

वार्षिक प्रतिवेदन  
Annual Report  
2013 - 14



राष्ट्रीय पोषण संस्थान  
National Institute of Nutrition  
(भारतीय आयुर्विज्ञान अनुसंधान परिषद्)  
(Indian Council of Medical Research)



# Annual Report

2013-14



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# SCIENTIFIC STAFF

**KALPAGAM POLASA, MSc, PhD, MBA**  
(SCIENTIST 'G' & DIRECTOR-INCHARGE)

## CLINICAL

R. Hemalatha, MD  
(Scientist 'F')  
Bharati Kulkarni, MBBS, DCH, MPH  
(Scientist 'E')  
G. Jagajeevan Babu, MBBS, MPH  
(Scientist 'E')  
K. V. Radhakrishna, MBBS, DCH  
(Scientist 'E')  
Sadhana Gupta, MSc, PhD  
Raja Sriswan Mamidi, MBBS

## PATHOLOGY

P. Uday Kumar, MD  
(Scientist 'F')  
SSYH. Qadri, MVSc  
(Scientist 'E')  
M.V.Surekha, MD

## MICROBIOLOGY & IMMUNOLOGY

R. Hemalatha, MD  
(Scientist 'F')  
M. Shiva Prakash, MSc, PhD  
(Scientist 'E')  
Devraj J. Parasannanavar, MSc, PhD  
A.Vijayendrachary, M.Pharm (SRF)  
N.Himaja, MSc (RF)  
D. Vasundhara, MSc (RF)

## LIPID CHEMISTRY

A. Vajreswari, MSc, PhD  
(Scientist 'F')  
S.Ahmed Ibrahim, MSc, PhD  
(Scientist 'E')  
S. M.Jeyakumar, MSc, MPhil, PhD  
P. Sujatha, MSc, PhD  
Anupama Tyagi, MSc (RF)  
S.Anil, MSc (RF)  
Ch. Anuradha, MSc (RF)  
V. Anantha Krishna, MSc (RF)  
J.Sugeetha, MSc (RF)  
G.Rajgopala Chary, MSc (RF)

## STEM CELL BIOLOGY

V.Vijayalakshmi, MSc, PhD  
(Scientist 'F')  
C. Suresh, MSc, PhD  
(Scientist 'E')  
A. Rajanna, MSc  
M.Soundarya, MSc (RF)  
Himadri Singh, MSc (RF)  
K Sowmya, MSc (RF)

## MOLECULAR BIOLOGY

Sudip Ghosh, MSc, PhD  
(Scientist 'E')  
Sanjay Basak, MSc, PhD  
M.Srivani, MSc (RF)  
B. Shankar Anand, MSc (RF)  
G. Venkateswarulu, MSc (RF)  
K. Sandeep Kumar, MSc (RF)

## MICRONUTRIENT RESEARCH

K. Madhavan Nair, MSc, PhD  
(Scientist 'F')  
P. Raghu, MSc, PhD  
P. Ravinder, MSc, PhD  
Swarnim Gupta, MSc (RF)  
A. Kiran Kumar, MSc (RF)  
N. Pallavi, MSc (RF)  
Y. Sravanthy, MSc (RF)  
P. Ravindranath, MSc (RF)  
M. Purna Chandra, MSc (RF)  
Dripta Roy Choudhary, MSc (RF)  
K. Archana, MSc (RF)

## WORK PHYSIOLOGY

Y. Venkataramana, MSc, PhD  
(Scientist 'F')  
Deethu Sara Varghese, MSc (RF)

## FOOD CHEMISTRY

T. Longvah, MSc  
(Scientist 'G')  
K. Bhaskarachary, MSc, PhD, PGDN & DM  
J. Sreenivasa Rao, MSc

S. Devendra, MSc, MPhil, PhD  
R. Ananthan, MSc, PhD  
J. Vahini, MSc (RF)  
Naveena Natrajan, MSc (RF)  
Daniella Chyne, MSc (RF)

#### ENDOCRINOLOGY & METABOLISM

M. Raghunath, MSc, PhD  
(Scientist 'G')  
Ayesha Ismail, MSc, PhD  
Jitendra Kumar Sinha, MSc (RF)  
Shampa Ghosh, MSc. MPhil (RF)  
Mehraj-ud-din, MSc (RF)  
N.Bindu, MSc (RF)  
G. Srividya, MSc (RF)  
Anju Elizabeth Joseph, MSc (RF)  
K. Nagabhushan Reddy, MSc (RF)

#### OCULAR BIOCHEMISTRY

G. Bhanuprakash Reddy, MSc, PhD  
(Scientist 'F')  
P. Suryanarayana, MSc, PhD  
(Scientist 'E')  
N.Saravanan, MSc, PhD  
V.Sudhakar Reddy, MSc (RF)  
Sara Sarin Jose, MSc (RF)  
K. Ashok Reddy, MSc (RF)  
Madhusudhan A. Patil, MSc (SRF)  
M. Sivaprasad, MSc (SRF)  
S.Vishwaraj, MSc (RF)  
Sneha Jakhotia, MSc (RF)  
K. Shruthi, MSc (RF)  
T. Shalini, MSc (RF)

#### FIELD DIVISION

A. Laxmaiah, MBBS, MPH  
(Scientist 'F')  
R. Harikumar, MBBS, DPH  
(Scientist 'E')  
N. Arlappa, MD  
(Scientist 'E')  
I. I. Meshram, MBBS, MD(PSM)  
K. Mallikharjuna Rao, MSc, PhD  
M. S. Radhika, MSc, PhD  
Sylvia Fernandez Rao, MA, PhD  
Ch. Gal Reddy, MSc

#### BIOSTATISTICS

K. Venkaiah, MSc  
(Scientist 'F')  
M. Vishnuvardhana Rao, MSc, PhD, MTech (IT)  
(Scientist 'F')  
N. Balakrishna, MSc, PhD  
(Scientist 'E')

#### PUBLICATION, EXTENSION & TRAINING

D. Raghunatha Rao, MSc, PhD, PG Dip. FNS  
(Scientist 'F')  
T. Vijaya Pushpam, MA, MPhil, PhD  
(Scientist 'E')  
M. Maheshwar, MCOM, MA, MCJ, LLB, MPhil  
G. M. Subba Rao, MA, PGDJ, PGDT, PhD  
K. Damayanthi, MSc, PhD

#### INSTRUMENTATION

V.Vijayalaxmi, MSc, PhD  
(Scientist 'F')

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

Kalpagam Polasa, MSc, PhD, MBA  
(Scientist 'G' & Director In-charge)

#### FOOD TOXICOLOGY

Arjun L Khandare, MSc, PhD  
(Scientist 'F')  
S.N.Sinha, MSc, PhD  
(Scientist 'E')  
J. Padmaja, MSc, PhD  
(Scientist 'E')  
V. Sudershan Rao, MSc, PhD  
(Scientist 'E')  
S. Vasanthi, MSc, PhD  
P. Amrutha Rao, MBBS, DPH  
V. Vakdevi, MSc (RF)  
Priyanka Shankar, MSc (RF)  
Agatha Betsy, MSc (RF)  
M.Ankulu, MSc (RF)  
Alekhya, MSc (RF)  
Nivedita, MSc (RF)

#### DRUG TOXICOLOGY

M.P. Rajendra Prasad, MBBS, MSc (AN), PhD  
(Scientist 'F')  
B. Dinesh Kumar, MSc (Pharma), PhD  
(Scientist 'F')  
K. Nirmala, MSc, PhD  
Chetan Nimgulkar, MSc (RF)  
M. Chalamaiah, MSc (RF)  
V.Varsha, MSc (RF)

#### NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

Kalpagam Polasa, MSc, PhD, MBA  
(Scientist 'G' & Director In-charge)  
P. Suresh Babu, MVSc  
(Scientist 'F')  
N. Hari Shanker, MSc, PhD  
K.Rajender Rao, MSc, PhD  
N.Muralidhar, MSc (JRF)

# ADMINISTRATIVE STAFF

(MINISTERIAL & SECRETARIAL)

## SENIOR ADMINISTRATIVE OFFICER

N. Sitaramanjaneyulu  
Wef 6.3.2014

## ADMINISTRATIVE OFFICER

A.Suryaprakash Rao  
M. Ashok Raj

## ACCOUNTS OFFICER

G. Krishna Reddy

## SECTION OFFICERS

M. Vijay Kumar  
G. Ramakrishna  
Abdul Rasheed Shaik  
Latha Kumarsswamy  
N.Muralikrishna  
M.Siva  
P.Dhanasekharan  
R.C.Padmini Mohan  
K.Ch.Ramayya Dora (on lien)  
D.Venkateswarlu (adhoc)

## PRIVATE SECRETARIES

Ch. Madhulatha [A.O (NNMB)]  
P.Venugopal  
Sudha Srinivasan  
D.V.Laxmi Rani

## ASSISTANTS

V.Laxminarayana  
T.Satyanarayana  
K.Sivarami Reddy  
M.Rajagopala Chary  
M.K.Koteshwara Reddy  
V.Elisha  
Alice Mary  
D.Sunil  
A.Narayana Reddy  
M.Babu  
C.Kalavathi  
D.Seetharamaiah  
E. Syama Sundari

## PERSONAL ASSISTANTS

G.Hanumantha Rao (PS adhoc)  
Malini V. Rao  
G.Prashanthi  
V.Swayam Prabha  
K.Sailaja  
G.Durga Prasad

## UPPER DIVISION CLERKS

Shakila Banu (Asst. NNMB)  
G.R. Srinivas (Asst. Adhoc)  
K.Jayamma  
P. Prabhavathi  
Mohd. Iliyas  
Shaik Jamaluddin  
C.Prabhu  
Mini Pramod  
T.Anuradha Jayalaxmi  
A.Narsing Rao  
G.S.Gautami  
A.Satyanarayana Prasad  
Y.Bala Narayana

## STENOGRAPHERS

G. Mahesh Kumar (PA Adhoc)

## LOWER DIVISION CLERKS

M. Rekha  
D. Ramanjaneyulu  
G.Y.Anita  
A. Venkataramana  
U. Somayya  
M. Raghuram

## RECEPTIONIST-CUM-TELEPHONE OPERATOR

M. Jawahar Joshua

## LIBRARY STAFF

### Asst. Library Information Officer

B.Narayana

### Library Information Assistant

Prakash Kulkarni

### Library Clerk

Ungarala Naidu

# TECHNICAL STAFF

## Technical Officer "B" (Tech. staff)

1. Ramachander Chaugule
2. S. Ananda Rao
3. Ch. Gal Reddy
4. Anil kumar dube
5. Sharad kumar
6. M. Ravindranath
7. K.Yadamma (adhoc)

## Technical Officer "B" (Engg. Support staff)

1. Bandam Ramulu

## Technical Officer "A" (Tech. staff)

1. G. Amarendra Reddy
2. A. Kasiviswaraja Mouli
3. V. Satish Babu
4. D.Sreeramulu
5. M.Satyavani
6. Virendra Vasant Panpatil
7. M. Krupadanam
8. R. Naveen Kumar
9. B.Narahari
10. Laxmi Rajkumar
11. K.Swaroopo Rani
12. Vani Acharya
13. C. Maniprabha
14. Abhay Kumar
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16. V. Vikas Rao
17. Kasinath Lingreker
18. R. Radhakrishna Sarma
19. V.Radhakrishna Rao
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21. D. Pandu Ranga Vittal
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23. Ch. Nagambika Prasad
24. S. Lopamudra
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32. D.Vijaya Bhaskara Rao
33. P. Ajey Kumar

34. Saleem Shariff
35. G.V. Narasimha Rao
36. N. Sreenivasa Chary
37. B. Venkateswara Rao
38. P.Krishnaswamy
39. G. Chenna Krishna Reddy
40. K. Subash

## Technical Officer "A" (Engg. Support staff)

1. Mota Chandrasekhara Rao
2. P. Moses Ashok Kumar
3. U.D. Awasthi
4. G. Mohan Rao
5. G.B. Walter
6. L. Vijaya durga
7. B. Om Prakash

## Technical Assistant (Tech. staff)

1. K. Narasimha Reddy
2. T. Nagasekhara Rao
3. Ch. Ranga Rao
4. K. Sharada
5. P. Sailaja
6. D. Prem Raj
7. B.R. Annapurna
8. Ch. Hanumantha Reddy
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10. V. Bhasker
12. P. Yadagiri Reddy
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14. S.A. Brinda
15. K. Vasudev
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17. K. Suryam Reddy
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19. B. Tulja
20. Narottam Pradhan
21. K. Swatantra Rao
22. Madhusudhana Chary
23. P.S.Rama Rao

## Technical Assistant (Engg. Support staff)

1. N. Satyanarayana
2. G. Janardhan
3. M. Asaithurai
4. Micheal Fernandez

5. A. Anjaiah
6. T. Shyam Sunder
7. Joseph Vijaykumar
8. YVL Narasimha Rao
9. Mohd. Younus
10. G.P. Narender

**Driver (Special Grade)**

1. P. Mahender

**Technician "C" (Tech. staff)**

1. R. Raghunath Babu
2. K. Usha Rani
3. C. Sai Babu
4. P. Satish Babu
5. C. Janardhan
6. G. Govindarajulu
7. P.S. Prashanthi
8. S.P.V. Prasad
9. K. Sree Ramakrishna (on deputation NNMB)
10. P. Anitha Chauhan
11. G. Madhavi
12. B. Giri Babu
13. G. Venkataraji Reddy
14. S. Ashok
15. G.I. Stephen
16. G.A. Rabbani
17. E. Sammi Reddy
18. K. Balaji
19. M. Sripal Reddy
20. Y. Salaiah

**Technician "C" (Engg. Support staff)**

1. K. Pavan Kumar
2. Syed Jalaluddin Hussaini
3. R. Sahadeva
4. J. Kumaraswamy
5. A.I. Goverdhan
6. K. Srenivasa Raju
7. N. Narasimha
8. Purnachandra Beshra
9. P. Dasarath
10. S. Devendran
11. Ramavath Ramsingh
12. Sriramulu Naidu
13. E. Srinivas
14. V. Bhuvaneswaran
15. Polishetty Naidu
16. P. Narender Kumar

**Technician "B" (Tech. staff)**

1. N. Peddi Reddy
2. Y. Agreepa Raju
3. P. Sashidharan Pillai

4. Gandamalla Narasimha
5. P. Bheem Shanker
6. J. Pochaiah
7. M. Balram
8. S. Chandraiah
9. B. Nagender rao
10. Prabhu Raj
11. P. Nagabhashunam
12. Nigala Yadagiri
13. E. Krishna
14. Bommaka Srinu
15. Neelakanta
16. L. Dasu
17. D. Dasaratha
18. J. Nageswara Rao
19. C. Chandramouli
20. Abdul Sattar
21. N. Rajaiah.
22. K. Rama Rao
23. V. Rajkumar

**Technician "B" (Engg. Support staff)**

1. B. Bal Reddy
2. J. Bhujender
3. K. Parthasarathy
4. D. Ravinder
5. N. Ramesh Kumar
6. Y.veeraiah

**Technician 'A' (Engg. support staff)**

1. Mahender Singh Jadav
2. E.Ganesh
3. M.Narasimha

**Driver (Grade – I)**

1. Zahid Ali Khan
2. K. Krishna
3. V. Kondaiah
4. Syed Mohd. Ali

**Driver (Grade – II)**

1. D. Amruthanathan
2. K. Jangaiah

**Junior Staff Nurse**

1. G. Rajakumari
2. B.V. Nancharamma
3. D. Threessamma
4. D. Rani
5. K. Venkataramana
6. S. Rojamani

### Auxiliary Nurse Midwife

1. K. Santhosham
2. Ch. Anitha
3. G. Tulasi Bai
4. V. Aruna Reddy
5. E. Sheela
6. G. Vijayalakshmi

### Nursing Attendant

1. R.Rajyalakshmi
2. Govada Bhavani
3. Valentina Teriscova
4. D.Swarupa

### Senior Cook (Isolated Post)

1. H.S. Ramu

### Attendant (Services)

1. K.B. Raju
2. C. Shankaraiah
3. Manga Narasaiah
4. M. Eshwar
5. Abdul Bhasid
6. G. Eswaraiah
7. G. Viswanatham
8. M. Suresh
9. Mohd. H. Yousuf
10. Mohd. Abdul Khader
11. Bondi Ramulu
12. J. Yadagiri
13. Syed Mohd. Iqbal
14. C. Rajaiah
15. Mabbu Ramulu
16. V. Shanker
17. Kompally Pochaiah
18. A. Narasaiah
19. Mukkera Krishna
20. Mohd. Mehboob
21. J. Lakshmaiah
22. K. Rajaiah
23. P.V. Poulous
24. Manupathi Bikshapathi
25. Dhanavath Saida
26. V. Dasaratham
27. Manmohan Meena
28. Srihari Ram
29. Ch.Guruswamy
30. Mohd. Maqbool
31. P. Shiva Shanker
32. S. Hanumantha Rao
33. K. Chandran

### Attendant (Services) Contd..

34. Mirza Ghouse Baig
35. G. Yadagiri
36. Mohd. Yaseen
37. K. Balraj
38. R. Narasimhulu
39. Mohd. Chand
40. Mohd. Maulana
41. Shaik Mukthar
42. K. Kasipathi
43. M. Leela
44. Manchikanti Krishna
45. Syed Asif Ali
46. K. Gopal
47. B. Eswaraiah
48. Mohd. Issamaiah
49. K. Harinarayana
50. E. Mallesh
51. Mohd. Sabeer
52. K. Narender
53. Y. Ramulu
54. M. Somaiah
55. G. Venkatesh
56. V. Somaiah
57. E. Marthamma
58. T. Govind
59. P. Srihari
60. Mohd. Habibuddin
61. A. Venugopal
62. M. Kisan
63. B. Nageswara Rao
64. P. Nagulu
65. M. Seenu
66. B.K.Mahadevaiah
67. A. Chandraprakash

### Attendant (Services) Contd..

68. M. Jayamma
69. D. Venkaesh
70. M. Satyamma
71. C. Sivaleela
72. G. Satyapual
73. A. Narsing Rao
74. A. Lakshmi
75. Majeed Shareef
76. M. Upender
77. R. Punna Reddy
78. K. Srinu
79. M. Narsing Rao
80. A. Shanker
81. P. Ravinder
82. D. Madhava Reddy
83. B.V. Sudershan Babu
84. I. Posheety
85. G. Yadagiri
86. M. Venkataiah
87. N. Bhasker
88. A. Jangaiah
89. P. Dasarath
90. S. Narahari
91. K. Venkatesh
92. P. Narasimha
93. E. Kondal Reddy
94. K. Venkat Reddy
95. G. Upender
96. M. Koumura Reddy
97. Ch. Shanker
98. G. Saraswathi
99. P. Balrajun

# RESEARCH HIGHLIGHTS

## 1. COMMUNITY STUDIES

### 1.1 Nutritional status of below five year old children in the state of Meghalaya

The Government of Meghalaya is contemplating to develop State Nutrition Policy and develop a plan of action for implementation of district level strategy, to improve the nutritional status of children. Therefore, a study was carried out in all the 7 districts of the State. Only 37% of the deliveries were institutional and rest of them (63%) took place at home and 11% of the babies born were with low birth weight (<2.5 kg). Half of the newborns were breastfed within an hour of delivery and none of the infants exhibited any nutritional deficiencies. The overall prevalence of underweight, stunting and wasting was 21%, 44% and 5% respectively, which were lower than the national figures.

### 1.2 Sensory evaluation and acceptability of locally produced ready- to-eat supplementary foods for beneficiaries of ICDS in the age group of 12-35 months: A study in the Ranga Reddy District of Andhra Pradesh

Two new skim milk based ready to eat supplementary food products with different combination of cereal's, pulse's, skim milk, sugar and fat ratios and fortified with micronutrients (Product 1 - 50:05:10:25:10 & Product 2 - 55:05:10:20:10), were developed by AP Foods Ltd., for management of undernutrition among young children. These products were assessed for their sensory properties and acceptability, in comparison to the currently existing soy based ready to eat supplementary food (Modified Therapeutic Food, MTF - 45:20:00:25:10) of the ICDS-WDCD, Government of Andhra Pradesh. The mean sensory scores given by Mother's Panel for all the sensory attributes including overall palatability was significantly ( $p<0.001$ ) higher for product 1, followed by product 2 as compared to MTF. Based on 3-criteria priori set for product's, product 1 appears to be more acceptable, suggesting its long term adherence over a 14-day period. It is recommended that this product should be supplied weekly in individual sealed package, and service providers need to impart education to caregivers on handling, usage and storage of the same. Accordingly, the Govt. of Andhra Pradesh is supplying the food for more than 20 lakh children daily.

### 1.3 A rapid assessment of nutritional status of under five year old tribal children and women of Attappady hills, Palakkad district of Kerala and causes of infant deaths by ICMR Verbal Autopsy Guidelines (Rapid Survey)

There were reports in the media about unusual number of infant deaths, occurring among tribal families in the Attappady Block of Palakkad District in Kerala in 2013. A rapid nutrition assessment was conducted, in May 2013, among children and mothers of the above tribe to ascertain the cause of infant deaths. The food and nutrient intake of the tribal population was lower than the recommended levels. The extent of deficit in micronutrients such as iron, vitamin A, riboflavin and free folic acid was higher. The prevalence of underweight, stunting and wasting among <5 year children was higher compared to other tribes as well as their rural counterparts. The study revealed that, high maternal undernutrition (48% had BMI<18.5). During one year preceding the survey, 10 abortions/still births, 11 neonatal deaths and 3 infant deaths were reported. The major cause of stillbirths and abortions was attributed to pregnancy induced hypertension (PIH) and diabetes, followed by premature delivery and accidental death. For neonatal deaths, the major cause revealed was PIH, premature delivery, and ante-partum haemorrhage, obstructed labour and congenital anomaly. Lapses in healthcare delivery system were identified and reported for corrective measures.

## 2. BIostatistics AND BIOinformatics

### 2.1 Meta-analysis approach on micro-nutrients, food fortification and its effect on health, social and economic factors- A statistical model building

- ☒ A meta-analysis of randomized, controlled iron fortified feeding trials, that evaluated hemoglobin (Hb) concentration using meta-regression analysis, indicated that the duration of the study was positively related to the effect size (Regression coefficient =0.368; (95% CI: 0.005, 0.731),  $p < 0.05$ ). The net pooled effect size marked after removing the confounders was 4.74 g/L (95% CI: 3.081, 6.399). Association was observed between intakes of iron fortified foods on Hb concentration in children less than 10 years of age. Iron fortified foods could be an effective strategy to mitigate iron deficiency anemia in children.

Further, when evidence from parallel and cross-over randomized controlled trials were combined, to assess the impact of iodine fortified foods on urinary iodine concentration (UIC) in children, meta-regression analysis indicated that dose of feeding was positively related to the effect size (regression coefficient=0.014; 95% CI 0.003, 0.026;  $p < 0.019$ ). There was an association between intakes of iodine fortified foods and UIC in children.

## 3. MICROBIOLOGY AND IMMUNOLOGY

### 3.1 Effect of fructo-oligosaccharide coated probiotic on fetal immuno-programming and other health benefits

This study explored fetal immune programming with probiotic and prebiotic (Synbiotic) supplementation in pregnant dams. The results indicated that, in the F0 generation there was a significant ( $p < 0.05$ ) improvement in the T cell proliferation with synbiotic (*Lactobacillus rhamnosus* GG and Fructo-oligosaccharide) supplementation compared to the control group. Interestingly, this improvement in the cell mediated immunity (T cell proliferation response) ( $p < 0.05$ ) continued even in the F1 (pups bred from F0 that received synbiotic) generation though the F1 pups did not receive synbiotic supplementation.

However, immune response against specific antigen (Hep-B surface antigen) showed significant ( $p < 0.05$ ) improvement only in those F1 (pups from F0 that received synbiotic) group that was given synbiotic supplementation though this improvement failed to appear in the group that was not supplemented with synbiotic though, their mothers received the supplementation during pregnancy. No significant improvement in cell mediated immune response was observed when compared to the control group both in treated and untreated probiotic groups of F1 generation. In the F1 generation (pups from F0 that received probiotics) which were given probiotic supplementation there was increased ( $p < 0.05$ ) immune response against specific antigen (Hep-B surface antigen) but only next to Synbiotic group; and this effect was observed even in the F1 (pups bred from F0 that received probiotics) generation that did not receive probiotic supplementation.

Cell mediated immunity showed intergenerational effect with synbiotic supplementation and this improvement in pups (F1 generation) was associated with Bifidobacterial colonization. In contrast, the antibody response to Hep-B surface antigen showed intergenerational effect in pups (F1 generation) born to probiotic supplementation suggesting fetal immune programming with probiotic supplementation.

### 3.2 Development of tools to identify and map IgE binding epitopes using synthetic peptides.

As part of the study, sequencing and transcriptome analysis of brinjal or eggplant (*Solanum melongena L*) fruit was done to identify allergenic genes and proteins. From the total RNA, unwanted RNAs (rRNA, tRNA and other small RNAs) were removed and qualified Brinjal fruit mRNAs (qualified by Qubit Analyzer) were used, to pair end library preparation and were sequenced on Illumina HiSeq 2500. There were a total of 89,763,638 raw reads in brinjal fruit. Of them, 149224 (Level 3 assembly) were found to be clean reads after filtering. On analyzing the assembly length distribution of brinjal fruit transcripts, most of the sequences were observed between 100-500 and, few sequences were 3000-4000 nucleotides length and the highest sequence length was 10795 (1 sequence). Of the

### Immune response in F1 generation

F0	<i>Lactobacillus rhamnosus</i> GG and Fructo-oligosaccharide (Synbiotic) supplementation in F0		<i>Lactobacillus rhamnosus</i> GG (probiotics) supplementation in F0	
F1 pups	Synbiotic supplemented	Synbiotic not supplemented	Probiotics supplemented	Probiotics not supplemented
CMI (T cell proliferation) in F1 pups	Increased	Increased	Comparable to control	Comparable to control
Antibody response to Hep-B surface antigen in F1 pups	Increased	Comparable to control	Increased	Increased

149224 sequences identified from brinjal fruit by transcriptome analysis, 6804 sequences were annotated and were found to be functional genes. Of the 6804 sequences, the exact functions were identified for 1053 sequences by mapping. From the total sequences, 72625 sequences had Basic Local Alignment Search Tool (BLAST) hits and were matching with existing databases. However, 68742 sequences were newly identified and did not have any Blast hits with existing databases.

### 3.3 Regulatory T cell (Cd4/ Cd25/ CD127-/ FOXP3) population and B cells with Cd23/ CD21 expression in pregnant women with vitamin D deficiency and their newborns

The study hypothesised that low levels of vitamin D would be associated with impaired regulatory T cell function and increased an amount of B cells with CD23/CD21 expression, resulting in higher risk of asthma/allergy. It explored Treg cell function, CD23/CD21 expression and VDR expression in pregnant women with vitamin D deficiency. The results indicated that of the total T cell population, the proportion of the regulatory T cell population (CD4/CD25/CD127-/FOXP3) was significantly ( $p < 0.05$ ) lower in vitamin D deficient subjects compared to sufficient and insufficient subjects. Of the total B cells (CD19) population, the proportion of B cell with CD23 expression levels was significantly higher in vitamin D deficient subjects compared to vitamin D sufficient and insufficient. B cells with CD21 expression levels were also significantly higher in vitamin D deficient subjects compared to vitamin D sufficient and insufficient subjects. VDR and FOXP3 gene expressions were down regulated and CD23 and CD21 genes were up regulated in the placenta of vitamin D deficient pregnant women. These results show impaired regulatory T cell function and increased IgE receptors expression, in cord and maternal blood and placenta of vitamin D deficient pregnant women.

### 3.4 Effect of probiotic supplementation on weight reduction and its impact on micronutrient and immune status in obese subjects

The indigenously prepared probiotic curd in combination with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* showed significant changes in the lipid profile parameters, upon supplementation to obese subjects. There was also an immunomodulation effect in the obese subjects, upon supplementation with probiotic curd.

## 4. BASIC STUDIES

### 4.1 Simultaneous determination of biochemical indicators of micronutrient status from the finger puncture blood sample by kits

Dried blood spot collection kit for vitamin A analysis by HPLC and ELISA based serum ferritin estimation kit: Two kits (i) Dried Blood Spot (DBS) for diagnosing Vitamin-A deficiency and (ii) ELISA for ferritin as Marker for assessing bioavailability of iron using Caco-2 cell line (Patent application no. 1927/DEL/2013) were developed. These kits were launched by the Union Health Minister on 20th Feb 2014.

