prevalence of Bitot spots was about 3.1%. Fever and ARI (32% each) and dysentery (7%) were the common morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 54% of children were underweight (weight for age  $<$  median-2SD), 43% were stunted (height for age  $<$  median-2SD) and 17% were wasted (weight for height  $<$  median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was significantly higher among the children of labourers, cultivators, illiterate parents, those living in semi pucca or kutcha houses, HHs using firewood as cooking fuel, not having electricity and sanitary latrine.
- ◆ Forty one percent of the houses were kutcha, 40% were semi pucca and 19% were pucca HHs. The major sources of drinking water were tube well (57%), followed by tap (34%).
- ◆ About 80% of women reportedly fed colostrum to the newborns. About two thirds (64%) of the women initiated breast feeding within three hours of delivery. About 43% of the women reportedly gave pre-lactal feeds, the most common ones being honey water (27%) and milk (12%).
- ◆ About 21% of 6-11 months children started receiving complementary foods by the age of 6 months. About 32% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 83% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 70% of the children were receiving complementary feeding  $\leq 3$  times a day, while 12% were receiving 4-5 times a day.
- ◆ Majority (91%) of 12-23 months children were completely immunized. About 98% and 91% received BCG and measles vaccines respectively.
- ◆ About one third (34%) of the HHs were found to be consuming adequately iodized ( $> 15$  ppm) salt.
- ◆ The prevalence of Bitot spots was about 0.8%. Fever (36%), diarrhoea (7.6%), dysentery (3%), and ARI (1%) were the morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 47% of children were underweight (weight for age  $<$  median-2SD), 35% were stunted (height for age  $<$  median-2SD) and 15% were wasted (weight for height  $<$  median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was significantly higher among children of SC and ST communities, landless labourers, illiterate parents, those living in semi pucca or kutcha houses, HHs not having electricity and sanitary latrine.

## 10. LATUR DISTRICT, MAHARASHTRA

- ◆ About a fifth of the HHs belonged to SC (23%) and ST (3%), while 64% belonged to OBC. About 54% of the HHs were joint families, while 28% were nuclear families. About 85% of the fathers and 76% of mothers of index children were literates. The average family size was 7.0. The major occupation of the head of HH in a majority of the HHs was labour (38%), followed by agriculture (36%), business (13%) and service (13%).



## 11. NANDURBAR DISTRICT, MAHARASHTRA

- ◆ More than three fourths (78%) of the households belonged to ST, 5% belonged to SC while about 7% belonged to OBC. About 40% each were joint and nuclear families. About 51% of the fathers and 38% mothers of index children were literates. The average family size was 6.0. The major occupation of the head of HH was labour (49.8%), followed by agriculture (33.6%), business (9%) and service (5.8%).
- ◆ Seventy nine percent of the houses were kutcha and 18% were semi-pucca and only about 3% were pucca. The major sources of drinking water were tube well (44.7%), followed by tap (42.7%).
- ◆ About 91% women reportedly fed colostrum to the newborns. Majority of the women (83%) initiated breastfeeding within three hours of delivery. About 15% of the women reportedly gave pre-lacteal feeds, the most common ones being honey water (7.3%) and plain water (1.7%).
- ◆ About 48% of the 6-11 months children started receiving complementary foods before 6 months of age. Seventeen percent of children were solely breastfed even after 11 months of age. Of the 12-23 months children covered, 85% were receiving complementary feeding in addition to breast feeding, while the rest were solely breastfed. About a third (31.6%) of the children were receiving complementary feeding  $\leq 3$  times a day, while about a half (48.5%) were receiving 4-5 times a day.
- ◆ About 87% of 12-23 months children were completely immunized. About 95% and 87% received BCG and Measles vaccines respectively.
- ◆ Only about a third (34.6%) of the HHs were found to be consuming adequately iodized ( $\leq 15$  ppm) salt.
- ◆ The prevalence of Bitot spots was about 1.1%. Fever (29.7%), ARI (7.6%) and diarrhoea (2.6%) were the morbidities experienced by the children during previous fortnight of the survey.

- ◆ About 63% of children were underweight (weight for age  $<$  median-2SD), 50% were stunted (height for age  $<$  median-2SD), and 20% were wasted (weight for height  $<$  median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was significantly high among the children of SC / ST communities, labourers, cultivators, illiterate parents, those living in semi-pucca or kutcha houses, HHs using firewood as cooking fuel, not having electricity and sanitary latrine.

## 12. KORAPUT DISTRICT, ORISSA

- ◆ About two thirds of the HHs belonged to ST, 17% of HHs belonged to SC, while 15% of HHs belonged to OBC. About 65% were nuclear families and 17% each were extended nuclear or joint families. About 36% of the fathers and 14% mothers of index children were literates. The average family size was 5.0. The major occupation of the head of HH was labour (65%), followed by agriculture (23%), business (5%) and service (4%).
- ◆ Sixty three percent of the houses were kutcha, 35% were semi-pucca and only 2% of HHs were pucca. The major sources of drinking water were tube well (81%), followed by either stream/canal/river (9%) or open well (8%).
- ◆ In a majority of the HHs (97%), firewood was the cooking fuel, while only 3% of the HHs used either LPG or coal. About 92% of the houses were not electrified and only 2% of the HHs were using sanitary latrine.
- ◆ Majority of women (98%) reportedly fed colostrum to the newborns and initiated breastfeeding within three hours of delivery.
- ◆ About 40% of 6-11 months children started receiving complementary foods by 6 months of age. About 27% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 95% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. In majority of instances, the type of complementary food given was either home



made semi-solids (67%) or solids (28%). About 92% of the children were receiving complementary feeding  $\leq 3$  times a day.

- ◆ About 91% of 12-23 months children were completely immunized. About 91% received measles vaccine and almost all the children received BCG vaccine.
- ◆ About one third of the HHs was found to be consuming adequately iodized ( $\geq 15$  ppm) salt. The prevalence of Bitot spots was 0.2%. Fever (28%), diarrhoea (10%) and ARI (14.3%) were the common morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 51% of children were underweight (weight for age  $<$  median-2SD), 35% were stunted (height for age  $<$  median-2SD) and 16% were wasted (weight for height  $<$  median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was relatively higher among the children of SC/ST communities, landless labourers, illiterate parents, those living in semi-pucca or kutcha Houses, HHs using firewood as cooking fuel, not having electricity and sanitary latrine.

### 13. TONK DISTRICT, RAJASTHAN

- ◆ Majority (55%) of the HHs belonged to OBC followed by SC (22%), ST (13%), and OC (10%). About 45% were joint families and 38% were nuclear families. About 74% of the fathers and 25% mothers of index children were literates. The average family size was 7.0. The major occupation of the head of HH was labour or agriculture (38% each), followed by business (11%) and service (7%).
- ◆ Forty five percent of the houses were kutcha, 34% were pucca and 21% were semi pucca. The major sources of drinking water in a majority of HHs was tube well (65%), followed by open well (22%) and tap (12%).
- ◆ About 67% women reportedly fed colostrum to the newborns and about 55% of the women initiated breast feeding within three hours of

delivery. About 57% of the women reportedly gave pre-lacteal feeds; the most common ones were sugar water (40%), milk (9%) and plain water (3.3%).

- ◆ About 32% of 6-11 months children started receiving complementary foods by 6 months of age. About 19% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 82% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 69% of the children were receiving complementary feeding  $\leq 3$  times a day, while 13% were receiving 4-5 times a day.
- ◆ About 74% of 12-23 months children were completely immunized. About 90% and 76% received BCG and measles vaccines respectively.
- ◆ About 36% of the HHs were found to be consuming adequately iodized ( $\geq 15$  ppm) salt.
- ◆ The prevalence of Bitot spots was about 0.3%. Fever (21%), diarrhoea (8%) and ARI (3%) were the morbidities experienced by the children during previous fortnight of the survey. About 57% of children were underweight (weight for age  $<$  median-2SD), 40% were stunted (height for age  $<$  median-2SD) and 19% were wasted (weight for height  $<$  median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was significantly higher in children of landless labourers, illiterate parents and HHs without sanitary latrine.

### 14. KRISHNAGIRI DISTRICT, TAMIL NADU

- ◆ A majority of the HHs (69.5%) belonged to OBC, while the rest belonged to SC (28.7%). More than half (58.4%) of the families were nuclear, while about a fourth (25.6%) were joint families. About 71% of the fathers and 67% mothers of index children were literates. The average family size was 5.0. The major occupation of the head of HH was labour



(53.8%), followed by agriculture (17.4%), service (10.6%), artisan (9.5%) and business (5.9%).

- ◆ Two thirds of the houses were semi-pucca, 21% were pucca and 13% were kutcha. The major source of drinking water in a majority of HHs was tap (94.3%), followed by Pond/Tank (2.9%) and Tube well (2.4%).
- ◆ About 89% women reportedly fed colostrum to the newborns. About seventy three percent of the women initiated breastfeeding within three hours of delivery. Only about a fourth (24.1%) of the women reportedly gave pre-lacteal feeds, the most common ones being sugar water (14.7%) and honey water (1.2%).
- ◆ About 47% of 6-11 months children started receiving complementary foods before 6 months of age. About 8% of children were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 44% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. Thirty eight percent of the children were receiving complementary feeding  $\leq 3$  times a day.
- ◆ About 98% of 12 - 23 months children were completely immunized. Almost all (99.5%) and 98% received BCG and Measles vaccines respectively.
- ◆ About two thirds (65.2%) of the HHs were found to be consuming adequately iodized ( $\geq 15$  ppm) salt.
- ◆ The prevalence of Bitot spots was 0.5%. Fever (18.8%), ARI (7.6%) and diarrhoea (2.8%) were the common morbidities experienced by the children during previous fortnight of the survey.
- ◆ About a third (33.4%) of children were underweight (weight for age  $<$  median-2SD), 30.5% were stunted (height for age  $<$  median-2SD), and 4.5% were wasted (weight for height  $<$  median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was significantly

higher in children of SC and ST communities, agriculture labourers, illiterate parents, those living in semi-pucca or kutcha houses, HHs using firewood as cooking fuel and not having electricity.

## 15. LALITHPUR DISTRICT, UTTAR PRADESH

- ◆ A majority of the HHs surveyed other BC (48%), followed by SC (24.9%) and ST (6.5%). About 70% were nuclear families, while 25% were joint families. About 63% of the fathers and 33% mothers of index children were literates. The average family size was 5.0. The major occupation of the head of HH in a majority of the HHs was agriculture (48.7%), followed by agricultural labour (36.9%), business (11%) and service (1.1%).
- ◆ A majority of the houses were semi-pucca (81%), 12% were kutcha and only about 7% were pucca in nature. The major sources of drinking water in a majority of Hhs were tube well (85.5%), followed by tap (14%).
- ◆ Almost all women (93.2%) reportedly fed colostrum to the newborns. Only about 40% of the women initiated breastfeeding within three hours of delivery. About 7% of the women reportedly gave pre-lacteal feeds; the most common ones were sugar water (2.5%) and goat milk (4.3%).
- ◆ About 17% of children started receiving complementary foods before the age of 6 months. A majority of children (82.8%) were solely breast fed even after 11 months of age. Of the 12-23 months children covered, 64% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 24% of the children were receiving complementary feeding  $\leq 3$  times a day, while 36% were receiving 4-5 times a day.
- ◆ About 85% of 12-23 months children were completely immunized. While 97% and 85% received BCG and measles vaccines respectively.
- ◆ About 28% of the HHs were found to be consuming adequately iodized ( $\geq 15$  ppm) salt.



- ◆ The prevalence of conjunctival xerosis was less than 1%. Fever (7%), ARI (4.5%) and diarrhoea (1%) were the morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 64% of children were underweight (weight for age <median-2SD), 50% were stunted (height for age <median-2SD) and 20% were wasted (weight for height <median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition was significantly higher among children of SC / ST communities, landless labourers, illiterate parents, those living in semi-pucca or kutcha houses, HHs using firewood as cooking fuel, not having electricity and sanitary latrine.

## 16. PURULIA DISTRICT, WEST BENGAL

- ◆ About 29% of the HHs belonged to SC, while 15.7% belonged to ST. Backward and OC accounted for about 27% each. Of the HHs covered, a majority (56.2%) were joint families, followed by nuclear (31.3%) and extended nuclear families (12.5%). About 68% of the fathers and 49% mothers of index children were literates. The average family size was 7.0. The major occupation of the head of HH was agriculture (43.2%), followed by labour (29.8%), business (11.6%) and service (3.9%).
- ◆ About 74% of the houses were kutcha in nature, followed by semi-pucca (15.4%) and pucca (10.8%). The major sources of drinking water were tube well (56.9%), followed by open well (39.8%).
- ◆ About 80% women reportedly fed colostrum to the newborn. About 69% of the women initiated breastfeeding within 3 hours of delivery. About 23% of the women reportedly gave pre-lacteal feeds, the most common ones being sugar water (14.2%) and honey water (4.6%).
- ◆ About 30% of 6-11 months children started receiving complementary foods before 6 months of age. About 27% of children were solely breastfed even after 11 months of age. Of the 12-23 months children covered, 98.8% were receiving complementary feeding in addition to breastfeeding, while the rest were solely breastfed. About 56% of the children were receiving complementary feeding 3 times a day, while 40.2% were receiving 4-5 times a day.
- ◆ About 92% of 12-23 months children were completely immunized. About 97% and 93.9% received BCG and Measles vaccines respectively.
- ◆ Majority (70%) of the HHs were found to be consuming adequately iodized (15 ppm) salt.
- ◆ The prevalence of Bitot spots was 0.2%. Fever (27.7%), ARI (27.6%) and diarrhoea (13.9%) were the common morbidities experienced by the children during previous fortnight of the survey.
- ◆ About 60% of children were underweight (weight for age <median-2SD), 38.2% were stunted (height for age <median-2SD) and 24.7% were wasted (weight for height <median-2SD).
- ◆ Significant ( $p < 0.05$ ) association was observed between nutritional status of children and different socio-economic variables. The prevalence of undernutrition (underweight and stunting) was significantly higher among children of SC and ST communities, HHs involved in labour activities, illiterate parents, those living in semi pucca or kutcha houses, Hhs using firewood as cooking fuel, not having electricity and among HHs, where sanitary latrine was not available / not using. The prevalence of wasting was significantly higher among illiterate parents and in HHs without electricity.



## II. CLINICAL STUDIES

### 1. DOUBLE BLIND, RANDOMIZED, CONTROLLED, CLINICAL TRIAL OF ZINC SUPPLEMENTATION TO FULL TERM INFANTS

Zinc is considered as one of the essential (type II) trace elements, which is not stored in the body and stunting in childhood is said to be an indirect evidence of Zinc deficiency. It is associated with primary growth failure and increased morbidity. Six to 24 months of age is the critical period of growth and development in life. Cereal based Indian weaning and complementary foods with low levels of zinc are high in phytates and lead to low bio-availability of dietary zinc. The prevalence of stunting in preschool children in India is around 50% with severe stunting around 23%. Acceleration of growth following zinc supplementation is considered as direct evidence of zinc deficiency. Zinc is also essential for lean body mass synthesis, has a role in essential fatty acid metabolism, is an integral part of many enzymes and also promotes defense mechanisms of the body.

Zinc supplementation studies carried out in India and elsewhere have shown 25-30% reduction in diarrheal morbidity (episodes and duration) and 41% reduction in lower respiratory tract infections.

With this background it was hypothesized that zinc supplementation in full term infants initiated after the age of 4 months should reduce the morbidity, promote growth, both ponderal and linear, thereby prevent stunting and help in attaining optimum body composition.

Sample size of (180 in zinc and 180 in control groups) was calculated initially taking the prevalence of stunting as 25% and aiming at reduction by 50% in supplemented group with 80% power at 0.05 significance level and adding 20% for dropouts.

Through a double blind study, Zinc was given, to select children through randomization,

5 mg/day, as zinc, in syrupy base with Riboflavine 0.5 mg / day, and control children received only Riboflavine 0.5 mg/day (since Riboflavine is not known to have significant effects on growth or morbidity and also for ethical considerations).

#### AIMS & OBJECTIVES

1. To Supplement Zinc (as elemental zinc 5mg/day ) plus Riboflavine 0.5 mg / day, to full term infants from 4 months to 18 months and Riboflavine 0.5 mg / day, both in syrupy base to control group children.
2. To record anthropometric data for body composition assessment at recruitment, and every 3 months till the end of the study.
3. To record morbidity data by 15 day recall every month.
4. To see the effects of supplementation on anthropometric indicators & morbidity and to see whether the effect if any persists even after the discontinuation of the supplementation for another 6 months i.e., till the age of 24 months.

The recruitment of full term newborns was started in October 2003, in an urban slum with a population of around 30,000. Supplementation was started by the age of 4 months in March 2004, after the children were randomly assigned to one of the 2 groups (A & B, either supplemented or control). It was a double blind randomized controlled trial. Morbidity data was collected by monthly visits through 15 day recall & anthropometric data collection was done at three months intervals. Quality control for interrater reliability and reproducibility was done every 3 months. Data regarding feeding practices and any other supplementation was recorded for all the children.

All children from the slum including the study children had access to a medical center run by NIN, a bi-weekly clinic run at the community center, but attempts were made not to interfere with the routine medical care provided by the local health authorities and private medical practitioners.

Since October 2003, a total of 475 consecutive cases were recruited and placed in one of the groups. All the study children have completed 2 year follow up. Data entry is in progress.

## RESULTS

Four hundred and seventy five children were recruited for the study and supplementation was given after randomization to all as described earlier. In 390 children supplementation was stopped after completion of 18 months. 5 children died at various ages (3 due to aspiration at <6 months of age, 1 due to road accident at 19 months of age and one following convulsions at the age of 23 months) and 79 dropped out of the study either mostly due to migration and some as they were not willing to continue in the study. Blood samples were collected from 100 children after completion of supplementation at 18 months of age, for estimation of Hemoglobin, Zinc, Copper and Vitamin A by standard procedures. Dietary intakes were also calculated by 24 hours food frequency questionnaire in these 100 children.

A total 20.7% of these children were LBW babies, males constituted 9.1% and females 11.6%. All the anthropometric measurements were significantly low among LBW babies at all ages. There was no significant difference in body fat percentage at 3<sup>rd</sup> and 18<sup>th</sup> months among low birth weight and normal babies (18.7% Vs 20.1 and 18.4 Vs 19.7 NS).

In the pooled sample 42.1% of children were undernourished (weight for age), 35.3% stunted and 25.1% were wasted at 18 months of age, and this proportion did not change at 24 months of age. There were no significant differences between zinc supplemented and control children in any of these parameters. Though, there were no significant differences in all the anthropometric parameters between the two groups there was an excess of height gain of 0.39 cms in supplemented children, which was not significant.

Mean body fat percentage was between 18% and 21% in all the pooled sample during the study period. Mean body fat percentage was

significantly higher in supplemented children at 12 months of age ( $20.2 \pm 5.34$  Vs  $19.0 \pm 4.4$ ,  $p=0.02$ ) at 9, 18, 21 and 24 months, however a trend was detected in this parameter (fat %) in the supplemented group ( $p=0.055$ ,  $0.075$ ,  $0.073$  and  $0.108$  respectively).

Mean number of episodes of diarrhea was not significantly different between supplemented and control groups (1.46 vs 1.62 per 100 days followup). Similarly mean diarrheal duration was 7.1 and 8.3 days respectively per 100 days follow up ( $p=0.039$ ). Similarly, mean number of episodes of LRI was 0.22 and 0.23 and duration of respiratory infection was 14.4 and 15.4 days respectively in supplemented and control children for 100 days follow up.

Mean hemoglobin, serum zinc, copper levels and vitamin A levels were not significantly different in these two groups. Mean dietary energy intakes at 1½ years of age was 599+287 calories per day in these children.