#### **4.2 Characterization of risk factors of anemia among infants and preschoolers from rural India**

Project Grow-Smart, a randomized controlled interventional trial, was carried out among infants and pre-schoolers in 26 villages of four state administrative blocks (mandals) from Nalgonda District of Andhra Pradesh (now in Telangana), India. The baseline data was analyzed for estimating anaemia prevalence and severity and to characterize maternal and child factors associated with anaemia in infants (N= 518) and preschoolers (N=326), in preparation for designing an effective anaemia control program. There was high prevalence of anaemia in infants (66.4 %) and preschoolers (47.8%). Determinants of anaemia in infants were male gender, maternal anaemia, low infant ferritin and high c-reactive protein. Maternal anaemia, maternal low education, child age, high c-reactive protein and soluble transferrin receptor were significant risk factors for anaemia in preschoolers. Family-based strategies need to be identified and enforced to tackle this nutritional problem among children.

#### **4.3 Dietary diversification of Indian vegetarian diet to improve iron bioavailability- Studies using Caco-2 Cell line model**

The focus of the study was to screen combinations of food groups to formulate a diversified diet high in bio available iron. To further, improve bioavailability of diversified diet Indian herbs were screened using Caco 2 cell line. Identified herbs had iron content ranging from around 10-60 mg/100 g. Among the herbs *B. diffusa*, *L. sativum* and *T. ammi* contained iron above 40 mg/100g but also high amount of tannin/ phytate. *A. racemosus* and *B. diffusa* were selected on account of high iron, contrasting inhibitor contents for testing iron absorption promoting activity. There was no difference in ferritin induction either in the presence or absence of FeCl<sub>3</sub>. However, mineral solutions of both the herbs with exogenous an incorporation of ascorbic acid, induced a 10 fold higher ferritin concentration, signifying that these herbs though rich in iron could not effect endogenous and exogenous iron bioavailability. A combination of wheat and rice at a ratio of 1: 3 had the highest iron dialyzability. Among pulses, green gram and lentil had higher percent dialyzability compared to bengal gram or red gram. Addition of these pulses to Bengal gram and red gram enhanced percent dialyzability significantly. These results suggest that the habitual Indian diets can be effectively diversified to enhance iron bioavailability.

#### **4.4 Foetal programming for neuro-musculoskeletal development in the rat offspring: Role of antenatal and perinatal magnesium deficiency**

The role of antenatal and perinatal magnesium deficiency in foetal programming, for neuro-musculoskeletal development in the rat offspring was evaluated. It was observed that in the embryos/ offspring of the Mg restricted WNIN rat dams, the brain development was impaired albeit transiently (but not at birth). This appears to negate any role of impaired brain / neuronal development in the changes observed in their body composition in later life. The differential gene expression of Leptin, adiponectin and 11 $\beta$ HSD1 and the corresponding change in DNA methylation in the embryos, at later time points appear to suggest that maternal Mg restriction induced changes in the body composition of the offspring may be programmed right during the intra uterine growth. The findings suggest that maternal Mg restriction induced alteration of promoter DNA methylation could be a mechanism underlying changes in the expression of relevant genes responsible for adiposity and associated stress.

#### **4.5 Role of the Ubiquitin-Proteasome pathway in vitamin D deficiency induced muscle atrophy and hypoinsulinemia**

Vitamin D deficiency leads to muscle wasting in both animals and humans. A vitamin D deficient rat model was created using Sprague Dawley male rats. The involvement of the ubiquitin proteasome and other proteolytic pathways in vitamin D deficiency induced muscle atrophy were studied. To delineate the effect of hypocalcemia, which accompanies D-deficiency a group of deficient rats were supplemented with high calcium alone. Total protein degradation in muscle was assessed by release of tyrosine; proteasomal, lysosomal and calpain enzyme activities were studied using specific substrates by fluorometry, and E2 enzyme expression was assessed by western blot analysis.

Muscle histology was done by myosin ATPase staining method, while 3-methylhistidine in the urine was estimated using HPLC. Muscle gene expression was measured by semi-quantitative RT-PCR. Total protein degradation in muscle and the level of 3-methylhistidine in urine were increased in the deficient group, compared to control group. Proteasomal enzyme activities, expression of the E2 ubiquitin conjugating enzyme and ubiquitin conjugates were increased in the deficient group, compared to controls. On the other hand, lysosomal and calpain activities were not altered. Type II fibre area; a marker for muscle atrophy was decreased in the deficient muscle compared to control muscle. Muscle atrophy marker genes and proteasomal subunit genes were up regulated, while myogenic genes were down regulated in D-deficient muscle. From the results it appears that the ubiquitin proteasome pathway is the major pathway involved in vitamin D deficiency induced muscle protein degradation and that calcium supplementation alone in the absence of vitamin D corrects the changes.

#### **4.6 Isolation and characterization of procyanidine-B2 as a novel antiglycating agent from cinnamon**

Previously, it was established that cinnamon has significant potential to inhibit advanced glycation endproducts (AGE) formation, under in vitro conditions. Based on bioassay-guided fractionation, we have isolated and characterized procyanidin-B2 as the active component of cinnamon that is involved in AGE inhibition. The data indicate that procyanidin-B2 enriched fraction, scavenges dicarbonyls. Further, procyanidin-B2 fraction of cinnamon inhibited the formation of glycosylated hemoglobin in human blood under ex vivo conditions.

#### **4.7 Amelioration of diabetic nephropathy in rats, by procyanidin-B2 from cinnamon through inhibition of AGE formation**

In this study, the potential of procyanidin-B2 (PCB2) isolated from cinnamon to prevent in vivo accumulation of AGE and to ameliorate renal changes in diabetic rats have been described. Feeding with PCB2 prevented glycation mediated RBC-IgG cross-links and HbA1c accumulation in diabetic rats. PCB2 also inhibited the accumulation of N-carboxy methyl lysine (CML), a prominent AGE in diabetic kidney. Interestingly, PCB2 prevented the AGE mediated loss of expression of glomerular podocyte proteins-nephrin and podocin. Inhibition of AGE by PCB2 ameliorated diabetes mediated renal malfunction in rats, as evidenced by reduced urinary albumin and creatinine.

#### **4.8 Effect of soluble lutein and soluble curcumin against diabetic cataract**

Lutein and zeaxanthin are present in macula and lens of the human eye to protect them from oxidative damage and reduce the risk of age related macular degenerations and cataracts. Previously, it was shown that lutein (1%) and curcumin (0.01%) in the diet delayed but did not prevent cataract in diabetic rats. Bioavailability is a major issue for the success of clinical utilization of compounds such as lutein and curcumin. Now, it has been demonstrated that soluble lutein and soluble curcumin are more effective in delaying diabetic cataract compared to their respective regular formulations at the same dose. Increased bioavailability of soluble formulations might explain the observed biological effects.

#### **4.9 Evaluation of WNIN/GR-Ob rat as a model for obesity associated type2 diabetic complications**

A series of animal experiments were conducted, with various rodent models to evaluate a suitable animal model to understand the molecular basis of obesity associated diabetic complications. Based on these studies, we report that neonatal-streptozotocin (nSTZ) WNIN-GR/Ob model could serve as a suitable model for studies on obesity associated diabetic complications, and also for conducting dietary intervention studies.

#### **4.10 Development of rapid assays for the detection of genetically modified foods**

Genetically modified (GM) crops are gaining popularity across the globe because of their improved agronomic properties and rapidly gaining entry into the food chain of human's and livestock. Consumption of genetically modified food and feed is highly debated as their safety, on long term use

is yet to be established. There is a need to verify presence of biomarkers in GM foods to comply with food regulatory authorities, labeling requirements, safeguard consumers, check unapproved varieties and environmental contaminant. In a Public-Private partnership model with Agilent Technologies, India, PCR-based multiplex and singleplex assays were developed to detect a number of commercially available genetically modified crops in India and abroad. Singleplex PCR assays were developed for the inserted transgenes like NOS, CaMV35S, FMV, cry1AC, Cry1AB, EPSPS and events of cotton (MON531, MON15985), Maize (GA21, Bt-176), Rice (LLrice62), Canola (GT73) and potato (EH 92-527-1). Single tube multiplex PCR assays were developed for cotton and maize. In addition, multiplex PCR assays were also developed to detect multiple GM crops in a single assay when presented as simple admixture e.g. canola and cotton, maize and cotton, rice and canola, rice and potato, canola and maize as well as complex admixture of cotton, potato, canola, rice and maize.

#### **4.11 Assessment of body composition in Indian females using different techniques**

The study was designed to validate the existing methods (Hydro-densitometry, Air Displacement Plethysmogram (ADP), Bio-electrical Impedance Analysis (BIA) and Skinfolts (SKF)) to assess body composition and to develop regression equations using skin fold measurement for accurate appraisal of body composition to suite Indian female population. The result of the study revealed that in Preadolescents, there is a good agreement between ADP and BIA when compared to ADP and SKF method. Based on the results, regression equations for fifteen different combinations of skinfold thickness were drawn, suitable to Indian female population.

#### **4.12 Ergonomic study to test the efficacy/suitability of Bicycle Driven Charkha among women spinners**

An Ergonomic study to test the efficacy/suitability of bicycle driven charkha among women spinners was a translational study that was carried out, at the request of Khadi and Village Industries Commission, GOI, wherein; the efficacy and suitability of newly developed bicycle driven charkha was tested as against traditional Hand Charkha based on the physiological efficiency, physical work performance and productivity. All the physiological parameters tested to assess the work efficiency of the two modules indicated that though the oxygen consumed ( $VO_2/\text{min}$ ) and over all energy expended (MET) is more in Cycle Charkha, the energy utilised to produce one gram yarn yield is less in cycle charkha (1.552 kcal) compared to hand charkha (2.698 kcal). It was observed that in Cycle Charkha, the women spinners were spending 1.552 kcal for producing one gram of thread as against 2.698 kcal in Hand Charkha for the same amount of produce. Further, it was observed that in Hand Charkha, the spinners could spin 2.25 hanks per hour, whereas, in Cycle Charkha it was increased to 4.99 hanks per hour resulting in 121% increase in their produce. The Cycle Charkha was found to be more efficient, convenient and ergonomically viable over the traditional hand driven charkha.

#### **4.13 Anti-inflammatory potential of dietary n-3 polyunsaturated fatty acids in experimental ulcerative colitis: Biochemical and molecular mechanisms**

The study investigated the impact of substitution of n-6 PUFA with n-3 PUFA ( $\alpha$ -linolenic acid/EPA&DHA) on inflammatory response in dextran sulphate sodium induced colitis (DSS) in rats. The results showed that substitution of n-6 PUFA with  $\alpha$ -linolenic acid (n-6:n-3 ratio of 2) or DHA&EPA (n-6:n-3 ratio of 10), mitigates the DSS induced colitis as evidenced by reduction in neutrophil infiltration, preservation of colonic architecture and reversal of the shortening of the colon length as well as improvements in the clinical symptoms of the colitis. The results of the study reinforce the current recommendations of increasing n-3 PUFA in the diet for the prevention of diet related chronic diseases including inflammatory bowel disease (IBD).

### **5. PUBLICATION, EXTENSION AND TRAINING DIVISION**

#### **5.1 Nutrition education for adolescents: An interventional approach to create awareness on "Eat right and play with might"**

The study was conducted to impart education on nutrition and physical activity through participatory approach and peer education using Oorja clubs (school nutrition clubs). After intervention, through

“Oorja club concept” a significant improvement in knowledge related to nutrition and importance of physical activity, was observed among the adolescent trainers. These trainers carried out peer education on nutrition, health and physical activity to their peers in schools. The results indicated a significant improvement in the knowledge on breakfast, importance of nutrition and physical activity. The study demonstrated that integrated nutrition education approach that combines interactive lectures and participatory approaches engaging students peer education can be effective in educating students in nutrition while underscoring the importance of physical activity.

## **5.2 Nutritional awareness among primary school children: Role of interventional approaches**

A study assessed nutritional awareness among primary school children using pictorial questionnaires and evaluated the use of interventional approaches with reiterative colorful imagery and demonstration of live samples of foods. The study showed that the intervention improved not only the children's ability to recognize various foods but also to differentiate between healthy and unhealthy options.

## **6. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE**

### **6.1 Prevalence and severity of fluorosis in Doda district, Jammu and Kashmir**

Fluorosis, which is a public health problem is endemic in 204 Districts of 21 States of India including the Doda District of Jammu and Kashmir. Scanty information is available on prevalence of fluorosis in Doda District. The fluoride levels were highest in the Golibagh followed by Malwas village resulting in high human intake of fluoride. Dental fluorosis was more common in girls than boys. High level of urinary fluoride excretion was observed in affected individuals. There was also kidney related (Creatinine, Urea) and bone related CALP, osteocalcin, PTH, 25OH vitamin D and 1,25(OH) vitamin D and liver related (AST and ALP) abnormalities in children exposed to fluoride.

### **6.2 Assessing the thermal stability of oxytocin in milk and digestive stability of oxytocin *in vitro* and *in vivo***

When the thermal stability of oxytocin in milk and digestive stability of oxytocin *in vitro* and *in vivo* was assessed, it was found that there was no thermal degradation of oxytocin due to boiling. *In silico* digestion analysis revealed that pepsin, chymotrypsin and intestinal proteases possess specific proteolytic cleavage sites in oxytocin amino acid sequence while trypsin has no such sites. Pancreatin could significantly digest oxytocin due to serine proteases. The studies also revealed that exogenous OT injections do not influence its content in milk and OT administered orally is rapidly digested in the intestine.

### **6.3 Fish egg protein hydrolysates as nutraceutical/ health food in promotion of immunomodulatory activities**

In the current investigation fish egg waste has been identified as potential source of protein which can become a value added product. The bioactive Protein hydrolysates has been successfully prepared from underutilized rohu fish by using pepsin trypsin and Alcalase enzymes. The compositional analysis of protein hydrolysates revealed that it has good amino acid profile along with essential micronutrients along with presence of  $\omega$ -3 fatty acids, especially docosahexanoic acid (DHA). The Molecular mass distribution of the hydrolysates showed, presence of low mass peptides below 10 kDa.

The hydrolysates dose dependent antioxidant activity in various *in vitro* models such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2'-azino-bis(3-ethylbenzthiazoline-6)-sulfonic acid (ABTS+) radical cation scavenging activity, ferric reducing antioxidant power (FRAP), and ferrous ion (Fe<sup>2+</sup>) chelating ability has been observed. The humoral and cell mediated immune responses in BALB/c mice after 45 days of oral administration was recorded. Rohu egg protein hydrolysates suggest an immune-modulatory effect. The study outcome demonstrate's that the fish egg protein hydrolysates has bioactive peptides, with potential health benefits and can become a value added product.

#### **6.4 Evaluation of the impact of genetic polymorphism on pharmacodynamic activity of commonly prescribed antihypertensive drugs (Thiazide diuretics, ACE inhibitors, CCBs and -blockers)**

The study aims to observe the current trends in prescribing patterns of antihypertensive drugs along with its pharmacodynamic activity and in understanding the role of genetic polymorphism. The first part of the study was to monitor the prescription and consumption profile of antihypertensive drugs in Tertiary care hospital. The results of the study demonstrated use of antihypertensive drugs such as monotherapy (57%), ditherapy (35.0%), and multitherapy (8.1%) in patients. The prescription profile indicated preferential order as BBs (atenolol), CCBs (amlodipine), ARBs (telmisartan) and ACEIs (ramipril) in monotherapy. Among the ditherapy, CCBs were most preferred choice of drug which were administered in combination with BBs, ACEIs and ARBs.

The second phase of the study is undertaken by recording all the prescription information and assessing the role of Single Nucleotide Polymorphism in the uncontrolled Blood pressure.

### **7. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES**

#### **7.1 Studies on anti-obesity properties of *Garcinia* species:**

Studies on anti-obesity properties of *Garcinia* species indicated that the maximum activity against hyperlipidemia was exhibited at high dose level of *Garcinia indica* (5%). It reduced body fat significantly, without any toxicity. Since this compound is anti-glycating, it helps in correcting insulin resistance. The results of the present study indicate that further studies can be conducted to explore its mechanism of action so that a good anti-hyperlipidemic and anti-glycating agent could be developed.

#### **7.2 Dose and time dependent effects of *Mucuna pruriens*, linn ethanolic seed extract on stress related parameters in WNIN/GR-Ob rats**

Dose and time dependent effects of *Mucuna pruriens*, linn ethanolic seed extract on stress related parameters in WNIN/GR-Ob rats was studied. Physical parameters like growth, food intake, feed efficiency ratio, activity was monitored for the experimental period of 45 days. Physiological parameters like body composition and bone mineral contents were measured. Biochemical parameters like cholesterol, triglycerides and stress markers like serum cortisol and *L-dopamine* were analyzed. Cortisol has many functions. It helps the body use sugar (glucose) and fat for energy (metabolism), and it helps the body to manage stress. By supplementing *Mucuna pruriens* which contains dopamine and improving environmental conditions the animals exhibited less stress levels.

#### **7.3 Evaluation of promising plant extracts and active constituents for anti-obese, anti-diabetic and hepato-protective properties in WNIN/GR-Ob rats**

The study evaluated promising plant extracts and active constituents for anti-obese, anti-diabetic and hepato-protective properties in WNIN/GR-Ob rats. There was a significant reduction in the body weights and food intake of animals treated with the plant extracts. Body composition estimation by TOBEC revealed that, there was a significant increase in the lean body mass and reduction in the total body fat content in treated animals compared to controls. However, no significant changes were noted in bone mineral content and density. Hypoglycemia was prominent in *A.indica* extract treated animals, compared to the other experimental animals. Lipid contents like cholesterol and triglycerides decreased significantly in all the treated animals. There was no toxicity observed in the liver of animals treated with the plant extracts. Varying degrees of degeneration of kidney tubules were observed in all the experimental animals as compared to controls and animals treated with *A. indica*.

#### **7.4 Effect of bio-active compounds isolated from *piper nigrum* on high fat diet induced obesity in SD rats – AMRI study**

Rats fed with high fat diet when treated with *Piper nigrum* have shown reduced physical parameters like food intake, growth. Significant increase was found in body composition parameters like lean body mass. Decrease in total body fat and % fat was observed in the HFD rats after being treated with

piperine extracts. DXA analysis revealed that there is a significant increase in bone mineral content and bone mineral density. Regional wise fat distribution analysis revealed that there is predominant deposition of fat at retroperitoneal region. The MRI analysis revealed that, there is a significant reduction in the adipose tissue volumes in the whole body and regional adipose tissue volumes i.e., subcutaneous, thoracic, and retro peritoneal. This study of effect of *Piper nigrum* on obesity can be explored further with respect to its mechanism of action to translation research.

## **8. PRECLINICAL TOXICOLOGY**

### **8.1 Acute toxicity study in mice and sub-chronic toxicity study in rats, *in-vitro* allergenicity test of seed & leaf of transgenic mustard containing Hybrid Dmh-11**

Transgenic *brassica juncea* containing barnase, barstar and bar genes has been developed by Centre for Genetic Manipulation of Crop Plants (CGMCP), University of Delhi South Campus (UDSC). As per the Guidelines for safety assessment of genetically engineered plants, 2008, prescribed by ICMR and the accompanying protocols by DBT, the edible plant parts which have the expression of the inserted genes, need to be tested for their safety profile. We have completed the investigations i. Assess potential allergenic Cross-Reactivity by The databases PubMed, Allergen Online version 12.0 Allergen Database and NCBI Entrez Protein Database were used to accomplish the bioinformatics searches. ii. Pepsin digestibility and thermal stability assay of recombinant Bar, Barnase and Barstar proteins, iii. Acute oral toxicity of Recombinant Bar, Barnase and Barstar protein in Swiss Albino Mice. The results of the study indicated a lack of any significant sequence similarity of Barnase, Barstar or Bar proteins to any allergenic proteins. The pepsin digestibility assay showed that all the three recombinant proteins were rapidly degraded by pepsin in SGF, and 90% digestibility was achieved within 0.5 mins. The Bar (1000mg/kg), Barnase (1000mg/kg) Barstar (1700mg/kg) proteins administered once orally at the above concentration to mice, did not show any adverse effect on any of the parameters studied. The subchronic investigations and compositional analysis are in progress to determine the safety of transgenic mustard containing Hybrid Dmh-11.

### **8.2 Pre-clinical bioavailability and safety evaluation of temozolamide co-crystal**

The role of Temozolomide in treatment of malignancies such as melanoma, sarcoma and astrocytomas at clinical level is well established. However, the limitation of usage is due to its very short stability. TMZ Crystalin Research Private Limited has developed, TMZ Co-crystal using Co-crystallization method. Since, this processing modifies physicochemical characteristics of active pharmaceutical ingredients, comparative bioavailability and safety profile is necessary as per the guidelines of Schedule Y of DCGI. The Pharmacokinetic and Toxicokinetic studies was conducted in Sprague Dawley Rats using cross over design. The Acute and Sub-chronic Toxicity test (28 days) has been conducted in healthy Sprague Dawley Rats. The test and reference standard drugs are found to be bio-equivalent with no significant change in C<sub>max</sub>, AUC and T<sub>max</sub> between test drug and reference standard drug. The toxicokinetic parameter suggest that, no adverse events based on liver, renal and hematology investigation. The NOAEL in rats has been recorded at 70mg/kg which is 2 times higher than the intended clinical dose. The project is completed and dossiers has been submitted for regulatory approvals to undertake clinical trials.



# COMMUNITY STUDIES

## 1. NUTRITIONAL STATUS OF BELOW FIVE YEAR OLD CHILDREN IN THE STATE OF MEGHALAYA

One of the millennium development goals is to reduce extreme poverty and hunger, halving under nutrition and reducing infant and maternal mortality by 2015. Despite rapid strides in agricultural and industrial sector, and consequent economic development, undernutrition continues to be a major public health problem in the developing world, including India. Larger diversity exists between the states of India with its specific socio-economic level, ethnicity, dietary pattern, and health infrastructure and communication facilities especially in North-Eastern region. Thus, the health and nutritional status of population shows significant variations, resulting from various factors.

Currently, the data on nutritional status of the under five year old children is available at state level. However, disaggregated data at the district level makes it mandatory for development of appropriate strategies and programmes for control and prevention of undernutrition in the community.

In this context, the Government of Meghalaya is contemplating to develop State Nutrition Policy and develop plan of action for implementation of district level strategy, in order to improve the nutritional status. Therefore, National Institute of Nutrition carried out survey in all the following 7 districts of the state, to assess the nutritional status of under 5 year old children and infant's and young child feeding practices.

Sl. No.	Districts
1	East Garo Hills
2	East Khasi Hills
3	Jaintia Hills
4	Ri-Bhoi
5	South Garo Hills
6	West Garo Hills
7	West Khasi Hills

The results of the study carried out in all seven districts were pooled. The details for Meghalaya State during the period December 2012 to March 2013, is presented here. The primary objective of the study is to assess the health and nutritional status of <5 year old children and infant and young child feeding practices among <3 year old children in the rural areas at the district level.

### RESULTS

A total of 4140 households from 210 Anganwadi centres were covered for the study. A total of 4529 children (Boys: 2307, Girls: 2222) of <5 years of age were covered for nutritional anthropometry, and for examination for presence of clinical signs of nutritional deficiency. In addition, socio-economic and demographic particulars were collected from all the households. A total of 1209 mothers having <12 months old children, and 1963 mothers, having 12-35 months old children were interviewed to assess Infant and Young Child Feeding Practices (IYCF), as well as for coverage of immunization, receipt of iron folic acid tablets and vitamin A status.

#### *Maternal characteristics*

##### ***Particulars of last pregnancy (mothers having <12 months children)***

About 91% had reportedly undergone antenatal check-up (ANC) during the last pregnancy, of which about 58% had undergone at least three ANCs. About 45% of mothers had registered for ANC before 12 weeks of gestation. About 49% of ANCs were conducted by ANM, and 37% by Medical Officers. About 91% of pregnant mothers reportedly received TT immunization, of whom 64% received two doses of TT.

About 89% of pregnant women received IFA tablets; mostly from ANM (68.7%), and Medical Officer-PHC (15.5%), about 62% received  $\geq 90$  tablets and 47% consumed  $\geq 90$  IFA tablets during the last pregnancy. About 90% of the pregnant women reportedly received ICDS supplements during the last stages of pregnancy.

### **Particulars of last delivery (Mothers having <12 months children)**

Only about 37% of the deliveries were institutional, either at PHC/Govt. hospitals (30%), sub-centre (1.8%) or private Hospital (5.3%), and remaining (63%) deliveries took place at home. Majority of the deliveries were attended by Untrained Dai/elders (47.7%) followed by ANM/ LHV/ TBA (28%), Medical Officer- PHC (16.7%), private doctors (4.5%). Only about 56% of mothers stated that the birth weight of their babies was recorded and mostly (45%) on the same day. As per the records, 11% of the newborn babies had low birth weight (<2.5 kg).

### **Infant and Young child feeding practices (IYCF)**

#### **0-11 month children**

Only 8% of the women reportedly gave pre-lacteal feeds like plain water/others, to their newborns. About 50% of the newborns were given breast feeding within an hour of delivery, while 39% received between 1-3 hours after delivery. Majority (94%) of the mothers interviewed reportedly fed colostrum to the newborn. About 77% infants of 6-11 months received complementary foods in addition to breast milk. About 30% were solely breast fed up to six months of age.

Among children who were currently receiving complementary foods in addition to breast milk (77%), the complementary feeding was initiated at 6 months of age in 38% of infants, while 22% received the same before 6 months of age. The commonly used complementary foods included homemade semi-solids (55.8%) homemade solids (34.8%), formula milk (24.4%), commercial baby foods (20.9%) and cow/buffalo milk (18.8%).

#### **12-35 month children**

About 64% of the 12-35 month children surveyed were currently receiving complementary foods in addition to breast milk and 35% of the children were not breast fed. The type of complementary food being currently given included homemade solids (88.6%), semi solids (36.5%), cow/buffalo milk (26.8%) and formula milk (18.6%).

#### **Care of the Child**

About 46% of mothers reportedly were taking care of their children by themselves at home, while 26% by mother-in-law, 9% by elder siblings and 8% of the mothers carried their children to the work spot.

#### **Personal Hygiene**

Only 63% of the mothers of 0-59 months children used soap for 'hand wash' before feeding the child and 75% were washing their hands with soap after defecation.

#### **History of Morbidity**

About 26% of the children suffered from one or more morbidities, the proportion of which was higher (36%) in the age group of 6-11 months children, and tended to decrease with increasing age to 18% in 48-59 months children. The common morbidities reported were fever (17.7%), followed by acute respiratory infections (12.1%) and diarrhoea (7.2%).

A majority of the mothers in general stated that, they consult PHC (57.1%), private practitioner (19.8%) or ANM/LHV (13.4%) when their children fall sick. About 7% of the children reportedly had diarrhoea during the previous fortnight and 3% of them received ORS from ANM/AWW. About 6% of the children who had acute respiratory infection received co-trimoxazole tablets.

#### **Participation in ICDS supplementary feeding programme**

About 97% of 6-59 months children were participating in the ICDS supplementary feeding programme, of whom 89% were regular. The extent of participation was similar in both the age groups.

### Coverage for Immunization under UIP

About 86% of the children were fully immunized, 10% were partially immunized and 4% were not immunized. Major source of information on immunization was from immunization card (80.5%), followed by parents input (17.7%).

### Coverage for massive dose of vitamin A supplementation

In general, about 88% of 12-59 months children reportedly received at least one dose of vitamin A in the preceding one year. In majority of cases, vitamin A was administered in sub-centres (37.4%), PHC (23.4%) and AWC (22.7%), mostly by ANM (83%). The major reason for non-receipt of massive dose of Vitamin A was "unaware of the need" (2.7%), 'not offered' (2.6%) or 'mother was busy' (2.4%).

### Coverage for iron and folic acid tablets supplementation

Only 68% of 12-59 months children reportedly received IFA tablets during the preceding year, mostly from ANM (58%). About 39% received and consumed 60-90 IFA tablets.

### Nutritional status of children (<5 years)

None of the infant had any sign of nutrition deficiency, while 16% of 36-59 months children had dental caries.

### Prevalence of undernutrition

The prevalence of underweight (Weight for age <median -2SD), stunting (Height for age <median -2SD) and wasting (Weight for Height <median -2SD) according to Standard Deviation classification using WHO child growth standards among <5 year old children according to age and gender were taken as guidelines.

### Underweight

The overall prevalence of underweight (<Median -2SD) was 21%, of which 5% were of severe underweight (<Median -3SD) and 16% had moderate underweight (-3SD to -2SD). The prevalence of underweight increased with increase in age, from 10.5% in the age group of 0-11 months to 28% in 36-59 months age group. The prevalence of underweight among boys was higher (22.5%) compared to girls (19%). The overall prevalence of underweight among <5 year children in the district was lower (21%) when compared to the figures reported for the State of Meghalaya, by NFHS-3 (49%).

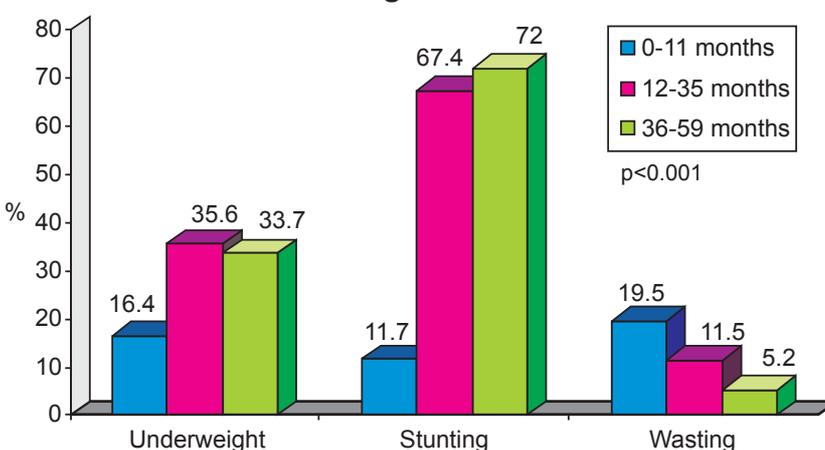
### Stunting

In general, about 44% of <5 year children were stunted (<Median-2SD). The prevalence of severe stunting was 19% (<Median -3SD) and moderate stunting (-3SD to -2SD) was 25%. The prevalence was lower among 0-5 months age group (8.5%) and highest among 48-59 months (61.6%) age group. The prevalence was marginally higher among boys (35%) than in girls (33%). The overall prevalence of stunting among <5 year children in the district (44%) was lower than that reported for the State of Meghalaya, by NFHS-3 (55%).

### Wasting

The overall prevalence of wasting (<Median -2SD) was 5%, of which 1% of children had severe wasting (<Median-3SD) while 4% had moderate wasting. Prevalence of wasting was lowest (2.4%) in 48-59 month's children and highest (6.2%)

Fig. Prevalence (%) of Undernutrition among <5 yrs Children according to SD Classification



in 0-5 months children. The prevalence was observed to be marginally higher among boys (11.7%) as compared to girls (10.6%). The overall prevalence of wasting among <5 year children in the district (11.2%) was lower than the figure reported for the State of Meghalaya, by NFHS-3 (31%).

### ***Performance of an Anganwadi Worker (AWW)***

In-depth interviews were conducted on 209 selected AWWs in all the 7 districts of Meghalaya, (30 AWWs in each district) regarding their knowledge, perception and practices related to ICDS.

### ***Knowledge of the AWWs***

Only 44% of the AWWs were aware of all the ICDS objectives and 60% had knowledge about all the services. Only about 31% of the AWWs were aware that colostrum protects the children from infection. About 88% AWWs opined that breast feeding alone is enough for proper growth of a child up to 6 months of age. About 79% AWWs were aware of oral rehydration therapy (ORT) and 72% were aware correctly about homemade ORS.

### ***Training of AWWs***

Majority of the AWWs interviewed (90.9%) had undergone induction training for various components. About 66% of the AWWs had undergone refresher training programme during the previous year and opined that it was useful for them.

### ***Service delivery by AWWs***

About 34% of the AWWs were serving the supplement using standardized cup and 33% used spoon, and 28% were doing it approximately to serve supplement to the beneficiaries. Almost all the AWWs were using pre-school teaching methods such as talks/ stories, recitations/songs, games & plays.

### ***Growth monitoring***

About 92% of the AWWs stated that they were aware that growth monitoring is useful to assess nutritional status of children, 72% stated it to be useful for early detection of growth faltering/ identification of risk to children, while 60% stated that this was used to educate the mother's regarding nutritional status of their children. Majority of AWWs (77%) stated that they have growth charts and were aware of correct definition of growth faltering & are showing the growth chart to mothers and are providing nutrition education.

### ***Referral services***

About 68% of AWWs referred the beneficiaries during previous one year, of which 37% were referred to MO-PHC, 17% to Govt. hospital. Majority of the referrals (66%) were promptly attended by the health functionaries and all given feedback verbally during meetings or visits of the health functionaries.

### ***Health and Nutrition Education (H & NE)***

Majority of AWWs (94%) were reportedly conducting health and nutrition education sessions for the pregnant and lactating women, mothers of 1-5 year children, adolescent girls and women of 15-45 years, through various methods such as person-to-person communication (81%), group discussions (92%) and posters (48%). About 58% of AWWs were imparting H & NE, monthly, through person to person discussions and 62% by group discussions.

### ***Supervision of AWCs by ICDS/Health functionaries***

About 44% of supervisors, 67% of ANMs and 8% of LHVs were visiting anganwadi centers once in a month, It was observed that in most of the times, the supervisors and CDPOs were check registers and the food stocks during their visits to the AWCs, while, ANM and MO used to talk to beneficiaries. About 85% of AWWs were making home visits along with ANM either monthly (63%) or quarterly (12%).

## **CONCLUSIONS**

Hence, it is concluded that the prevalence of undernutrition, especially chronic undernutrition is still a public health nutritional problem in Meghalaya. Though the current prevalence in the state is relatively

lower than that reported for the state of Meghalaya by NFHS-3 (2006), undernutrition continues to be a public health problem in Meghalaya. It was observed that the prevalence of stunting was higher with very low prevalence of wasting among children, may have certain implications for the role of cultural and ethnic diversity in the health and nutritional disparity in India especially in the north east. Therefore, there is a need to strengthen the existing national nutrition intervention programmes along with promotion of better infant and young child feeding practices, health and nutrition education for parents and health care and sanitation practices for overall improvement of health and nutritional status of children. There is also a need to strengthen the programmes aimed at income generation, so as to enhance HH food and nutrition security.

## **2. SENSORY EVALUATION AND ACCEPTABILITY TRIALS OF LOCALLY PRODUCED READY-TO-EAT SUPPLEMENTARY FOODS FOR BENEFICIARIES OF ICDS IN THE AGE GROUP OF 12-35 MONTHS: A STUDY IN THE RANGA REDDY DISTRICT OF ANDHRA PRADESH**

Undernutrition is both preventable and manageable. Management of under-nutrition among young children is a public health priority and requires special nutrition support in most of the developing countries including India. Ready to use milk based supplementary foods is suggested as an alternative to fortified cereal-pulse/legume based blended flours as it has more advantages in terms of better amino acid profile, good contributor of bio-available calcium and potassium, does not contain anti-nutrients and is contributory towards linear growth.

In view of these, the State Nutrition Expert Committee comprising of technical experts from National Institute of Nutrition (NIN), Department of Women and Child Development (DWCD), GoAP, Andhra Pradesh Foods (APF), a state government enterprise, UNICEF-Andhra Pradesh and Acharya N.G.Ranga Agricultural University (ANGRAU), Andhra Pradesh formulated two skim milk based ready to eat supplementary food products (RTESFs; Product 1 and Product 2) using locally available ingredients, with different cereal to pulse ratios and fortified with micronutrients (Table 1). These are intended to be used in the management of 6-35 month old children who are beneficiaries of the ICDS program in the State of Andhra Pradesh and is required to be consumed as a supplemental food in addition to the child's regular diet. The standardization, shelf life, sensory evaluation and acceptability of the products remain to be tested before efficacy trials and subsequent consideration for inclusion in the government food security programme. Therefore, NIN with financial support from APF/ Government of Andhra Pradesh carried out a research study in two phases. In phase I, the sensory properties, and in phase II, the acceptability of the two formulations was assessed among children 12-35 months of age in comparison to the currently existing food model of APF, i.e., Modified Therapeutic Food (MTF), under the Supplementary Nutrition Programme (SNP) that is provided through the Integrated Child Development Services Scheme in the State of Andhra Pradesh (Table 1).

### **Hypothesis**

The ready to eat supplementary foods are equally accepted as the MTF by 12-35 months old ICDS beneficiaries.

### **Primary Objective**

To assess the sensory properties and acceptability of RTESFs in comparison to MTF, among 12-35 months old child beneficiaries of ICDS.

## Secondary Objectives

- o To assess sensory properties by semi-trained sensory panellists from NIN.
- o To conduct sensory evaluation trial in mothers/primary care givers of ICDS children.
- o To assess quantity of the supplement that can be consumed in one meal by 12-35 months children over a specified duration using a centre-based approach.
- o To conduct quantitative and qualitative assessments of product use, to the children by deploying a Take Home Ration (THR)/ home use approach.
- o To assess adverse effects on consumption of the supplements if any, such as diarrhoea, vomiting, fever, ARI, GI disturbances, etc.
- o To explore perceptions of mothers/primary care givers and service providers (AWWs) towards successful use of the food supplements for their children in future and identify barriers/obstacles, if any.

## METHODOLOGY

### Study food products:

The production of the two novel food supplements (Product 1 and 2) and the existing food supplement (MTF) were done by AP Foods based on recommendations of nutrition expert group, for use in the study. The food and nutrient composition of the food products are given in Table 1.

**Table 1. Composition of the two novel as well as the existing (MTF) supplementary food products used in the study**

Ingredients (g)	Product 1 (New)	Product 2 (New)	MTF (Existing)	Gol Norms
Roasted Wheat Flour	50	55	45	
Roasted Bengal Gram Flour	5	5	0	
Skimmed Milk Powder	10	10	0	
Sugar	25	20	25	
Refined Palmolein Oil	10	10	10	
Roasted Full Fat Soya Flour	0	0	20	
<b>Total (g)</b>	<b>100</b>	<b>100</b>	<b>100</b>	
<b>Nutrients</b>				
Energy (Kcal)	414.2	411.3	429.4	500
Protein (g)	11.0	11.6	14.1	10-15
Fat (g)	11.1	11.2	14.7	-
<b>Fortified with micronutrients at the level of 50% of RDA<sup>7</sup></b>				
Vitamin A (µg)	200	200	200	200
Thiamine (mg)	0.4	0.4	0.4	0.4
Riboflavin (mg)	0.4	0.4	0.4	0.4
Niacin (mg)	4	4	4	4
Vitamin C (mg)	20	20	20	20
Folic Acid (µg)	18	18	18	18
Calcium (mg)	200	200	200	200
Iron (mg)	8	8	8	8

### **Sensory evaluation studies**

The sensory properties of all the 3 food products was assessed by the semi-trained sensory panel (n=50) from NIN using a 9-point hedonic scale at NIN as well as by the primary caregivers of the ICDS children, using a 5-point smiley hedonic scale (Mothers panel, untrained panel; n=50), at study field sites. Sensory evaluation was repeated three times, with an interval of one week between each evaluation. Inclusion criteria for sensory panellists comprised of adults in the age-group of 20-49 years from NIN (NIN Panel; employees, research scholars and M.Sc. Applied Nutrition students) and mothers of ICDS children (Mothers panel), with no reported taste/odour perception disorders, colour blindness, denture defects, minor infections of nose, throat, no current illness such as fever, GI disturbances, food intolerance/allergies, and willing to participate during the three time points.

### **Food acceptability studies**

The acceptability of the food products, in terms of proportion of supplement consumed by ICDS children (n=30) was assessed using the AWC centre based 3-day test feeding trial followed by a 2-week Take Home Ration trial (THR, Home use trial), for each of the food product by adopting a randomized controlled cross-over study design. Three focus group discussions with the primary care givers and four in-depth interviews with AWWs were also conducted to further understand acceptability, compliance, adherence and willingness to continue with the food products in the long term. Inclusion criteria for study children comprised of ICDS children in the age group of 12-35 months and their mothers/primary care givers, consuming solid foods and the existing supplement (MTF) for at-least the past 30 days, apparently healthy i.e., not suffering from any chronic diseases/ other medical complications, living in the study area for at least 6 months, no known allergy to milk products, soy products, wheat products etc., moderate underweight, stunting and wasting to normal grade children (WAZ, HAZ and WHZ  $\geq -3$  z score), having written informed consent for participation in the study from their parents and planning to remain in the study area and continue for the entire study duration of 6 months.

### **Compliance**

The field studies were carried out in 2 AWC villages in the Ranga Reddy district of Andhra Pradesh. Liaisoning with AP Foods was done to ensure supply, distribution and consumption of food supplements during the study period. Compliance and adherence to consumption of supplements was monitored by the project field staff every day by weighing of the leftovers and direct observation at the AWC during test meal trial and by assessing remaining product at weekly home-visits during THR trial. The AWC staff was involved to coordinate and facilitate the consumption of supplements on a daily basis. The care givers and AWC staff were instructed that the food supplements should be fed only to the study children and not shared with other members of the family or with other children. Surprise checks by the research staff of NIN were conducted to monitor compliance.

### **Study approvals**

Ethical clearance from the Institutional ethical committee of NIN, approval from the Scientific advisory committee of NIN, informed written consent from sensory panelists and care givers of study children, permission from Commissioner, DWCD, GoAP and concerned village heads was obtained before initiating the studies.

### **Data analysis**

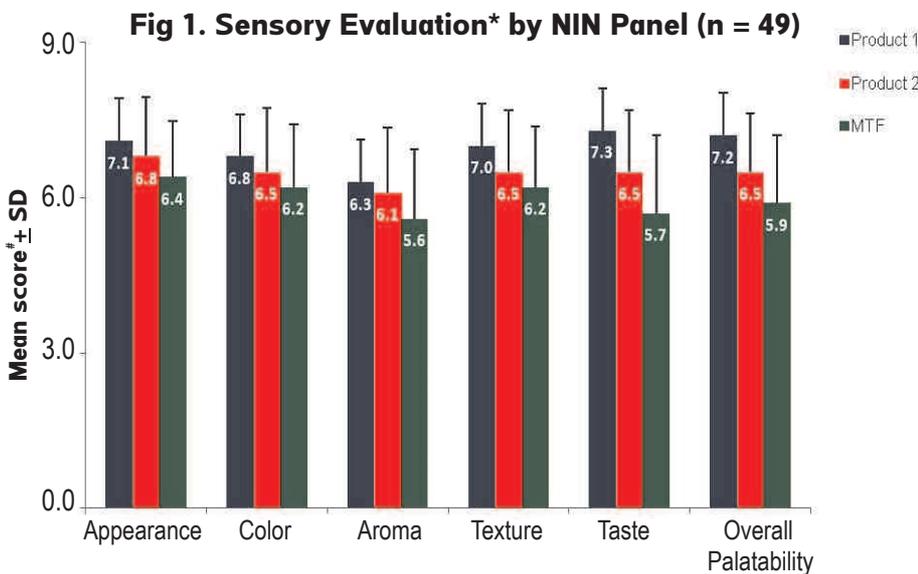
The data was analyzed using SPSS version 19.0. Descriptive statistics included means, standard deviation and 95% confidence interval for continuous data and proportions for categorical data. Means were compared using two way ANOVA for all the sensory attributes. The longitudinal data in the acceptability studies, i.e., the differences in total quantity consumed, between food products, between time points and interaction between product x time was analyzed using mixed model analysis. The associations between proportions were compared using Chi square test. Non-parametric multiple comparisons using Friedman pair-wise ranking test followed by post hoc Tukey's Honestly Significant Difference (HSD) test was used to analyze the data on preference for food products by NIN panel and simple ranking test for the Mothers Panel. The significance level set was  $p < 0.05$ .

## RESULTS

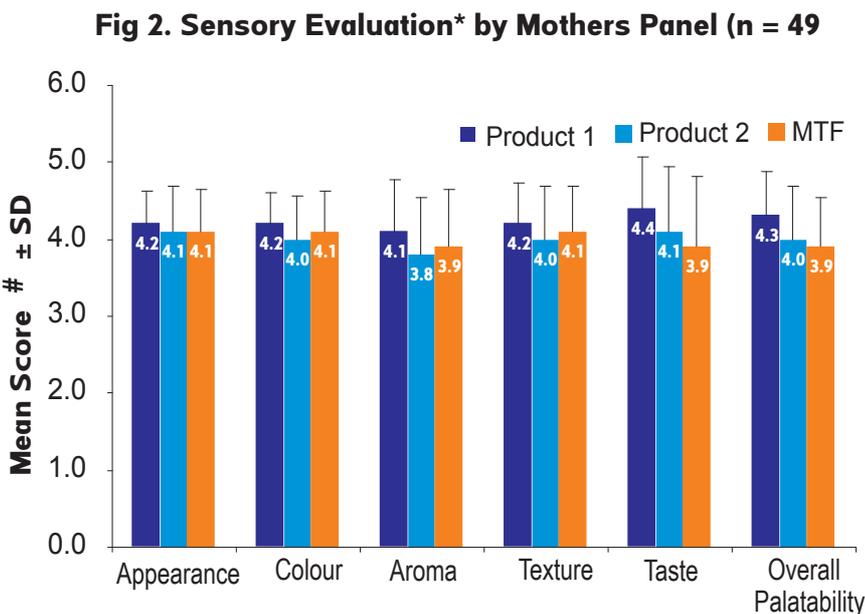
The results of the study are described briefly below,

### Sensory evaluation studies

- The mean age of NIN panel was 26.9 ± 4.22 years with 52% of them being women and that of the Mothers Panel was 24.4 ± 3.50 years. Maternal illiteracy was 10%.
- About 49 of 50 panelists (each in the NIN panel & Mothers panel) completed the study.
- The mean sensory scores given by NIN Panel for all the sensory attributes including overall palatability was significantly ( $p < 0.001$ ) different between products while the difference in scores given by the Mothers Panel was significant for aroma and texture ( $p < 0.01$ ) and taste & overall palatability ( $p < 0.001$ ). Product 1 was superior followed by product 2 compared to MTF, as assessed by both the panels (Fig 1 & 2).
- The sensory scores were similar between time-periods and both the panels preferred product 1 ( $p < 0.001$ ) for young children in comparison to product 2 or MTF.



All values are Mean ± SD; \* 9 Point Hedonic Scale: 9. Like extremely ... 5. Neither like nor dislike ... 1. Dislike extremely; # All values are pooled for time periods; Difference between time periods was not significant for any of the attributes ( $p$  value: 0.079 to 0.797; Interaction (products x time) was not significant for any of the sensory attributes ( $p$  values: 0.517 to 0.940).



All values are Mean ± SD; \* 5 Point Hedonic Scale: 5. Like very much ... 3. Neither like nor dislike ... 1. Dislike very much; # All values are pooled for time periods; Difference between time periods was not significant for any of the attributes ( $p$  value: 0.425 to 0.790); Interaction (products x time) was significant only for the attributes of appearance ( $p = 0.036$ ) and taste ( $p = 0.031$ ).

### **Food acceptability studies**

- The mean±SD age of the study children was 19.9±5.34 months and in 86.7% of the households mother was the primary care giver. Maternal illiteracy was only 7%.
- About 27 of 30 children completed the test meal trial and 28 of 30 children completed the Take Home Ration/Home use trial.
- The test meal feeding trial indicated that the mean±SD percentage of the offered amount of supplementary food consumed by children per day was about 74.7±27.56 for product 1, 72.1±26.84 for product 2 and 68.3±30.52 for MTF. The percent consumption did not differ by food product ( $p=0.327$ ) or time period ( $p=0.206$ ), suggesting all the three products appeared to be equally acceptable.
- The take home ration/ Home use trial revealed that the mean±SD (95% CI) percentage of the recommended amount of supplementary food consumed per day was about 85.5±26.42 (77.26, 93.66) for product 1, 80.2±34.74 (71.95, 88.35) for product 2 and 77.7±32.85 (69.52, 85.92) for MTF. The percent consumption did not differ by food product ( $p=0.405$ ) or time period ( $p=0.578$ ).
- However, based on the 3-criterion *priori* set for considering a food product acceptable (% consumption should be  $\geq 75\%$  of the recommended amount,  $SD \leq 30\%$  and the lower limit of 95% CI should be  $>50\%$ ), product 1 appears to be more acceptable as all the 3 criteria are satisfied, while for product 2 and MTF, the SDs are higher than acceptable (35% and 33% respectively), suggesting its long term adherence over a 14-day consecutive period.
- The focus group discussions held with mothers/primary care givers brought out three very important factors that (1) the product B was more preferred by the children because it was sweeter and tasted better than the other two, (2) the supply of food products in individual packets favoured regular consumption as they were perceived to be hygienic and (3) education by the project staff on handling, usage and storage of the supplements improved adherence and compliance. They were willing to continue using the food product in the future.
- The AWWs also shared that the mothers told them that their children liked the sweeter supplement (product B) better. They also opined that it would be logistically more favourable to distribute individual sealed packets to the beneficiaries both from the point of hygiene as well as acceptability and adherence on a long term basis.

### **CONCLUSIONS**

- The novel product 1 is the most accepted supplementary food for children aged 12-35 months, based on its superior sensory properties, preference and acceptability followed by product 2 compared to the existing MTF.
- The FGDs highlighted important aspects that food product should be supplied in individual packets for each child and the service providers need to impart education to the mothers on handling, usage and storage of the supplement to ensure good compliance.
- The study warrants for further randomized controlled efficacy and effectiveness trials on the use of the new milk based ready to eat supplementary food among young children, through the government food security program to study long term adherence as well as effects on growth and nutritional status.

### **3. A RAPID ASSESSMENT OF NUTRITIONAL STATUS OF UNDER FIVE YEAR OLD TRIBAL CHILDREN AND WOMEN OF ATTAPPADY HILLS, PALAKKAD DISTRICT OF KERALA AND CAUSES OF INFANT DEATHS BY ICMR VERBAL AUTOPSY GUIDELINES (RAPID SURVEY)**

Three ethnic tribal groups i.e., Irula, Muduga and Kurumba are living in the hilly areas of Attappady, which is one of the most backward revenue block of palakkad district of Kerala. There were reports in the media, about unusual number of infant deaths occurring among tribal families of Attappady, since one year, attributed mostly to undernutrition and inefficient health care. Hence, a rapid nutrition assessment among women and children, and cause of infant deaths was carried out in the select villages of Attappady in the month of May 2013. The list of villages/hamlets, where the infant deaths were reported was obtained from project officer, ITDA, Agali, Palakkad. All the 17 villages/hamlets, where infant deaths reported, during past 6 months, were surveyed. All the households (HHs) in the selected villages/hamlets, mothers of <5 year old children were investigated. Majority of the HHs (72.6%) covered were nuclear families, followed by extended nuclear (14.6%) and joint families (12.8%). The average family size was 4.3. About 78% of fathers and 76% of mothers of indexed children were literate. The major occupation of the fathers was labour (85%) and mothers mostly housewives (72%). About 5% of fathers and mothers engaged in private /govt. services. A small proportion of them (1-2%) were engaged in agriculture. The average monthly per capita income (PCI) of HHs was only ` . 787.00.

About 35% of the families were living in pucca houses, which were mostly constructed under Indira Awas Yojana (IAY) programme, while 59% living in semi-pucca and 6% living in kutchha houses. The major source of drinking water was tap water (58.1%), followed by stream/river (25.2%), and open well (15.1%), tube well (1.7%). Only 1% of the HHs were using LPG for cooking purpose, while, 99% were using firewood. About 86%, 60% and 33% of the HHs had separate kitchen, electricity and sanitary latrine respectively. Eighty three percent of the HHs were availing Targeted Public Distribution System (TPDS), 28% Mahatma Gandhi National Rural Employment Guarantee (MGNREG) Programme. In general, the food and nutrient intake of tribal population was lower than the suggested levels of ICMR 2010. The extent of deficit in the nutrient intakes was relatively higher, especially in micronutrients such as iron, vitamin A, riboflavin, free folic acid. Higher prevalence of conjunctival xerosis (4.7%) and Bitot spots (1.2%) indicated that vitamin A deficiency among <5 year old children is a public health problem as per the WHO criteria. The prevalence of underweight, stunting and wasting among <5 year old children was 48.5%, 49.7%, and 23.7% respectively. The prevalence was higher compared to other tribes (NNMB Kerala 2009) as well as their rural counterparts of Kerala (NNMB Kerala 2012).

Information on infant and young child feeding practices obtained from the mothers of <3 year children, indicated that about 12% of the women reportedly gave pre-lacteal feeds to the newborn, which included mostly glucose water, honey or cow/goat milk etc. About 84% percent of the mothers reportedly fed colostrum to their newborn. About 71% of the newborn were given breast feeding within an hour of birth while 18% were fed between 1-3 hours. Only 35% of the children were fully immunized, 57% were partially immunized, while about 8% did not receive any immunization at all. In general, only 15% of 12-35 months old children reportedly received at least one dose of vitamin A during the previous one year. About 27% of mothers stated that they have health facility in their villages/hamlets. For 36% of villages/hamlets, the distance of health facility was >5 Km, while 24% had 3-5Km distance, 11% were 1-3 km and 2% were <1Km of distance. The type of health personnel approached for treatment, was usually allopathic doctor (83%). During past one year, 10 abortions/still births, 11 neonatal deaths and 3 infant deaths were reported. The major cause of stillbirths and abortions was pregnancy induced hypertension (PIH) and diabetes, followed by premature delivery and accidental death. For neonatal deaths, the major cause of death was PIH (4 deaths), premature delivery (4 deaths), and ante-partum haemorrhage (APH),

obstructed labour and congenital anomaly (one each). Low birth weight and premature delivery are the reasons for infant deaths. Maternal undernutrition may also have influenced to aggravate of the problem.

The study revealed that, the food basket of Attappady tribes mainly consists of boiled rice and there is not much dietary diversity. The consumption of protective foods such as GLV, other vegetables, milk and milk products, fats and oils and sugar and jaggery was below the recommended levels of ICMR, leading to high levels of micronutrient undernutrition (hidden hunger) such as iron deficiency anaemia, vitamin A, iodine deficiency disorders, especially among children and women of reproductive age groups. Faulty infant feeding practices like feeding of pre-lacteals to the new born, exclusive breast feeding, delayed complementary feeding and sub-optimal complementary feeding in terms of quantity, quality and frequency were observed. In addition, high prevalence of low birth weight, frequent morbidities etc, are also associated with undernutrition.

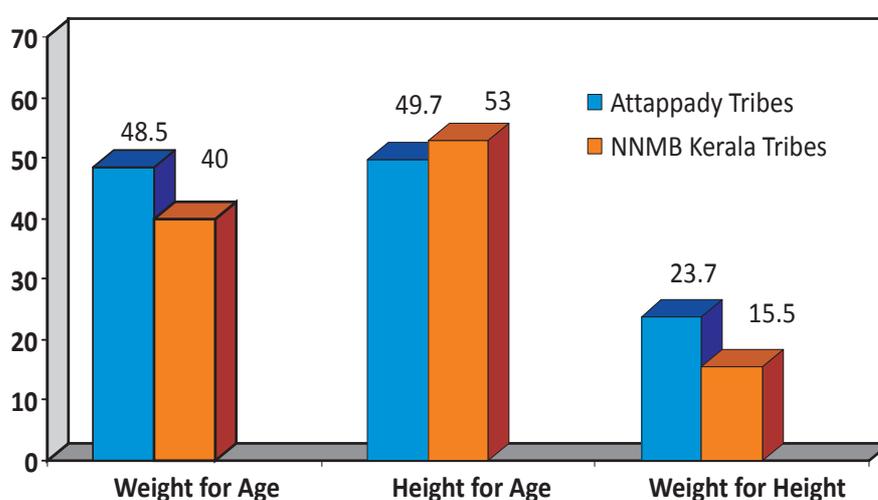
The prevalence of underweight, stunting and wasting among <5 year old children was 48.5%, 49.7%, and 23.7% respectively. The prevalence was higher compared to other tribes (NNMB Kerala 2009) as well as their rural counterparts of Kerala (NNMB Kerala 2012) (Fig1). The study shows that high maternal undernutrition, that is 48% of adult women had chronic energy deficiency (CED: BMI<18.5) and 9% were overweight/obese. The prevalence of CED was marginally higher among Attappdy women (48%) compared to other tribal women of Kerala (44%) (Fig 2).

The implementation of national nutrition and health intervention programmes like iron & folic acid, massive dose of Vitamin A supplementation and immunization are poor. Overall, it can be concluded that the adaptability of these tribes to changes in their lifestyles, because of influence of settlers, leading to some positive and negative effects in their lives, need's to be thoroughly examined.