It is possible that with calorie deficits of 500 – 600 Kcal micronutrient supplements may not be able to impact growth or morbidity in infants from this socio-economic group.

## 2. ASSESSMENT OF PREVALENCE OF OSTEOPOROSIS IN ADULT POPULATION IN INDIA - INDIAN COUNCIL OF MEDICAL RESEARCH TASK FORCE STUDY

Osteoporosis is emerging as one of the most important problems of aging and Bone Mineral Density (BMD) values are increasingly used for screening population. BMD is correlated with fracture risk and therefore is being used as a surrogate marker for osteoporosis. However, BMD guidelines for diagnosis of osteopenia or osteoporosis in Caucasians may not be suitable for Indian population.

Therefore, there is a need for studies from different regions of the country to establish a data base of PEAK BONE MASS at different sites and the age at peak bone mass. It is also necessary to establish bone densities at different age groups in



relation to the dietary calcium intakes and other related factors to study the prevalence of osteopenia and osteoporosis.

### Rationale

The ICMR Expert Group on Osteoporosis recommended that an epidemiological, multicentric, nationally representative study should be carried out on both men and women to assess the incidence and prevalence of osteoporosis in India in four centres, National Institute of Nutrition (NIN) is one of the important ICMR centre. Besides, there is a need to have standards of normal bone density in India of spine, hip and forearm.

### OBJECTIVES

I. To establish peak Bone Mineral Density (BMD) reference values for Indian men and women (Group-1).

II. To assess the prevalence of osteopenia and osteoporosis in Indian populations (Group-2).

### To Establish BMD Reference Values in Indian Men and Women (Group-1)

To establish reference values of BMD, (age group of 20-30 years), men and women who "have no constraints" to growth and bone mineralization during childhood & adolescence were recruited. A check list defined the 'no constraints' group.

Subjects from the upper socio-economic group with normal BMI (18.5-25.0) from a locality which is known to have people from the upper socio economic group (i.e. Grade A colony), and where the selected subjects have been living for at least 10+ years were recruited.

Exclusion criteria which are likely to interfere with bone growth/bone mass were identified while recruiting the subjects for the study.

### Sample size

Assuming a mean BMD of 0.9 with a confidence interval of 95% and with a relative precision of 5%, a sample size of 100 was fixed to give a stable estimate for BMD. Hence, each centre has selected 100 men and 100 women.

The following information was collected from each individual selected.

1. Dietary assessment of calcium intakes by the food frequency method.
2. Biochemical tests by standard procedures. A fasting blood sample was drawn between 9.30a.m. - 11.00 am. for the following tests
  - Haemoglobin
  - Serum Albumin
  - Serum calcium
  - Serum phosphorus
  - Serum alkaline phosphatase
  - Plasma 25 hydroxy vitamin D3
  - Serum parathormone
  - Urine fluoride levels
3. Physical activity assessment
4. Sun exposure for vitamin D status

BMD measurements were carried out at the following sites using DXA

- Left hip
- Left forearm for right handers and vice versa
- Spine (L1-L4) AP
- Whole body mineral content and body composition.

### To assess the prevalence of osteopenia and osteoporosis in Indian population (Group-2)

AGE GROUP: 30 years and above upto 70+ years for both men and women, and divided into 5 age groups 30-40, 40-50, 50-60, 60-70, 70+ years.

**Socio-economic groups:** The following three socio-economic groups were included for the study.

1. High income group
  2. Middle income group
  3. Population living in the urban slum.
1. **High Income group:** Criteria given earlier was followed. The 10 years rule however, was not operative.
  2. **Middle Income group :** Population living in a known middle class locality.
  3. **Urban slum :** Those living in the well demarcated urban slums.



## Sample size

Three areas to represent urban upper income/middle income and slum area was selected. Ten thousand population in each of the areas was identified and enumerated. Based on the population proportion of each age group from 30 to 70 +years, 250 men and 250 women were selected giving proportionate representation to the population in each age group.

Simple random sampling was used to select the households to cover the required sample.

A sample of 250 was fixed for each of the income and sex groups with the assumption of 20% prevalence and relative error of 25% and a level of confidence of 95%.

Biochemical tests : was carried on a fasting blood sample in 20% of the above sample. The list of tests are as given earlier for the Group I.

## RESULTS – PREVALENCE GROUP

A total of 1630 adults in the age group of 30 – 70+years were recruited for the study from the 3 socio-economic groups. The table-2 gives a breakup of the distribution of men and women in each group. Their mean ages ranged from 50+ years in the HIG men to 45+ years in the LIG.

The weights and heights of the men and women from the HIG were greater than the weights and heights of the LIG, whereas these values for the MIG were in between. There was a secular increase in heights of the HIG and MIG men and women and also in the case of LIG men, that is the older generation was shorter when compared to the younger generation, however, there was no secular increase in heights of the women from the LIG.

The mean heights of the younger (30-40 years) men and women from the HIG were less than the 50<sup>th</sup> percentile of the NCHS standards. However, the mean BMI's of the HIG was in the over weight range and that of the MIG and LIG was normal except for the MIG women who had mean BMI's of 25.9.

The calcium intakes from food sources ranged from 974 mg/day in the HIG which is close to the Western RDA, 665 mg in the MIG and 297 mg /day in the slum population which is much below the Western RDA. It was not different in the men and women from the same socio-economic group.

The surface area exposed to sunlight varied from 20% (in men) to 35% (women). This did not vary across the 3 groups. The time of exposure was however, 1 hour (HIG and MIG men) to 2 hours (LIG men) when compared to about half an hour in HIG and MIG women and 1 ½ hours in LIG women.

Pooled analysis of the data on bone parameters will be carried out by the ICMR.

## REFERENCE GROUP

As mentioned above, young men and women in the age groups of 20-30years, with no constraints to growth during their growth phase and with no present or past health problems known to affect growth were recruited for the study. The total sample size was 210 (male 104, female 106) and the mean (SD) age was 24.1±3.13years, and this was not different in the two gender groups. Their mean (SD) heights (cm) were 174.1±6.19 in men and 159.9±5.61 in women. Mean (±SD) weights (kg) in men were 69.7±8.06 and 57.6±11.9 in women. Calcium

**Table-2. Anthropometric data of the subjects from different socio economic groups**

Income group/ Sex	Age (yrs)	Weight (kg)	Height (cm)	BMI
High M (255)	50.56±13.144	76.6±12.03	169.5±6.90	26.9 ±3.77
F (328)	49.05 ±11.726	66.9±11.12	155.7±6.55	28.1 ± 4.22
Middle M (253)	47.41±12.618	68.0±11.53	165.9±6.18	24.6 ( 3.60
F (262)	45.81(12.055)	59.6(10.69)	151.8(5.60)	25.9 (4.32)
Low M 283)	45.12(11.078)	55.8(10.36)	161.8(6.22)	21.3 (3.71)
F (249)	45.30(11.040)	52.2(11.5)	149.7(5.96)	23.2(4.54)



(mg) intakes in men was  $1074.7 \pm 379.0$  and women  $991.3 \pm 348.0$ .

Exposure to sunlight – Both men and women had similar body surface areas exposed to sunlight and it was around 24%, however, the duration of exposure was  $76.5 \pm 59.88$  minutes in men and only  $45.0 \pm 48.43$  minutes in women.

The population recruited for the reference group had normal blood biochemistry and there were no differences between the two sex groups (Hb:  $13.4 \pm 1.46$  gm/dl); serum albumin:  $4.5 \pm 0.19$  g/dl; serum calcium:  $9.8 \pm 0.70$  mg/dl; serum phosphorus :  $4.4 \pm 0.45$  mg/dl. Men had a higher vitamin D levels (Men:  $24.3 \pm 14.63$  ng/ml, women :  $18.6 \pm 15.06$  ng/ml ( $P < 0.05$ )) but the PTH levels were not different (Mean :  $38.2 \pm 25.17$  pg/ml). All the subjects had normal urinary fluoride levels.

#### **The bone parameters were as follows:**

Using the western cut-off levels the mean Z scores for BMD at neck of femur was  $-0.22 \pm 0.872$ , Tronchanter (Z scores  $-0.15 \pm 0.787$ ), hip BMD (Z scores  $-0.17 \pm 0.745$ ), forearm BMD (Z scores  $-0.40 \pm 0.826$ ), spine (Z scores  $-0.73 \pm 0.823$ ). There was a wide variation in the Z scores, with Z scores for men slightly worse off than that for women but they were not significantly different except in the case of spine and forearm where men had poorer Z scores ( $P < 0.05$ ). Men had a mean SD whole body BMC of  $2472 \pm 314$  gms of mineral content whereas, women's whole body mineral content was  $2026 \pm 205$  gms, and they were significantly different in the two groups ( $P < 0.05$ ).

Since, the age group of the study sample was 20-30 years, it was felt that the peak bone accretion would have occurred only in late 20s. To explore this possibility the subjects were further

divided into  $< 25$  years &  $> 25$  years. When the data was analysed for men & women separately in these age groups there were no differences in the bone parameters in the two age groups except for the spine BMD which was better after the age of 25 years in the case of women ( $0.961 \pm 0.081$  g/cm<sup>2</sup> at  $< 25$  years, &  $1.016 \pm 0.145$  g/cm<sup>2</sup> at  $> 25$  years  $P < 0.014$ ) and a trend towards better T scores at this site ( $P < 0.063$ ). In addition, women had higher levels of parath hormone after the age of 25 years ( $34.85 \pm 21.27$  pg/ml at 20–25 years and  $46.178 \pm 31.516$  pg/ml after 25 years  $P < 0.042$ ). Surprisingly the forearm bone parameters and whole body BMD were significantly better after the age of 25 years in men but not in the case of women.

Using the Z score it is obvious that the bone parameters of young adults from these backgrounds are very close to the western standards. The peak bone mass is achieved at the age of 20-25 years in this population with a plateau after that, however, the PBM at spine for women and forearm for men appears to occur after 25 years. When the bone parameters were correlated with variables such as weight, height, BMI and biochemical parameters, it was significant that weight and BMI in females and men correlated with densities at all the sites ( $P < 0.05$ ).

In addition higher the extent of activity status, higher the BMDs at all sites except the forearm. The activity score of 3 appeared to be optimum, for bone density increase. Any further increase in activity score did not impart further benefit. Infact higher activity levels appears to have resulted in poorer BMD in these groups of youngsters.

Sunlight exposure was not correlated with any



# III. MICROBIOLOGY & IMMUNOLOGY

## 1. ROLE OF ZINC – IN THE TRIAD OF TRUNCAL ADIPOSITY, INSULIN SENSITIVITY & PROINFLAMMATORY CYTOKINES : BASELINE DATA

Coronary Heart Disease (CHD) is known to be a major cause of morbidity and mortality world wide, and is assuming an increasing role as a significant cause of morbidity and mortality in developing countries. 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% shows no major risk factors. Higher trygliceride, lower HDL and increased visceral fat and insulin resistance have been proposed as reasons for the higher risk of CHD and type 2 diabetes. There seems to be a role for sub clinical micronutrient deficiency in this phenotype of central obesity and increased insulin resistance in Indians of the micronutrients, zinc has been shown to promote insulin sensitivity and protein synthesis and also reduce fat deposition apart from modulating chemokine response. Subclinical zinc deficiency could be prevalent in India, which might be contributing to insulin resistance.

Recent studies have shown adipose tissue to be an endocrine organ that actively secretes a number of proinflammatory cytokines and chemokines; that are also called adipokines. Most of these adipokines such as TNF-, ICAM 1, resistin, leptin reduce insulin sensitivity and contribute to endothelial dysfunction. On the other hand adiponectin, improves insulin sensitivity and is antiatherogenic. In the present study the prevalence of insulin resistance and its association with adipokines and CHD risk factors in young adults was studied.

### METHODS

#### Subjects & Study design

Eighty five apparently healthy men and women aged twenty to thirty years, were enrolled

for the study. A fasting blood sample was collected from these subjects for measuring zinc, adiponectin, resistin, blood glucose, insulin concentration and lipid levels.

### BODY COMPOSITION & ANTHROPOMETRY

Anthropometrical measurements include weight (to the nearest 0.1 kg ), height (to the nearest 0.1 cm) The body mass index (BMI) will be calculated as body weight divided by body height squared.

### BIOCHEMICAL PARAMETERS

High Density Cholestrol (HDL) & triglycerides were measured by kits. Insulin in the fasting sample was measured by radioimmunoassay using Human Insulin EIA kit (DAKO Diagnostics Ltd.,). Insulin resistance was calculated using the homeostasis model assessment (HOMA). The HOMA index was calculated as fasting insulin concentration ( $\mu\text{U}/\text{mL}$ ) x fasting glucose concentration ( $\text{mmol}/\text{L}$ ) / 22.5.

BMI equal to or greater than  $23 \text{ kg}/\text{m}^2$  was considered as cutoff point for overweight. Serum zinc was assessed by AAS. Adiponectin and resistin were measured by ELISA.

### RESULTS

3.5% of the subjects under study had high serum glucose values ( $> = 110 \text{ mg}/\text{dl}$ ), however a significant proportion (43.8%) of them had poor insulin sensitivity ( $\text{HOMA} > = 3.16$ ). A proportion of 36.3% had  $\text{BMI} > = 23.0$ . cholesterol and triglyceride concentrations were higher than the normal values in 7.1% and 8.2% respectively while HDL was less than the normal value ( $> = 35 \text{ mg}/\text{dl}$ ) in 58.8% of the subjects. 7.2% of the subjects had Zinc deficiency ( $< 70 \mu\text{g}/\text{dl}$ ). Significantly higher proportions of males had higher BMI and higher HOMA compared to females. ( $P < 0.05$ ) and the mean values of Zinc ( $85.4 \mu\text{g}/\text{dl}$ ) was significantly ( $P < 0.05$ ) lower in males.

BMI was significantly associated with HOMA ( $P < 0.05$ ) and as expected was also associated with high glucose and high insulin concentration



( $P < 0.05$ ). Lipid levels were however comparable between the two BMI groups (BMI  $< 23.0$  and BMI  $\geq 23.0$ ). Of the two adipokines tested, adiponectin was inversely associated with BMI while resistin was comparable (Table 3).

**Table 3. Association of cardiovascular risk factors with BMI in young adults**

Parameter	BMI $< 23.0$	BMI $\geq 23.0$
HOMA	2.98 $\pm$ 0.21 (51)	4.14 $\pm$ 0.35* (29)
Insulin (mU/L)	13.0 $\pm$ 0.79 (51)	17.4 $\pm$ 1.37* (29)
Glucose (mg/dl)	92.7 $\pm$ 1.08 (51)	96.6 $\pm$ 1.71* (29)
TG (mg/dl)	90.3 $\pm$ 7.72 (51)	95.5 $\pm$ 6.10 (29)
cholesterol (mg/dl)	145.6 $\pm$ 4.85 (51)	147.2 $\pm$ 7.78 (29)
HDL (mg/dl)	33.1 $\pm$ 1.64 (51)	37.2 $\pm$ 2.61 (29)
Adiponectin ( $\mu$ g/ml)	3.4 $\pm$ 0.18 (49)	2.5 $\pm$ 0.26* (29)
Resistin (ng/ml)	3.73 $\pm$ 0.338 (51)	3.03 $\pm$ 0.490 (29)

Values are Mean  $\pm$  SE.

Number in parentheses denotes number of samples.

\*  $p < 0.05$

When the subjects were stratified based on HOMA, subjects with HOMA  $\geq 3.16$  had

significantly lower Zinc and adiponectin compared to subjects with HOMA  $< 3.16$  group (Table 4). The lipid levels and resistin were not associated with HOMA.

**Table 4. Association of Zinc and adiponectin with HOMA in young adults**

Parameter	HOMA $< 3.16$	HOMA $\geq 3.16$
BMI	21.1 $\pm$ 0.40 (45)	23.8 $\pm$ 0.55* (35)
Zinc ( $\mu$ g/dl)	91.0 $\pm$ 2.32 (37)	82.1 $\pm$ 3.45* (18)
TG (mg/dl)	86.9 $\pm$ 7.85 (49)	94.9 $\pm$ 5.97 (36)
Cholesterol (mg/dl)	147.6 $\pm$ 5.01 (49)	139.8 $\pm$ 6.65 (36)
HDL (mg/dl)	33.7 $\pm$ 1.72 (49)	34.5 $\pm$ 2.23 (36)
Adiponectin ( $\mu$ g/ml)	3.3 $\pm$ 0.19 (48)	2.7 $\pm$ 0.24* (35)
Resistin (ng/ml)	3.80 $\pm$ 0.398 (49)	3.18 $\pm$ 0.334 (36)

Values are Mean  $\pm$  SE. Number in parentheses denotes number of samples. \*  $p < 0.05$

## CONCLUSION

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 & higher BMI associated with low zinc status.



## IV. BASIC STUDIES

### 1. VALIDATION OF IRON BIO-AVAILABILITY USING THE CACO-2 CELL MODEL

Poor density and bioavailability of dietary iron from staple foods are the major etiological factors for the widespread prevalence of iron deficiency in India. Increasing the density and bioavailability of iron either by chemical fortification and biofortification are being considered as strategies to control and prevent anemia. The Department of Biotechnology, Ministry of Science and Technology has initiated the crop-biofortification project with the aim to increase the density of iron, zinc and  $\beta$ -carotene in major staple crops such as rice, wheat and maize. In this context screening for iron bioavailability of various germplasm and breeder lines is necessary to select elite source of iron. Although human studies are described as gold standard for assessing the bioavailability of iron, in the recent years coupled *in vitro* digestion/Caco-2 cell model has been used as a screening tool to assess the bioavailability of iron and carotenoids from various kinds of food sources including biofortified varieties. Thus, it is envisaged to establish the screening facility and also validate the same for bioavailability of iron, zinc and  $\beta$ -carotene.

#### METHODS

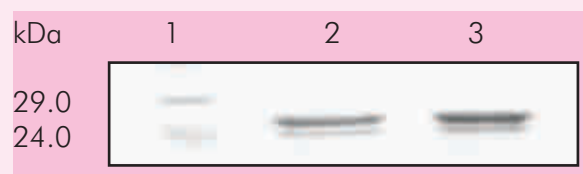
##### Validation of Caco-2 cell for iron bioavailability

To validate the Caco-2 cell model for iron bioavailability ferrous sulphate and purified pea seed ferritin (Fig. 1) as sources of iron in the presence and absence of ascorbic acid and phytic acid was tested. Simulated *in vitro* digestion was performed. Caco-2 cells of passages 15-20 at 12-14 d post seeding in a 6 well tissue culture trans well plate was used for testing the bioavailability.

Briefly, 100 $\mu$ L of stock purified pea phytoferritin (780 $\mu$ g of iron/2.5mg protein/mL) or equivalent amount of iron from ferrous sulphate was subjected to peptic and intestinal phases of

digestion. Two mL of this digest was placed in apical chamber of the transwell plate for a period of 2h. Subsequently the apical chambers were removed and the cells were further incubated for 22h. Cell extract was prepared and ferritin was assayed using a specific ELISA method as a surrogate marker for iron bioavailability and expressed as ng ferritin /mg protein.

**Figure 1. Characterization of purified pea ferritin: 12.5% SDS-PAGE of pooled fractions obtained after gel filtration chromatography on Sepharose-6B column. Lane 1, molecular weight markers; Lane 2 and 3, 5 and 10g of purified pea seed ferritin**



##### Gastric stability of pea ferritin

Purified pea ferritin was incubated in the presence and absence of pepsin either at pH 7.2 or at 2 for a period of 2h and the integrity of the protein was monitored by native and SDS-PAGE. To assess the secondary and quaternary structure of pea ferritin at gastric pH, pea ferritin was incubated for a period of 30min at room temperature at pH 2 and 7.2 followed by spectropolarimeter (JASCO-810) and gel filtration chromatography on TSK2000S column in an HPLC. The percentage of secondary structure was calculated using the web-based program K2D.

Each experiment was conducted in triplicate and analyzed in duplicates. The ferritin data were log transformed and the means were compared using one-way ANOVA followed by least significant differences (LSD) post-hock test (SPSS-11).

#### RESULTS

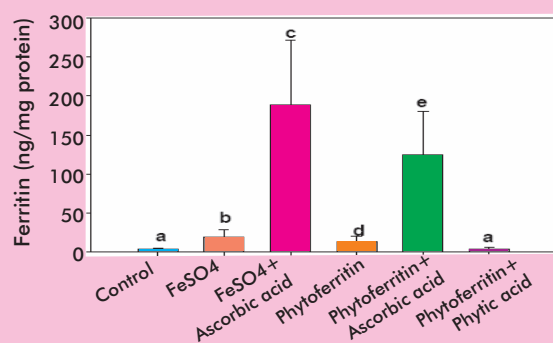
##### Validation of Caco-2 cell line for iron bioavailability

Iron from the sources, FeSO<sub>4</sub> and phytoferritin (purified pea ferritin), increased cell ferritin content significantly ( $P < 0.001$ ) compared to the



control. The increase in ferritin was significantly lower with pea ferritin than that of FeSO<sub>4</sub>. The cell ferritin content in the presence of ascorbic acid (molar ratio of 1:5) increased significantly with both the sources of iron compared to their respective controls (FeSO<sub>4</sub> and phytoferritin) (P<0.001). However, the ferritin formation was significantly higher in FeSO<sub>4</sub> treated cells compared to pea ferritin (<0.003) in the presence of ascorbic acid. Further, addition of phytic acid along with pea ferritin (molar ration 1:1) dramatically reduced the caco-2 cell ferritin formation compared to pea ferritin alone (P<0.001). These results are in line with the enhancing and inhibitory effects of ascorbic acid and phytic acid, respectively, on iron absorption in humans and thus validating the caco2 cell line for iron bioavailability studies.

**Figure 2. Caco-2 cell ferritin formation was measured by ELISA. Control (saline), FeSO<sub>4</sub> (78 g iron) and pea ferritin (78 g iron in 0.24 mg protein) in the presence or absence of ascorbic acid (1:5 ratio) and pea ferritin in presence of phytic acid (1:1 ratio) Means that do not share common letter differ significantly, P<0.001**

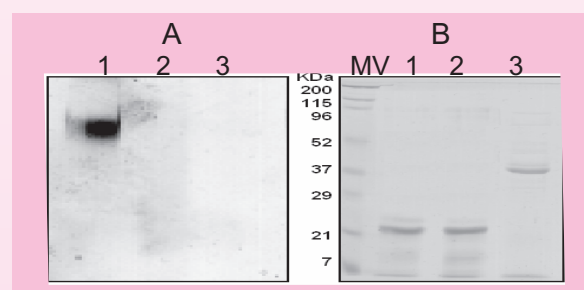


### Gastric stability of pea ferritin

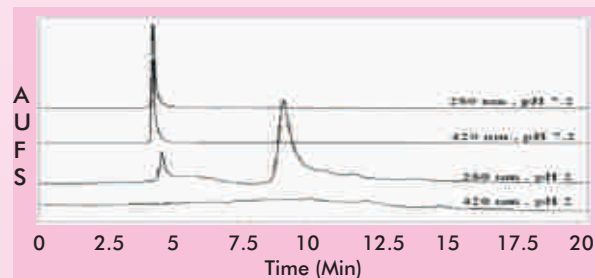
Pea ferritin moved as a single band on native-PAGE at pH 7 while no band was observed either in the absence or presence of pepsin at gastric pH 2.0 (Fig 3A). Upon SDS-PAGE, pea ferritin moved as 26 and 28 kDa bands at both pH 7 and 2 and no visible band in the presence of pepsin except for the enzyme band at 36kDa (Fig. 3B). These results suggest that the pea ferritin is highly sensitive to gastric pH and undergoes dissociation and peptic digestion.

In order to confirm the nature of pea ferritin dissociation at gastric pH, pea ferritin was subjected to gel filtration chromatography on TSK-2000 column (Fig. 4). At pH 7.0, pea ferritin moved as a single peak in the void volume of the column and was detected both at 280 (protein) and 420 nm (protein bound iron), confirming the integrity of the protein. Incubation of pea ferritin at pH 2 led to a reduction in the peak area at the high molecular weight region and a concomitant increase in protein eluting at low molecular weight region with no absorption at 420 nm. These observations confirm the dissociation of pea ferritin at acidic pH and release of protein bound iron.

**Figure 3. Digestive stability of pea ferritin: 6% PAGE (A) and 12.5% SDS-PAGE (B) Purified pea ferritin at pH 7.2 (lane 1) and at pH 2 in the absence (lane 2) and in presence of pepsin for 2 h at 37°C (lane 3)**



**Figure 4. The elution profile of purified pea ferritin either at pH 7.2 or pH 2 fractionated on TSK 2000 column. The protein monitored at 280 nm and protein bound iron at 420 nm.**

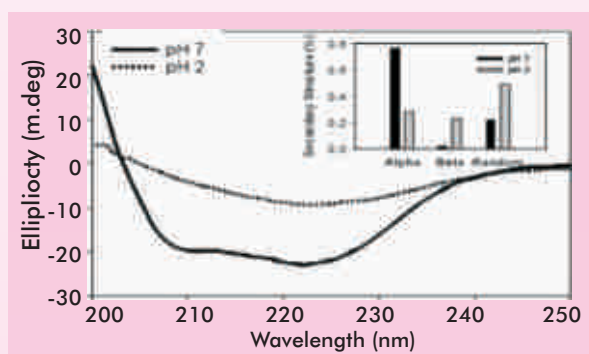


To further elucidate the effects of acidic pH on pea ferritin, far UV spectral analysis was carried out (Fig 5). At native pH, purified pea ferritin showed a typical-helical spectrum & acidification led to a significant loss in secondary structure. Analysis of the CD data using K2D programme



indicated a high content of  $\alpha$ -helix (76%) in the protein followed by  $\beta$ -sheet (2%) and random coil (22%) conformation at pH7. However, acidification of pea ferritin reduced the  $\alpha$ -helical content (28%) and increased the  $\beta$ -sheet (23%) and random coil (49%) conformation (Fig. 5, inset). These results strongly suggest loss of quaternary and secondary structure of pea ferritin at gastric pH and complete dissociation of protein bound iron.

**Figure 5. Far UV CD-spectra recorded in the range of 200–250 nm. Inset, the percentage of secondary structure calculated from the CD data points using K2D program: Purified pea ferritin (1 mg/mL) at pH 7 or at pH 2**



## CONCLUSIONS

Caco-2 cell line is a good tool for assessing iron bioavailability. The plant ferritin is susceptible to gastric digestion and therefore, the released iron interacts with dietary factors such ascorbate and phytate leading to increase or decrease in its bioavailability.

## 2. COMMERCIALIZATION OF DOUBLE FORTIFIED SALT

The Technical Committee constituted by the Government of India under the Chairmanship of Dr. M. K. Bhan, Secretary of DBT, GOI on “Formulation of Guidelines for use of double fortified Salt (DFS) as a measure to reduce prevalence of anaemia” has recommended for introducing NIN-DFS in nutritional programmes for vulnerable groups. As per the recommendations of the ICMR Expert Committee on commercialization of DFS, advertisement with

regard to technology transfer of NIN-DFS was released in the following newspapers: (i) The Times of India (English, Throughout India), (ii) Nav Bharat Times (Hindi, Throughout India), (iii) Dinamalar (Tamil, Tamil Nadu), (iv) Eenadu (Telugu, Andhra Pradesh), (v) Divya Bhaskar (Gujarathi, Gujarat) and (vi) Dainik Bhaskar (Hindi, Rajasthan). 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.

### (A) Service activities of the Regional Iodine Monitoring Laboratory (Southern Region):

#### 1. Field Kit for testing iodine in iodized salt (IS)/double fortified salt (DFS)

The demand for the Filed Kit for testing iodine in iodized salt and double fortified salt is increasing day by day. Recently the Andhra Pradesh Government and the Salt commissioner, GOI have requested for the supply of large number of this kit. Therefore, the large-scale production of this kit was taken up. The colour gradation card of the kit is replaced by a colour gradation sticker with instructions for use, which is pasted on the plastic dropper bottle of the test solution. By this process the cost of the kit is reduced by 4 rupees per kit. Each kit can estimate 250-300 salt samples. About 400 kits were sent to the Salt Commissioner, GOI for use in field conditions and feed back is excellent. Some kits were given to the UNICEF, Hyderabad for use in the field.

#### 2. Field Kit for testing iron in iron fortified salt (IFS)/double fortified salt (DFS)

There is a demand for the Filed Kit for testing iron in iron fortified salt and double fortified salt in the country. Recently the UNICEF officials from Chhattisgarh and Andhra Pradesh have requested for the supply of large number of this kit. Therefore, the large-scale production of this kit was taken up. The colour gradation card of

the kit is replaced by a colour gradation sticker with instructions for use, which is pasted on the plastic dropper bottles of the test solutions. By this process the cost of the kit is reduced by 4 rupees per kit. Each kit can estimate 250-300 salt samples. About 20 such kits were sent to the UNICEF, Chhattisgarh.

### 3. DEVELOPMENTAL ORIGINS OF ADIPOSITY AND INSULIN RESISTANCE: ROLE OF MATERNAL AND POSTNATAL MAGNESIUM STATUS

Maternal undernutrition is hypothesized to predispose the offspring to various diseases including insulin resistance syndrome (IRS) in adult life. It was reported earlier that maternal dietary vitamin / mineral restriction altered the body fat content, plasma lipids and insulin secretion, suggesting their predisposal to IRS. This was associated with oxidative stress and changes in adipokine expression in the offspring. It was also observed that maternal mineral restriction induced alterations in body fat % and fat / glucose metabolism in the offspring were somewhat irreversible by rehabilitation compared to those caused by vitamin restriction. Therefore it was considered pertinent to identify the causative mineral(s) important in the foetal programming for adiposity and hyperglycemia / insulin resistance in the offspring.

Deficiencies of Fe, Ca, and trace elements are common in Indian population and it is true that despite universal supplementation of iron and folate during pregnancy, incidence of low birth weight continues to be high (~ 33%) among Indians. Prevalence of high body adiposity, IR and its associated diseases has been on the rise in India. Therefore, it was considered that maternal trace element deficiency could be a factor responsible for this. Magnesium, the fourth most common cation in the body is involved in varied biological processes. As a cofactor for several enzymes, it modulates carbohydrate and lipid metabolism. Data on prevalence of Mg deficiency are scarce. Incidence is reported to be

25–39% in patients with diabetes mellitus and approximately 45% in pregnant women in developing countries such as India. Nevertheless, the role of maternal Mg deficiency in predisposing the pups to adiposity has not been assessed. In the light of earlier studies, the study was carried out in Wistar/NIN (WNIN) rats to validate the hypothesis that maternal Mg restriction *per se* predisposes the pups to increased body adiposity / insulin resistance later in their life.

#### OBJECTIVES

1. Assess the role of maternal magnesium deficiency in predisposing the offspring to adiposity and IR in later life.
2. Determine the preventability/delay of the effect by appropriate rehabilitation of the mothers/offspring and also decipher the probable biochemical basis of IR *vis a vis* the role of magnesium status.

#### Experimental

Female, weanling Wistar/NIN (WNIN) rats were divided into two groups. One group of rats was fed for twelve weeks, a casein based control diet (AIN – 93G) with 70% restriction of magnesium. The other group of rats served as the pair fed controls for the restricted group. After assessing their magnesium status, the rats were mated with control males and maintained on their respective diets throughout gestation. At parturition, one third of the restricted dams were shifted to control diet (MgSP) and the remaining restricted dams continued on restricted diet throughout the lactation. While control (MgC) and MgSP pups were weaned on to control diet, half of the restricted pups were also weaned on to control diet (MgSW) and the other half of the restricted pups continued on restricted diet (MgR). To avoid the possible effects of estrus cycle on glucose/fat metabolism and IR, only male offspring have been included in these studies.

Glucose tolerance test, GTT (area under curve, AUC for glucose and insulin), Insulin resistance (Homeostasis Model assessment, HOMA index & Glucose AUC : Insulin AUC), Body composition (as determined by total body electric conductivity) and plasma lipid profile



were analyzed in the offspring at regular intervals. The probable biochemical basis of the effect was monitored by assessing the (i) Parameters of oxidative stress (Malondialdehyde and protein carbonyls) and antioxidant status (reduced glutathione, and activities of catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the liver of the offspring at 6 and 18 months of age, (ii) expression of appropriate adipocytokines (TNF $\alpha$ , Leptin and adiponectin) and (iii) lipid metabolic enzymes (Fatty acid synthase, FAS & Fatty acyl transport protein 1 : FATP 1) by ELISA / western blot in appropriate tissues.

## RESULTS

Chronic dietary Mg restriction had no significant effect on the diet intake of WNIN female rats or their body weight gain, although significant decrease was observed in the plasma levels of magnesium in the restricted rats. The glucose tolerance, insulin resistance status and plasma lipid levels were comparable between rat dams of control and Mg restricted groups.

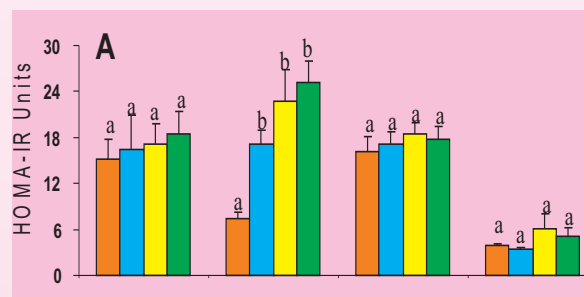
Weight gain during pregnancy and litter size were also comparable between the two groups and the birth weights of the offspring were comparable between the two groups. It appears from these results that Mg deficiency *per se* at the level generated in this study may not affect glucose tolerance, adiposity, insulin sensitivity / resistance and reproductive performance of the WNIN female rats.

However, when maternal Mg restriction, continued post-natally through lactation and weaning, body weight of the offspring was decreased at weaning and thereafter, indicating the importance of Mg in the neonatal and postnatal nutrition, in the development of rat offspring. In line with earlier report that multiple mineral restriction in rat dams increased the body fat and plasma triglyceride levels in the offspring at 3 months of age, MgR offspring also had significantly higher body fat % and plasma triglycerides in addition to lower LBM and FFM as compared to MgC rats on post natal day 90. These results not only suggest their probable predisposal to insulin resistance but also that maternal Mg deficiency significantly decreased

muscle mass in the offspring, an insulin sensitive tissue important in post prandial glucose clearance. At this time point however, there was no significant effect on fasting glucose, insulin, HOMA-IR (insulin resistance) or glucose tolerance in restricted offspring. Interestingly, MgSW but not MgSP offspring had significantly higher fasting glucose at this time point again indicating the importance of Mg during lactation is modulating glucose metabolism in offspring.

On post-natal day 180, fasting plasma insulin levels and HOMA-IR were significantly higher in MgR offspring compared to controls indicating the importance of maternal Mg deficiency and its postnatal continuation in the development of insulin resistance in the offspring at 6 months of age (Fig. 6).

**Figure 6. HOMA values of the rat offspring of different groups on postnatal 3, 6, 12 and 18 months**



Each bar represents a mean  $\pm$  SEM ( $n=6$ ) At a given age, means with different superscripts are significantly different ( $p<0.05$ ) by one way ANOVA.

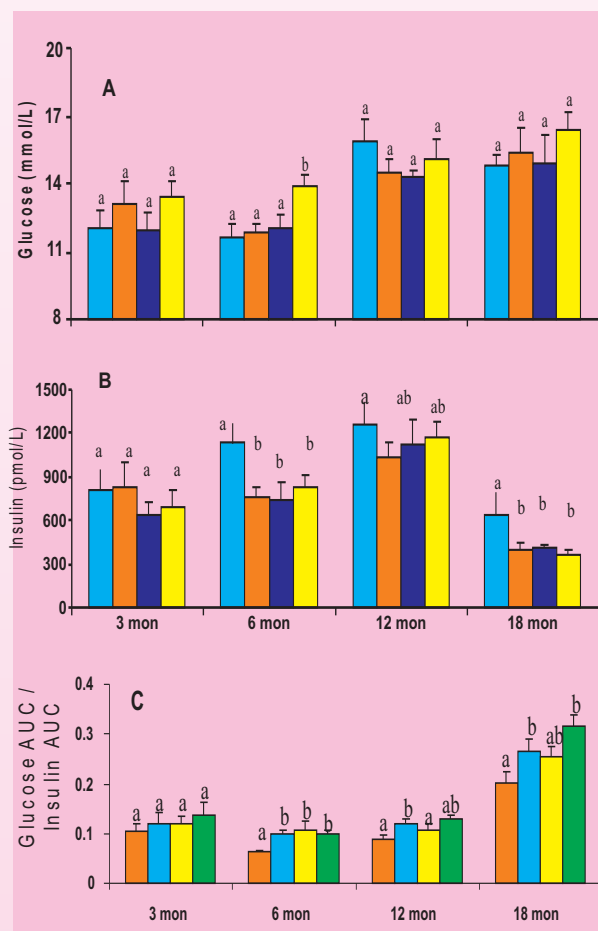
That rehabilitation of MgR mothers from parturition and their offspring from weaning or weaning MgR offspring on to control diet had no effect (in fact the fasting insulin levels increased further) on these indices not only indicates the importance of maternal Mg deficiency in predisposing the offspring to insulin resistance and the irreversibility of the defect by rehabilitation from as early as parturition but also that Mg supplementation from as early as birth may worsen the IR in offspring, an observation in agreement with Barker's hypothesis.

On postnatal day 180, bioavailability as indicated by area under curve (AUC) glucose was comparable between MgC and MgR offspring.



Rehabilitation of MgR offspring from weaning but not parturition significantly increased AUC glucose suggestive of impaired glucose tolerance in the MgSW and emphasizes that neonatal/ perinatal Mg nutrition is important in modulating glucose tolerance and/or metabolism in the offspring (Fig.7).

**Figure 7. Glucose AUC (panel A), Insulin AUC (panel B) and ratio of glucose AUC: insulin AUC (panel C) the rat offspring of different groups on postnatal 3, 6, 12 and 18 months**

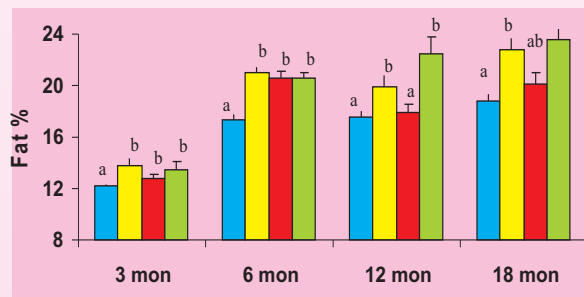


Each bar represents a mean SEM (n=6) At a given age, means with different superscripts are significantly different (p<0.05) by one way ANOVA.

Interestingly, AUC for insulin was significantly lower in MgR offspring than controls and either rehabilitation regimen had no effect on it. Taken together with the fasting hyper insulinemia in these rats, these findings indicate the impaired

capacity of  $\beta$  cells to secrete insulin to a glucose challenge. Whether this is due to the lower number/ mass of the  $\beta$  cells and/or the possible exhaustion of  $\beta$  cells in these rats need to be delineated. The ratio of AUC glucose: AUC insulin was also significantly increased in MgR offspring compared to controls and rehabilitation did not mitigate this change (Fig.7 PanelC). These findings are in general, similar to those seen in type 2 diabetes. Interestingly, the increase in body fat % (Fig 8)& decrease in LBM & FFM (Fig.9)observed in the MgR offspring at 3 months of age were persistent at this time point and were also not corrected by either rehabilitation regimen.