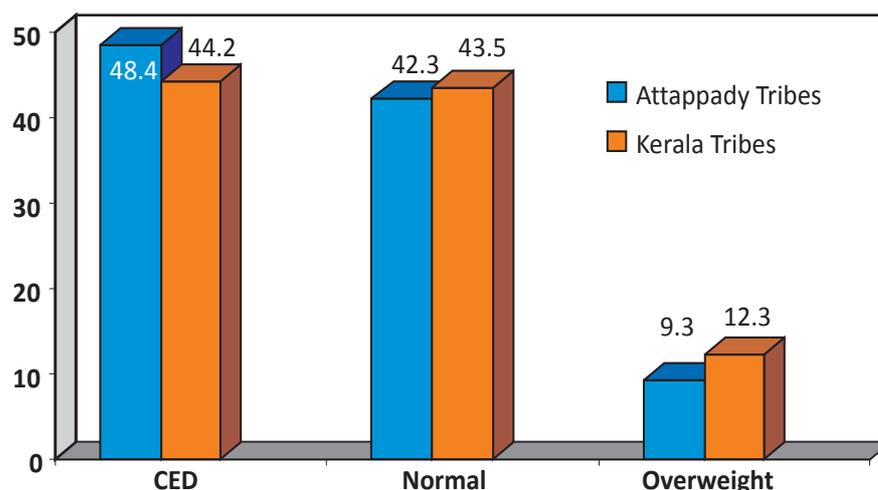
## CONCLUSIONS

- Awareness campaign should be organized by strengthening IEC activities on utilization of various national nutrition intervention programmes, explaining its availability and benefits of each scheme and rights of the beneficiaries.

**Fig 1. Prevalence (%) of undernutrition (<Median - 2SD among <5 year children according to SD classification**



**Fig 2. Distribution (%) of Reproductive age women (15-45 years) according to BMI Classification**



- Educate the pregnant and lactating women on optimal infant and young child feeding (IYCF) practices and improve maternal health and nutrition.
- Educate and motivate all the tribal pregnant and lactating mothers to utilize health services to the maximum level and also motivate them to stay in the hospitals for required time to complete the treatment.
- Referral fund should be provided to the Anganwadi centres in order to refer any emergency cases/sick patients to the health functionaries/hospitals.
- Need to improve the health care both in the accessibility, availability and utilization of the same by regular home visits by health personnel.
- Ensure the regular supply and distribution of micronutrient supplementation, like iron and folic acid and massive dose of vitamin A.
- National Rural Employment Guarantee Programme needs to be strengthened and provide maximum employment opportunities especially during lean periods of the year.
- Hospitals may be well equipped and deploy experienced health personnel to manage potentially risky pregnancies.
- Encourage development of kitchen gardens for growing green leafy vegetables and other vegetables in their backyards, to improve dietary consumption in order to control micronutrient deficiencies.

## II. BIostatistics and Bioinformatics

### META-ANALYSIS APPROACH ON “MICRO-NUTRIENTS FOOD FORTIFICATION AND ITS EFFECT ON HEALTH, SOCIAL AND ECONOMIC FACTORS”- A STATISTICAL MODEL BUILDING APPROACH

Nutrition science aims to create new knowledge, but scientists rarely sit back to reflect on what nutrition research has achieved in recent decades. Meta-analysis, a statistical method of combining data from multiple sources, has been used for many decades in the nutritional sciences, social sciences, environmental sciences, and in agriculture. In early 1990's, meta-analysis also started to gain traction in medicine as a valid and useful technique to combine the results of randomized controlled studies. Researchers trying to summarize the constantly growing body of research in the social, nutritional, behavioral, and health sciences are increasingly using this technique. Meta analysis provides an entire set of statistical methods for aggregating and comparing the results from several relevant studies, allowing researchers to determine whether a treatment is actually effective overall, and whether the effectiveness of a treatment depends on certain study and/or subjects characteristics (moderators). Potentially relevant studies to determine the most promising directions for future researchers are indicated.

#### OBJECTIVES

- ❖ To combine evidence from randomized controlled trials to assess the effect of iron fortified foods on mean hemoglobin concentration in children.
- ❖ Impact of iodine fortified foods on urinary iodine concentration among children: A meta-analysis combining parallel and cross-over randomized controlled trials.
- ❖ Meta-analysis was carried out to assess the effect of fortified foods with vitamin A on retinol concentration in children.

#### Objective 1

A meta-analysis of randomized, controlled iron fortified feeding trials that evaluated hemoglobin (Hb) concentration was conducted. The weighed mean difference (WMD) was calculated for net changes in Hb by using random effects models. Meta-regression and covariate analyses were performed to explore the influence of confounders on net pooled effect. Trials were identified through a systematic search from PubMed, Cochrane Library and secondary references. Eighteen studies covering 5142 participants were identified. The duration of feeding of fortified foods ranged from 6-12 months in these studies. Eighteen studies were included and evaluated in the meta-analysis. Overall pooled estimate of Hb concentrations showed significant increase (WMD: 5.09 g/L; (95% CI: 3.23, 6.95),  $I^2=90%$ ,  $\tau^2=18.37$ ,  $p<0.0001$ ). Meta-regression analysis indicated that the duration of the study was positively related to the effect size (regression coefficient =0.368; (95% CI: 0.005, 0.731),  $p<0.05$ ). The net pooled effect size after removing the confounders was 4.74 g/L (95% CI: 3.081, 6.399). An association was observed between

intakes of iron fortified foods on Hb concentration in children of <10 years of age. Iron fortified foods could be an effective strategy for reducing iron deficiency anemia in children.

### **Objective 2**

The purpose of this analysis was to combine evidence from parallel and cross-over randomized controlled trials to assess the impact of iodine fortified foods on urinary iodine concentration (UIC) in children. A structured search for studies on iodine intervention studies on MEDLINE, ProQuest, and the Cochrane Library from Jan, 1990 to Dec, 2012 was carried out. Carry-over effect was estimated by general linear model. Two methods were explored to pool continuous outcomes in a meta-analysis by combining parallel and cross-over trial designs. The standard mean difference (SMD) was calculated for net change in UIC. Fixed or random-effects models were used to summarise fortified food response data. Meta-regression and covariate meta-analysis were performed to explore the influence of confounders on the net pooled effect on UIC. The overall pooled estimate which combine parallel with cross-over trials in the absence carry-over effect of UIC from 9 studies showed a significant increase in the fortified group compared with the control group (N=3448; SMD=2.02 µg/L; 95% CI 1.30, 2.73; I<sup>2</sup>=99%, τ<sup>2</sup> =1.81, P<0.0001). Meta-regression analysis indicated that dose of the feeding was positively related to the effect size (regression coefficient=0.014; 95% CI 0.003, 0.026; p<0.019). The net pooled effect size after removing the confounders was 1.591 (95% CI 0.953, 2.229) µg /L. There was an association between intakes of iodine fortified foods and UIC in children. These results suggest that, we can combine parallel with cross-over trials for meta-analysis, provided that the iodine absorption is high and this effect becomes evident in children.

### **Objective 3**

Both PubMed and the Cochrane Library databases from 1990 up to 2012, and also reviews and the reference lists of the articles were searched. Used the keyword 'food forti?cation' paired with 'Vitamin A' or 'retinol' or 'dual forti?cation' or 'triple forti?cation' or 'multiple micronutrient forti?cation' and 'forti?cation trial'. All relevant articles were retrieved and a systematic review, fixed and random effects meta-analysis approaches were performed on the outcome measure (retinol). Out of 639 published full texts thirteen studies were included and evaluated. A pooled estimate showed less likely overall effect of iodine fortification on children (N5562; SMD: 0.49; 95% CI: 0.15, 0.54). However, substantial heterogeneity of estimate on effect (I<sup>2</sup>=97%, τ<sup>2</sup>=0.81, p=0.0001) was evident. This study suggested that, consumption of vitamin A forti?ed foods had signi?cant impact on children.

## II. MICROBIOLOGY AND IMMUNOLOGY

### 1. EFFECT OF *FRUCTO-OLIGOSACCHARIDE* COATED PROBIOTIC ON FETAL IMMUNO-PROGRAMMING AND OTHER HEALTH BENEFITS

It is becoming increasingly clear that the gut microbiome plays an important role in a host of activities including digestion, protection from potentially pathogenic organisms, and the regulation and development of immune system. We are beginning to understand that exposure to microorganisms before conception and during gestation, and in neonatal period's have profound effects on the developing immune system of fetus. Maternal exposure to microbes, their sequential colonization of the gastrointestinal tract after birth, the influence on colonization by the environmental conditions in the first few weeks of life, and the influence of prebiotics and probiotics, all support the adaptation of "fetal programming" hypothesis to a host-microbe corollary, explaining the specific interactions between the microbes and host during critical periods of immune development and which may have consequences well into adulthood. Epidemiological studies indicate that lack of early childhood exposure to microbial agents, may contribute to increased susceptibility to the development of allergic diseases. Moreover, environments characterized by a diverse and concentrated microbial milieu such as traditional farming sites may protect from allergic diseases. Moreover, when *Lactobacillus rhamnosus GG* was supplemented to pregnant women, children had lesser incidence of allergenic symptoms. In the current study the effect of supplementation of *Lactobacillus rhamnosus GG* (Probiotic) and *Fructo-oligosaccharide* (Prebiotic) in pregnant mice was studied on immune responses of F0 and F1 generation offspring.

#### METHODOLOGY

Twenty-one day old Swiss albino mice were obtained from National Center for Laboratory Animal Sciences (NCLAS) and after acclimatization for 15 days the animals were divided into 3 groups Control, Probiotic and Probiotic+Prebiotic (Pro+Pre) with 8 females and 4 males in each group. This is indicated as F0 Generation. The animals were subjected to food and sterile water ad libitum and were maintained at 24 hr light/dark cycles. The Control, Probiotic and Prebiotic + Probiotic group were then supplemented with 0.2ml sterile saline,  $2 \times 10^9$  cells of *Lactobacillus rhamnosus GG* and 0.0026 gm of Prebiotic (*Fructo-oligosaccharide*)+  $2 \times 10^9$  cells of LGG respectively. The treatment was continued for 3 months and then animals were put for mating and the respective treatments were continued even during gestation, lactation. After the pups were weaned the mothers were sacrificed for performing the immune parameters.

F1 generation pups, thus obtained from treated dams of F0 generation post weaning from all groups were subdivided and 50% pups among all groups were either supplemented with their respective supplements as that of their parents or left unsupplemented. At the age of 3 months they were then injected intramuscularly with 0.1ml Hep-B vaccine (Revac, Bharath biotech) on 0<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> day. Blood with drawl was performed one week after administration of booster dose, for serum separation and one week later the animals were sacrificed for performing immune related parameters.

## Results of various Immune parameters performed in the treated mothers (F0 generation):

### Enumeration of selective gut microbiota among F0 generation Probiotic and Probiotic + Prebiotic supplemented mothers:

- There was a significant improvement in the colonization of *Lactobacilli* in the probiotic group compared to Control and Pro + Pre groups.
- *Bifidobacterial* colonization was significantly highest in the Pro+ Pre group compared to Control and Probiotic group.

**Table 1. Population of *Lactobacilli* and Bifidobacteria in the F0 generation Probiotic and Probiotic+Prebiotic supplemented mothers**

Cecal colonization	Control	Probiotic	Pro+Prebiotic
<i>Lactobacilli</i>	2.80 ± 1.96	73.6* ± 27.6	22.6 ± 17.5
Bifidobacteria	26.2 ± 13.7	21.0 ± 8.50	140.6* ± 50.8

Values are Mean± SE. \*Indicates significance between the groups.

### Lymphocyte Function among F0 generation Probiotic and Probiotic + Prebiotic supplemented mothers:

Lymphocyte proliferation was significantly highest in the Pro + Pre group of F0 generation compared to Control and Probiotic groups.

**Table 2. Lymphocyte function in the F0 generation Probiotic and Probiotic + Prebiotic supplemented mothers**

Lymphocyte Function	Control	Probiotic	Pro+Pre
Lymphocyte proliferation index	12.85 ± 1.83	15.6 ± 2.81	30.8 * ± 1.81

Values are Mean± SE. \*Indicates significance between the groups.

### Leukocyte Function among F0 generation Probiotic and Probiotic + Prebiotic supplemented mothers:

- ✎ Leukocyte function was significantly improved in the Pro+Pre group treated animals compared to control and probiotic groups.

**Table 3. Leukocyte function in the F0 generation Probiotic and Probiotic + Prebiotic supplemented mothers**

Leukocyte Function	Control	Probiotic	Pro+Pre.
Leukocyte phagocytic ability	38.58 ± 3.33	43.12 ± 1.08	74.68*± 3.43

Values are Mean± SE. \*Indicates significance between the groups.

## Results of various Immune parameters performed in the pups born to treated dams (F1 generation)

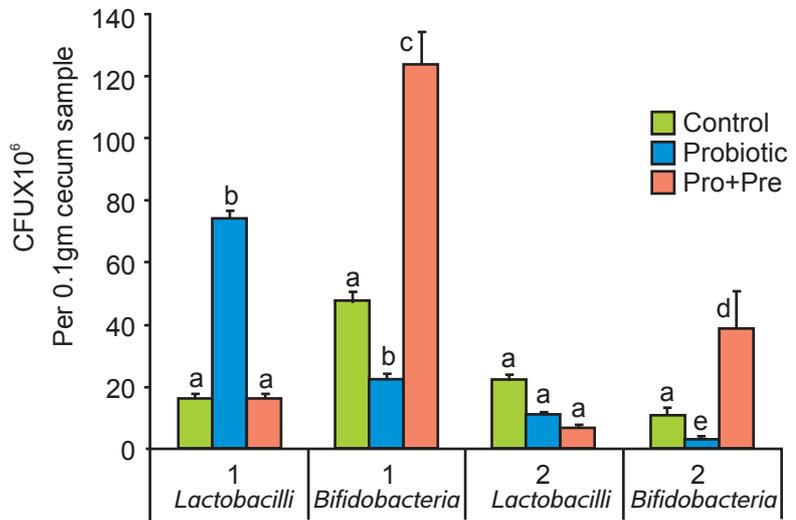
Animals of F1 generation that have been challenged with Hepatitis-B surface antigen (HBsAg) were sacrificed two weeks after the booster dose was administered and the following Microbiological and Immunological parameters were performed.

**Body Weights of F1 generation:** Body weights were found to be comparable, among the groups both in the supplemented and unsupplemented pups of F1 generation born to treated F0 generation.

**Enumeration of selective gut microbiota among F1 generation:**

The *Lactobacillus* population significantly ( $p < 0.05$ ) increased in the supplemented probiotic group of F1 generation when compared to control and Pro+Pre owing to the treatment with *Lactobacillus rhamnosus* GG. However, in the F1 animals that were not supplemented *Lactobacilli*, population was still comparable among the groups. Similarly, Bifidobacterial population was significantly high in the Pro+Pre group of supplemented F1 generation owing to the presence of prebiotic along with probiotic (*Lactobacillus. rhamnosus*GG). In the F1 generation unsupplemented groups, unlike the *Lactobacillus* population Bifidobacteria continued to thrive in the Pro+Pre group even in unsupplemented group which is indicated by the significant improvement in the Bifidobacterial concentration in the Pro+Pre group when compared to control and probiotic groups.

**Fig. 2. Population of *Lactobacilli* and *Bifidobacteria* in the F1 generation pups**

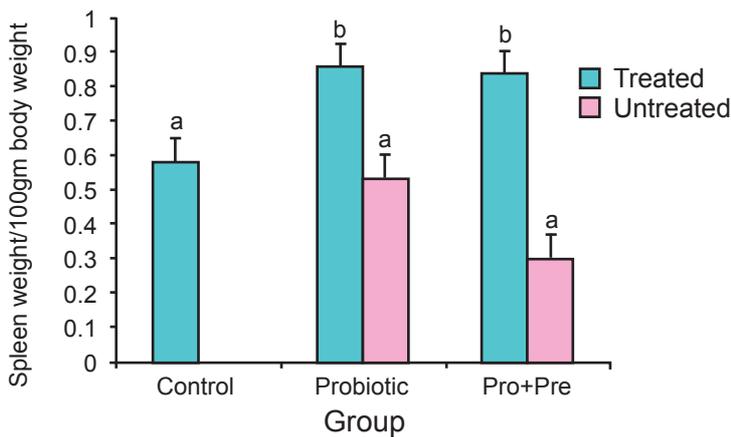


Values are Mean  $\pm$  SE, Same alphabets indicate no significance and different alphabets indicate significance. Values are significant if  $p < 0.05$ .

Represents the pups of F1 generation that were treated with similar supplementation as their respective mothers (F0 generation).

Represents the pups of F1 generation that were not supplemented with Probiotic or Probiotic+Prebiotic supplementation.

**Fig 3. Spleen weights of the animals belonging to F1 generation pups**



Values are Mean  $\pm$  SE, same alphabets indicate no significance and different alphabets indicate significance. Values are significant if  $p < 0.05$ .

Treated F1 generation represents the pups of F1 generation that were treated with similar supplementation as their respective mothers (F0 generation).

Untreated F1 generation represents the pups of F1 generation that were not supplemented with Probiotic or Probiotic + Prebiotic supplementation.

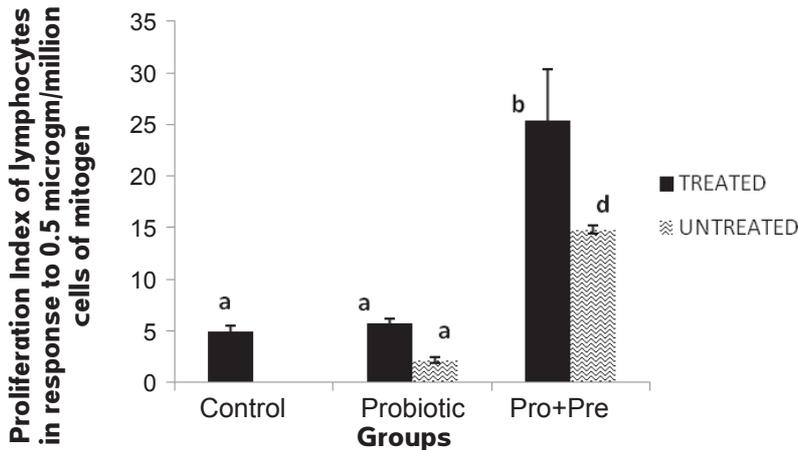
**Spleen weights of F1 generation:**

There was a significant improvement in spleen weights among the F1 animals that were supplemented with respective supplementations post weaning. However, in the animals that were unsupplemented the spleen weights remained comparable to control.

**Lymphocyte proliferation Index of F1 generation:**

There was a significant improvement in lymphocyte function as indicated by the increased lymphocyte proliferation index in response towards mitogen (Concavalin-A) at concentration of 0.5  $\mu$ gm/ million cells among the supplemented F1 generation probiotic+prebiotic group when compared to control and probiotic groups. In the F1 animals that were not supplemented with their respective treatments post weaning, there was a significant ( $p < 0.05$ ) improvement in the lymphocyte proliferation of Pro+Pre group compared with probiotic group.

**Fig 4. Lymphocyte function in F1 generation pups**



Values are Mean± SE, Same alphabets indicate no significance and different alphabets indicate significance. Values are significant if  $p < 0.05$ .

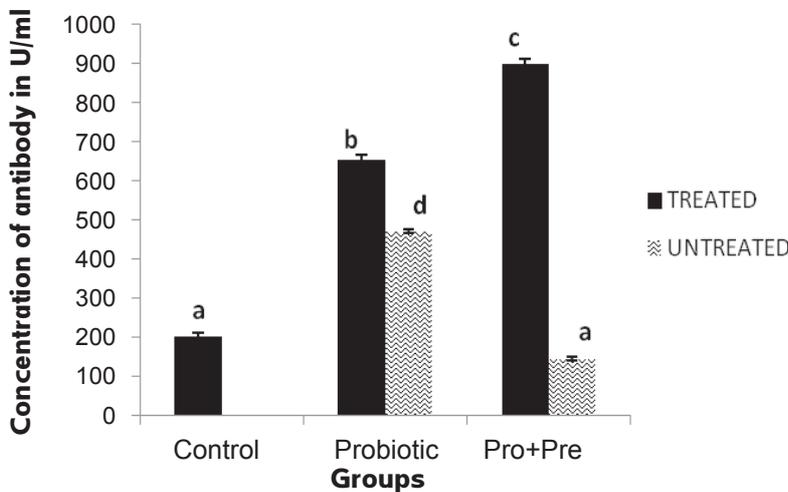
Treated F1 generation represents the pups of F1 generation that were treated with similar supplementation as their respective mothers (F0 generation).

Untreated F1 generation represents the pups of F1 generation that were not supplemented with Probiotic or Probiotic + Prebiotic supplementation.

**ELISA values of Anti HBsAg Antibody concentration in U/ml among F1 generation:** There was a significant ( $p < 0.05$ ) improvement in the antibody concentration against specific antigen in the probiotic group when compared to control but highest concentration was observed in the Pro+Pre group of F1 generation that was treated with respective supplementation post weaning.

In the animals that were discontinued with the treatment after weaning there was significant rise in the specific antibody concentration in the probiotic group when compared with control and Pro+Pre groups.

**Fig 5. Serum Anti HBsAg antibody response in Hepatitis-B immunized F1 generation pups**



Values are Mean±SE, same alphabets indicate no significance and different alphabets indicate significance. Values are significant if  $p < 0.05$ .

Treated F1 generation represents the pups of F1 generation that were treated with similar supplementation as their respective mothers (F0 generation).

Untreated F1 generation represents the pups of F1 generation that were not supplemented with Probiotic or Probiotic + Prebiotic supplementation.

## CONCLUSIONS

- ✍ There was a significant improvement in the leukocyte and lymphocyte activities in the Probiotic+Prebiotic treated group of F0 generation.
- ✍ Cecal colonization showed increase in the population of *Lactobacilli* in probiotic treated group, and Bifidobacteria was significantly highest in the probiotic+prebiotic group of F0 generation.
- ✍ These results suggest that Probiotic+prebiotic (synbiotics) treatment beneficially affects the immune response as indicated by higher spleen weights, improved lymphocyte proliferation and greater antibody response against specific antigen (Hepatitis-B surface antigen) in the supplemented F1 pups.

- ✎ Probiotic supplementation also improved immune response although to a lesser extent compared to Probiotic+prebiotic (Synbiotic) supplementation in the supplemented F1 generation pups.
- ✎ Beneficial effects like improved antibody production towards specific antigen was also observed in the probiotic group of F1 generation animals that were not given treatment indicating an important and vital finding that humoral response is being transferred across generations. This effect was also observed in the case of cell mediated immunity as indicated by enhanced lymphocyte function in the Pro+Pre group among unsupplemented F1 animals.
- ✎ More studies are required to further explore the mechanisms exhibited by the probiotics and prebiotics in immune programming of off springs born to the supplemented parents.

## 2. DEVELOPMENT OF TOOLS TO IDENTIFY AND MAP IGE BINDING EPITOPES USING SYNTHETIC PEPTIDES

### 2.1 SEQUENCING AND TRANSCRIPTOME ANALYSIS OF BRINJAL OR EGGPLANT (*SOLANUM MELONGENAL*) FRUIT

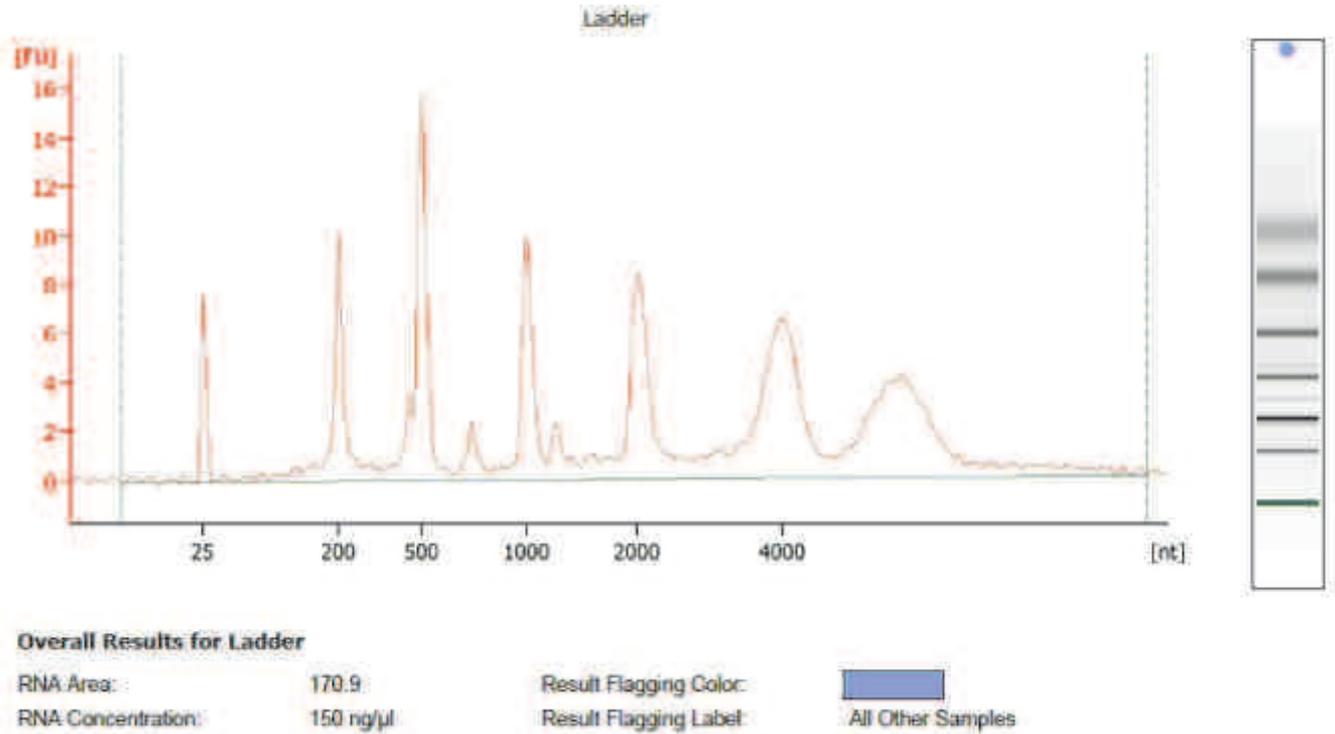
The Solanaceae is one of the plant families most involved in our daily lives; it includes economically important crops such as tomato, potato, pepper, and eggplant and these are commonly consumed throughout the world. Eggplant and the closely related Solanum species are some of the most important vegetable crops in Asia, the Middle and Near East, Southern Europe, and Africa (Daunay and Lester, 1988). World production of eggplant has been growing by the year during the last two decades and reached 32 million tons in 2007, which was roughly one-fourth of the total tomato production (FAOSTAT, <http://faostat.fao.org/>). Allergic reactions to tomato, potato, and bell pepper have been widely reported in the medical literature, and several allergens have been identified in these vegetables. Most of the allergens from tomato and potato have been well characterized. The preliminary data showed that the brinjal (eggplant) as one of the important vegetables causing allergic reactions. However, no allergens from brinjal were characterized till now. In the present study, whole transcriptome of brinjal fruit to identify allergenic genes and their proteins were sequenced and analyzed.

#### METHODOLOGY

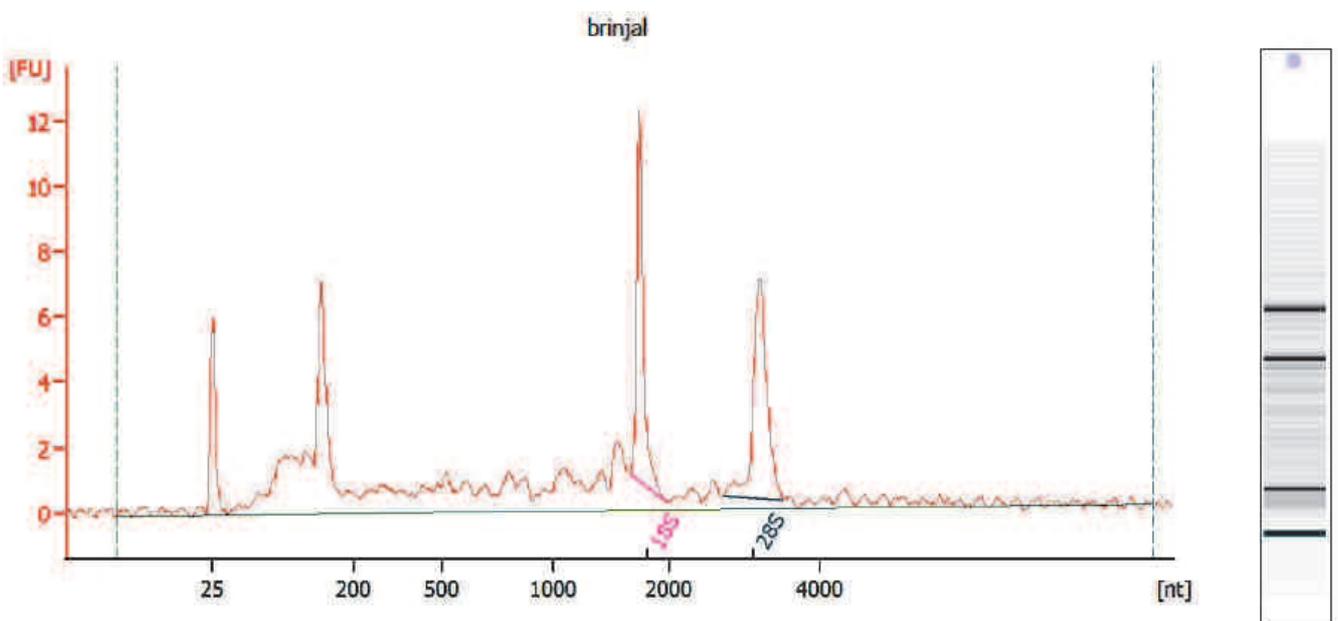
- Field fresh brinjal fruit samples were collected in RNA later solution and the total RNA was extracted by Trizol method.
- **Electropherogram summary of (A) Ladder, (B) Brinjal total RNA samples:** The RNA samples were analyzed by Agilent 2100 bio-analyzer. The quantity and RNA integrity number (RIN) are shown below as Electropherogram. The total RNA extracted was from Eukaryotes as evidenced by 18S and 28S RNA fragments. After detection, the ratio of the fragment areas was calculated and displayed in figure. To calculate the concentration of the RNA, the area under the entire RNA electropherogram was determined. The ladder, which provides the concentration/area ratio, is applied to transform the area values into concentration values ( Fig. 1A & B).
- Unwanted RNAs (rRNA, tRNA and other small RNAs) were deleted and qualified brinjal fruit mRNAs (qualified by Qubit Analyzer) were used to pair end library preparation and were sequenced on Illumina HiSeq 2500. Reads were filtered based on quality values (Phred Quality scores) and trimmed (known adaptor sequences were removed).