**Figure 8. Body fat % (Panel A), lean body mass (Panel B) & fat free mass (Panel C) of the offspring of different groups on postnatal 3, 6, 12 and 18 months as determined by TOBEC.**



Each bar represents a mean SEM (n=6) At a given age, means with different superscripts are significantly different (p<0.05) by one way ANOVA

These findings are in agreement with literature, which suggests that changes in adiposity precede IR in general. However, there were no differences among the four groups of offspring in their oxidative stress, antioxidant status or glucose uptake by diaphragm at this time point. Though not significant, insulin stimulated glucose uptake was lower in diaphragm of MgR than MgC. MgSP but not MgSW seemed to reverse the effect again stressing the importance of Mg during lactation in regulating glucose clearance / metabolism. Although plasma adiponectin levels were comparable among the four groups, plasma leptin levels were significantly reduced in MgR.

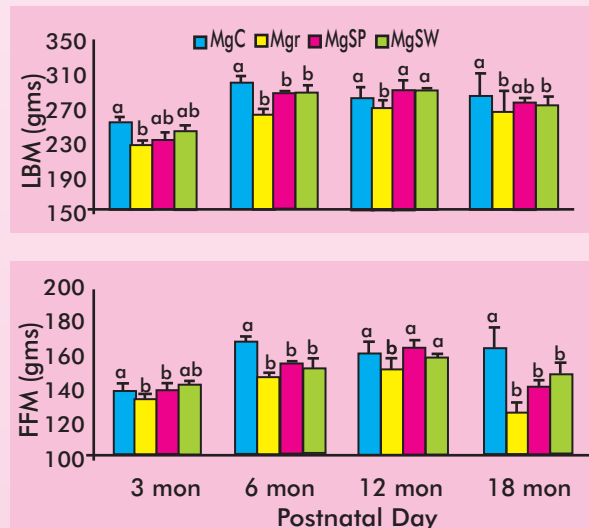


That MgSP but not MgSW offspring had leptin levels corrected but not body fat appears to suggest that hypoleptinemia and/or leptin resistance may co exist in them. Thus the findings emphasize that maternal and postnatal Mg nutrition is important in modulating adiposity, insulin resistance and glucose tolerance and / or metabolism in the offspring.

Literature evidences indicate that adiposity changes and insulin resistance precede the development of type 2 diabetes and related abnormalities. In line with these reports, it was observed that maternal Mg restriction increases adiposity in the rat offspring at 3 months and insulin resistance at 6 months of their age. Whether or not these changes observed at 3 & 6 months of age will be sustained and / or precipitate in type 2 diabetes and / or associated disorders in the offspring at later time points was assessed in the offspring at 9, 12, 15 and 18 months of age.

Interestingly, changes in body adiposity observed in MgR offspring on postnatal day 90 were sustained till 18 months of age and neither rehabilitation regime could correct it (Fig.8).

**Figure 9. Lean Body Mass (panel A) and Fat free mass (panel B) of the offspring of different groups on postnatal 3, 6, 12 and 18 months as determined by TOBEC**



Values are mean  $\pm$  SE ( $n=6$ ). Means at an age without a common letter are significantly different at  $p < 0.05$  by one way ANOVA.

These observations not only indicate that maternal and postnatal Mg nutritional status probably is important in the long term programming of body adiposity in the offspring, but also that they were irreversible by rehabilitation from as early as birth. The increase in adiposity appears to be due to the increase in TNF $\alpha$  levels (but not due to significant hypoleptinemia) in MgR offspring than MgC, although their adiponectin levels were comparable (Table 5).

**Table 5. Adipocytokine levels in the rat offspring of different groups at 6 and 18 months of age**

Adipo-cytokine	PN day	MgC	MgR	MgSP	MgSW
Adiponectin ( $\mu\text{g/mL}$ )	6 mon	3.82 $\pm$ 0.26	3.86 $\pm$ 0.22	3.36 $\pm$ 0.22	3.49 $\pm$ 0.27
	18 mon	3.74 $\pm$ 0.13	3.78 $\pm$ 0.13	3.61 $\pm$ 0.26	3.23 $\pm$ 0.28
Leptin (ng/mL)	6 mon	3.12 $\pm$ 1.101	0.52 $\pm$ 0.233	2.79 $\pm$ 1.502	0.98 $\pm$ 0.195
	18 mon	2.31 $\pm$ 0.716	0.74 $\pm$ 0.126	1.83 $\pm$ 0.715	0.51 $\pm$ 0.187
TNF $\alpha$ (pg/mL)	6 mon	54.7 $\pm$ 34.4	129 $\pm$ 94.8	37.9 $\pm$ 17.8	33.5 $\pm$ 9.83
	18 mon	39.2 $\pm$ 28.7	65.7 $\pm$ 44.2	38.8 $\pm$ 16.3	116 $\pm$ 34.2

Values are mean SE ( $n=6$ ). Means without a common letter are significantly different at  $p < 0.05$

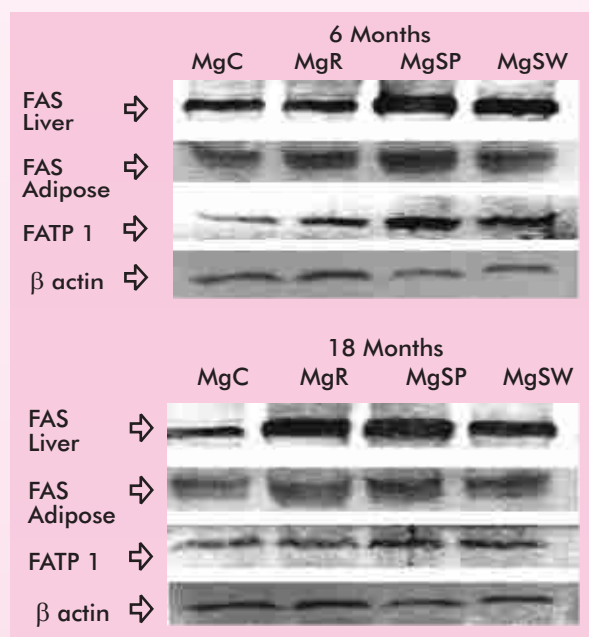
That rehabilitation from parturition (not weaning) although corrected the leptin levels to that of controls, did not correct the increased body fat appears to suggest the co- existence of both hypoleptinemia and leptin resistance. Whether this has any role in modulating body adiposity in the MgR offspring need to be deciphered. In line with these observations, expression of the lipogenic enzyme, fatty acid synthase (FAS), was significantly increased in liver and adipose tissue at 6 and 18 months of age as also the expression of FATP1 (Fig.10). It appears from these observations that maternal Mg restriction continued postnatally causes hypoleptinemia and / leptin resistance in the offspring. This probably increased fatty acid synthesis and transport and thus may increase body fat % in the offspring. Interestingly these



changes seem to be irreversible by rehabilitation.

The significant insulin resistance (increase in HOMA IR) that was observed at 6 months of age did not persist in any of these three groups at the later time points tested and so was the tissue oxidative stress / antioxidant status which were comparable among the four groups.

**Figure 10. Fatty acid synthase and Fatty acyl transport protein 1 expression in liver and adipose tissue of 6 & 18 months old offspring**



Values are mean  $\pm$  SE (n=4). Means without a common letter are significantly different at  $p < 0.05$

However, glucose stimulated insulin secretion continued to be low in MgR till 18 months of age and appeared not correctable by either rehabilitation regime. Taken together, these observations suggest that Mg deficiency during the gestation and lactation results in fasting hyperinsulinemia (an increase in HOMA IR) perhaps due to target tissue insulin resistance (as suggested from the decreased insulin stimulated glucose uptake by diaphragm albeit not significant, Table 6) by six months of age. This probably exhausted the  $\beta$  cells which failed to respond to a glucose challenge with insulin. Alternatively the decreased insulin response to a glucose challenge could be due to a decrease in islet cell number/ mass due to maternal / postnatal Mg deficiency. That Mg supplementen-

tation from parturition and weaning could not correct the animal's insulin response to glucose challenge till 18 months of age indicates that it may be programmed irreversibly by the maternal/ postnatal Mg deficiency.

That the significant decrease in LBM & FFM observed at 3 / 6 months persisted till 18 months in MgR rats indicate that maternal & postnatal Mg deficiency decreased muscle mass in offspring. That this could not be corrected by rehabilitation suggests the role of Mg in programming the muscle mass of offspring and its irreversibility. Although insulin stimulated glucose uptake by diaphragm was not altered in MgR offspring at 18 months, basal glucose uptake was significantly decreased (Table 6). Taken together with significantly decreased muscle mass, the significant decrease in muscle's capacity to take up glucose suggests that post prandial clearance of glucose by muscle is severely impaired in MgR rats than MgC and this is irreversible by rehabilitation.

**Table 6. Glucose uptake in diaphragm of 6 & 18 months old offspring**

	Post-natal day	MgC	MgR	MgSP	MgSW
Basal	6 months	0.15 $\pm$ 0.05	0.21 $\pm$ 0.03	0.17 $\pm$ 0.02	0.16 $\pm$ 0.01
	18 months	0.19 $\pm$ 0.079 <sup>a</sup>	0.07 $\pm$ 0.008 <sup>b</sup>	0.08 $\pm$ 0.017 <sup>ab</sup>	0.07 $\pm$ 0.014 <sup>b</sup>
Insulin stimulated	6 months	0.24 $\pm$ 0.11	0.21 $\pm$ 0.07	0.23 $\pm$ 0.06	0.17 $\pm$ 0.03
	18 months	0.22 $\pm$ 0.075 <sup>a</sup>	0.13 $\pm$ 0.074 <sup>a</sup>	0.06 $\pm$ 0.016 <sup>b</sup>	0.20 $\pm$ 0.038 <sup>a</sup>
Insulin stimulated/ basal	6 months	1.4 $\pm$ 0.15	0.93 $\pm$ 0.20	1.32 $\pm$ 0.17	1.07 $\pm$ 0.30
	18 months	1.61 $\pm$ 0.42	1.94 $\pm$ 0.36	0.91 $\pm$ 0.47	2.68 $\pm$ 0.96

Values are mean SE (n=4). Means without a common letter are significantly different at  $p < 0.05$ . Units : nmol/hr/gm tissue

## CONCLUSION

Chronic maternal Mg restriction appears to predispose the offspring to irreversible increase in body fat content, decreased muscle mass and their capacity to clear glucose from circulation and also impair their insulin response to a glucose challenge. The decreased insulin response to a glucose challenge appears to be due to insulin resistance (increased HOMA IR)



and the consequent fasting hyperinsulinemia which probably exhausts the  $\beta$  cells of islets or alternatively the number of islets could be reduced due to maternal Mg deficiency. The results stress the importance of Mg status during pregnancy and lactation in modulating the adiposity and insulin secretion / glucose metabolism in the offspring in its later life.

#### 4. ANTICATARACTOGENIC EFFECT OF EMBLICA OFFICINALIS AND ITS TANNOIDS AGAINST STREPTOZOTOCIN - INDUCED DIABETIC CATARACT IN RATS

Chronic hyperglycemia is a major determinant in the development of secondary complications of diabetes, including diabetic cataract. Activation of polyol pathway is one of the major mechanisms implicated in the development of diabetic cataract. Aldose reductase (AR), the key enzyme of polyol pathway and its activity is increased several folds under hyperglycemia, reduces excess sugars to sugar alcohols. Accumulation of these sugar alcohols in the lens leads to cataract formation. So inhibition of AR is one of the important events in the prevention/delay of long-term complications of diabetes. Earlier, it was shown that aqueous extract of emblica and its active principles, tannoids, inhibited rat lens AR as well as hyperglycemia induced lens opacification in organ culture.

##### OBJECTIVE

Therefore, the aim of the present study was to determine whether emblica and tannoids of emblica prevent/delay the onset and maturation of diabetic cataract induced by streptozotocin (STZ) in rats.

##### METHODOLOGY

WNIN male rats (2-3 months old) with an average body weight of  $231 \pm 11$  g were used in the study. Diabetes was induced in these animals by giving single intraperitoneal injection of STZ (32 mg/kg) in 0.1 M citrate buffer, pH 4.5. Another set of rats, which received only vehicle served as control (group I;  $n = 8$ ). After 72 h of STZ injection, fasting blood glucose levels were

monitored. Animals having blood glucose levels  $>140$  mg/dL were considered as diabetic and divided into three groups (groups II-IV); Group II (Diabetic Control;  $n=15$ ; AIN 93 diet), Group III (Diabetic + Tannoids;  $n=15$ ; AIN 93 diet + 0.2% Emblica tannoids), Group IV (Diabetic + Emblica;  $n=15$ ; AIN 93 diet + 2% Emblica powder). These animals were maintained on the respective diets for 8 weeks.

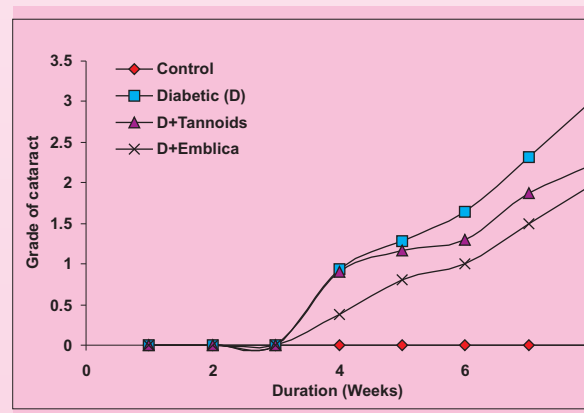
During this experimental period daily food intake, weekly blood glucose levels and body weights were monitored. Onset, progression and maturation of cataract were monitored by slit lamp microscope. At the end of 8 weeks animals were sacrificed and the lenses were stored at  $-80^{\circ}\text{C}$  for further analysis.

##### RESULTS

The increased blood glucose levels and decreased insulin in diabetic animals were not prevented by Emblica or tannoids of Emblica.

Slit lamp examination results indicated that there was a delay in the onset, progression and maturation of cataract in Emblica fed diabetic animals when compared to diabetic control group (Fig-11). Whereas there was a delay in the progression and maturation of cataract, but not the onset in tannoids fed diabetic animals. Emblica appears to be more potent in delaying cataract than its active principles tannoids.

Figure 11. Effect of Emblica and its tannoids on average stage of streptozotocin-induced cataract as a function of time.



The specific activity of AR and sorbitol levels were significantly higher in group II animals when



compared to group I. Interestingly, AR activity and sorbitol levels were decreased in diabetic rats fed with Emblica and its tannoids compared to untreated diabetic rats (Table 7). However, the decrease in sorbitol levels due to Emblica or its tannoids was not completely normalized (Table 7). This may be one of the reasons for partial effects of Emblica and its tannoids against diabetic cataract. Sorbitol dehydrogenase (SDH) activity was not altered (Table 7) in any of these group animals.

**Table 7. The Effect of Emblica and its tannoids on polyol pathway in rat lens**

Enzyme	Group I	Group II	Group III	Group IV
AR	24.96 ± 1.04	30.98 ± 1.27 <sup>#</sup>	26.74 ± 0.16 <sup>\$</sup>	27.85 ± 1.69 <sup>\$</sup>
SDH	3.59 ± 2.07	3.57 ± 1.00	3.35 ± 1.02	3.12 ± 1.09
Sorbitol	0.24 ± 0.02	5.74 ± 0.77 <sup>#</sup>	3.63 ± 0.47 <sup>\$</sup>	4.02 ± 0.27 <sup>\$</sup>

The data are the mean ±SD (n=4).

AR activity was expressed as μmoles NADPH oxidized/h/100 mg protein

SDH activity was expressed as μmoles NADH oxidized/h/100 mg protein.

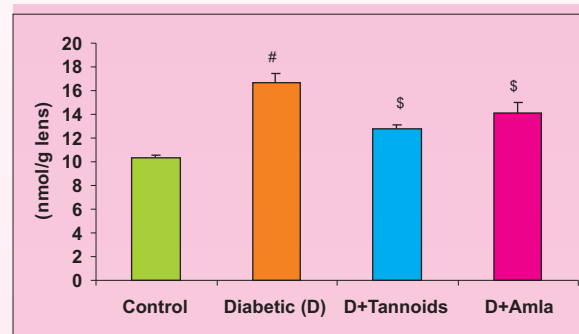
Sorbitol was expressed as μmoles/g lens

# Symbol denotes significantly different from Group I

\$ Symbol denotes significantly different from Group II

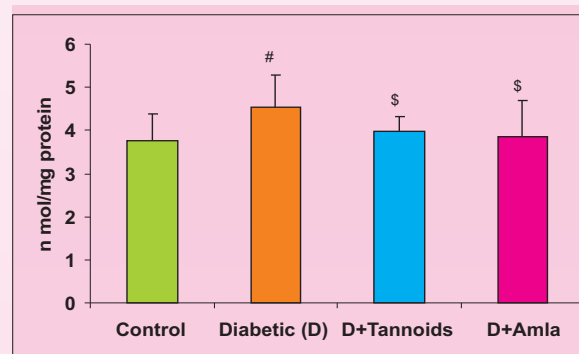
There is accumulating evidence showing the contribution of oxidative stress to the development of diabetic cataract. In the present study increased TBARS levels and protein carbonyls in group II rats compared with the control group indicate increased oxidative stress in the lens (Figs 12 & 13). Interestingly, feeding of Emblica and its tannoids to the diabetic rats significantly inhibited TBARS levels and protein carbonyls in group III and IV indicating inhibition of lipid peroxidation and protein oxidation in Emblica and tannoids treated groups (Figs 12 & 13). Similarly, increased specific activities of SOD, GPx and the marginal decrease in GST observed in the lenses of group II animals compared with group I, further substantiate the role of oxidative stress in cataractogenesis due to

**Figure 12. The effect of Emblica and its tannoids on hyperglycemia-induced lipid peroxidation in the lens.**



# Indicates statistically significant from control and \$ indicates significantly different from diabetic group.

**Figure 13. The effect of Emblica and its tannoids on hyperglycemia-induced protein oxidation in the lens.**



# Indicates statistically significant from control and \$ indicates significantly different from diabetic group.

hyperglycemia (Table 8). Emblica and tannoids treatment partially prevented the altered activities of antioxidant enzymes. These data clearly demonstrate that Emblica and tannoids not only inhibited osmotic stress but also prevented hyperglycemia-mediated lenticular oxidative stress.

During the development of cataract, alterations in lens protein and insolubilization have been considered to be the one of the major changes that results in lens opacification. In the present study, there was a significant decrease in both total and soluble protein in diabetic group compared to control group (Table 9). Feeding of Emblica and tannoids to the diabetic rats prevented the loss of total and soluble protein in the lens.



**Table 8. The Effect of Emblica and its tannoids on activities of SOD, GPx, and GST in rat lens.**

Enzyme	Group I	Group II	Group III	Group IV
SOD	26.81± 0.62	36.88± 6.95 <sup>#</sup>	31.15± 7.65 <sup>§</sup>	26.84± 0.67 <sup>§</sup>
GPx	17.01± 0.52	18.72± 0.75 <sup>#</sup>	17.84± 0.40 <sup>§</sup>	18.77± 0.20
GST	24.82± 0.55	22.18± 0.48 <sup>#</sup>	22.11± 1.86	23.37± 0.35

The data are the mean ± SD (n = 3). SOD activity is expressed as units/min/100 mg protein, and the activity of GPx and GST is expressed as μmoles NADPH oxidized/h/100 mg protein and μmoles CDNB-GSH conjugate formed/h/100mg protein respectively.

# Symbol denotes significantly different from Group I  
§ Symbol denotes significantly different from Group II

**Table 9. The Effect of Emblica and its tannoids on protein content of rat lens**

Parameter	Group I	Group II	Group III	Group IV
Total protein (mg/g lens)	470± 27.88	414± 45.96 <sup>#</sup>	458± 29.37 <sup>§</sup>	467± 36.05 <sup>§</sup>
Soluble protein (mg/g lens)	392± 29.91	209± 86.00 <sup>#</sup>	330± 46.00 <sup>§</sup>	340± 47.01 <sup>§</sup>
Soluble protein (%)	83.40	50.48	72.05	72.81

The data are the mean ± SD (n = 4).

# Symbol denotes significantly different from Group I  
§ Symbol denotes significantly different from Group II

The distribution profile of major lens crystallins, evidenced by HPLC analysis, showed that there is an appearance of a high molecular weight (HMW) aggregate peak and decrease in β- and γ-crystallin peaks in untreated diabetic animals (Table 10), suggesting modification of all the three major crystallins due to diabetes induced hyperglycemia. The decrease in β & γ-crystallins suggests that protein degradation in diabetic cataract lens may be involved in the formation of HMW aggregates due to cross-linking or aggregation. However, these alteration in the α, β & γ-crystallins due to diabetes were minimized in the group III and IV animals fed with Emblica and tannoids prevented the decrease in protein content but also aggregation and insolubilization

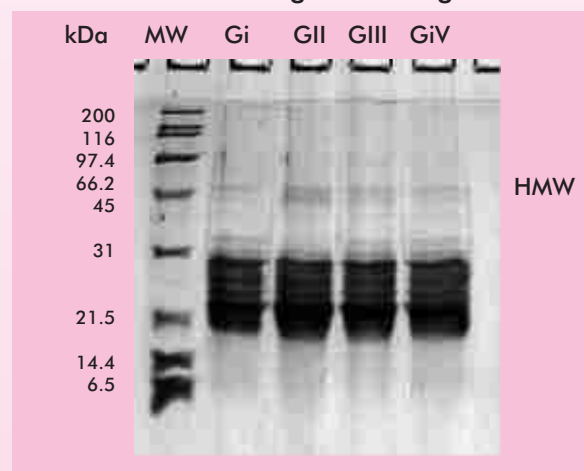
of lens proteins due to hyperglycemia as assessed by SDS-PAGE (Fig. 14).

(Table 10). Most importantly, Emblica and tannoids treatment not only Table 10. 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
HMW Peak	9691	25855	14152	16473
α-Crystallin	225162	217374	221874	220147
β-Crystallin	370472	289659	317144	300241
γ-Crystallin	338580	284713	309900	298967

Data are arbitrary units for absorbance at 280 nm.

**Figure 14: Effect of Emblica and its tannoids 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**



## CONCLUSIONS

The results 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.



## 5. MOLECULAR CHAPERONE FUNCTION OF $\alpha$ -CRYSTALLIN UNDER HYPERGLYCEMIC CONDITIONS: MODULATION BY DIETARY FACTORS – MODULATION OF CHAPERONE ACTIVITY OF $\alpha$ -CRYSTALLIN UNDER HYPERGLYCEMIC CONDITIONS BY ANTIGLYCATING AGENT MYB2

The eye lens structural protein is composed of the family of proteins known as crystallins. The most abundant among these is  $\alpha$ -crystallin which accounts for 30% of total lens protein.  $\alpha$ -Crystallin is a multimeric protein and made up of two homologous gene products, A and B-crystallins of each 20 kDa.  $\alpha$ -Crystallin is a representative member of small heat shock protein family, which acts as molecular chaperone. Molecular chaperones are a class of proteins that are known to interact with partially unfolded states of other proteins and prevent off pathway aggregation and inactivation, thus keeping them in a folding competent state. The chaperone-like activity of  $\alpha$ -crystallin in preventing aggregation of other lenticular proteins is particularly important in the context of maintaining lens transparency.

Owing to lack of cell turnover in the nucleus of the lens, the lenticular proteins are accumulated from the birth and are susceptible to several post-translational modifications (PTM). Most of the PTM of  $\alpha$ -crystallin are demonstrated to result in the loss of chaperone activity. Some of the PTM, particularly non-enzymatic glycation, are accelerated under diabetic condition. Indeed  $\alpha$ -crystallin from diabetic rat and human lenses has shown a substantial loss in its chaperone function.

It was earlier demonstrated that chaperone activity of  $\alpha$ -crystallin is compromised under diabetic conditions where as curcumin treatment modulated chaperone activity of  $\alpha$ -crystallin under diabetic conditions (*Annual Report-2004*). A number of dietary sources for their antiglycating potential for combating diabetic complications

including diabetic cataract were screened. Antiglycating ability of a dietary source (code name: MYB2) using *in vitro* protein glycation system was demonstrated.

### AIM AND OBJECTIVES

The main objective of the study was to investigate the effect of MYB2 on  $\alpha$ -crystallin chaperone activity under diabetic condition *vis-à-vis* its anticataractogenic potential.

### METHODOLOGY

Two-month-old male WNIN rats with average body weight of 190 g were used for this study. All the animals were fed AIN-93 diet ad libitum through out the study. The control rats (Group I; n=8) received 0.1 M citrate buffer pH 4.5 as vehicle, where as the experimental rats received a single intraperitoneal injection of streptozotocin (STZ; 32 mg/kg) in the same buffer. After 72 h, fasting blood glucose levels were monitored and animals having blood glucose levels less than 140 mg/dl were excluded from the experiment and rest were distributed into three groups. Animals in these groups received either only AIN-93 diet (Group II; n=8) or received AIN-93 diet containing 0.5% (Group III; n=6) and 2.5% of MYB2 (Group IV; n=6). Animal care and protocols were in accordance with and approved by the Institutional Animal Ethics Committee. Cataract progression due to STZ-induced hyperglycemia in treated and untreated rats is monitored by slit lamp biomicroscope.

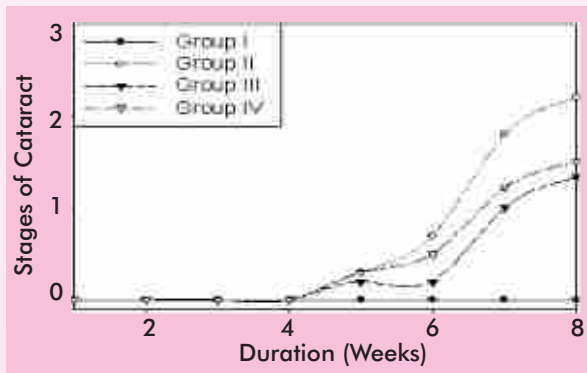
After 8 weeks of STZ injection rats from all the four groups were sacrificed by CO<sub>2</sub> asphyxiation and lenses were enucleated and lenses from three rats in each group were pooled. Water-soluble proteins were analyzed on a Sephacryl S-300 gel filtration column and fractions corresponding to  $\alpha$ H-,  $\alpha$ L-,  $\beta$ - and  $\gamma$ -crystallins were pooled separately. Chaperone activity of  $\alpha$ -crystallin (both  $\alpha$ H and  $\alpha$ L) was assessed by aggregation and enzyme inactivation assays. Far- and near-UV CD spectra of  $\alpha$ H and  $\alpha$ L-crystallin were recorded. Intrinsic tryptophan fluorescence and fluorescence of protein bound 8-anilino-1-naphthalene-sulfonic acid (ANS) was also measured.



**RESULTS**

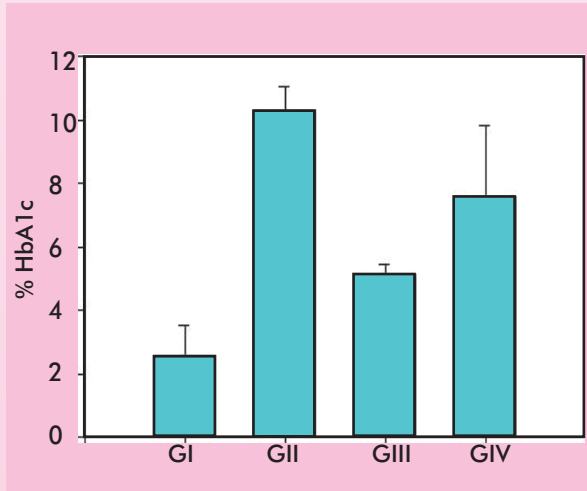
1. Feeding MYB2 to diabetic rats did not prevent the STZ-induced hyperglycemia.
2. Interestingly, MYB2 delayed the progression and maturation of STZ induced diabetic cataract. The effect was more pronounced with MYB2 at 0.5% (Figure 15).

**Figure 15.** Effect of MYB2 on progression and maturation of streptozotocin-induced cataract. G1-control rats, GII-diabetic rats, GIII-diabetic rats fed with 0.5 % MYB2 and GIV-diabetic rats fed with 2.5%.



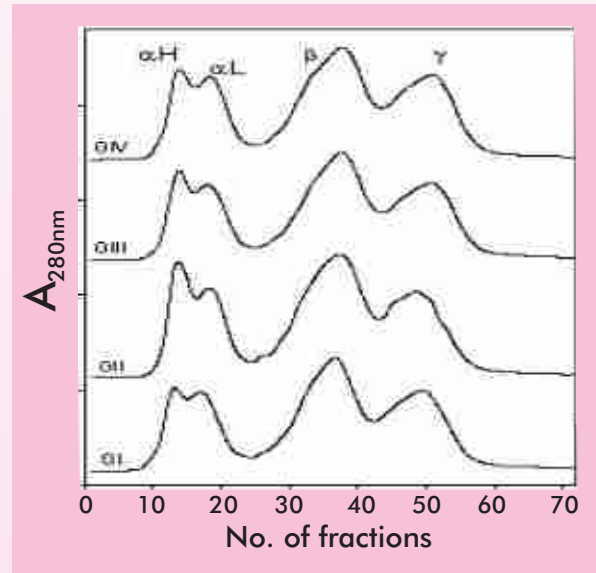
3. Percentage of glycated hemoglobin (HbA1c) is more in untreated diabetic rats (Group II) compared to Group I rats. Whereas feeding of MYB2 resulted in the decrease in percentage of HbA1c in dose dependent manner (Figure 16) substantiating MYB2 antiglycating property.

**Figure 16.** Estimation of glycated protein. G1-control rats, G II- diabetic rats, G III-diabetic rats fed with 0.5 % of MYB2 and G IV-diabetic rats fed with 2.5% MYB2



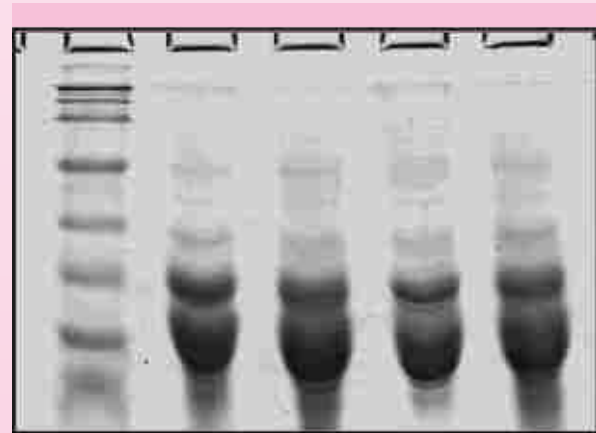
4. There was a marked difference in the relative distribution of crystallins between the groups.  $\alpha$ H-crystallin peak elevated in diabetic rat lens (Group II) compared to control rat lens (Group I). Feeding of MYB2 (Group III and IV) reverted the altered crystallin profile in a dose dependent manner (Figure 17).

**Figure 17.** Separation profile of crystallins on gel filtration column (Sephacryl S300 HR). G1-GIV corresponds to Group 1-IV, respectively

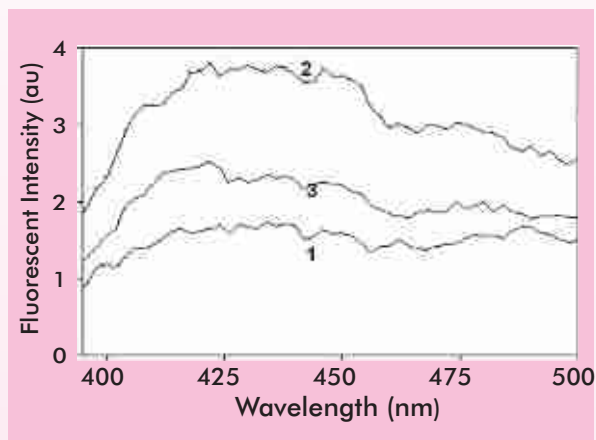


5. MYB2 treatment prevented the formation of high molecular weight aggregates due to hyperglycemia as assessed by SDS-PAGE (Figure 18).

**Figure 18.** SDS-PAGE profile soluble lens protein: Lane 1-control rats, lane 2- diabetic rats, lane 3-diabetic rats fed with 0.5 % of MYB2 and lane 4-diabetic rats fed with 2.5% MYB2



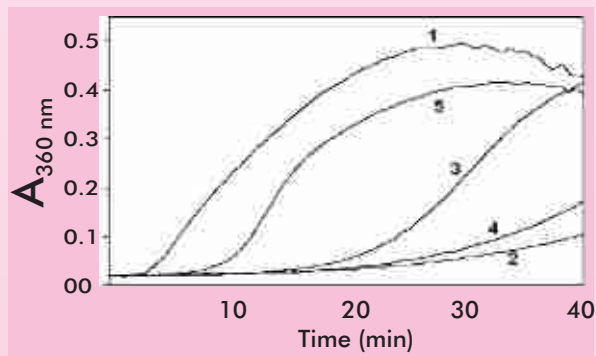
**Figure 19. AGE fluorescence of  $\alpha$ L-crystallin from control (trace 1), diabetic (trace 2) and diabetic rats fed with 0.5%MYB2 (trace 3)**



6. AGE fluorescence of  $\alpha$ L-crystallin from diabetic rat lens is elevated than  $\alpha$ L-crystallin from control lens where as MYB2 0.5% feeding decreased the AGE fluorescence (Fig. 19).

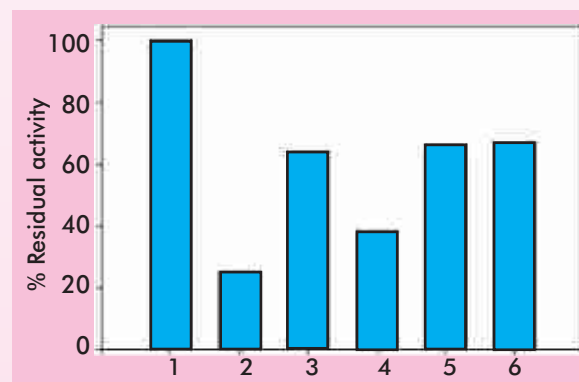
7.  $\alpha$ L-Crystallin from Group II rat lens showed loss of chaperone activity in suppressing the heat-induced aggregation of  $\beta$ -crystallin when compared to the activity of  $\alpha$ L-crystallin from Group I (Figure 20). Interestingly, chaperone like activity of  $\alpha$ L from Group III was improved than Group II rat lens. Unexpectedly,  $\alpha$ L from Group IV showed decreased chaperone activity than  $\alpha$ L from Group II (Fig.20). Similar results were observed in heat-induced aggregation assay of citrate synthase.

**Figure 20: Chaperone activity of  $\alpha$ L-crystallin against heat induced aggregation of  $\beta$ L-crystallin at 60°C.  $\alpha$ L-crystallin is incubated in the absence (trace 1) or in the presence of  $\alpha$ L-crystallin from Group I (trace 2), Group II (trace 2), Group III (trace 3) & Group IV (trace 4)**



8. Similar to aggregation assays, the ability of  $\alpha$ L-crystallin from Group II to prevent heat-induced inactivation of G6PD was declined as compared to Group I (Fig. 21). Surprisingly  $\alpha$ L-crystallin from both Group III and IV showed improved chaperone activity compared to Group II in prevention of heat-induced inactivation of G6PD (Fig. 21).

**Figure 21. Chaperone activity of  $\alpha$ L-crystallin in enzyme inactivation assays. Protection of heat-induced inactivation of G6PD at 42°C by  $\alpha$ L crystallin. Bar 1-G6PD alone, bar2- heat inactivated G6PD, bars 3-6, are G6PD plus  $\alpha$ L-crystallin from Groups I-IV, respectively. Data are average of three independent assays**



9.  $\alpha$ L from Group II showed lesser ANS binding when compared with  $\alpha$ L from Group I where as ANS fluorescence of  $\alpha$ L-crystallin from Group III is greater than  $\alpha$ L from Group II. Decreased chaperone activity of  $\alpha$ L from Group II correlated with decreased ANS binding.

10. Far- and near-UV CD signal and tryptophan fluorescence for  $\alpha$ -crystallin isolated from Group II rats lens decreased compared to Group I lens indicating altered secondary and tertiary conformation to the  $\alpha$ -crystallin from diabetic rat lens. MYB2 treatment at 0.5% has modulated the far - UV CD spectra and tryptophan fluorescence in significant manner.

## CONCLUSION

In the present study it was demonstrated that MYB2 delayed progression of STZ induced diabetic cataract. The delay in progression of cataract could be due to modulation of chaperone activity particularly in the rats fed with



MYB2 0.5%. Modulation of chaperone activity MYB2 0.5% was attributed to antiglycating ability of this compound under hyperglycemia.

## 6. SCREENING AND TESTING OF ALDOSE REDUCTASE INHIBITORS & ANTIGLYCATING AGENTS FROM DIETARY SOURCES – SPECIFICITY OF DIETARY ALDOSE REDUCTASE INHIBITORS

Diabetes mellitus and its disabling complications, which include cataract, nephropathy, retinopathy, and myocardial infarction, affect millions of people annually. The complications arise from various pathways; one of them is polyol pathway, where the elevated levels of glucose in tissues such as the nerve, kidney, retina and lens are converted to sorbitol by aldose reductase (ALR2). Because sorbitol does not easily cross cell membranes and its subsequent conversion to fructose via sorbitol dehydrogenase is slow, it accumulates inside certain cells resulting in changes in osmotic pressure, alterations in the redox state (i.e. increased NADH/NAD<sup>+</sup> ratio) and depleted intracellular levels of myoinositol. Inhibitors of ALR2 may control these biochemical changes, which have been linked to diabetic complications. Although many ALR2 inhibitors (ARI) have been extensively developed with promising results, none have demonstrated sufficient efficacy in human clinical trials without undesired side effects. 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 (EC 1.1.1.2), member of the aldo-keto reductase super family that coexists with ALR2 in most tissues. Many ARI are shown to equally interact with ALR1. During the last three years many dietary sources for their potential to inhibit ALR2 and found significant inhibition with some dietary sources were screened. Therefore, aim of the present study was to investigate the specificity of these dietary ARI against ALR1.

## METHODOLOGY

Aqueous extracts of freeze-dried dietary sources were prepared and tested for the inhibition against rat lens aldose reductase, recombinant human aldose reductase (rALR2) and aldehyde reductase (ALR1). Common active principles present in the most effective extracts were also tested against rALR2 and ALR1.

### PURIFICATION OF ENZYMES

#### RAT LENS ALR2

Crude ALR2 was obtained by homogenizing the WNIN rat lens in 10 volumes of 100 mM potassium phosphate buffer pH 6.2. The homogenate was centrifuged at 15,000g for 30 min at 4°C and the resulting supernatant was used as the source of ALR2.

#### HUMAN RECOMBINANT ALR2

Human rALR2 was overexpressed in *E. coli*. Enzyme from expression cultures was extracted and purified by affinity chromatography over AffiGel Blue followed by gel filtration.

#### LIVER ALR1

ALR1 was purified from goat liver by subjecting the tissue homogenate to (45 – 75 %) ammonium sulfate fractionation. Supernatant was loaded on to the ion exchange (DEAE-column) column. Fractions having ALR1 activity were pooled and subjected to gel filtration column. The fractions were concentrated, dialyzed and purity was assessed by SDS-PAGE.