- Unwanted reads were also removed from the raw reads and three levels of assemblies were done using Velvet/Oasis, CD-hit, CAP3 softwares (Fig2).
- Functional annotations were performed using BLASTx and BLAST2GO algorithms (Fig 3 & 4). Contigs were used as queries to search protein databases using the BLASTx programme. The queried databases included the NCBI non redundant protein database (nr) and UniProtKB-SwissPRot.

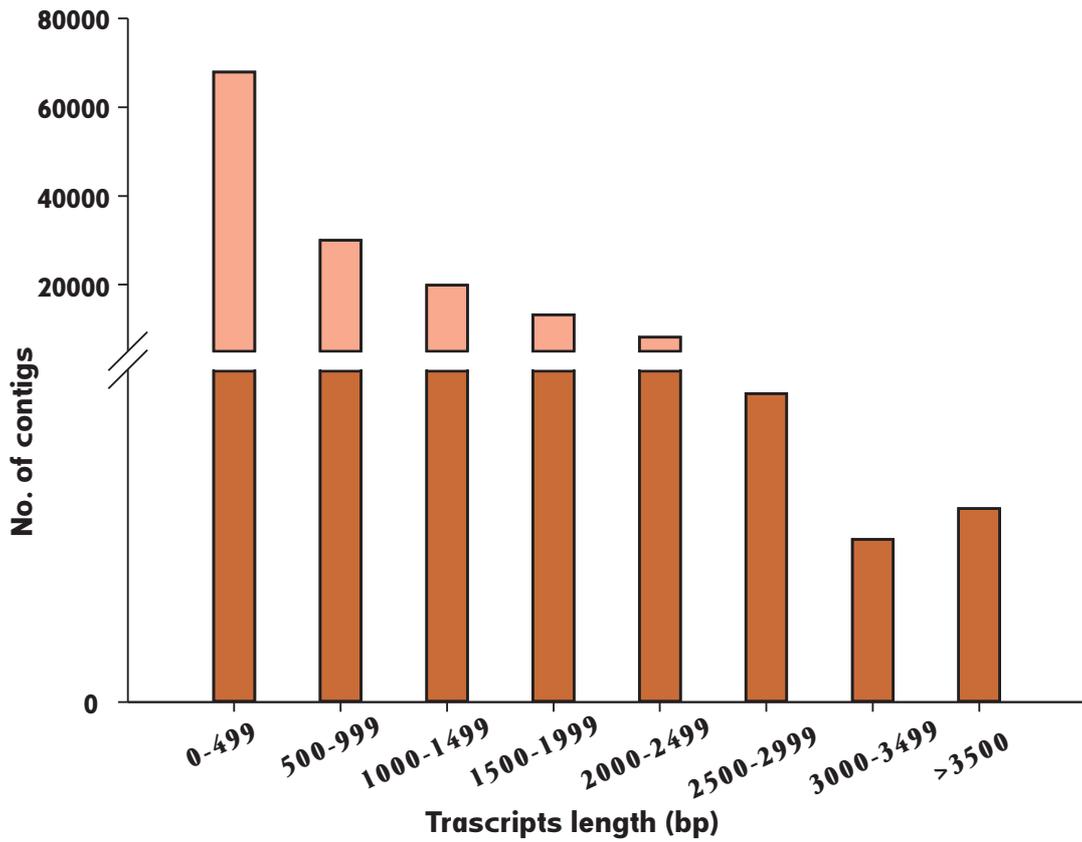
**Fig 1A. Electropherogram of RNA ladder**



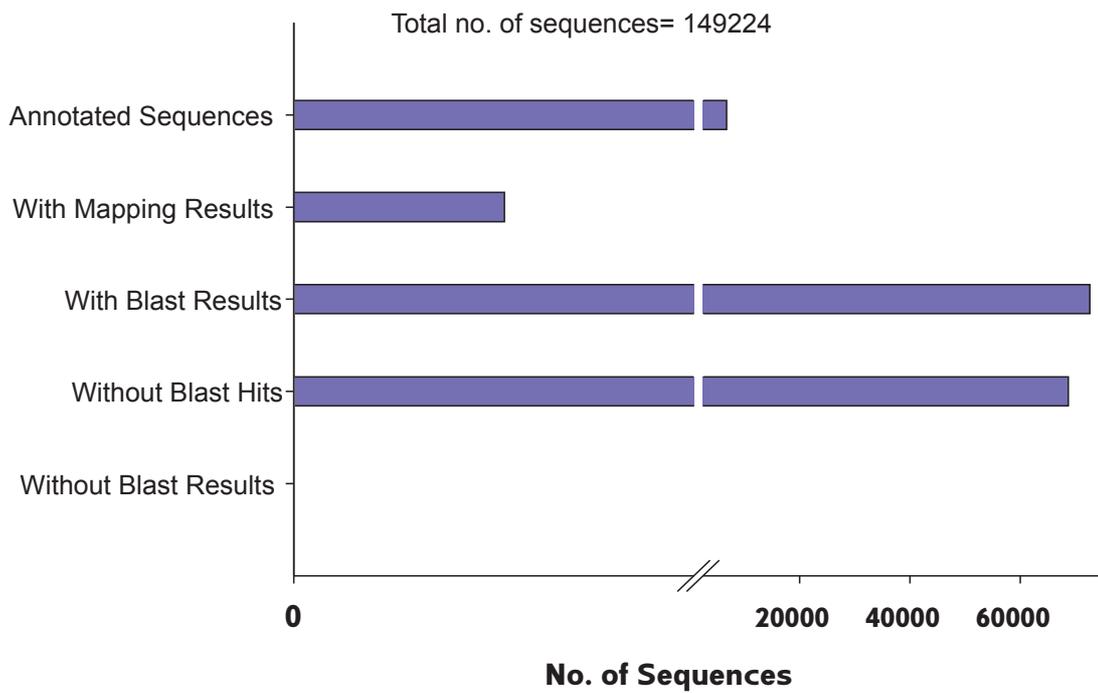
**Fig 1B. Electropherogram of Brinjal total RNA**



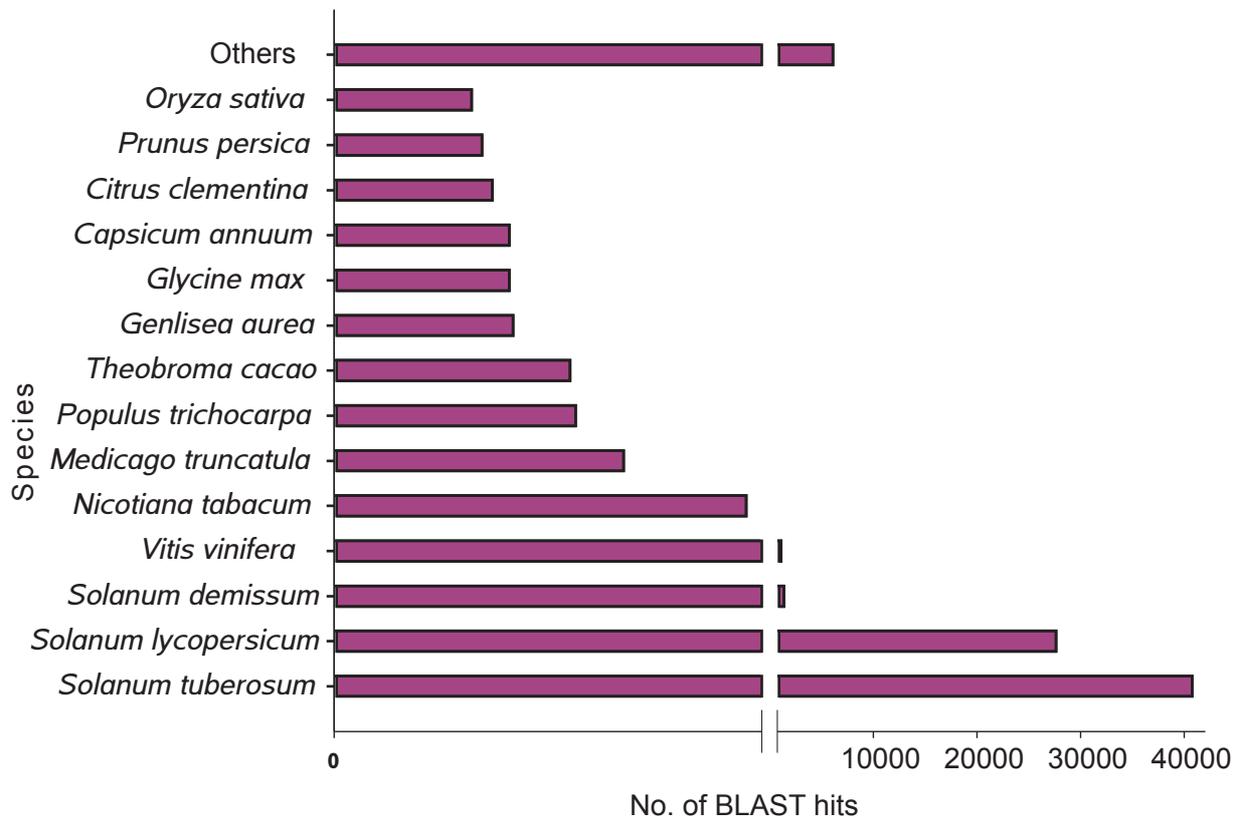
**Fig 2. Brinjal assembly length distribution**



**Fig 3. Brinjal BLAST statistics**



**Fig 4. Annotations and BLAST top hits of brinjal transcripts with other species**



## RESULTS

- Transcriptome sequencing was performed for brinjal fruit that gave 89,763,638 raw reads.
- Of the 89,763,638, the clean reads were 149224 (Level 3 assembly) after filtering.
- *De-novo assembly length distribution of brinjal fruit transcripts.* On analyzing the assembly length distribution of brinjal fruit transcripts, most of the sequences were between 100-500 and few sequences were 3000-4000 nucleotides length and the highest sequence length was 10795 (1 sequence) (Fig 2).
- *The summary of BLAST statistics.* Of the 149224 sequences identified from brinjal fruit by transcriptome analysis, 6804 sequences were annotated and were found to be functional genes. Of the 6804 sequences, the exact functions were identified for 1053 sequences by mapping. From the total sequences 72625 sequences had BLAST hits and were matching with existing databases. However, 68742 sequences were unknown and did not have any Blast hits with existing databases (Fig 3).
- *Gene ontology or annotation of brinjal fruit transcripts (Top hit species distribution).* This histogram shows the distribution of the BLAST top hits against various species among the NCBI data using BLASTx and Blast2GO algorithms (Fig 4).

## 2.2 SEQUENCING AND TRANSCRIPTOME ANALYSIS OF ONION BULB (*ALLIUM CEPA* L)

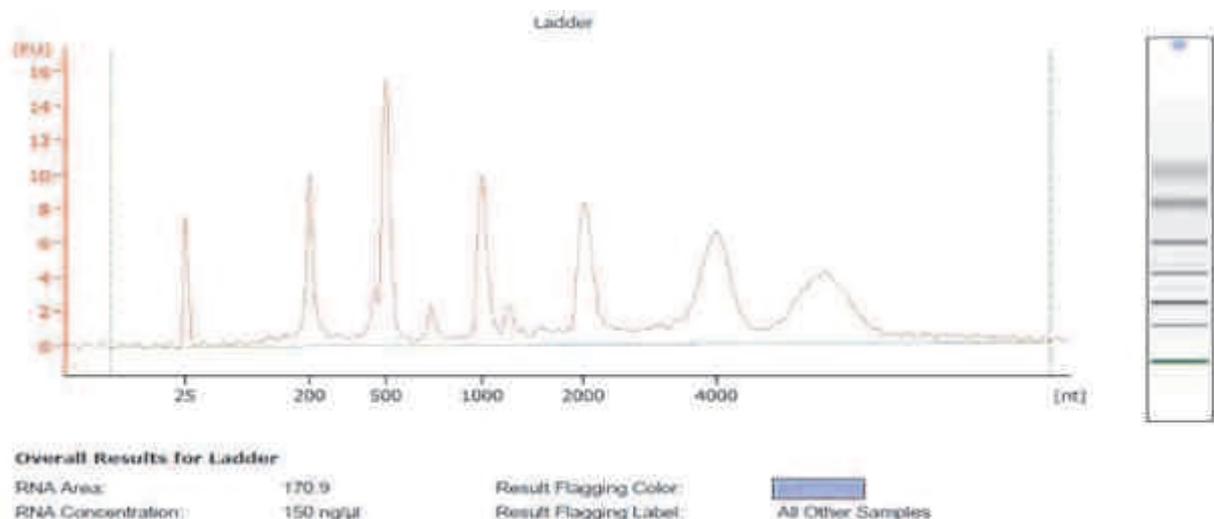
Bulb Onion (*Allium Cepa*) is one of the highly consumed vegetable worldwide, that may be grown throughout the year at different places in the world. Onions contain phenolics and flavonoids that have potential anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties. Though, they are known for many health benefits, onions are also well known for causing allergies in people. Recent advances in genomic technologies allow for the development of genomic tools to enable detection of those allergy causing proteins and their genes which are called as food allergens.

Thus in the present study sequencing was conducted and whole transcriptome analysis of Onion bulb RNAs to identify allergenic genes and their proteins.

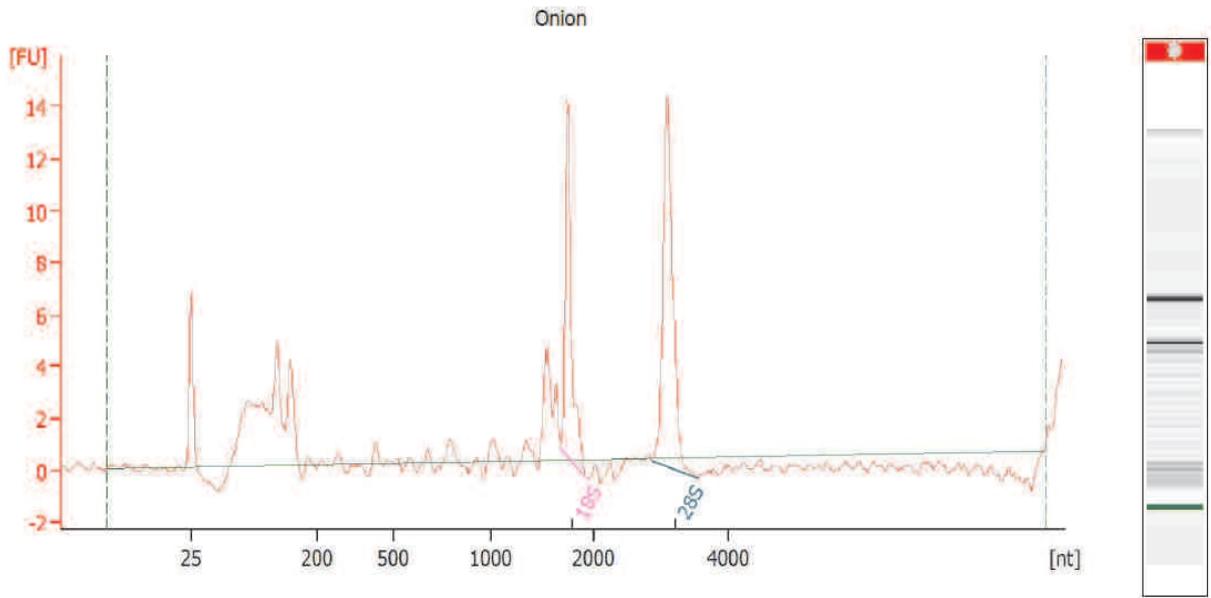
### METHODOLOGY

- ❖ Field fresh Onion bulb samples were collected in RNA later solution and total RNA was extracted by Trizol method.
- ❖ *Fig. 1 Electropherogram summary of (A) Ladder and (B) Onion total RNA samples:* The RNA samples collected in RNA later solution were analyzed by Agilent 2100 bio-analyzer. The quantity and RNA integrity number (RIN) are shown below as Electropherogram. The total RNA extracted was from eukaryotes as evidenced by 18S and 28S RNA fragments. After detection, the ratio of the fragment areas was calculated and displayed in figure. To calculate the concentration of the RNA, the area under the entire RNA electropherogram was determined. The ladder, which provides the concentration/area ratio, is applied to transform the area values into concentration values.
- ❖ Unwanted RNAs (rRNA, tRNA and other small RNAs) were eliminated and the qualified Onion RNAs (qualified by Qubit analyzer) were used to pair end library preparation and were sequenced on Illumina Hiseq 2500. Reads were filtered based on quality values (Phred Quality scores) and trimmed (known adaptor sequences were removed).
- ❖ Unwanted reads were also removed from the raw reads and three levels of assemblies were done using Velvet/Oasis, CD-hit, CAP3 softwares (Fig2).
- ❖ Functional annotations were performed using BLASTx and BLAST2GO algorithms (Fig3 & 4). Contigs were used as queries to search protein databases using the BLASTx programme. The queried databases included the NCBI non redundant protein database (nr) and UniProtKB-SwissPRot.

**Fig. 1 A. Electropherogram of RNA ladder**



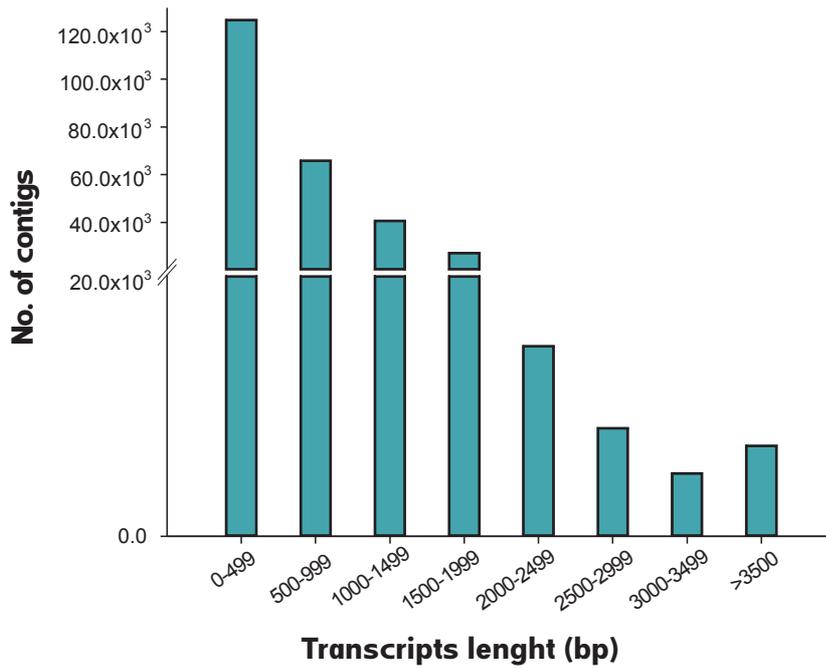
**Fig. 1 B. Electropherogram of Onion RNA**



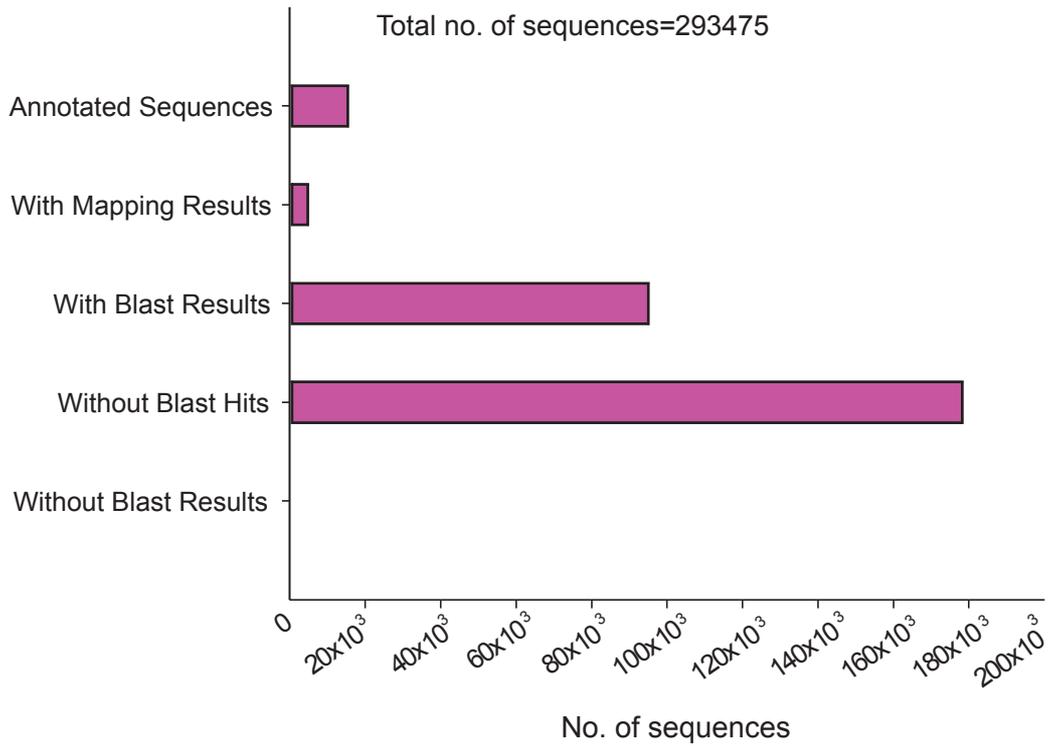
**Overall Results for sample 3 : Onion**

RNA Area:	68.2	RNA Integrity Number (RIN):	N/A (B.02.08)
RNA Concentration:	60 ng/ul	Result Flagging Color:	<span style="background-color: #cccccc; border: 1px solid black; display: inline-block; width: 20px; height: 10px;"></span>
rRNA Ratio [28s / 18s]:	1.4	Result Flagging Label:	RIN N/A

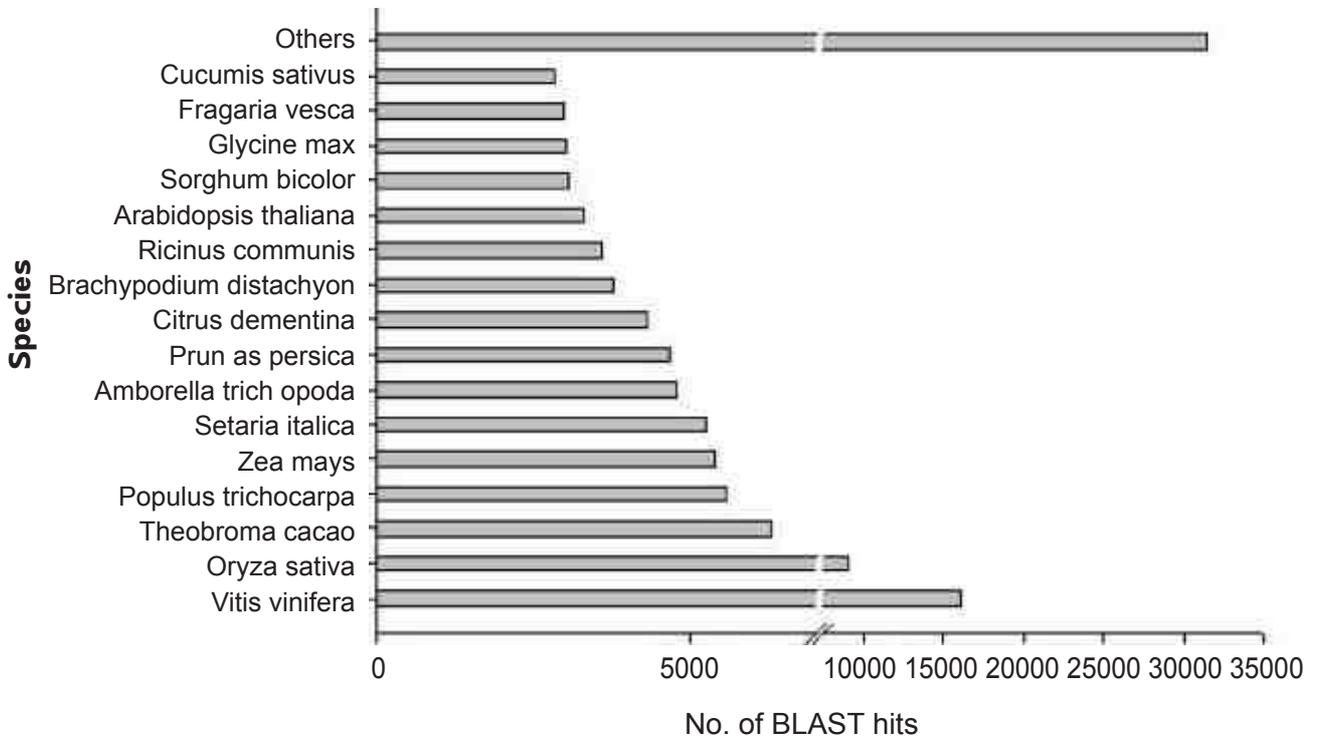
**Fig. 2. Onion assembly length distribution**



**Fig 3. Onion BLAST statistics**



**Fig. 4. Annotations and BLAST top hits of onion transcripts with other species**



## RESULTS

- ❖ By using transcriptome sequencing 99,074,309 raw reads were found in onion bulb.
- ❖ Of the 99,074,309 raw reads, 293475 (Level 3 assembly) reads were found to be clean after filtering.
- ❖ *Fig 2. De novo assembly length distribution of Onion bulb transcripts.* On analyzing the assembly length distribution of onion bulb transcripts, most of the sequences were between 100-500 and few sequences were 3000-4000 nucleotides length and the highest sequence length was 12635 (1 sequence).
- ❖ *Fig 3. The summary of BLAST statistics of Onion bulb transcripts.* Of the 293475 sequences identified from Onion bulb by transcriptome analysis, 15434 sequences were annotated and were found to be functional genes. Of the 15434 sequences, the exact functions were identified for 4780 sequences by mapping. From the total sequences 95037 sequences had BLAST hits and were matching with existing databases. However, 178224 sequences were unknown and did not have any Blast hits with the existing databases.
- ❖ *Fig 4. Gene ontology or annotation of Onion bulb transcripts (Top hit species distribution).* This histogram shows the distribution of the BLAST top hits against various species among the NCBI data using BLASTx and Blast2GO algorithms.

## 3. REGULATORY T CELL (Cd4/ CD25/ Cd127-/ FOXP3) POPULATION AND B CELLS WITH Cd23/ CD21 EXPRESSION IN PREGNANT WOMEN WITH VITAMIN D DEFICIENCY AND THEIR NEWBORNS

Patients with chronic inflammatory diseases are usually deficient in 25-hydroxyvitamin-D (25-D) and therefore consuming greater quantities of vitamin D, which elevates 25-D levels, alleviates inflammation. However, there are suggestions that by persistently activating the Vitamin D receptor with vitamin D3 supplements, other natural proteins are prevented from reacting with the vitamin D receptor. This might undermine the immune system.