### ENZYME ASSAYS

Rat lens ALR2, rALR2 and liver ALR1 activity was assayed by standard procedures described previously. The change in the absorbance at 340 nm due to NADPH oxidation was followed in a Cary Bio 100 spectrophotometer.

## RESULTS

1. Aqueous extracts of diet sources that have inhibited rat lens AR with IC<sub>50</sub> values less than 0.5 mg/ml are considered as effective sources of ALR2 inhibition and are shown in Table 11.



**Table 11. List of the dietary materials screened for the inhibition of rat lens ALR2 along with their IC<sub>50</sub> values.**

S. No.	Common Name	IC <sub>50</sub> values* (mg/ml)	Rank
1	Amla Tannoids	0.006	1
2	Curcumin	0.06	2
3	Spinach	0.1	3
4	Cumin	0.22	4
5	Fennel	0.23	5
6	Lemon	0.25	6
7	Black pepper	0.27	7
8	Bittergourd	0.29	8
9	Ocimum	0.31	9
10	Cinnamon	0.5	10

\*IC<sub>50</sub> values are average of three assays

- Extracts showing effective inhibition against the rat lens ALR2 were further tested for their inhibition against rALR2 (Table 12). Most of the dietary sources that inhibited rat lens ALR2 also inhibited rALR2 with equal efficiency or even better (Table 12). Dietary extracts that have inhibited rALR2 with IC<sub>50</sub> < 0.25 mg/ml are considered for further analysis.

**Table 12. List of the dietary materials tested for the inhibition against rALR2 along with their IC<sub>50</sub> values**

S.No.	ARI	IC <sub>50</sub> mg/ml	Rank
1	Amla Tannoids	0.010	1
2	Curcumin	0.052	2
3	Ocimum	0.125	3
4	Cumin	0.15	4
5	Spinach	0.15	5
6	Cinnamon	0.20	6
7	Fennel	0.21	7
8	Black Pepper	0.22	8
9	Bitter gourd	0.25	9

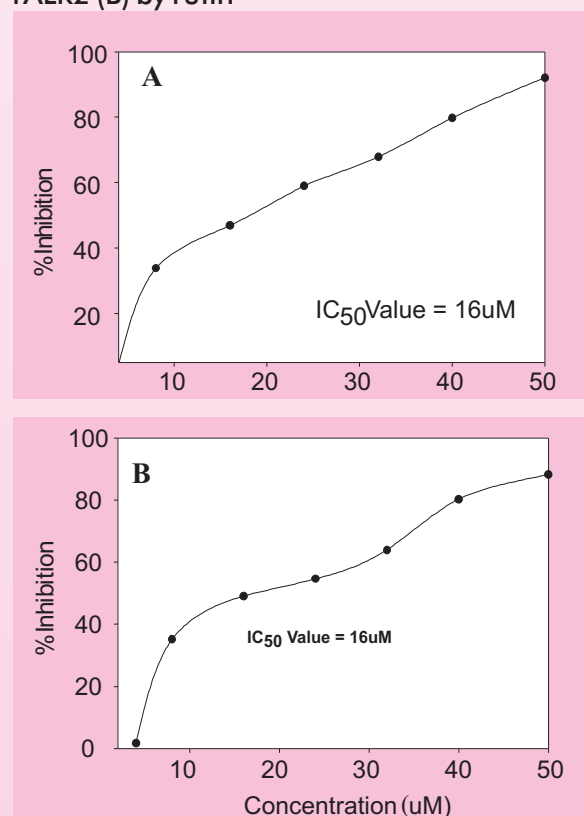
- Specificity of most effective dietary ARI towards ALR2 was demonstrated by their ability to inhibit liver ALR1. Interestingly, these dietary ARI could not inhibit goat liver ALR1 even at the concentrations 10 fold higher than their IC<sub>50</sub> values against ALR2 (Table 13)

**Table 13. List of dietary ARI screened for their specificity against goat liver ALR1**

S. No.	ARI	IC <sub>50</sub> Values (mg/ml)
1	Tannoids	0.28
2	Cinnamon	> 4.0
3	Ocimum	> 4.0
4	Spinach	> 4.0
5	Cumin	> 4.0
6	Bitter gourd	> 4.0
7	Fennel	2.6
8	Black Pepper	2.46

- Common active principles present in the most effective dietary sources were studied for the inhibition of ALR2. Rutin is known to be present in many of these dietary sources which exhibited ARI potential.
- Rutin was the most effective of all with IC<sub>50</sub> value 16 μM against rat lens ALR2 (Figure 22). Similar results were obtained for rutin with rALR2, with IC<sub>50</sub> value of 16 μM Fig (Fig.22). Other active components, which have IC<sub>50</sub> values close to rutin with respect to ALR2, are listed in Table 14.

**Figure 22. Inhibition of rat lens ALR2 (A) and rALR2 (B) by rutin**



6. Further specificity of rutin towards ALR1 was evident as it inhibited ALR1 20 times to that of its IC<sub>50</sub> value with ALR2 (>320 μM).

**Table 14. IC<sub>50</sub> value of some common active principles present in the diet sources which showed inhibition against rat lens ALR2**

S.No.	Active principle	IC <sub>50</sub> Value
1	Rutin	16 μM
2	Quercetin	33 μM
3	Caffeic acid	250 μM
4	Coumarin	350 μM
5	p- Coumaric acid	365 μM

## CONCLUSIONS

Specific inhibition of ALR2 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. Studies are in progress to evaluate their efficacy in preventing the diabetic complications in animal models.

## 7.IMPORTANCE OF α-CRYSTALLIN HETEROPOLYMER IN THE EYE LENS: CHAPERONE-LIKE ACTIVITY AND PHYSICO-CHEMICAL PROPERTIES

Impaired function of the lens due to partial or complete opacification is called *cataract*. α-crystallin belongs to the family of small heat shock proteins. α-Crystallin is abundant in the eye lens of almost all vertebrates, reaching levels up to 50% of lens soluble proteins. Apart from its structural role, α-crystallin is known to have chaperone like activity. In particular, the chaperone function is suggested to be instrumental in the prevention of cataract formation in the ocular lens. α-crystallin is normally isolated as large heteropolymer, composed of two related sub units, A and B, each with a molecular mass of 20 kDa, sharing a

sequence homology of about 57 %. Depending on species and age, the aggregate size can range from 300 kDa to over 1,000 kDa. α-crystallin is shown to exist as a homopolymer in non-lenticular tissues as either αA- or αB-homopolymer. Interestingly, in most of the vertebrate lens the ratio of αA and αB subunits is 3:1 and the significance of this ratio at present is not known. Understanding the importance of αA and αB-crystallin in 3:1 ratio is important to understand the chaperone function of α-crystallin, as this particular ratio may have a direct role to play in the chaperone like property of α-crystallin.

## OBJECTIVE

The main objective of the study was to understand the significance of 3:1 ratio of αA/αB crystallin in terms of chaperone function.

## METHODOLOGY

### Purification of recombinant αA and αB crystallins

Recombinant αA and αB-crystallins were expressed in *E. coli* and purified by ion exchange followed by gel filtration according to previously reported methods.

### Chaperone activity assays

Chaperone like activity of α-crystallin heteropolymer with various ratios of αA and αB-crystallins & homopolymers was assessed by measuring the ability of these proteins to protect aggregation prone substrate proteins. Heat induced aggregation of βL-crystallin (0.2 mg/ml) at 60°C in the absence and presence of α-crystallin homo and heteropolymers (0.05 mg/ml) was monitored. UV-induced aggregation of γ-crystallin (0.25 mg/ml) was carried at 300nm using a Xe arc lamp fitted to a monochromator in the presence and absence of crystallin homo and heteropolymers (0.1 mg/ml). Heat-induced aggregation of citrate synthase (50 μg/ml) was monitored in the presence and absence of crystallin homo and heteropolymers (0.03 mg/ml) by heating the reaction mixture at 45 °C. Aggregation in all these assays was monitored in a UV/ Vis Spectrophotometer at 360nm. Chaperone activity of α-crystallin hetero-polymer was also studied by its ability to prevent the heat-



induced inactivation of glucose 6-phosphate dehydrogenase (G6PD). Assay was performed by incubating G6PD (0.5U/ml) and  $\alpha$ -crystallin at 44°C for 30 min. Increase in absorption due to NADP reduction was monitored at 340 nm in a UV/Vis Spectro-photometer.

### Fluorescence Measurements

Hydrophobicity of crystallin homo and heteropolymers (0.1 mg/ml) was studied in Jasco FP-6500 spectrofluorometer by following binding of ANS (8-Anilino-1-naphthalene-1-Sulphonic acid). 100  $\mu$ M ANS was incubated with the corresponding protein samples for 30 min at room temperature. Fluorescence of protein bound dye was measured by excitation at 390 nm and emission spectra were recorded between 450 and 550 nm. Tryptophan fluorescence was monitored by exciting  $\alpha$ -crystallin (0.1 mg/ml) at 295 nm and the following emission spectra was recorded between 300 and 380 nm.

### Secondary and tertiary structure

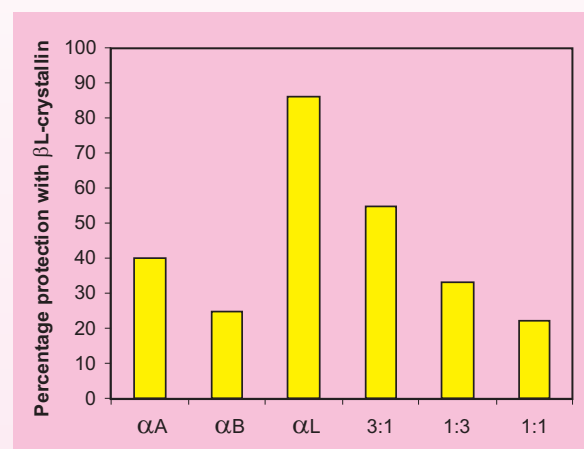
Far- and Near- UV CD spectra of  $\alpha$ -crystallin (0.2 mg/ml and 1.0 mg/ml respectively) was recorded using a Jasco-810 spectropolarimeter. Percentage alpha helical and beta sheet content of the protein was measured by Jasco J-810 software.

## RESULTS

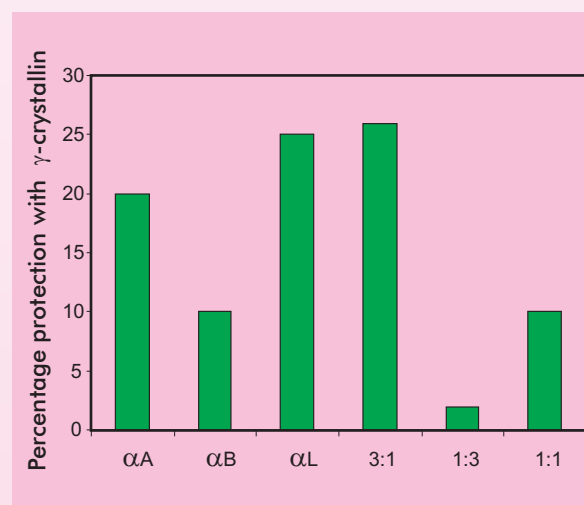
Recombinant  $\alpha$ A and  $\alpha$ B-crystallins were mixed accordingly and incubated at 4°C for 30 min to get the desired ratios. Goat  $\alpha$ L-crystallin was taken as a reference. Recombinant heteropolymer  $\alpha$ A to  $\alpha$ B 3:1 and goat  $\alpha$ L-crystallin (native variant of A to B 3:1 ratio) displayed higher chaperone activity than homopolymers and heteropolymers with other ratios i.e., 1:1, 1:3 in the  $\beta$ L-crystallin and  $\gamma$ -crystallin aggregation assay (Fig. 23 & 24).

However, in citrate synthase aggregation assay and G6PD heat inactivation assay protection with 3:1 was no better than other ratios and homopolymers (Fig. 25 & 26). Studies are underway to assess the chaperone activity of homo & hetero-polymers under physiologically relevant conditions such as cell cytotoxicity and apoptosis assays.

**Figure 23. Heat induced aggregation of  $\beta$ L-crystallin in the presence of  $\alpha$ -crystallin**



**Figure 24. UV light induced aggregation of  $\gamma$ -crystallin in the presence of  $\alpha$ -crystallin**



Hydrophobicity has been implicated as a major factor in chaperoning function. Hence, hydrophobicity of homo- and heteropolymers was assessed. While,  $\alpha$ B homopolymer appears to be more hydrophobic than  $\alpha$ A homopolymer, hydrophobicity of heteropolymer with  $\alpha$ A to  $\alpha$ B 3:1 ratio is intermediate to that of  $\alpha$ A and  $\alpha$ B-crystallin (Fig 27). Though, homo- & heteropolymers do not differ in their secondary structure, a significant difference was observed with homo- and heteropolymers of  $\alpha$ -crystallin with respect to tertiary structure (Fig 28 & 29; Table 15). The difference in tertiary structure may influence the chaperone activity.



Figure 25. Heat induced aggregation of citrate synthase in the presence of  $\alpha$ -crystallin

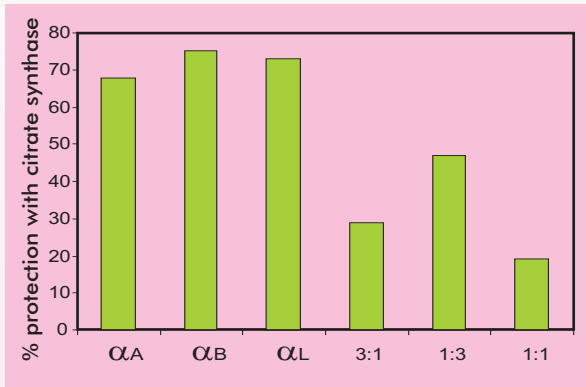


Figure 26. Heat-induced inactivation of G6PD in the presence of  $\alpha$ -crystallin

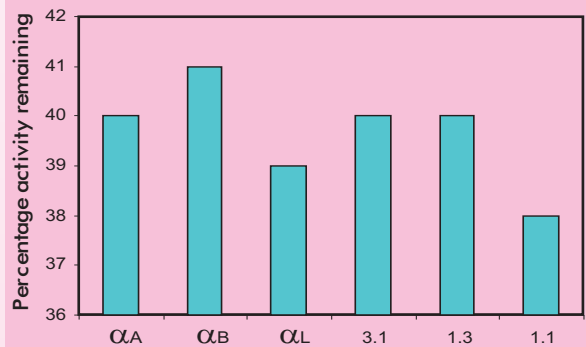


Figure 27. Hydrophobicity of hetero and homo polymers of  $\alpha$ -crystallin

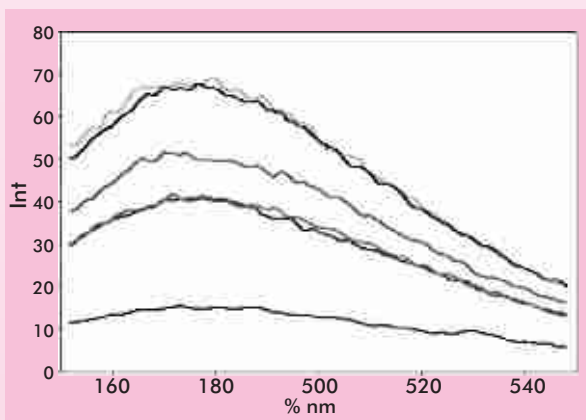


Figure 28. Tertiary structure of hetero and homo polymers of  $\alpha$ -crystallin

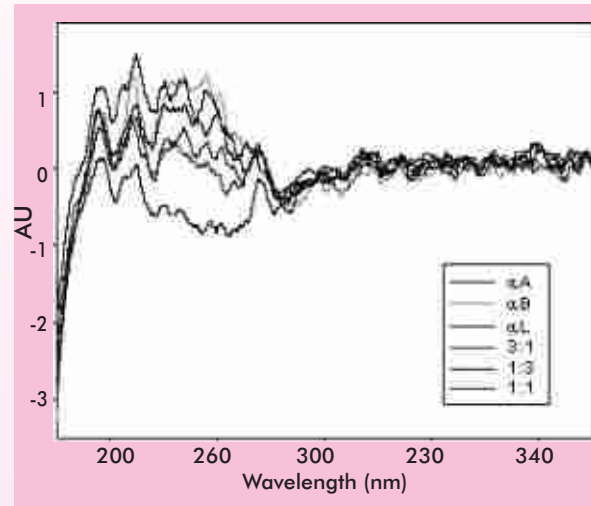


Table 15. Content of secondary structural elements of hetero and homo polymers of  $\alpha$ -crystallin

Sample	Alpha	Beta	Random	Coil
$\alpha$ A	28.1	32.0	24.9	15
$\alpha$ B	24.4	23.1	27.5	25
$\alpha$ L	29.8	24.4	21.3	24.5
3:1	26.3	25.9	28.1	19.7
1:3	21.8	30.2	26.7	21.3
1:1	25.7	25.8	27.2	21.3

## CONCLUSIONS

$\alpha$ -Crystallin heteropolymer with 3:1 A to B ratio was more effective in preventing the aggregation of other lens proteins but non-lenticular proteins. However, hydrophobicity is not related to the higher chaperone activity of heteropolymer and tertiary structure appears to play role in chaperone activity.



## 8. ACUTE EFFECT OF AOA RICH LEGUME SPROUT SALADS ON OXIDATIVE STRESS AND ANTI-OXIDANT STATUS OF ADULT HUMAN VOLUNTEERS

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. It also involves assessing the acute effect of the consumption of these recipes on AO status of human volunteers.

### AIMS AND OBJECTIVES

To assess acute effect of the consumption of sprout salads recipes on AO status of human volunteers.

### RESULTS

Effect of different kinds of domestic processing on AOA and PC of chosen plant foods (Annual Report 2004-2005) showed that AOA was higher in the sprouts of green gram, Bengal gram and moth beans compared to all other types of processing done, which had variable but not generally significant effect either on AOA.

The AOA of salad prepared with green gram sprouts with lemon, salt and pepper was the highest among the different salad recipes tested and the one prepared from Bengal gram sprouts was the next best.

Acute effect of consuming the two AOA rich salads prepared from green gram sprouts (ggs) and Bengal gram sprouts (Bgs) on the oxidative stress and antioxidant status of human volunteers was determined in eleven apparently healthy, adult male volunteers, 25-60 years of age. Milk bread was used as the negative control for this purpose. The effect of consumption of the salad recipes made from the sprouts of 50 grams of raw green gram or Bengal gram or 75 grams of milk bread, which had comparable amounts of calories, was studied. The composition of the two recipes consumed by the subjects is given in Table 16 and their phenolic content and AOA given in Table 17.

It is apparent from Table 17 that the two sprout salads were very rich in AOA whereas the negative control bread had very less AOA. Similarly, bread had the least PC among the three foods tested (Table 18).

Plasma MDA (TBARs) levels in the study subjects were comparable at fasting as well as up to three hours after the consumption of the recipes or bread indicating the lack of any acute effect of the recipes on the oxidative stress of the subjects. (F ratio by one way ANOVA was not significant between the three recipes at any point tested). The results are given in Table 19. Also, the difference in plasma MDA levels with time in subjects after the consumption of milk bread or the legume sprout based recipes was also no significantly different (Table 19).

Table 16. Composition of the legume sprout based recipes

Specifications	Negative Control	Recipe 1	Recipe 2
Recipe	White Bread	Green gram sprout salad with salt, lemon and pepper	Bengal gram sprout salad with salt, lemon and pepper
Quantity (g)	75	50 (Raw legume)	50 (Raw legume)
Calories (K Cal)	184	167	180



**Table 17. AOA and PC of the recipes and milk bread (negative control)**

Diets	Recipe	Antioxidant Activity*	Phenolic Content**
Control	Bread	11.4 ± 0.93	42.2±3.49
Experimental – 1	Green Gram Sprout Recipe	0.30 ± 0.02	106 ± 9.7
Experimental – 2	Bengal Gram Sprout Recipe	0.31 ± 0.00	61.6± 7.39

\* AOA expressed as mg of food required for 50% inhibition of auto-oxidation of β-carotene in the β-carotene & linoleic acid mixture.

\*\* PC expressed as mg of gallic acid equivalent in 100g of raw foodstuff. Values given are Mean ± S.D. (n=3)

**Table 18. Plasma malondialdehyde (MDA) levels (n moles/ml) in subjects consuming milk bread and legume sprout salads**

	Bread	Green gram sprout salad	Bengal gram sprout salad	F ratio (ANOVA)
N	11	10	11	
0 min	9.01 ± 2.72	7.83± 1.82	9.84± 6.95	0.560
60 min	12.11± 2.33	9.23± 3.91	13.02± 8.92	1.220
120 min	9.20 ± 3.22	8.82± 2.13	9.02± 3.81	0.064
180 min	9.03 ± 3.91	9.40± 2.73	9.51± 4.73	0.060

P value : NS Values given are Mean ± S.D.  
NS : Not significant.

**Table 19. Change in plasma MDA with time in subjects consuming milk bread and legume sprout salads**

MDA	F ratio (ANOVA)	P value
0 vs 60 min.	1.064	NS
0 vs 120 min.	0.757	NS
0 vs 180 min.	1.483	NS

**Table 20. Plasma ferric reducing antioxidant power (FRAP) (n moles/ml) in subjects Consuming milk bread and legume sprout salads**

	Bread	Green gram sprout salad	Bengal gram sprout salad	F ratio (ANOVA)	P value
N	11	10	11		
0 min	1195 ± 228.9	957 ± 156.8	1012 ± 191.2	4.334	P<0.05
60 min	995 ± 356.2	958 ± 161.6	1047 ± 189.4	0.323	NS
120 min	970 ± 247.8	968 ± 163.7	1040 ± 186.9	0.442	NS
180 min	1058 ± 215.4	976 ± 167.6	1067 ± 198.4	0.664	NS

Values given are Mean ± S.D. NS : Not significant.

The plasma FRAP levels of subjects at different time points after the consumption of the two salad recipes or the white bread are given in Table 20. The consumption of milk bread decreased the anti-oxidant status of the subjects as evident from a progressive decrease in their plasma FRAP values. It was interesting that consumption of legume sprout salads prevented this progressive deterioration of their anti-oxidant status. Indeed at all the three time points tested, the decrease in plasma FRAP with time (as compared to the fasting value) was significantly higher on consumption of milk bread than the two legume sprout based salad recipes (Table 21).

Although not statistically significant, plasma resistance to CuSO<sub>4</sub> induced oxidation ex vivo was higher in subjects consuming legume sprout based salads than milk bread and this was true at all the three time points tested after their consumption (Table 22, 23).

Notwithstanding that only the differences in plasma FRAP were statistically significant on consumption of AOA rich legume sprout based recipes, but not their resistance to exogenously induced oxidation ex vivo, the results from the acute feeding study, seems to be consistent with



**Table 21. Change in plasma FRAP in subjects consuming milk bread and legume sprout salads**

Frap	F ratio (ANOVA)	P value	Significant Difference
0 vs 60 minutes	3.864	P < 0.05	Bread vs Gg and Bg salads
0 vs 120 minutes	6.880	P < 0.05	Bread vs Gg and Bg salads
0 vs 180 minutes	17.700	P < 0.05	Bread vs Gg and Bg salads

**Table 22. Resistance of plasma to CuSO<sub>4</sub> induced oxidative stress ex vivo**

	Bread	Green gram sprout salad	Bengal gram sprout salad	F ratio (ANOVA)	P value
N	11	10	11		
0 min	1.50* ± 0.66	1.52 ± 0.59	1.64 ± 0.55	0.185	NS
60 min	1.74 ± 0.83	1.27 ± 0.70	1.34 ± 0.37	1.57	NS
120 min	1.64 ± 0.55	1.49 ± 0.92	1.44 ± 0.40	0.284	NS
180 min	1.87 ± 0.51	1.33 ± 0.58	1.43 ± 0.52	3.12	NS

Values given are Mean ± S.D.

NS : Not significant

\* Values given are MDA with CuSO<sub>4</sub> - without CuSO<sub>4</sub>(n moles/ml)

**Table 23. Time course of CuSO<sub>4</sub> induced change in oxidative stress in subjects consuming bread (control) & legume sprout salads**

Frap	F ratio (ANOVA)*	P value
0 vs 60 min.	2.756	NS
0 vs 120 min.	0.536	NS
0 vs 180 min.	2.168	NS

NS : Not significant

\* One way ANOVA among Δ60 – 0; Δ120 – 0 & Δ180 – 0 values of plasma MDA on consumption of the three recipes.

the hypothesis that feeding AOA rich recipes may be one of the effective ways to improve the AO status of the subjects and also protect them against exogenously induced oxidative stress. These preliminary observations seem not only to stress the importance of this approach in improving the AO status of individuals but also indicate the need for more detailed and in depth studies in this direction with more number of such AOA rich recipes.

## 9. HEALTH BENEFICIAL EFFECTS OF FOODS

### I. ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF CEREALS, MILLETS, LEGUMES AND PULSES

Natural antioxidants have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic values. Literature on health beneficial effects of Indian plant foods is scanty. Hence, studies have been taken up to generate database on antioxidant activity of plant foods commonly consumed in India and correlate them with their phenolic content. Earlier, antioxidant activities of green leafy vegetables, fresh and dry fruits were reported by Annual Report 2000 and 2006. In continuation, the phenolic content and antioxidant activity (by three different methods: FRAP, DPPH radical scavenging and reducing power) of cereals, millets, legumes and pulses commonly consumed in India is reported.

#### MATERIAL AND METHODS

Commonly consumed varieties (identified based on the NNMB survey) of cereals, millets, legumes and pulses were purchased from three



different local markets of the twin cities of Hyderabad and Secunderabad. It is observed that about 90% of the Indians consume less than 100 foods among the various foods available in the country. This observation is derived by calculating median quantity of food item consumption divided by number of consumers.

Standard extraction methods and estimation protocols were adopted. While the phenolic content was determined by the Folin Ciocalteu method, the antioxidant activity (AOA) was determined by three different methods :1. FRAP (Ferric Reducing Antioxidant Power at pH 3.9), 2.DPPH Radical scavenging activity and 3. reducing power .

#### a. CEREALS AND MILLETS

Mean phenolic content and AOA (by three different methods) of the samples are given in Table 24.

1. Phenolic content ranged from 47.64 to 373 mg/100g of edible portion. Ragi had the highest amount of (373 mg/100g) total phenolic content and this was followed by wheat semolina (139 mg /100g). The mean phenolic content of cereals and millets was 120 mg/100g.

2. DPPH radical-scavenging activity ranged from 0.24 to 1.73 (Trolox equivalent) with the highest activity being seen in Ragi (1.73) followed by Maize (1.39) and the mean value was 0.95 mg/ 100g .
3. FRAP activity showed a wide range from 16.21 to 472 M / g, and the mean value was 112 M/g. The highest activity was seen again in Ragi (472M/g).
4. Reducing power ranged from 4.54 to 0.36 mg / g. Ragi had the highest activity (4.54) followed by wheat semolina (0.3) and the mean value was 1.31 mg/g.
5. Correlation analysis showed that in cereals and millets phenolics may contribute maximally to FRAP ( $r^2 = 82.53\%$ ) and reducing power ( $r^2 = 82.38\%$ ) but only around 20% to DPPH scavenging activity.

#### Correlation between phenolic content & AOA in cereals and millets

PC Vs	r	r <sup>2</sup> %
DPPH	0.45	20.49
FRAP	0.91	82.53
Reducing power (mg/g)	0.90	82.38

Values are Mean  $\pm$  SD; n = 3

**Table 24. Antioxidant activity of commonly consumed cereals and millets**

Food grain	Botanical name	Phenolic content (mg/100g)	DPPH (% Inhibition trolox equivalent) mg/g	FRAP ( $\mu$ M/g)	Reducing power (mg/g)
Rice (milled)	<i>Oryza sativa</i>	47.64 $\pm$ 0.82	1.23 $\pm$ 0.03	60.93 $\pm$ 8.76	0.79 $\pm$ 0.02
Rice parboiled (milled)	<i>Oryza sativa</i>	50.87 $\pm$ 1.99	0.75 $\pm$ 0.00	67.48 $\pm$ 10.98	0.84 $\pm$ 0.13
Wheat flour (whole)	<i>Triticum aestivum</i>	109.34 $\pm$ 23.71	0.24 $\pm$ 0.08	33.29 $\pm$ 2.40	0.61 $\pm$ 0.002
Jowar	<i>Sorghum vulgare</i>	57.55 $\pm$ 2.72	1.27 $\pm$ 0.04	66.90 $\pm$ 6.65	0.82 $\pm$ 0.13
Rice puffed	<i>Oryza sativa</i>	56.61 $\pm$ 3.06	0.49 $\pm$ 0.11	62.98 $\pm$ 5.57	0.93 $\pm$ 0.08
Bajra	<i>Pennisetum typhoideum</i>	133.63 $\pm$ 2.33	1.26 $\pm$ 0.18	145.69 $\pm$ 19.36	1.73 $\pm$ 0.09
Finger millet (Ragi)	<i>Eleusine cora cana</i>	373.15 $\pm$ 70.07	1.73 $\pm$ 0.03	471.71 $\pm$ 33.48	4.54 $\pm$ 0.81
Semolina	<i>Triticum aestivum</i>	138.72 $\pm$ 9.58	0.27 $\pm$ 0.09	16.21 $\pm$ 2.37	0.36 $\pm$ 0.10
Maize	zeamays	112.68 $\pm$ 0.43	1.39 $\pm$ 0.50	86.29 $\pm$ 21.90	1.20 $\pm$ 0.13
	Mean	120.02 $\pm$ 101.0	0.95 $\pm$ 0.53	112.38 $\pm$ 137.0	1.31 $\pm$ 1.26



## b. LEGUMES AND PULSES

1. Phenolic content of pulses and legumes showed a wide range (62.4 to 418 mg/100g). Black gram dhal had the highest total phenolic content (418 mg/100g) followed by Rajmah (333mg/100g). The mean phenolic content was 199 mg/100g (Table 25).
2. DPPH scavenging activity ranged from 0.26 to 1.07 mg/g (trolox equivalent) with the highest activity seen in Rajmah (1.07) and the least in Bengal gram dhal (0.26) and the mean value being 0.60 mg/g.
3. FRAP activity in pulses and legumes ranged from 52.9 to 373  $\mu$ M/g. The highest activity was in Rajmah (373 $\mu$ M/g) and the least in green gram dhal (52.9  $\mu$ M/g). The mean value was 120  $\mu$ M/g.
4. Reducing power ranged from 0.77 to 4.89 mg/g with the mean value being 1.70mg/g. The highest activity was found in Rajmah

(4.89mg/g) and the lowest in Black gram dhal (0.77 mg/g).

5. Compared to cereals and millets, pulses and legumes in general, showed poor correlation ( $r^2=2-19\%$ ) between PC and AOA.

The data generated on the AOA of cereals, millets, legumes and pulses commonly consumed in India indicates that phenolics in plant foods may contribute to their AOA only in some foods but not all.

### Correlation between phenolic content & AOA in legumes and pulses

PC Vs	r	r <sup>2</sup> %
DPPH	0.16	2.56
FRAP	0.44	19.39
Reducing power (mg/g)	0.33	10.91

Values are Mean  $\pm$  SD; n = 3

**Table 25. Antioxidant activity of commonly consumed pulses and legumes**

Food grain	Botanical name	Phenolic content (mg/100g)	DPPH (% Inhibition trolox equivalent) mg/g	FRAP ( $\mu$ M/g)	Reducing power (mg/g)
Red gram dhal	<i>Cajonus cajan</i>	229.33 $\pm$ 3.34	0.61 $\pm$ 0.18	102.65 $\pm$ 12.51	1.63 $\pm$ 0.24
Green gram dhal	<i>Phaseolus aureus Roxb</i>	62.35 $\pm$ 4.31	0.30 $\pm$ 0.10	52.85 $\pm$ 10.09	1.05 $\pm$ 0.31
Blackram dhal	<i>Phaseolus mungo Roxb</i>	418.34 $\pm$ 12.57	0.27 $\pm$ 0.07	61.75 $\pm$ 9.89	0.77 $\pm$ 0.28
Bengal gram dhal	<i>Cier arietinum</i>	149.37 $\pm$ 10.35	0.49 $\pm$ 0.12	84.12 $\pm$ 7.74	1.50 $\pm$ 0.18
Lentil	<i>Lens esculenta</i>	112.91 $\pm$ 3.19	0.44 $\pm$ 0.13	67.98 $\pm$ 7.09	1.03 $\pm$ 0.25
Green gram (whole)	<i>Phaseolous aureus Roxb</i>	327.64 $\pm$ 8.26	0.86 $\pm$ 0.07	152.36 $\pm$ 15.01	1.60 $\pm$ 0.11
Bengal gram dhal (rosted)	<i>Cier arietinum</i>	131.19 $\pm$ 4.62	0.26 $\pm$ 0.01	70.96 $\pm$ 10.13	1.33 $\pm$ 0.16
Bengal gram (whole)	<i>Cicer arietinum</i>	194.90 $\pm$ 9.60	0.53 $\pm$ 0.08	128.32 $\pm$ 12.58	1.88 $\pm$ 0.06
Peas green (dry)	<i>Pisum sativum</i>	126.63 $\pm$ 35.00	0.88 $\pm$ 0.05	97.35 $\pm$ 7.44	1.54 $\pm$ 0.34
Soya bean	<i>Glycine maxmerr</i>	100.54 $\pm$ 1.08	0.97 $\pm$ 0.02	130.57 $\pm$ 6.50	1.52 $\pm$ 0.53
Rajmah	<i>Phaseolus vulgaris</i>	332.98 $\pm$ 8.06	1.07 $\pm$ 0.21	372.76 $\pm$ 17.72	4.89 $\pm$ 1.14
	Mean	198.74 $\pm$ 114.72	0.60 $\pm$ 0.29	120.15 $\pm$ 89.57	1.70 $\pm$ 1.10



## II. ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF SPICES COMMONLY CONSUMED IN INDIA

Plant foods are good sources of antioxidants and phenolic compounds are potent antioxidant substances present in all plant foods. Since spices form important ingredients of Indian diets and a wide variety of spices are used in the Indian culinary preparations, the present study was conducted to assess antioxidant activity of commonly consumed spices of India and assess the contribution of their phenolic content to AOA.

### MATERIAL AND METHODS

Twenty two, commonly consumed spices used in curries, legumes, snacks and cereal foods prepared in India were studied. Three samples of each of the commonly consumed varieties of

spices were purchased from each of the three different local markets of the twin cities of Hyderabad and Secunderabad. Samples bought from one market were pooled and analysed as one samples. Thus for each of the spice, three pooled samples (each made of three samples) have been analysed.

Standard extraction and estimation protocols were adopted. While the phenolic content was determined by the Folin Ciocalteu method, the antioxidant activity (AOA) was determined by two different methods :1. DPPH Radical scavenging activity and 2. Inhibition of auto oxidation of carotene in a mixture of carotene and linoleic acid *in vitro* by the spice extract.

### RESULTS

The phenolic content and anti oxidant activity of the spice samples are given in Table 26 and the salient findings are as follows.

**Table 26. Antioxidant activity and phenolic content (expressed as mean  $\pm$  SD) in commonly consumed spices of India**

Botanical names	English common names	Antioxidant activity		Phenolic content <sup>#</sup>
		$\beta$ C-LA*	DPPH <sup>~</sup>	
Brassica nigra	Mustard seeds	1.276 $\pm$ 0.761	4.17 $\pm$ 0.44	207.0 $\pm$ 9.7
Cuminum cyminum	Cumin seeds	0.994 $\pm$ 0.264	7.43 $\pm$ 1.04	328.0 $\pm$ 98.6
Trigonella foenum graecum	Fenugreek seeds	1.542 $\pm$ 0.322	4.88 $\pm$ 1.89	224.6 $\pm$ 33.7
Capsicum annum	Red chillies	0.345 $\pm$ 0.103	1.93 $\pm$ 0.21	295.4 $\pm$ 45.3
Ferula foetida	Asafoetida	0.477 $\pm$ 0.347	1.29 $\pm$ 0.57	228.8 $\pm$ 125.3
Sajeera	Sajeera	0.419 $\pm$ 0.045	9.47 $\pm$ 0.80	444.5 $\pm$ 107.2
Cinnamomum zeylanicum	Cinnamon	0.207 $\pm$ 0.026	80.30 $\pm$ 16.98	3107.1 $\pm$ 189.5
Syzygium aromaticum	Clove	0.055 $\pm$ 0.005	165.32 $\pm$ 47.4	10418.7 $\pm$ 536.9
Elettaria cardamomum	Cardamom small	0.511 $\pm$ 0.279	1.70 $\pm$ 0.13	116.8 $\pm$ 20.9
Elettaria cardamum	Cardamom black	0.241 $\pm$ 0.033	3.71 $\pm$ 0.15	288.1 $\pm$ 28.0
Piper nigrum	Pepper	0.245 $\pm$ 0.022	4.02 $\pm$ 0.70	261.3 $\pm$ 17.7
Laurus nobilis L	Bay leaves	0.289 $\pm$ 0.094	35.6 $\pm$ 8.67	2021.2 $\pm$ 581.2
Myristica fragrans	Mace	0.124 $\pm$ 0.036	13.18 $\pm$ 6.40	627.1 $\pm$ 74.8
Myristica fragrans	Nutmeg	0.150 $\pm$ 0.016	14.38 $\pm$ 5.11	721.5 $\pm$ 122.6
Illicium verum	Staranise	0.194 $\pm$ 0.012	61.74 $\pm$ 12.48	1224.6 $\pm$ 269.6
Curcuma domestica	Turmeric	0.090 $\pm$ 0.038	10.88 $\pm$ 0.48	817.7 $\pm$ 61.8
Coriandrum sativum	Coriander seeds	1.431 $\pm$ 0.937	2.63 $\pm$ 0.25	207.4 $\pm$ 48.2
Papaver somniferum	Poppy seeds	5.531 $\pm$ 1.689	0.47 $\pm$ 0.02	16.1 $\pm$ 1.3
Chadila	Chadila	5.442 $\pm$ 0.869	2.23 $\pm$ 0.32	218.9 $\pm$ 75.0
Foeniculum vulgare Mill	Fennel seeds	0.448 $\pm$ 0.198	7.24 $\pm$ 1.32	364.0 $\pm$ 16.5
Zinziber officinale	Ginger dry	0.332 $\pm$ 0.029	3.77 $\pm$ 1.87	336.0 $\pm$ 23.1
Trachyspermum ammi	Bishops weed	0.176 $\pm$ 0.005	8.08 $\pm$ 2.16	932.0 $\pm$ 113.6

\* expressed as mg of food required for 50% inhibition of the autooxidation of  $\beta$ -carotene in the  $\beta$ -carotene and linoleic acid mixture *invitro* ~ expressed as mg Trolox equi/g of food<sup>#</sup> expressed as mg of gallic acid equivalent in 100g of raw food stuff



1. Antioxidant activity (AOA) analyzed by  $\beta$ CLA method (expressed as mg of food required for 50% inhibition of the autooxidation of  $\beta$ -carotene in the  $\beta$ -carotene and linoleic acid *in vitro*) was the highest in clove (0.055) and the lowest in poppy seeds (5.531).
2. Antioxidant activity as assessed by DPPH radical scavenging activity (expressed as mg trolox equivalent / g spice) showed that clove has the highest AOA (165.32) where as poppy seeds (0.47) had the lowest activity.
3. Total phenolic content (expressed as mg of gallic acid equivalent in 100g of raw foodstuff) was also the highest in clove (10,318.7) followed by cinnamon (3,107.1), Bay leaves (2,021.2) and the lowest was in poppy seeds (16.1).
4. Significant negative correlation (Spearman rho) was found between phenolic content (PC) vs  $\beta$ CLA (- 0.77 ), and PC vs DPPH (- 0.88), suggesting that PC contributed significantly to AOA of the spices studied as analysed by  $\beta$ CLA and DPPH methods used in the present study.