Vitamin D Receptor (VDR) is a member of the steroid superfamily of receptors, recognises the active vitamin D (1,25 dihydroxyvitamin D<sub>3</sub>), which results in the phosphorylation of the 1,25 dihydroxyvitamin D<sub>3</sub>-VDR complex. This phosphorylated complex then combines with the retinoic acid receptor to form a heterodimer that, in turn, interacts with a specific vitamin D responsive element in the target nuclei, leading to the mRNA synthesis for proteins such as osteopontin and osteocalcin. The 1,25 dihydroxyvitamin D<sub>3</sub> is produced not only in the kidneys but also in the placenta during pregnancy; similarly, the VDR are present not only in bone, but also expressed widely in the intestine, skin, kidneys, pituitary, parathyroids, pancreatic beta cells, gonads, skeletal muscles, circulating monocytes and activated T and B lymphocytes, thus suggesting wider roles, beyond bone, for VDR. There are many binding sites for the VDR along the length of the genome. These have been shown to be concentrated near genes associated with susceptibility to autoimmune conditions such as multiple sclerosis, Crohn's disease, SLE, rheumatoid arthritis and cancers such as chronic lymphocytic leukaemia and colorectal cancer.

Placenta and decidua provide a direct connection between mother and infant. We hypothesize that cord blood vitamin D status, VDR expression would modify CD23/CD21 interaction and regulatory T cell function in placenta and cord blood.

In the present study we propose to determine the link between serum vitamin D<sub>3</sub>, VDR expression, CD23/CD21 interaction and regulatory T cell function.

## OBJECTIVES

1. To determine the maternal blood vitamin D3 levels, VDR expression and their link with CD23/CD21 expression and Treg cell function.
2. Cord blood vitamin D, VDR and CD23/CD21 expression and Treg cell function and their association with wheezing, allergic asthma, atopic skin allergy in infants was followed for 6 months.
3. Impact of vitamin D and various T cell surface molecules *in vitro*.

## METHODOLOGY

Pregnant women (38 weeks) were recruited in Gandhi Hospital. Maternal blood was collected, and cord blood and placenta tissue was collected at the time delivery. Vitamins D was estimated by using HPLC technique, VDR expression in placenta tissue, by real time PCR, CD23 /CD21 expression in maternal blood and cord blood was done by flowcytometry. Regulatory T cell (CD4+/CD25+/CD127-/FOXP3) was expressed by flowcytometry, Th1, Th2 cytokines like IL-4, IL-5, IL-10, TGF- $\beta$ , IFN- $\gamma$  and TNF- $\alpha$  was estimated by Milliplex and was based on the luminex xMAP technology, Total IgE and Total Histamine was estimated by using ELISA method.

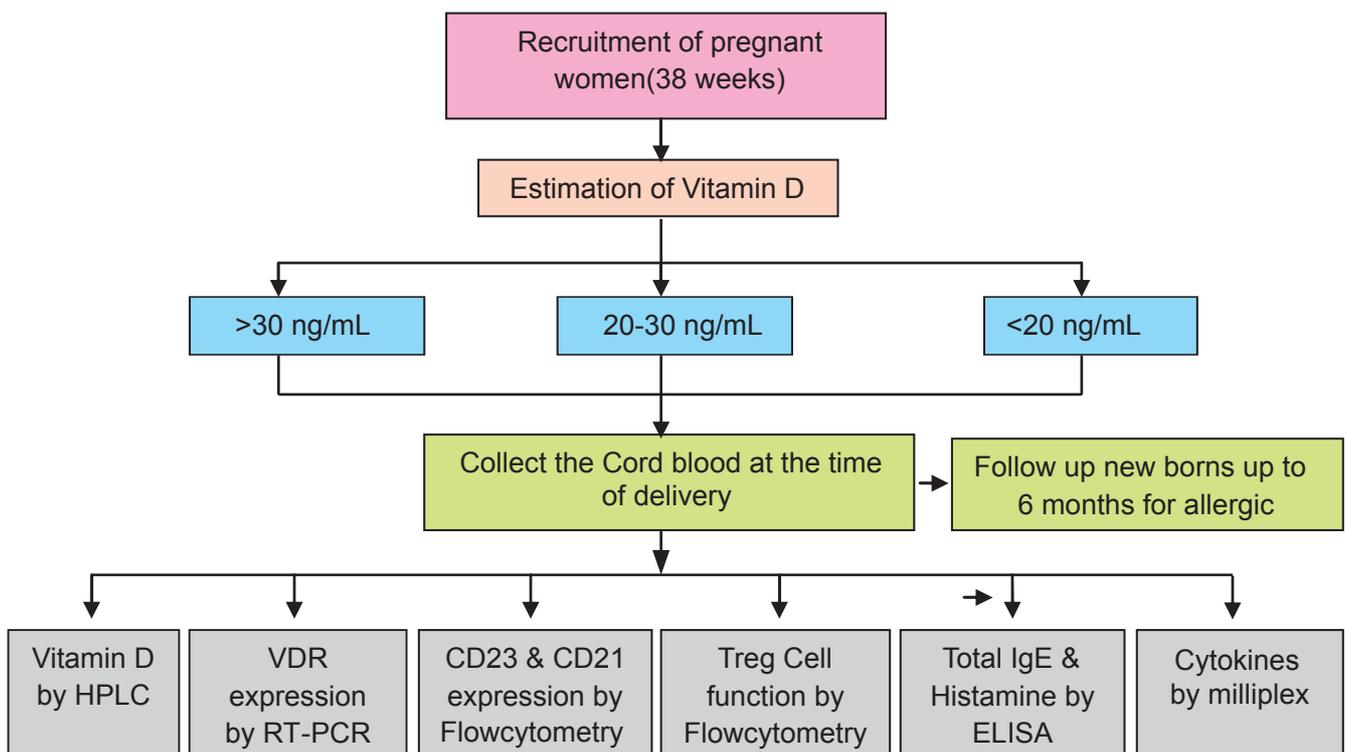
## ELIGIBILITY CRITERIA FOR PREGNANT WOMEN

### Inclusion Criteria:

- Age 18-35Yrs
- Pregnant women willing to participate in the study
- Signed informed consent

### Exclusion Criteria:

- Use of probiotics (preceding one month)
- Use of medication influencing the immune system
- Lactose intolerance
- Infection (preceding one month)



## WORK DONE

- Subject screening recruitment and Sample collection was completed for 18 vitamin D sufficient, 51 insufficient and 82 deficient subjects respectively.
- In these subjects we collected patient information like demographic profile, socioeconomic profile and collected samples like maternal blood, cord blood, placenta tissue and also included anthropometry measurements of newborn.
- Maternal and cord blood Vitamin D levels were estimated.
- Regulatory T cell (CD4+/CD25+/CD127-/FOXP3+) expression on T cells was done by flow cytometry.
- CD23 and CD21 expression on the B cells was done by flow cytometry.
- We have searched the corresponding nucleotide accession number from NCBI and designed primers in bioinformatics software (generous pro 5.4.6).
- Studied various genes expression in placenta tissue like Vitamin D receptor (VDR), CD23, CD21, and FOXP3 which was carried out by conventional PCR technique.

## RESULTS

- Hundred and fifty three pregnant women visiting the hospital from rural or urban areas for antenatal check-up were studied. Most women completed higher secondary schooling, and a few were illiterate; and were either labour workers or homemakers (Table2). Their milk intake or exposure to sunlight was dismally low. Majority of the women in the study had insufficient milk intake and less exposure to sunlight (Table 2). The mean±SD age of the pregnant women was 24.5±2.6 years and mean±SD gestational age was 39±1.5 weeks. The new-born mean±SD birth weight was 2.59±0.40 (Table 1).
- When women were categorized into three groups based on their vitamin D status, such as vitamin D sufficient (>30ng/mL), insufficient (20-30ng/mL) and deficient groups (<20ng/mL), of the 153 pregnant women, 18 were sufficient, 51 were insufficient and 82 were deficient in vitamin D. The Mean± SE values of

**Table 1. Demographic and clinical profile of pregnant women (153)**

Characteristic	Values
Mean age(Yrs)	24.5+ 2.6
Gestational age(Weeks)	39.0 ± 1.5
Hb (%)	10.4+ 3.7
BMI	21.5+ 1.4
Birth weight(kg)	2.59+ 0.4
Head circumference (cm)	34.2+ 2.2
Birth length (cm)	58.4+ 4.5
Sunlight exposure duration(%)	
<60min	82.0
>60min	18.0
Milk consumption (%)	
<500mL	76.0
>500mL	24.0

Values were expressed mean±SD, otherwise % of subjects

**Table 2. Socioeconomic status of the pregnant women (153)**

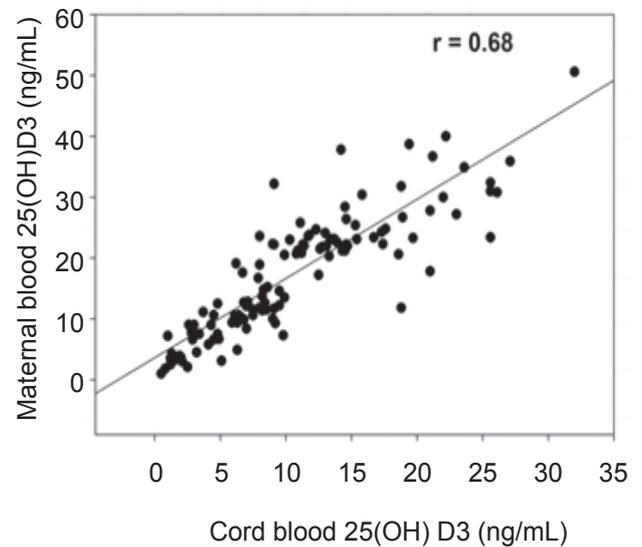
Socio economic variables	Proportion (Number)
<b>Locality</b>	
Urban	38 (58)
Rural	62 (95)
<b>Religion</b>	
Hindu	75.16(115)
Muslim	24.84(38)
<b>Education</b>	
Illiterate	12 (19)
Primary School	35 (53)
High School	31 (48)
Post high school	22 (33)
<b>Occupation</b>	
Not working (House wife)	44 (68)
Agriculture	24 (37)
Other labour work	32 (48)
<b>Socio-economic status</b>	
Upper	4 (6)
Upper middle	18 (28)
Middle/Lower middle	34 (52)
Lower/Upper lower	14 (22)
Lower	31 (47)

Values were given in % of subjects and in the parenthesis No. of subjects

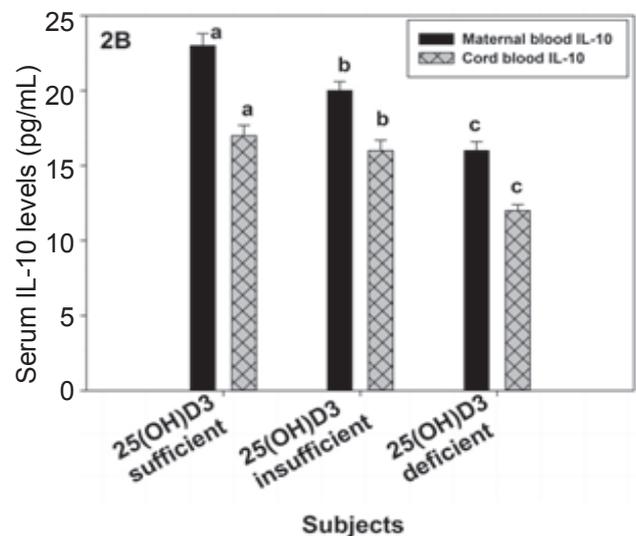
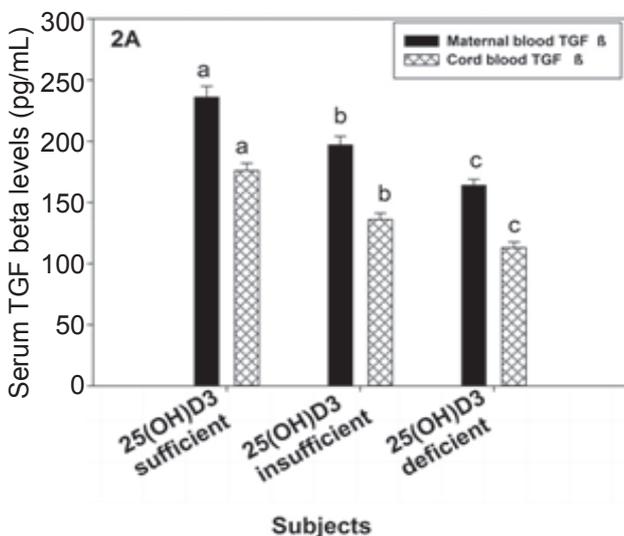
maternal vitamin D was  $37.04 \pm 2.74$ ,  $23.16 \pm 3.05$  and  $9.5 \pm 5.19$  ng/ml and cord blood vitamin D mean  $\pm$ SD values were  $16.12 \pm 3.01$ ,  $16.12 \pm 3.01$  and  $6.8 \pm 5.2$  in vitamin D sufficient, insufficient and deficient subjects respectively (Table 3). Significant correlation was found between maternal and cord blood vitamin D status (Fig. 1).

- The proportion of the (mean(%)  $\pm$ SD) regulatory T cell population was significantly ( $p < 0.05$ ) higher in vitamin D sufficient ( $0.42 \pm 0.2$ ) subjects compared to insufficient ( $0.2 \pm 0.1$ ) and deficient ( $0.18 \pm 0.1$ ) subjects and of the total T cell population in cord blood, the proportion of the (mean %)  $\pm$ SD) regulatory T cell population was significantly ( $p < 0.05$ ) higher in vitamin D sufficient ( $2.55 \pm 0.45$ ) subjects compared to insufficient ( $2.01 \pm 0.34$ ) and deficient ( $0.9 \pm 0.36$ ) subjects (Fig. 3). Regulatory cytokines like TGf beta and IL 10 were also significantly ( $P < 0.05$ ) decreased in vitamin D deficient pregnant women (Fig.2).
- The proportion of B cell with CD23 expression levels (mean (%)  $\pm$ SD ) was significantly ( $P > 0.05$ ) lower in vitamin D sufficient ( $0.2 \pm 0.13$ ) subjects, compared to insufficient ( $0.3 \pm 0.1$ ) and deficient ( $0.4 \pm 0.1$ ) subjects. The proportion of B cell with CD23 expression levels (mean(%)  $\pm$ SD ) in cord blood was also significantly ( $P > 0.05$ ) lower in vitamin D sufficient ( $0.2 \pm 0.13$ ) subjects compare to insufficient ( $0.3 \pm 0.1$ ) and deficient ( $0.4 \pm 0.1$ ) subjects (Fig. 1 & 3). Similar to B cells with CD23 expression, B cells with CD21 expression (mean (%)  $\pm$ SD) was significantly lower ( $p < 0.05$ ) in vitamin D sufficient group ( $0.4 \pm 0.13$ ) than in vitamin D insufficient ( $0.6 \pm 0.1$ ) and vitamin D deficient group ( $1.2 \pm 0.1$ ). The proportion of B cells with CD21 expression (mean (%)  $\pm$ SD) was significantly lower ( $p < 0.05$ ) in vitamin D sufficient group ( $0.3 \pm 0.13$ ) than in vitamin D insufficient ( $0.55 \pm 0.21$ ) and vitamin D deficient group ( $1.65 \pm 0.3$ ) (Fig. 5).

**Fig. 1:** Spearman's rank correlation between maternal and cord blood 25(OH)D3 ( $r = 0.68$ ;  $p = 0.001$ )

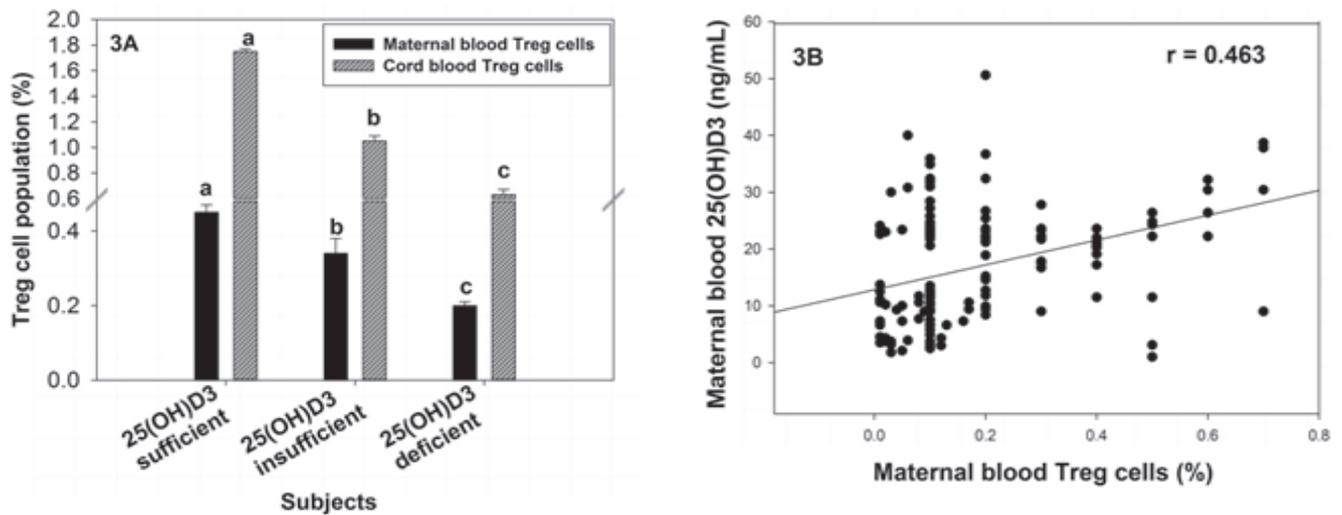


**Fig. 2:** 2A) TGF  $\beta$  concentration (pg/ml) in maternal and cord blood. 2B) IL-10 concentration (pg/ml) in maternal and cord blood. Different superscripts indicate significant difference at  $p < 0.05$ . Values are expressed as mean  $\pm$  SE.

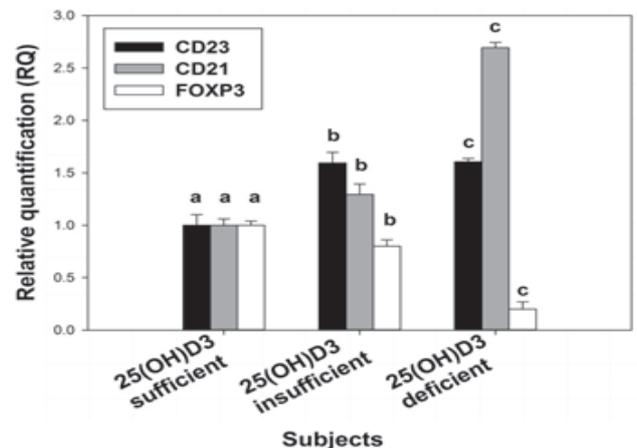


- In representative sample of 8 subjects from each group (vitamin D sufficient, insufficient and deficient), placenta tissues were collected and gene expression was studied. Vitamin D receptor (VDR), and FOXP3 (Treg cells) genes were up regulated in vitamin D sufficient and were significantly different from the in insufficient and deficient subjects. In contrast, CD23 and CD21 were down regulated in vitamin D sufficient subjects than in vitamin D insufficient and deficient subjects (Fig. 4). 2.5 Analysis of expression of genes related to inflammation by Reverse Transcriptase PCR.
- Vitamin D metabolizing enzymes (VDR, CYP27B1 CYP2R1, CYP24A1, VDBP and RXR) mRNA expression were altered in vitamin D deficient pregnant women (Fig.6).
- The mean±SE value of serum calcium levels were 9.11±2.1, 9.1±1.8 and 8.91 mg/dl in the vitamin D sufficient, insufficient and deficient subjects respectively and were comparable among the three group of subjects.

**Fig.3:** 3A) Treg cell population in maternal and cord blood. 3B) Spearman's rank correlation between maternal 25(OH)D3 and Treg cell population ( $r = 0.463$ ;  $p = 0.001$ ). 3C) Spearman's rank correlation between cord blood 25(OH)D3 and Treg cell population ( $r = 0.512$ ;  $p = 0.001$ ). Different superscripts indicate significant difference at  $p < 0.05$ . Values are expressed as mean ± SE.



**Fig. 4:** mRNA expression (RQ) of CD23, CD21 and FOXP3 genes in placenta tissue of women with 25(OH)D3 deficiency. 25(OH)D3 sufficient group was taken as calibrator and GAPDH was used as internal control. Different superscripts indicate significant difference at  $p < 0.05$ . Values are expressed as mean ± SE.

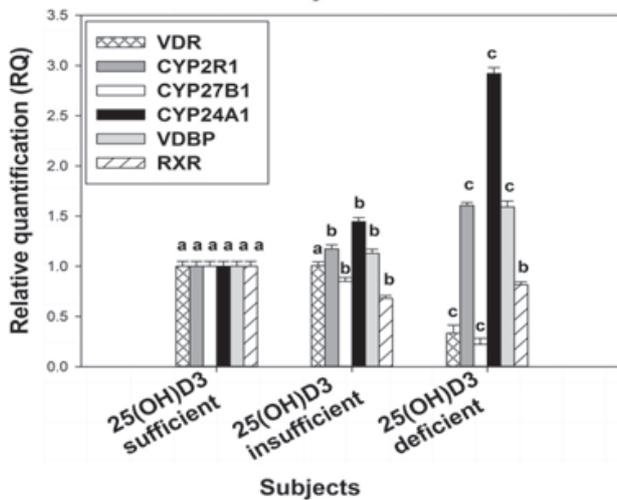
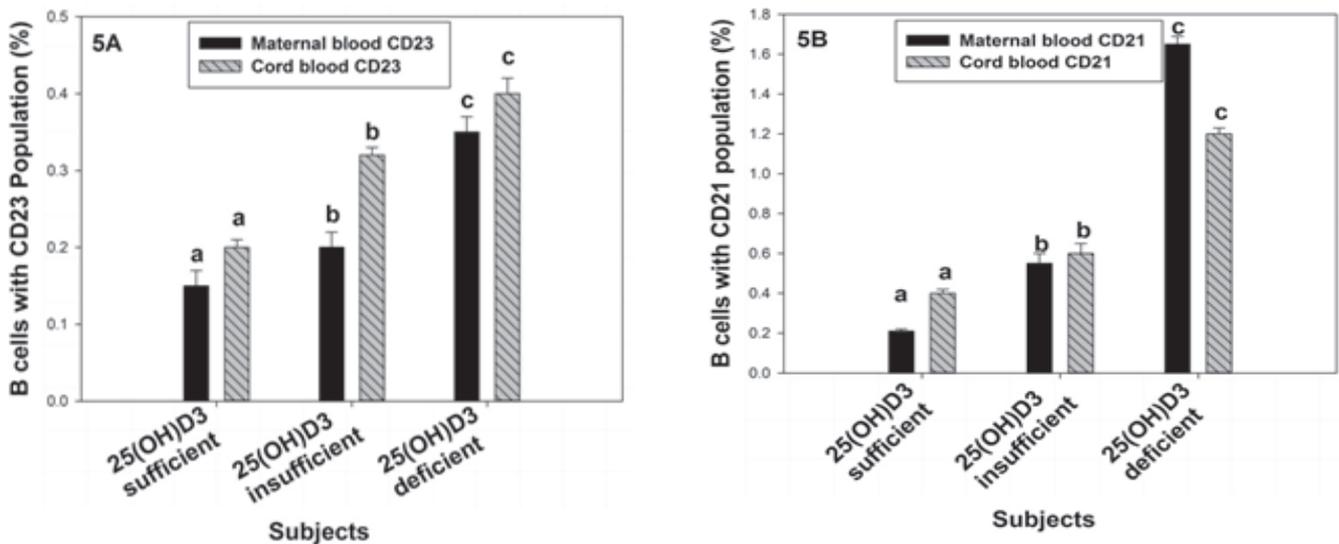


**Table 3. Vitamin D levels in maternal and Cord blood**

Subjects(153)	% subjects	Maternal Vitamin D (ng/mL)	Cord Vitamin D (ng/mL)
Sufficient (18)	11.7	37.04±2.74	16.12± 3.01
Insufficient(53)	34.6	23.16±3.05	12.58±3.7
Deficient(82)	53.5	9.5±5.19	6.8±5.2

Values were expressed as Mean± SD  
 >30 ng/mL considered as Vitamin D sufficient  
 20-30 ng/mL considered as Vitamin D Insufficient  
 <20 ng/mL considered as Vitamin D Deficient

**Fig. 5:** 5A) B cells with CD23 expression in maternal and cord blood. 5B) B cells with CD21 expression in maternal and cord blood. Different superscripts indicate significant difference at p < 0.05. Values are expressed as mean ± SE.



**Fig. 6:** mRNA expression (RQ) of VDR, CYP27B1 CYP2R1, CYP24A1, VDBP and RXR genes in the placenta tissue of women. Vitamin D sufficient group was taken as calibrator and GAPDH was taken as internal control. Different superscripts indicate significant difference at p < 0.05. Values are expressed as mean ± SE.

**CONCLUSION**

These findings suggested that in impaired immune regulation and increased receptors for IgE in pregnant women with vitamin D deficiency.

## 4. EFFECT OF PROBIOTIC SUPPLEMENTATION ON WEIGHT REDUCTION AND ITS IMPACT ON MICRONUTRIENT AND IMMUNE STATUS IN OBESE SUBJECTS

Probiotics are living micro-organisms that when consumed in sufficient number provides health benefit according to FAO / WHO report (2005). A number of beneficial effects of Lactobacilli species and strains were observed, for their ability to produce antibiotic like substances, synthesis of vitamin B-complexes, ability to deconjugate bile salts thus lowering cholesterol absorption, degradation of N-nitrosamines, thereby, reducing colon cancers and improve digestion of lactose in lactase deficient persons in addition to their immunomodulation effect.

Over weight is a major problem in developing and developed countries particularly in India and US etc. The lowering in body weight may lower blood pressure and reduces risk of developing diabetes, which can be achieved by changing the eating habits and physical activity etc. Earlier studies have shown that obese persons have poor immune response. Therefore probiotic treatment may help in the improvement of immune functions. Hence such studies are required to know the effect of probiotics on immune response among obese population. Probiotics helps in controlling body weight by reducing glucose absorption from the intestine and enhancing the metabolic use of glucose. It is often suggested that dairy calcium may also reduce the body fat. The anti - obesity activity is due to conjugated linoleic acid (CLA) produced by some of the probiotic micro flora. The present study was focused to study the effect of probiotic curd supplementation on the lipid profile and to study the immunomodulation, using Adenosine Deaminase (ADA) as an immunoenzyme marker in obese subjects.

### OBJECTIVES

- To observe changes in the body weights as a result of probiotic supplementation in overweight and obese subjects.
- To study the beneficial effect of probiotic supplementation on lipid profile and immune status in the above subjects.

### MATERIALS AND METHODS

**Inclusion criteria:** One Hundred (100) subjects with a Body Mass Index (BMI) above 25 were recruited for the study from local population of Hyderabad city, Andhra Pradesh, India. The subjects were categorized into overweight and obese based on their Body Mass Index (BMI) status. The subjects with BMI in between 25 to 28 were considered as overweight whereas those with 28 and above as obese respectively. The institutional ethical committee approval was obtained before the commencement of the study and the informed consent was also obtained from all subjects.

**Exclusion criteria:** Those individuals having secondary health complications like Hypertension, Diabetes and Cardio vascular or Thyroid diseases etc apart from obesity were excluded from the study.

### STUDY DESIGN

Considering 95% Confidence Interval (CI), 80 % Power, SD of BMI of overweight / obesity is 3.2 at an expected difference of 2.0 and also considering 20% dropout the required sample size is 100 subjects that were recruited for each group i.e. Group A - Probiotic with 50 were supplemented, (experimental) Group B with 50 subjects were given same quantity of pasteurized milk i.e.200 ml /day supplemented obese are kept as controls.

**Intervention:** The subjects were divided into two groups namely Group A as Experimental. Group and B as control. The Group A individuals were supplemented with 200 gms/ per day of freshly prepared curd by fermentation of pasteurized toned milk with known mixed bacterial flora viz. *Lactobacillus bulgaricus* (*delbrukii* subsps UBLB-38) and *Streptococcus thermophilus* (UBST-50) were obtained at free of cost

from Unique Biotech Company Private Limited located in Hyderabad. Group B subjects were advised to continue their intake of milk and were considered as controls. All the participants (Group A and B) were asked to take their regular diet, and to carry out their routine work and exercise. The details of their food habits and other information have been recorded in a special structured proforma.

The subjects were asked to consume probiotic curd at a quantity of 200gms/day/individual in one sitting ideally to take along with their lunch for a period of 30 days. The subjects were also advised not to take any fermented food products during the period of supplementation especially alcohol which may interfere in the investigation. However subjects were not restricted to commonly consumed food items like *Idly*, *Dosa* and/or *Uthappam* as these food items do not contain any live micro flora after cooking, though these are categorized as fermented foods.

### Preparation of probiotic curd

The fresh milk about 2-3 liters was procured from Mother Dairy and toned using cream separator machine in the metabolic kitchen at our institute. The milk was pasteurized and cool down to 37° c to 40° c and dispensed in to 200 ml steel cups and fermented with specific Probiotic *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. After incubation for about 5-6 hours the curds formed was tested for its acceptability by human volunteers from NIN using a questionnaire.