## 10. PRO12 ALA POLYMORPHISM IN PPAR $\gamma$ AND ITS ASSOCIATION WITH METABOLIC SYNDROME IN A COHORT FROM SOUTH INDIA

The etiology of T2DM has a strong genetic component and variations in several candidate genes have been widely implicated in predisposition to this disorder. Among these candidate genes, PPAR $\gamma$ , a nuclear hormone receptor, controls adipocyte differentiation and regulates a number of genes associated with energy homeostasis. The common variant Pro12Ala (rs1801282) of PPAR $\gamma$  has been consistently associated with T2DM among the Western populations, however, such associations have been inconsistent among the Asians. Another silent mutation in the exon 6 of PPAR $\gamma$  gene, C1431T (rs3856806), studied in several populations, was also found to be in linkage disequilibrium with the Pro12Ala variant. Intriguingly, the prevalence of diabetes in Hyderabad (South India) was found to be significantly high (16.6%) along with a higher

**Table 27. Anthropometric profile of the study subjects according to Diabetes and Hypertension status**

	Diabetes			Hypertension		
	Diabetics	Non-diabetics	P	Hypertensives	Normotensives	P
No. (699)	350	349	-	351	348	-
BMI	24.92±5.35	25.88±5.88	<0.05	25.73±5.39	25.39±6.18	NS
Waist circumference (cm)	93.79±9.011	90.23±9.43	<0.0001	92.91±8.71	91.11±9.95	<0.05
Systolic BP (mmHg)	134.68±25.88 (N=285)	136.32±28.94 (N=113)	NS	141.69±27.08 (N=213)	127.61±24.36 (N=185)	<0.001
Diastolic BP (mmHg)	83.90±13.73 (N=285)	84.10±14.45 (N=113)	NS	87.65±13.73 (N=213)	79.70±12.92 (N=185)	<0.001
Total Cholesterol (mg/dl)	329.43±286.53 (N=323)	218.16±128.96 (N=168)	<0.0001	328.80±277.46 (N=266)	247.16±204.50 (N=225)	<0.001
Total Triglyceride (mg/dl)	225.56±108.37 (N=323)	185.12±83.84 (N=175)	<0.0001	224.30±114.62 (N=267)	196.38±83.39 (N=231)	<0.05



**Table 28. Allele, genotype and haplotype frequencies of Pro12Ala and C1431T variants with respect to Type 2 Diabetes, Hypertension, BMI and Waist circumference**

	Diabetics (N=350)	Non Diabetics (N=349)	OR (95%CI)	Hypertensives (N=351)	Normotensives (N=348)	OR (95%CI)	BMI≥23 (N=453)	BMI<23 (N=246)	OR (95%CI)	High WC (N=419)	Low WC (N=280)	OR (95%CI)
<b>Pro12Ala</b>												
Pro/Pro	76.3%	77.7%	1.08 (0.76-1.54)	74.4%	79.6%	1.34 (0.95-1.92)	78.1%	74.8%	0.83 (0.58-1.19)	77.3%	76.4%	0.95 (0.66-1.36)
Pro/Ala+Ala/Ala Ala allele	23.7% 12.9%	22.3% 11.9%		25.6% 13.7%	20.4% 11.1%		21.9% 12.1%	25.2% 12.8%		22.7% 12.4%	23.6% 12.3%	
<b>C1431T</b>												
CC	71.7%	75.0%	1.19 (0.85-1.66)	70.0%	76.7%	1.41 (1.0-1.97)	74.0%	72.4%	0.92 (0.65-1.31)	71.1%	76.8%	1.34 (0.95-1.90)
CT+TT	28.2% 15.6%	25.0% 12.3%		30.0% 15.8%	23.3% 12.3%		26.0% 14.0%	27.6% 14.6%		28.9% 15.4%	23.2% 12.5%	
<b>Haplotypes</b>												
Pro-C	0.820	0.834	0.485	0.818	0.839	0.278	0.828	0.827	0.984	0.819	0.839	0.342
Ala-T	0.097	0.089	0.598	0.103	0.080	0.131	0.087	0.102	0.371	0.095	0.086	0.579
Pro-T	0.056	0.045	0.341	0.051	0.049	0.878	0.052	0.044	0.517	0.060	0.039	0.083
Ala-C	0.027	0.032	0.561	0.028	0.032	0.723	0.032	0.026	0.516	0.025	0.036	0.276



impaired glucose tolerance (IGT) (29.8%) when compared with urban Indian populations living in other metropolitan cities. Though, the Pro12Ala and the C1431T SNPs have been associated with a higher prevalence of diabetes in the western and some Asian populations, its effect remains to be seen in the Indian population, which has a higher prevalence of diabetes. Additionally, there is a lack of information pertaining to the C1431T SNP in Asian Indian population. Therefore, in the present study the roles of the Pro12Ala and the C1431T variants in a large cohort from Hyderabad with T2DM, obesity and hypertension was investigated and also haplotype analysis on these disease phenotypes from this urban population was carried out.

## CONCLUSION

Isolated genomic DNA from a cohort comprised of 699 unrelated subjects were presented at the Medici Hospital, Hyderabad, India between August 2004 and August 2006. Subjects were diagnosed for T2DM (mean age  $61.8 \pm 11.25$  years) based on their past history and being on anti-diabetic or hypoglycemic drug. Subjects over 50 years of age (mean age  $61.93 \pm 10.40$  years) with normal glucose levels or elevated glucose levels without glycosylated hemoglobins were enrolled as controls. Subjects were further categorized based on hypertension and obesity. The variants in the PPAR $\gamma$  were identified by using a PCR-RFLP method and the mutations were further validated by automated DNA sequencing (Table 27). Based on an earlier epidemiological study, the prevalence of Type 2 diabetes was highest (16.6%), with a very high prevalence of impaired glucose tolerance (29.8%) in Hyderabad, among the major metropolitan cities in India. Interestingly, the frequency of the minor Ala allele in our cohort (12.8%) was comparable to the other report from Chennai in Southern India (11%) and Caucasians (12%). However, this frequency was significantly higher than other Asian populations from Taiwan (4%), Korea (4.1%), Malaysia (3.2%), China (1%) and Japan (3%). On the other hand, the frequency of the T allele (C1431T) in the cohort was 14.2%, which was again similar to

Caucasian populations from UK (11.9%) and France (13.3%). Linkage disequilibrium (LD) between these two SNPs was almost similar across different phenotype categories such as, diabetes ( $D'=0.719$ ), hypertension ( $D'=0.713$ ), BMI ( $D'=0.713$ ) and waist circumference ( $D'=0.716$ ). Four different haplotypes were generated for these SNPs with respect to these phenotype categories. The estimated haplotype frequencies were not significantly different among the cohorts with and without the clinical traits within these phenotype categories (Table 28). These observations clearly point to a genetic proximity, at least with respect to Ala allele and T allele of Indian population to the Caucasians. No association of C1431T with T2DM, BMI and waist circumference was found, while there was a weak association with hypertension ( $p=0.049$ ). Since, the allele frequencies of both the variants in Indians are closer to that of Caucasian populations rather than other Asian populations, indicating that their implications are very different. This may be due to other genetic variants in candidate genes that are as yet uncharacterized in the Indian population.

## 11. UNDERSTANDING THE MECHANISM OF ACTION OF PPAR $\gamma$ IN REGULATION OF GLUCOSE METABOLISM

Insulin resistance and type 2 diabetes is emerging fast as a major health risk in developing countries like India. Epidemiological observations suggest a strong pathophysiological correlation of obesity to type 2 diabetes, however, the molecular link between these two conditions remained elusive. In a mice model, resistin, a cysteine rich secretory protein, which is down regulated by anti-diabetic drugs like thiazolidinediones (TZDs), has been implicated as the link between type 2 diabetes and obesity. However, the role of this protein in the etiology in humans is highly debated. Several studies have demonstrated a lack of correlation between levels of resistin and obesity and insulin resistance.



Figure 29. Truncated promoter constructs of human resistin

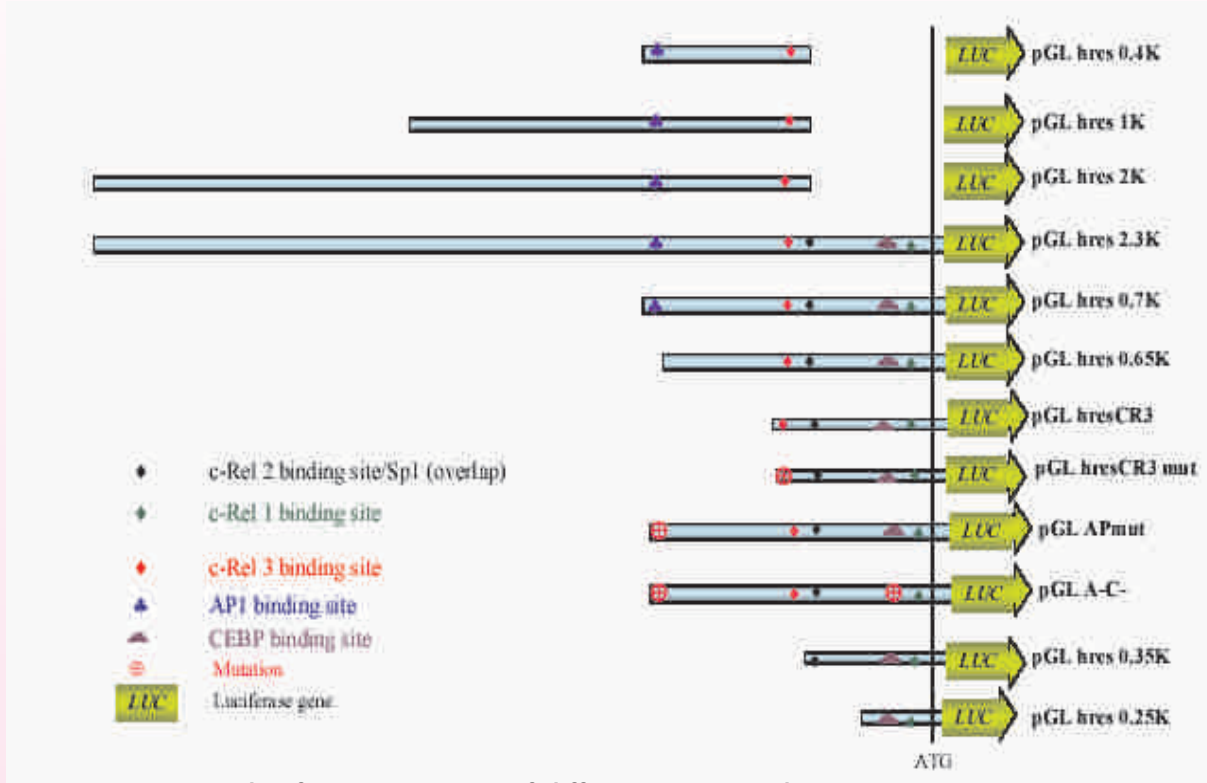
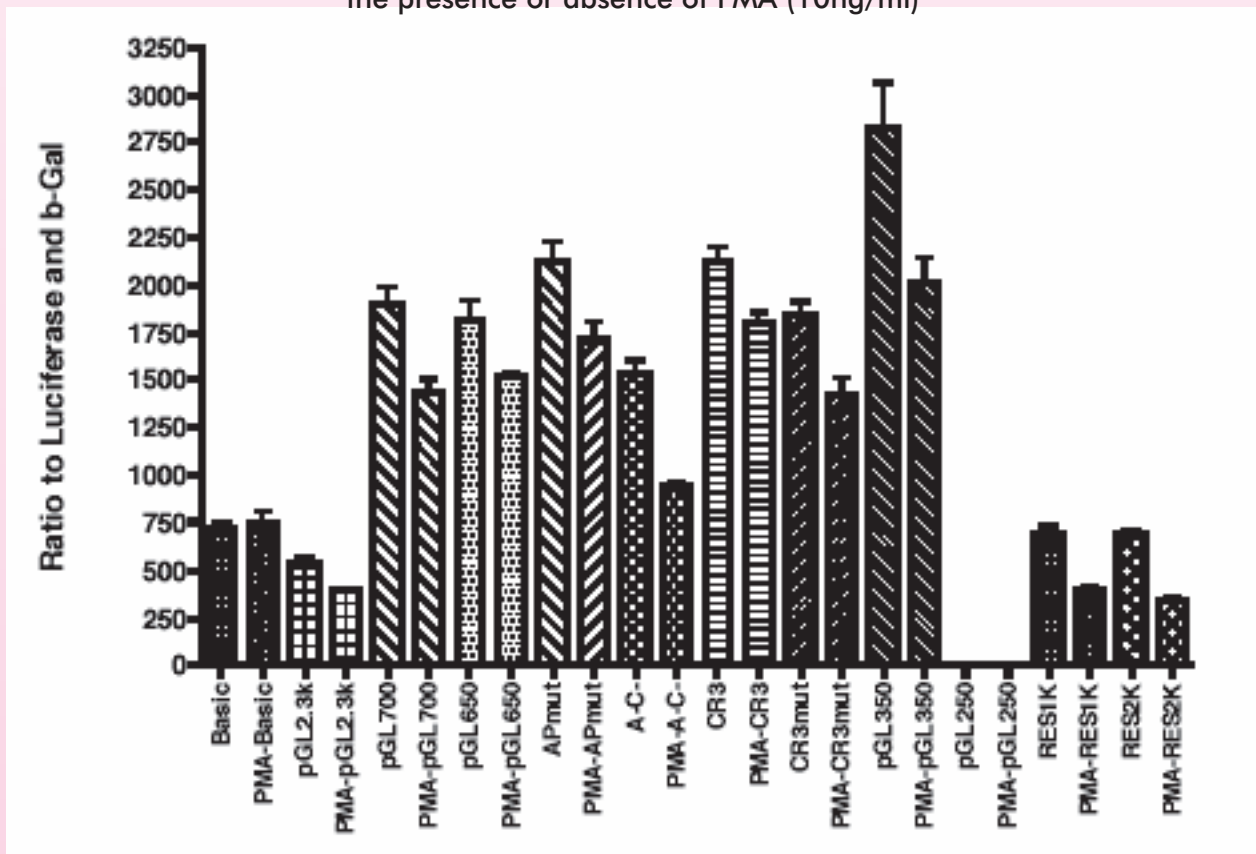


Figure 30. luciferase activities of different truncated promoter constructs in the presence or absence of PMA (10ng/ml)



Also, it appears that the resistin in humans play a role in inflammation rather than insulin resistance. Earlier observations indicated that the differences in the genetic organization of mouse and human may account atleast in part the difference of their expression patterns as well as levels. Therefore, much detailed study was undertaken in human resistin to understand the molecular mechanism of its regulation in humans.

## CONCLUSION

To understand the transcriptional regulation of human resistin performed *in silico* analysis was performed to identify putative transcription factors that may interact with the human resistin promoter. A promoter region of about 2.0 kb upstream to the transcriptional start site was cloned into a luciferase reporter vector. The 2.0 kb promoter was systemically deleted from the 5' region to generate a series of clones to study the importance of different promoter regions and the cognate binding proteins in regulating the transcriptional activities from the human resistin promoter (Fig.29). These constructs were

transfected into U937 monocytic cell lines and measured the respective reporter activities (Fig.30). Using *in silico* analyses, it was found that NF-B, AP-1, C/EBP and c-Rel are the major transcription factors present in the minimal promoter of human resistin. Since, transfected studies revealed that the resistin gene transcription can be modulated by PMA, a pharmacological inducer AP1 and NFkB, we examined the binding of both the AP-1 and NF-B using EMSA with oligonucleotides native to their binding sites on the promoter. It was found that c-rel and the Ap1 positively interacts with their cognate motifs in nuclear extracts prepared form U937 cells. To further elucidate the physiological relevance of the binding of c-Rel and AP-1 to the resistin promoter Chromatin immunoprecipitation (CHIP) of U937 cells treated with PMA and PDTC with anti C/EBP, anti Phospho-ATF2 and anti phospho-cJun antibodies was performed. These antibodies were found to immunoprecipitate the human resistin promoter sequences, indicating their functional interaction with the resistin promoter *in vivo*.



# V. PATHOLOGY

## EDAX – RAY ANALYSIS OF OIL SEEDS SESAME (TIL), MUSTARD, GROUNDNUT, CASTOR, COTTON AND COCONUT

### OBJECTIVE

SEM is a useful instrument for studying the microstructure of a variety of food properties like quality, nutrients composition pattern etc. The EDAX-ray analysis, elemental composition pattern of the oil seeds is reported in the present study.

### MATERIALS AND METHODS

Dry oil seeds *Sesame (Til)*, *Mustard*, *Groundnut*, *Castor*, *Cotton* and *Coconut* were collected from commercial markets of Hyderabad. Fractured samples of the above oilseeds were placed on SEM stubs with double adhesive tape and coated with gold 300 Å thick in a Hitachi high vacuum evaporator HUS-5G, were analyzed in link ISIS-300 oxford model of EDAX-ray accessory attached to Hitachi SEM-S-520, operated at 10 kV.

### RESULTS & DISCUSSION

C, O, Mg, Al, Si, P, S, and K was found only in *Castor* and *Coconut*, P and S content is high in

*Mustard* when compare to other oilseeds. Na was not present in *Sesame*, *Mustard* and *Cotton* seeds (Table 29).

In *coconut* carbon content was 67.47% and Oxygen content was 29.92%, Saturated fatty acid (SFA) content was 88% unsaturated fatty acid (USFA) 9% In *castor* saturated fatty acid content was 2% unsaturated fatty acid content was 98%. Carbon content 65.86%, Oxygen content was 30.80 (Table. 30). However, no correlation can be drawn between SFAs, USFAs content with oxygen and carbon content in these oil seeds. It was popularly believed that the consumption of excess mustard oil leads to skin diseases because of sulphur (1.61%) content in the oil.

Table 30. Comparisons with Fatty acids

Sample	Fatty acids in %			
	SFA	USFA	Carbon	Oxygen
Sesame	13	86	62.45	35.96
Mustard	11	89	57.80	38.24
Groundnut	21	79	64.39	32.94
Cotton	26	71	57.42	39.20
Castor	2	98	65.86	30.80
Coconut	88	9	67.47	29.92

SFA: Saturated Fatty Acids USFA: Unsaturated Fatty Acids

Table 29. Elemental Dispersive X-ray (EDAX-Ray) Analysis of oilseeds  
(All elements in percentage %)

S.No.	OIL SEEDS	Carbon C	Oxygen O	Sodium Na	Magnesium Mg	Aluminum Al	Silicon Si	Phosphorous P	Sulphur S	Chloride Cl	Potassium K
1.	SESAME	62.45	35.96	---	0.32	0.05	0.12	0.46	0.28	---	0.37
2.	MUSTARD	57.80	38.24	---	0.27	0.04	0.02	1.14	1.61	---	0.88
3.	GROUNDNUT	64.39	32.94	0.19	0.28	0.28	0.20	0.20	0.30	---	1.22
4.	COTTON	57.42	39.20	---	0.45	0.45	0.08	1.41	0.64	---	0.80
5.	CASTOR)	65.86	30.80	0.32	0.31	0.18	0.18	0.97	0.18	0.16	1.04
6.	COCONUT	67.47	29.92	0.04	0.24	0.09	0.03	0.29	0.42	0.36	1.11