The sensory evaluation was carried out using hedonic scale. A total of 80 % acceptability was obtained and therefore the supplementation was initiated to obese subjects under study. Probiotic micro flora was expressed as CFU/gm of the product and adjusted to 10<sup>7</sup> cfu/gm. Simultaneously, viability was tested upon storage at 4° to 8° c and at different time point's i.e. 1, 2, 3, and 4 weeks. It was found that viability was retained only up to one week time. However, to avoid risk we have supplemented with freshly prepared Probiotic curd i.e., not more than one day old.

### Laboratory investigations

About 3ml of overnight fasting venous blood samples were collected from all the subjects for lipid profile analysis at '0' & '30' days. Total blood cholesterol was estimated by enzymatic method of Liberman (1985) triglycerides were estimated using GPO method of Fossati and Lorenzo (1982) and HDL-C was estimated by enzymatic method of Demacker and Hifman (1980). The Adenosine deaminase (ADA) activity was measured by the method of Guisti (1984).

### Statistical analysis

The statistical analysis was carried out by students. T test, and ANOVA using SPSS software package (version 12.0) to find the level of significance between test results.

## RESULTS

The characteristics of the subjects under study are shown in Table 1. The results on lipid profile are depicted in the Table 2. It can be observed that the mean value of cholesterol at '0' day in group-A and group B were close by i.e. 161.24± 33.87 and 152 ± 18.0 which was not statistically significant. After supplementation there was a significant decrease in the cholesterol levels to 138.21 ±64.93 in the experimental group and on the contrary in controls there was a tremendous increase in the cholesterol levels 180 ± 18.0 which were significant upon comparison (P<0.05). The values for serum Triglyceride levels which showed a similar trend as that of cholesterol, where there was a significant decrease after 30 days and in the control group, there was a significant increase in the serum triglyceride levels after 30 days period (P<0.05). The result for the levels of HDL cholesterol presented showed that there was a significant and tremendous increase in the HDL in the supplemented group at 30 days when compared to their initial values (P<0.05). No significant changes were observed in the controls. The mean values of serum calcium were 8.95±0.53 mg/dl and 9.98±3.14 /dl both at before and after supplementation respectively (P<0.05). However this trend was not observed in the controls. This phenomenon was not observed in the unsupplemented group.

The mean values of serum phosphorous were  $3.87 \pm 0.53$  mg/dl and  $3.3 \pm 0.66$  mg/dl did not showed any difference in phosphorus levels both in supplemented and in non-supplementation groups. The results for ADA values (immune status) in both the experimental and control groups are depicted in Table 3 i.e. before (day 0) and after supplementation period of 30 days are presented. The experimental group was divided into low, normal and High ADA levels based on their normal values (15-25U/dl). In case of low ADA (below normal) the levels were significantly elevated on day 30 from  $10.05 \pm 3.14$  to  $18.08 \pm 2.51$ . In contrary in high ADA group (abnormal levels) the level of ADA significantly decreased to nearly normal levels  $33.82 \pm 9.40$  to  $25.71 \pm 6.26$  at day 30 ( $P < 0.05$ ). The normal ADA group retained the same levels without any significant change.

**Table 1. Characteristics of the subjects studied**

No	Characteristics	Experimental group	Control group
1	Number of subjects	42	42
a	Males	21	21
b	Females	21	21
2	Age (mean value - SD)	$39.45 \pm 6.2$	$37.26 \pm 5.7$
3	Weight (kgs) (mean value $\pm$ SD)	$86.16 \pm 0.83$	$87.56 \pm 14.24$
4	Height (cms) (mean value $\pm$ SD)	$167.66 \pm 10.13$	$165.8 \pm 11.09$
5	BMI (mean value $\pm$ SD)	$33.64 \pm 2.30$	$32.20 \pm 2.81$

**Table 2**

Parameters (mg/dl)	Experimental group (Lactobacillus bulgaricus + Streptococcus thermophilus) (Probiotic curd) n=42 Mean $\pm$ SD		Control group (Milk) n=42 Mean $\pm$ SD	
	"0" Day	"30" day	"0" Day	"30" day
Total Cholesterol	$161.24 \pm 33.87^a$	$138.21 \pm 64.93^b$	$152.0 \pm 28.0^b$	$180 \pm 18.0^b$
Triglycerides	$133.59 \pm 94.85^a$	$108.66 \pm 69.97^b$	$179.46 \pm 88.89^a$	$190.16 \pm 23.33^b$
High density lipoproteins	$29.67 \pm 8.20^a$	$40.70 \pm 7.75^b$	$31.74 \pm 4.9^a$	$34.84 \pm 6.95^a$
Calcium	$8.959 \pm 0.53^a$	$9.98 \pm 3.14^a$	$7.38 \pm 1.63$	$8.96 \pm 0.93$
Phosphorous	$3.871 \pm 0.53$	$3.3 \pm 0.66$	$4.12 \pm 2.10$	$4.08 \pm 1.22$

\*Differences in the superscript indicates statistically significant difference of  $P < 0.05$

**Table 3. Adenosine deaminase level of obese subjects before and after supplementation and withdrawal**

Category	Number of Subjects	ADA Levels (U/L) Mean $\pm$ SD	
		0 Day	30Day
Experimental group (Lactobacillus bulgaricus + Streptococcus thermophilus) (Probiotic curd) n=50	15 (Low ADA)	$10.05 \pm 3.14^a$	$18.08 \pm 2.51^b$
	15 (High ADA)	$33.82 \pm 9.40^b$	$25.71 \pm 6.26^c$
	12 (Normal ADA)	$19.90 \pm 3.11^a$	$23.06 \pm 5.49^a$
Control group (Milk) n=50	42	$23.02 \pm 4.44^a$	$22.24 \pm 3.85^a$

Normal Values of ADA: 15-25 U/L \* Differences in the superscripts indicate significant at  $P < 0.05$  level

## CONCLUSIONS

- The indigenously prepared probiotic curd with combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* showed significant changes in the lipid profile parameters upon supplementation in obese subjects.
- There was an immunomodulation effect in the obese subjects upon supplementation as assessed by Adensine deaminase (ADA) activity as an immune enzyme marker.

# IV. BASIC STUDIES

## 1. SIMULTANEOUS DETERMINATION OF MICRONUTRIENT STATUS FROM FINGER PRICK BLOOD SAMPLE

### ***Dried Blood Spot (DBS) collection kit for vitamin A analysis by HPLC***

Vitamin A deficiency is the most common and widespread nutritional disorder in Indian children. Vitamin A estimation is the only definitive way of assessing vitamin A deficiency in the population. Serum vitamin A analysis involves collection of venous blood, separation of serum, transportation to the laboratory under cold chain. NIN has developed a convenient and field-friendly method of blood collection for vitamin A analysis. The present method involves a ready-to-use kit for collection of small amounts of blood from finger puncture on to a special type of filter paper, which is air-dried and transported to the laboratory. Vitamin A is extracted from this and estimated by HPLC analysis. DBS sample collection kit coupled with HPLC analysis is a useful tool for assessing the sub clinical vitamin A deficiency in general population. Using this method, sample can be collected from remote geographical locations and eliminates the need for venous blood drawing. The vitamin A activity in the DBS samples is stable at  $-20^{\circ}\text{C}$  for a period of one year.

Current status: The kit has been launched by ICMR on February 20, 2014. License Agreement has been signed between ICMR and M/s Soothe Healthcare, Greater Noida to transfer this technology for commercialization.

### ***In-house ELISA for ferritin as marker for assessing bioavailability of iron using Caco2 cell line***

Anaemia is the most common and widespread nutritional disorder in India. Most National surveys have shown over 60 % prevalence of anaemia in Indian population and 40-50 % have iron deficiency requiring some form of iron therapy. In India, anaemia control strategies are based on haemoglobin levels. However, estimation of serum ferritin has been found to be a specific diagnostic tool for early detection of iron deficiency anaemia. Therefore, a ferritin test helps to diagnose with certainty, the extent of iron deficiency in the body thus avoiding unnecessary iron supplementation. An indigenous ELISA based kit has therefore been developed and validated at NIN for analysis of ferritin in blood. This has been exploited further for measuring the human intestinal cell based (Caco-2 cell) ferritin as a surrogate measure of iron bioavailability. This technology is cost effective, accurate and convenient compared to other commercially available technologies. It has a potential application in screening of iron bioavailability of fortified foods, premixes and pharmaceuticals. Additionally, the method can be used for screening of iron status of the population and patients who require repeated blood transfusions.

Current status: The kit has been launched by ICMR on February 20, 2014. Patent has been filed by ICMR. License Agreement has been signed between ICMR and M/s Soothe Healthcare, Greater Noida to transfer this technology for commercialization.

**Fig 1. DBS collection kit for vitamin A analysis**



**Fig 2. ELISA based serum ferritin estimation kit**



**Fig 3. Release of the kits by the ICMR**



## 2. CHARACTERIZATION OF RISK FACTORS OF ANEMIA AMONG INFANTS AND PRESCHOOLERS FROM RURAL INDIA

National databases indicate an alarming anemia prevalence of 80% among 6-35m children and 58% among 36-59m old children (NFHS-3, 2007). Characterizing anemia and the maternal and child factors associated with it during these critical stages of child growth and development forms the basis for development of effective strategies for anemia control.

### OBJECTIVES

To estimate anemia prevalence and severity and to characterize maternal and child factors associated with anemia in infants 6-12 months and pre-schoolers 29-59 months of age.

### METHODS

Project Grow-Smart, a randomized controlled intervention trial, registered as NIH, USA NCT 01660958, was carried out among infants and preschoolers in 26 villages of four state administrative blocks (Mandals) from Nalgonda district in the state of Telangana erst- while Andhra Pradesh. About 518 infants and 326 preschoolers were enrolled from rural Andhra Pradesh, India into a randomized controlled trial of simultaneous early learning opportunities, and at home or preschool-based fortification with multiple micronutrient powders. At baseline, information on factors such as socio-economic status, age, anthropometry and maternal characteristics were recorded. Blood was collected from 476 infants and 316 preschoolers and their respective mothers. Blood samples were analyzed for hemoglobin and plasma samples of the children were analyzed for ferritin, soluble transferrin receptor, zinc, folate, vitamin B-12 and C-reactive protein.

### STATISTICS

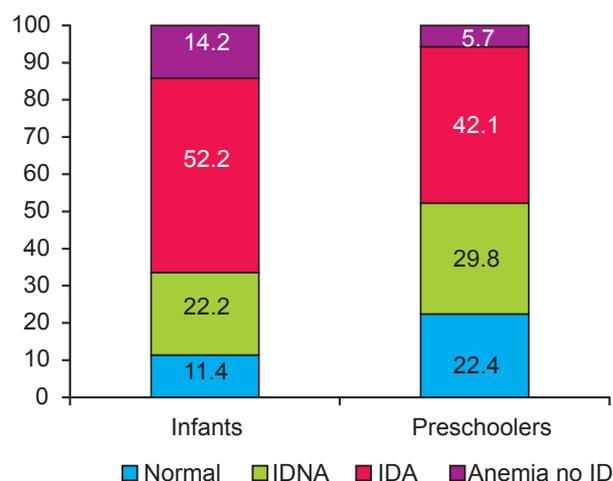
The factors associated with anemia were analyzed in infants and preschoolers independently because the two samples differed in recruitment strategy. For risk factors, a logistic regression model was built using social and biological factors and odds ratios were calculated.

### RESULTS

#### *Anaemia prevalence, iron deficiency and micronutrient status*

- Anemia was recorded in 66.4% of the infants and 47.8 % of preschoolers. Moderate anemia occurred in 41% of infants while it was 24.1 % in preschoolers. Mild anemia occurrence was close to 25 % in both groups. One-third of infants, and more than half of preschoolers had normal haemoglobin concentrations.
- Infants suffered from deficiencies of iron (27.6 % for ferritin and 69 % for sTfR) and vitamin B12 (20.6%). In preschoolers 42 % had low ferritin, 67% had high sTfR, 11% had zinc deficiency, 4.1 and 5.4% suffered inadequacies of vitamin B12 and folate respectively.
- ID without anemia was found in 22 % of infants and 30% of preschoolers while iron deficiency with anemia was recorded in 52 & 42 % respectively. There were 14 % Iron Deficiency with No Anaemia (IDNA) in infants and 5.7 % in preschoolers (Fig 1).

**Fig 1. Classification of iron deficiency**



Normal= Hb>110 g/L, ferritin >12 µg/L and sTfR<2.5 mg/L. Iron deficiency with no anaemia (IDNA) =Hb>110 g/L, ferritin <12 µg/L or sTfR>2.5 mg/L or both. Iron deficiency anaemia (IDA) = Hb<110 g/L, ferritin <12 µg/L or sTfR>2.5 mg/L or both. Anaemia without iron deficiency = Hb<110 g/L, ferritin >12 µg/L and sTfR<2.5 mg/L.

### Factors associated with anemia

- Infants were more likely to have anaemia if they had high sTfR/log ferritin index or their mothers had anaemia. Preschoolers were more likely to have anaemia, if while still young, they had elevated CRP or elevated sTfR/log ferritin index or if their mothers had anaemia. We also observed a trend ( $P=0.095$ ) in DDS of  $\leq 3$  among infants (Table 1). Among the food groups, fortified foods alone showed an association with anaemia among infants (62.1% anaemia in infants who received fortified foods compared to 70% anaemia in infants who did not receive fortified foods,  $P=0.039$ ). A trend was also observed with respect to low birth weight ( $P=0.085$ ) and maternal education ( $P=0.058$ ) among preschoolers.

**Table 1. Factors associated with anemia among infants and preschoolers**

Variables	Risk	Infants N=445		Preschoolers N = 263	
		OR (95% CI)	P	OR (95% CI)	P
<b>Child factors</b>					
Sex	Boy	1.42 (0.91-2.21)	0.126	1.23 (0.69-2.19)	0.488
Age	Infants (9-12 mo) Preschoolers (29 - 35mo)	1.20 (0.73-1.96)	0.471	2.00 (1.10-3.63)	0.023
Any illness symptom in past 15 days	Yes	0.89 (0.57-1.38)	0.603	0.64 (0.33-1.25)	0.192
Zinc	<10.0 $\mu\text{mol/L}$			1.76 (0.71-4.37)	0.220
C-Reactive protein	$\geq 5$ mg/L	1.69 (0.91-3.13)	0.095	4.21(1.63-10.92)	0.003
sTfR/log ferritin	$\geq 1.9$	2.21(1.39-3.54)	0.001	5.37 (2.67-10.78)	<0.0001
Vitamin B-12	<148 pmol/L	1.33 (0.77-2.29)	0.313	0.89 (0.22-3.64)	0.876
WAZ	<-2 SD	0.93 (0.50-1.746)	0.826	1.58 (0.80-3.14)	0.192
HAZ	<-2 SD	1.46 (0.79-2.70)	0.231	0.77 (0.39-1.53)	0.461
Dietary diversity score	$\geq 3/\leq 7^*$	1.51(0.93-2.46)	0.095	1.14 (0.63-2.05)	0.667
Birth weight	<2.5 kg	0.98 (0.53-1.83)	0.954	1.95 (0.91-4.15)	0.085
<b>Maternal/social factors</b>					
Standard of living index	<24	0.86 (0.51-1.46)	0.584	1.00 (0.53-1.86)	0.989
Maternal anaemia	<120 g/L**	3.31 (2.10-5.23)	<0.0001	4.19 (2.22-7.91)	<0.0001
Maternal BMI	<18.5 kg/M <sup>2</sup>	1.21 (0.78-1.88)	0.403	0.80 (0.44-1.45)	0.453
Maternal education	Illiterate and primary level education	1.38 (0.83-2.32)	0.217	1.83 (0.98- 3.43)	0.058
Family size	>4	0.74 (0.44-1.26)	0.270	1.15 (0.54-2.45)	0.721
Food security	Not secure	1.40 (0.71-2.74)	0.335	0.65 (0.27-1.55)	0.330

sTfR, soluble transferrin receptor; HAZ, height-for-age Z score; WAZ, weight-for-age Z score; BMI, body mass index. \* $\leq 3/\leq 7$  for infants and preschoolers respectively. The results are based on logistic regression analysis. § low prevalence of plasma zinc <10.0  $\mu\text{mol/L}$  to include in the model to compute OR. \*\* Hb cut-off of 110 g/L for pregnant women.

### CONCLUSION

Anaemia prevalence was high among infants and preschoolers, indicating a problem of severe public health significance, for which iron deficiency is the major contributor. The roles of maternal anaemia and inflammation in addition to that of iron, in anaemia among infants and preschoolers are highlighted.

### 3. DIETARY DIVERSIFICATION OF INDIAN VEGETARIAN DIET TO IMPROVE IRON BIOAVAILABILITY- STUDIES USING CaCo-2 CELL LINE MODEL

There is paucity of evidence on iron availability from individual foods and their combinations and the utility of Indian herbs rich in iron and /or hematinic to facilitate dietary diversification. Previously, we have reported the development of rapid and sensitive screening method to estimate accessibility (dialyzable) of iron from food stuffs using Phen Green SK (a fluorescent probe) in 6 well plates set up (Annual Report 2011-12). The present study attempted to utilize the screening method to screen a number of combinations of food groups, to formulate a diversified diet high in bio available iron and the utility of Indian herbs to further improve bioavailability of diversified diet.

#### AIMS AND OBJECTIVES

- To screen & select iron rich and/ or hematinic herbs using a human enterocyte cell line model, Caco-2 cells.
- To formulate diversified Indian meals using PGSK based *in vitro* dialyzability for screening food combinations.

#### METHOD

Eight Indian herbs, considered being *iron rich and/or hematinic* namely *B. diffusa*, *T. ammi*, *A. paniculatus*, *L. sativum*, *M. sativa*, *A. racemosus*, *S. indicum* and *P. longum* were selected for the study. Mineral composition and phytate and total polyphenol content were analyzed.

#### Testing of bioavailability and absorption promoting property of iron in herbs using Caco-2 cell line model

Screening herbs for iron bioavailability was carried out using Caco-2 cell line model coupled to *in vitro* digestion for all the herbs. In order to assess the iron absorption promoting property, two contrasting herbs in terms of inhibitor contents *B. diffusa* and *A. racemosus* were selected and tested in the presence and absence of FeCl<sub>3</sub> (1 μmole) and ascorbic acid (1:20 molar ratio).

#### Formulation of diversified Indian meals and screening for *in vitro* dialyzability

Habitually consumed food stuffs from 6 food groups were identified. Indian bread (chapati) was prepared from whole wheat flour and bajra flour while rice and pulses were pressure cooked. Cooked samples were homogenized and lyophilized thereafter. Briefly, the pH of the food homogenate was adjusted to 2 and digested with 0.1 mL of pepsin in 6-well plate for 2 h at 37°C. The insert fitted with a dialysis membrane was introduced and filled with 2 mL of 0.15M PIPES pH 6.3 and incubated for 30 min at 37°C, followed by the intestinal digestion with 0.5 mL of pancreatin-bile mixture, and incubated at 37°C for 2 hr. Dialysate from the top chamber of the insert was collected and analyzed for iron by PGSK method (Annual report 2011-2012).

Total iron in food samples was estimated in moisture free sample (0.5 g) by wet microwave digestion (MARS XPRESS, CEM Corporation, US) followed by iron estimation using bathophenanthroline method. Means were compared using one way ANOVA followed by LSD Post Hoc test. The results were considered significant if p value was < 0.05.

#### RESULTS

Identified herbs had iron content ranging from 10-60 mg/100 g. Based on iron, phytate and tannin contents, *B. diffusa*, *L. sativum* and *T. ammi* contained iron above 40 mg/100g and high amount of tannin/ phytate. *A. paniculatus*, *M. sativa*, *P. longum*, *S. indicum* had low iron content (10-15 mg/ 100g) with high phytate and tannin. *A. racemosus* had 38 mg/ 100g iron and low phytate and tannin content. Among all the

herbs, *B. diffusa* had highest iron and calcium content followed by *T. ammi* with highest amount of zinc (5.9 mg/100g). *A. paniculatus* had lowest iron and tannin content while *M. sativum* and *P. longum* had highest phytate and tannin content respectively. *A. racemosus* had lowest zinc, copper, phytate contents). *A. racemosus* and *B. diffusa* on account of high iron, contrasting inhibitor contents were selected for testing iron absorption promoting activity.

### Iron bioavailability and absorption promoting property of herbs

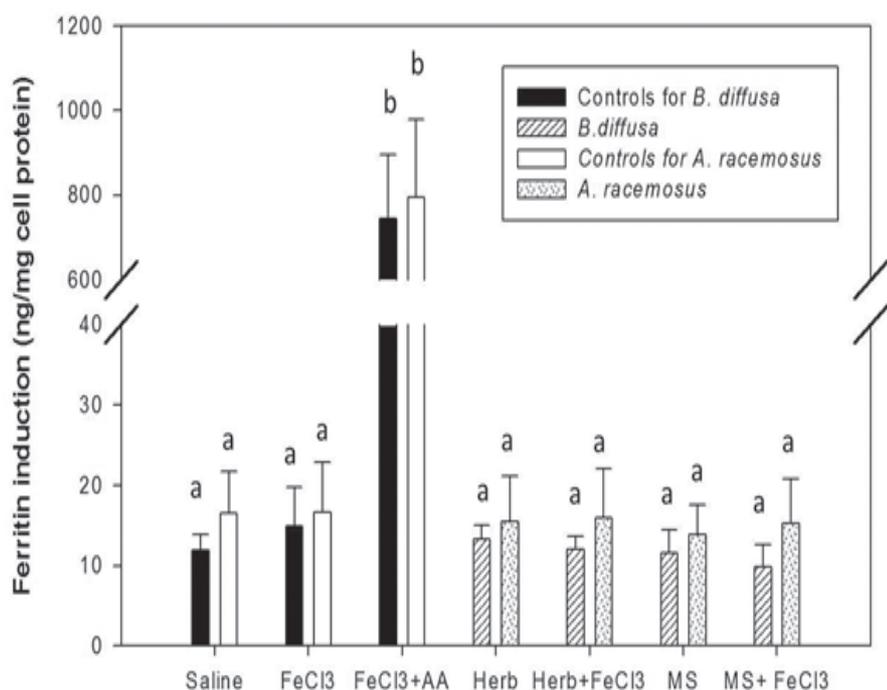
All the eight herb digests showed similar induction of ferritin in Caco-2 cells (14-20 ng/ mg cell protein) to that in saline control (17 ng/ mg cell protein). There was no difference in ferritin induction either in the presence or absence of FeCl<sub>3</sub> with *A. racemosus* or *B. diffusa* herb powder and mineral extract (P>0.05) (Fig 1). However, mineral solutions of both the herbs with exogenous ascorbic acid induced 10 fold higher ferritin concentration (P<0.05; Fig 2) while herbs as such did not induce ferritin (20 ng/mg cell protein) in Caco-2 cells.

### Iron dialyzability from combination of cereals

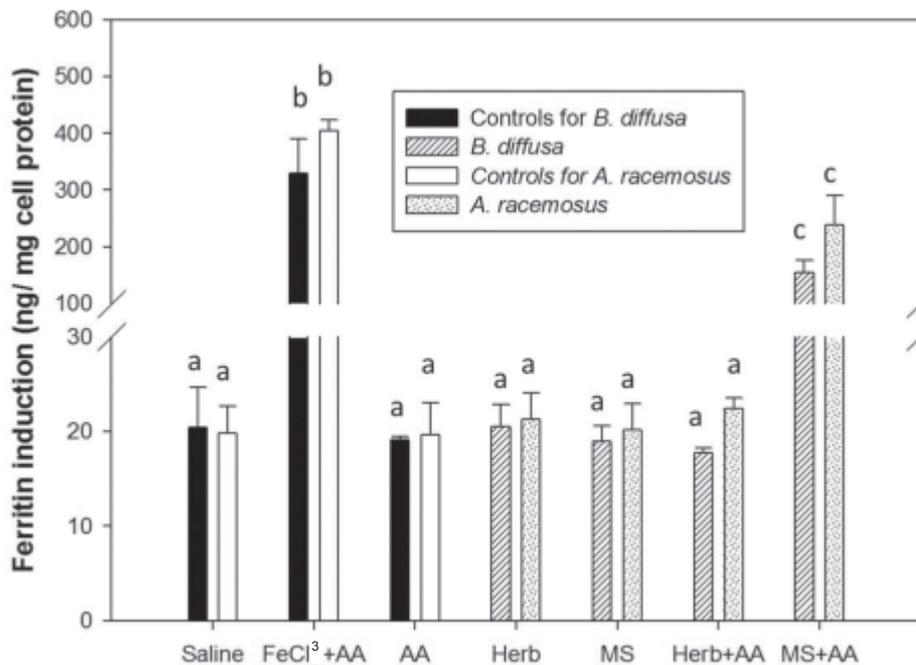
The percent iron dialyzability from cereals was in the range of 1.13-2.91 % total iron and their combinations ranged from 1.24-4.21. The percent dialyzability was higher from rice (2.9 %) compared to wheat (1.1%) and bajra (1.6%). Rice with bajra at the ratios of 1:1 (1.73% of total iron) and 3:1 (1.76 %) showed significantly higher percent dialyzability compared to wheat with bajra. The net dialyzable iron was higher when all the three were in equal proportions (1:1:1). Highest per cent iron dialyzability (4%) was observed with wheat: rice ratio of 1:3.

### Iron dialyzability from pulses and lentil and their combinations

Among the four selected food stuffs, lentil and green gram showed higher dialyzability (about 5.5%) than bengal gram and red gram (3.8%). Lentil and greengram when added to the pulses with low dialyzable iron (bengal gram or red gram), lead to an increase in the percent iron dialyzability from the respective pulses. Addition of green gram was effective in increasing the iron dialyzability of red gram at all the three ratios tested, while from bengal gram it could enhance only at the highest proportion (Table 1). With a combination of more than 2 pulses, no further increase in percent dialyzability of iron was observed.



**Fig 1.** Induction of ferritin in Caco-2 cells in response to herb powder, mineral solution of *B. diffusa* (leaf) and *A. racemosus* (root) in the presence/absence of exogenous iron. FeCl<sub>3</sub>- Ferric chloride, FeCl<sub>3</sub>+ AA- Ferric chloride and ascorbic acid, MS- Mineral solution. Exogenous iron was added in the form of FeCl<sub>3</sub> to the sample prior to gastric digestion. Experiment was done in triplicate and replicated once to generate six observations. Bars represent mean+SD of ferritin induction expressed as ng/mg cell protein. Comparison has been made in respect to a particular herb and its controls. Bars not sharing common superscript are significantly different at P<0.05 by one-way ANOVA and post-hoc't' test.



**Fig 2.** Induction of ferritin in Caco-2 cells in response to herb powder, mineral solution of *B. diffusa* (leaf) and *A. racemosus* (root) in the presence/absence of exogenous ascorbic acid. FeCl<sub>3</sub>- Ferric chloride, FeCl<sub>3</sub>+AA- Ferric chloride and ascorbic acid, AA- Ascorbic acid, MS- Mineral solution. Experiment was done in triplicate and replicated once to generate six observations. Bars represent mean  $\pm$  SD of ferritin induction expressed as ng/mg cell protein. Comparison has been made in respect to a particular herb and its controls. Bars not sharing common superscript are significantly different at P<0.05 by one-way ANOVA and post-hoc't' test.

**Table 1. Dialyzability of iron (percent of total iron) from combinations of pulses**

Pulse combinations	Dialyzability	Pulse combinations	Dialyzability
Bengal gram	3.83 $\pm$ 0.062 <sup>a</sup>	<b>3:1 ratios</b>	
Red gram	3.73 $\pm$ 0.267 <sup>a</sup>	Bengal gram+red gram	4.51 $\pm$ 0.085 <sup>b</sup>
Green gram	5.39 $\pm$ 0.677 <sup>b</sup>	Green gram+lentil	5.25 $\pm$ 0.479 <sup>c</sup>
Lentil	5.5 $\pm$ 0.183 <sup>b</sup>	Bengal gram+green gram	3.97 $\pm$ 0.206 <sup>a</sup>
<b>2 Pulse combination</b>		Bengal gram +lentil	4.92 $\pm$ 0.498 <sup>c</sup>
<b>1:1 ratios</b>		Red gram +Green gram	5.14 $\pm$ 0.265 <sup>c</sup>
Bengal gram+ red gram	4.45 $\pm$ 0.086 <sup>a</sup>	Red gram+lentil	4.46 $\pm$ .069 <sup>b</sup>
Green gram+lentil	5.0 $\pm$ 0.350 <sup>b,c</sup>	<b>1:3 ratios</b>	
Bengal gram+green gram	4.21 $\pm$ 0.102 <sup>a</sup>	Bengal gram+red gram	4.89 $\pm$ 0.272 <sup>a</sup>
Bengal gram+lentil	4.84 $\pm$ 0.541 <sup>b</sup>	Green gram+lentil	5.23 $\pm$ 0.640 <sup>a,c</sup>
Red gram + green gram	5.3 $\pm$ 0.421 <sup>c</sup>	Bengal gram+green gram	5.04 $\pm$ 0.518 <sup>a,c</sup>
Red gram+lentil	4.26 $\pm$ 0.194 <sup>a</sup>	Bengal gram+lentil	5.44 $\pm$ 0.405 <sup>c,d</sup>
		Red gram+ green gram	5.84 $\pm$ 0.379 <sup>d</sup>
		Red gram+lentil	4.9 $\pm$ 0.337 <sup>a</sup>

Values are mean  $\pm$  SD, n=6

Values not sharing common superscript are significantly different at P<0.05 by one-way ANOVA and post-hoc't' test.

## CONCLUSIONS

Endogenous iron from the selected herbs was not bioavailable in Caco-2 cells. The two contrasting herbs, *B. diffusa* and *A. racemosus*, are devoid of any iron absorption promoting activity. Thus, incorporation of these herbs in the diet may ensure an increase in the total iron content but not bioavailability of endogenous and exogenous iron.

A combination of wheat and rice at a ratio of 1: 3 had the highest iron dialyzability. Among pulses, green gram and lentil had higher percent dialyzability compared to bengal gram or red gram. Addition of these pulses to bengal gram and red gram enhances percent dialyzability significantly.

## 4. FOETAL PROGRAMMING FOR NEURO MUSCULO-SKELETAL DEVELOPMENT IN THE RAT OFFSPRING: ROLE OF ANTENATAL AND PERINATAL MAGNESIUM DEFICIENCY

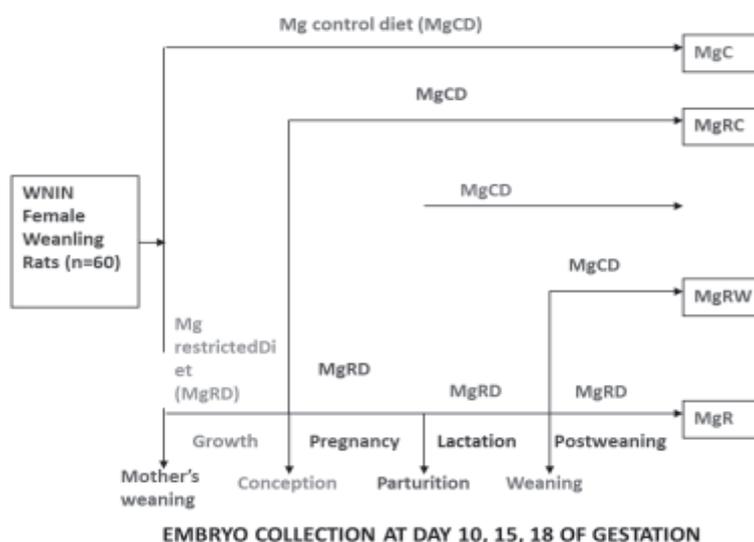
Maternal under nutrition, programmes the offspring for abnormal development and function of nervous tissue which impairs/alters muscle and adipose tissue development, leading to alterations in the body composition of the offspring. Substantial evidence supports that nutritional insult *in utero* modulates gene expression in the offspring by epigenetic regulation. Our recent study on maternal magnesium (Mg) restriction *in utero*, demonstrated the predisposal of the offspring to increased body fat % and decrease in lean body mass (LBM), fat free mass (FFM), basal glucose uptake by the muscle and glucose stimulated insulin secretion. These changes were not correctible by rehabilitation of Mg restricted mothers/ offspring from parturition/ weaning. Considering the importance of Mg in bone and muscle development and the impact of its restriction *in utero* on body adiposity and myogenesis, the present study was conducted to validate/ negate the following hypothesis.

Antenatal and peri-natal Mg deficiency alters the body composition (adiposity, muscle mass and function) of the offspring and is associated with impaired development/ function of the nervous system and these changes in turn are associated with epigenetic mechanisms such as promoter methylation, histone acetylation of the affected genes and changes in the expression of microRNAs etc.

### AIMS AND OBJECTIVES

- To develop a rat model of Mg deficiency.
- To assess the effect of maternal Mg deficiency on fetal neuronal and glial development (expression of genes involved in neurogenesis and the neuronal markers by immunoblotting/ RT-PCR in the neuronal ectoderm and correlate the brain weight at birth.
- To determine whether impaired neuronal and/ or glial development precedes the changes in body composition. (This will be done by monitoring the development/differentiation of these cells by assessing the expression of appropriate markers for these tissues appropriately at differentiation/ development during embryogenesis, foetal growth and postnatally by immunoblotting and/ or RT-PCR or / and histochemically).
- To determine whether altered expression of the genes/proteins is epigenetically regulated.

### Experimental Design



## Progress of work

### Important Highlights of results:

#### Mothers

- Mg restriction *per se* did not affect the growth characteristics like food intake, body weight gain and body mass index in female weaning Wistar/ NIN (WNIN) rats.
- Mg restriction *per se* alters the expression of some cytokines like MCP1 which reflect a condition of stress in the mothers (before mating). Plasma cortisol levels were significantly high in the MgR group.
- Mg restriction *per se* seems to decrease the methylation pattern in Leptin and 11 $\beta$ HSD1 indicating higher expression of the same. But at the protein level no such changes were observed.

#### Placenta

- The placental tissue of MgR at E18 showed an increase in methylation pattern in Leptin Gene promoter whereas the embryos at the corresponding time point showed a decrease in methylation in MgR and it was not corrected by rehabilitation.
- The placenta of MgR at 18 day of gestation showed a decrease in methylation in the 11BHS1 gene promoter indicating a severe condition of stress. Interestingly, adipose tissue in the rehabilitated offspring showed a similar decreased pattern of methylation at several loci, when tested at various time points.

#### Offspring adipose development

- Adiponectin and Leptin showed varied expression but the expression of PPAR and 11 $\beta$  HSD1 was significantly higher in MgR embryos on day 10 and 15 of gestation and was interestingly corrected, upon rehabilitation.
- There was higher methylation in the adiponectin gene promoter in MgR embryos at 10th day of gestation and rehabilitation did not correct this change. Indeed, the rehabilitated groups continued to show high methylation pattern in liver and adipose tissues even at later stages of adult life.
- Maternal dietary Mg restriction in WNIN rats increased body fat % and decreased % of LBM and FFM in both male and female offspring and also affected body fat distribution differently in male (increased visceral adiposity) and female (increased tissue associated fat).
- Maternal dietary Mg restriction resulted in a significant difference in HDL cholesterol, insulin and HOMA IR in MgR offspring of both the genders and these effects appeared to be corrected by rehabilitation.
- Maternal dietary Mg restriction altered the expression of several adipocytokines like Leptin, Mcp1 and IL1 $\beta$  in circulation which appeared to suggest a proinflammatory state in general. Rehabilitation in general, appeared to create/ aggravate stress in the offspring as evident from the increased cortisol levels.

#### Offspring brain development

- Genes involved in neural development (NeuroD2, NCAM, Neurofilament and GFAP) was upregulated in MgR embryos, as early as day 10 & 15 of gestation and rehabilitation from conception did not correct the change.
- MgR offspring (only males) had significantly lowered brain weight than controls albeit on postnatal day 10 but not earlier or later.

#### Offspring muscle and bone development

- Expression of muscle development genes (DESMIN, MYOGENIN) was down regulated on day 10 and 15 of gestation and was also not corrected by rehabilitation.
- Dlx5 gene showed varied expression at 10 and 15 day of gestation. However there was a decreased methylation found in the MgR 15 day embryos in Dlx5 gene promoter and was corrected by rehabilitation.

- Maternal dietary Mg restriction in WNIN rats decreased % of LBM and FFM in both male and female offspring.
- Maternal dietary Mg restriction did not affect bone weight but it appeared to increase BMC and BMD in offspring of both sexes.

## CONCLUSION

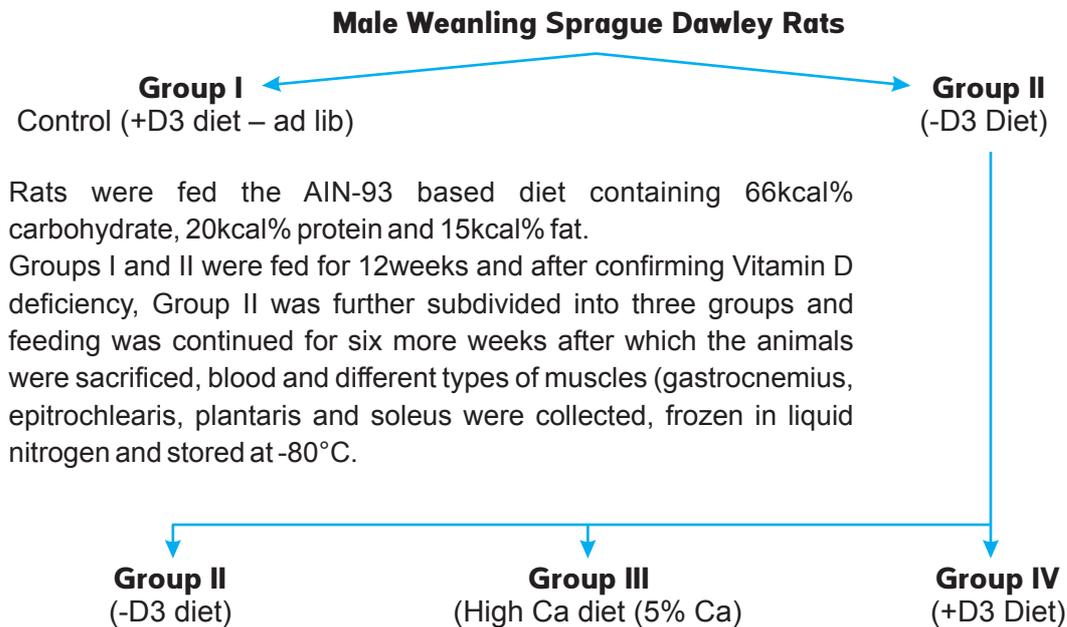
The observations in the embryos/ offspring of the Mg restricted WNIN rat dams suggest's that brain development was impaired *albeit* transiently (and not at birth), appears to negate any role of impaired brain/ neuronal development in the changes observed in their body composition in later life. The differential gene expression of Leptin, adiponectin and 11 $\beta$ HSD1 and the corresponding change in DNA methylation in the embryos and at later time points appear to suggest that maternal Mg restriction induced changes in the body composition (increased body fat %/ visceral adiposity and decreased % of LBM, FFM and BMC) may be programmed right during the intra uterine growth of the offspring. The epigenetic mark is being passed on from mother to offspring. Increased stress seems to be the mechanism underlying changes in body composition. Rehabilitation at the time of conception corrected changes in gene expression and promoter DNA methylation. However, rehabilitation at later time points (Parturition and Weaning) showed adverse effects for the same. The study findings suggest that, maternal Mg restriction altered promoter DNA methylation and this could be one of the underlying mechanisms for the changes in the expression of relevant genes underlying altered adiposity and associated stress. Further analysis is being done to correlate the promoter methylation pattern and gene/protein expression as this may prove useful in the development as a marker for magnesium deficiencies.

## 5. ROLE OF THE UBIQUITIN-PROTEASOME PATHWAY IN VITAMIN D DEFICIENCY INDUCED MUSCLE ATROPHY AND HYPOINSULINEMIA

The active hormonal form of vitamin D-1,25 (OH)<sub>2</sub>D<sub>3</sub> is essential for the maintenance of calcium homeostasis and bone health in humans. Vitamin D deficiency leads to bone loss and muscle wasting or atrophy in both animals and humans. On the other hand, studies in humans and experimental animals have demonstrated that vitamin D deficiency leads to insulinopenia or low levels of circulating insulin. Muscle atrophy or muscle wasting occurs when there is an imbalance between protein synthesis and protein breakdown pathways. Loss of body protein stores is a common feature observed in many systemic diseases such as cancer, diabetes mellitus, AIDS, uremia and sepsis. The major pathway involved in the degradation of proteins is the ubiquitin proteasome pathway (UPP). Accelerated muscle protein degradation in many disease states, leads to muscle wasting. However, the other two pathways in muscle namely: the lysosomal and calpain pathways are also known to contribute to muscle wasting in some disease conditions. The role of the different proteolytic systems in vitamin D deficiency associated muscle protein degradation is not known. Studies on the presence of the VDR in muscle tissue in different species are controversial. Therefore, it is unclear if the muscle abnormality seen in D-deficiency is a direct consequence of impaired vitamin D actions in muscle via the VDR, or as a result of secondary systemic changes such as hypocalcemia or elevated PTH in the circulation. Calcium is known to play an important role in muscle function and metabolism. The role of calcium *per se* in the absence of vitamin D in improving the muscle wasting observed in a vitamin D deficient state is not known. Hence, the present study was conducted to delineate the role of vitamin D and calcium on the muscle atrophy observed in a vitamin D deficient state.

## METHODOLOGY

The schematic diagram for the experiment is shown below.



## RESULTS

- a) Vitamin D deficiency was confirmed by undetectable serum 25(OH)D<sub>3</sub> levels (indicator of vitamin D status) and hypocalcaemia. In addition, the serum 1,25(OH)<sub>2</sub>D<sub>3</sub> levels (biologically active hormonal form of vitamin D<sub>3</sub>) was significantly ( $P \leq 0.001$ ) decreased (~10pg/ml) in both the vitamin D deficient and high calcium intake groups confirming their D-deficient status. On the contrary, the other two groups fed diets containing vitamin D, had normal (>40 pg/ml) serum 1,25(OH)<sub>2</sub>D<sub>3</sub>.
- b) Type II muscle fibre area but not number, was observed to be significantly reduced in the deficient group compared to control group (Fig 1A-1C). Supplementation with vitamin D could reverse this change; however supplementation with calcium in the absence of vitamin D could not correct it. On the other hand there was no difference in the type I fibre area between the groups (data not shown).
- c) Muscle protein degradation was increased by 40% in epitrochlearis muscles of deficient group compared to controls. Supplementation with vitamin D could reverse this change, however rescue with high calcium diet resulted in 18% increase in TPD compared to controls, suggesting a partial correction with calcium alone (Fig 2A,  $P \leq 0.01$ ). Further, 3-MH is a minor amino acid of actin and myosin, and upon release from myofibrillar protein it is neither degraded nor reutilized for protein synthesis. We observed a 53% increase in the 3-MH content in urine of deficient rats compared to control rats (Fig 2B). Supplementation with vitamin D reversed the 3-MH content comparable to that of controls. On the other hand a 17% decrease was observed in muscle protein synthesis in the deficient group compared to control (Fig. 2C,  $P \leq 0.01$ ).
- d) The expression of muscle atrophy markers Atrogin-1 and MuRF1 was increased by two fold ( $P \leq 0.05$ ) in the deficient muscle compared to control. Similarly, expression of the proteasomal subunit genes: PSC2 & PSC8 was also increased in the deficient muscle compared to controls (Fig 3A). On the other hand, there was a twofold ( $P \leq 0.05$ ) decrease in the expression of the myogenic genes: MyoD & MyoG in the deficient group than control group. Myf5, another myogenic factor was also significantly reduced ( $P < 0.05$ ) in the deficient group (Fig 3B). Interestingly, both supplementation with vitamin D and high calcium feeding could normalize the gene expression to that of controls. Expression of myostatin, a negative regulator of muscle growth was not altered between the groups (Fig 3B). As expected the expression of lysosomal enzyme genes (cathepsin B & L) and calpain enzyme genes (m &  $\mu$  calpain) were not different in the muscle of different groups (Fig 3C).

**Fig 1. Histochemical analysis of gastrocnemius muscle in the different experimental groups**

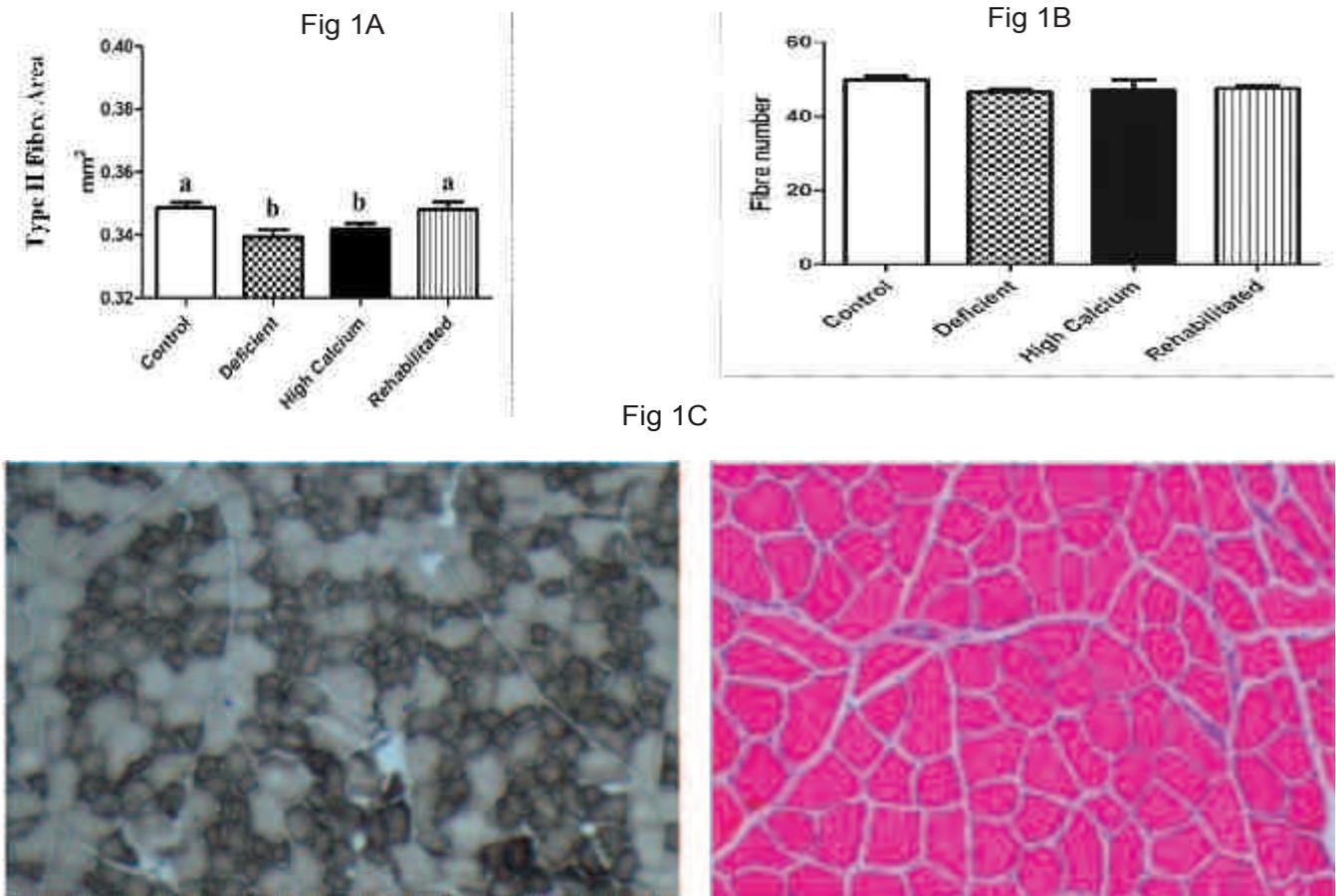
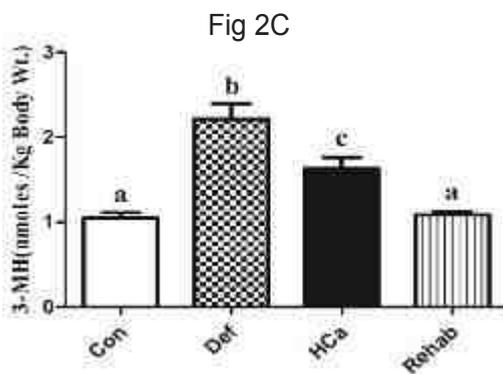
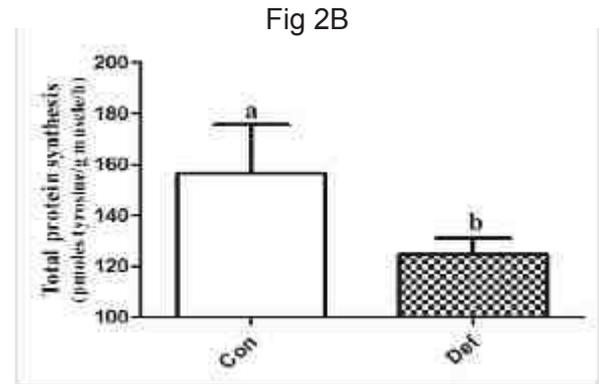
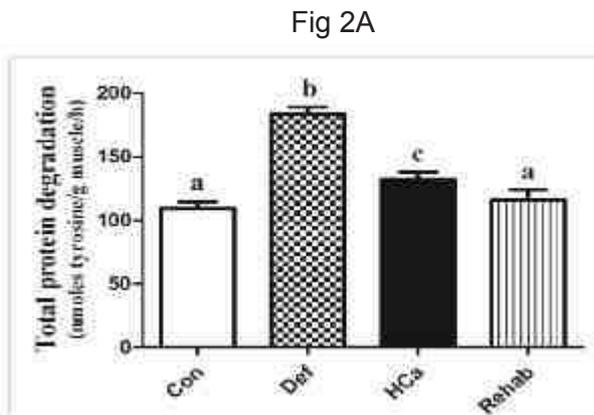


Fig 1C

Gastrocnemius (GM) muscle was quickly dissected out from 21 week old rats from the four different groups. Sections were taken from muscles frozen in isopentane cooled liquid nitrogen as described in methods section. Sections were stained either with hematoxylin/eosin (H/E) or ATPase as described. Data is expressed as mean  $\pm$  SEM. Fig1A and 1B shows the type II fibre area and number respectively in the different groups, whereas Fig1C depicts representative pictures of ATPase and H/E staining. Data is shown as mean  $\pm$  SEM of measurements from six rats in each group. Data with different superscripts are significantly different from each other at  $P \leq 0.05$ . (Control group- Con; Deficient group - Def; High calcium group - HCa; Supplemented with D group - SD)

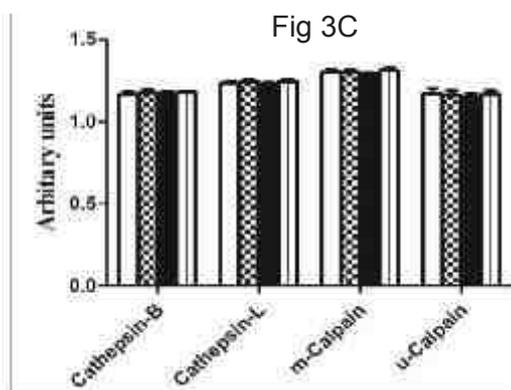
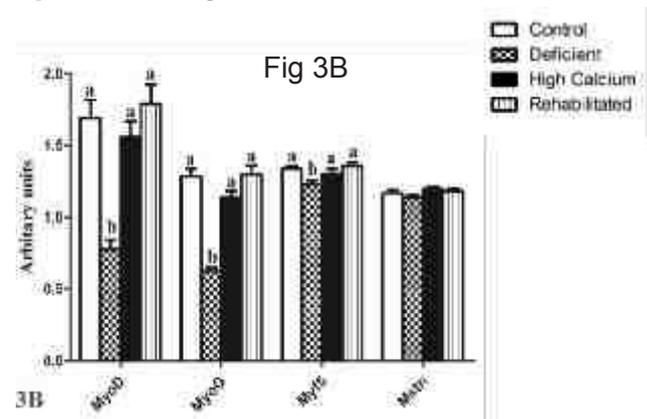
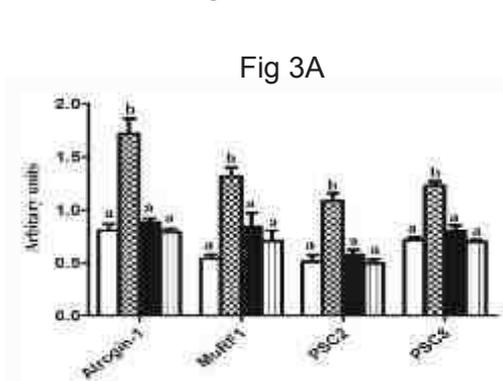
- e) Vitamin D deficient group had significantly higher Ch-L (10%), T-L (7%) and Cp-L (7%) activities compared to vitamin D sufficient group in the mixed fibre gastrocnemius muscle (Fig 4A-4C). On the other hand only the Ch-L activity was significantly increased (7%) in the soleus muscle of the deficient group compared to control group (Fig 4D-4F), and this was reversed both by supplementation with vitamin D and with the rescue diet containing high calcium.
- f) Vitamin D deficiency caused a significant increase (60%,  $P \leq 0.05$ ) in the protein expression of the 14-kDa E<sub>2</sub> ubiquitin-conjugating enzyme in epitrochlearis muscle compared to controls. Both supplementation with vitamin D and rescue with high calcium diet could reverse this change (Fig 5A & 5B). High molecular weight ubiquitin conjugates were significantly increased (230%,  $P < 0.05$ ) in deficient muscle compared to control muscle. Supplementation with vitamin D could reverse this change completely (125%), while rescue with high calcium diet could partially (150%) correct it (Fig 5C & 5D).
- g) Lysosomal and calpain activities were studied in the gastrocnemius and soleus muscles using fluorogenic substrates specific to the two enzymes. Both the lysosomal and calpain enzyme activities were not different between the groups (Fig 6A-6D) in either the gastrocnemius or soleus muscles.

**Fig 2. Vitamin D deficiency increases muscle protein degradation and decreases protein synthesis**



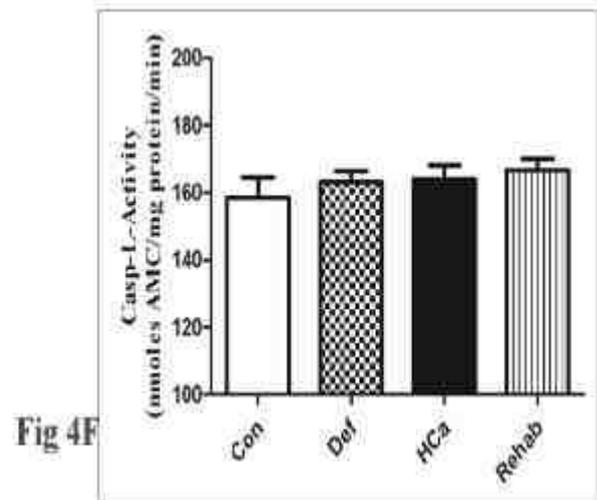
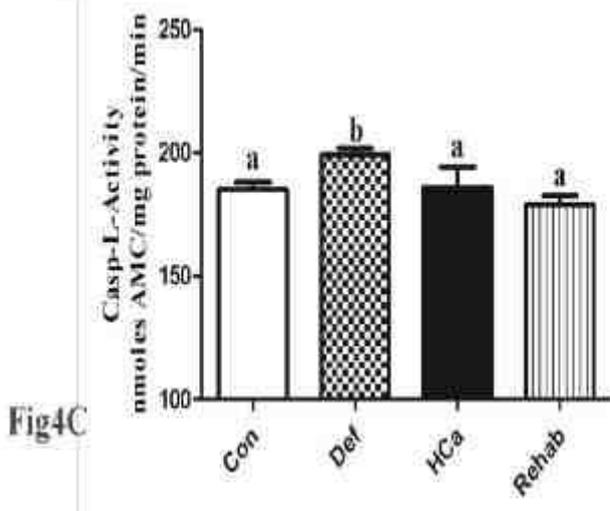
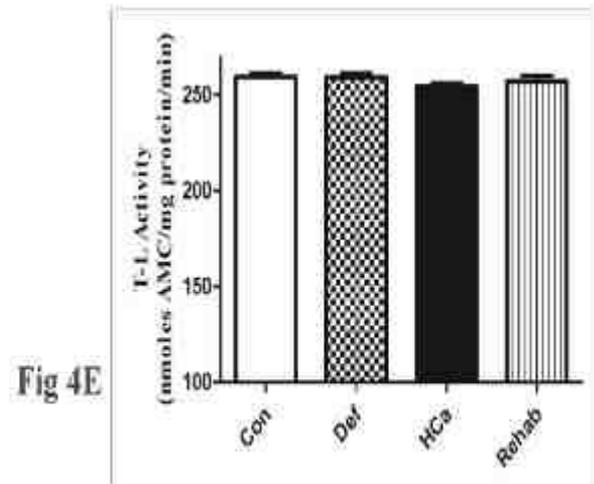
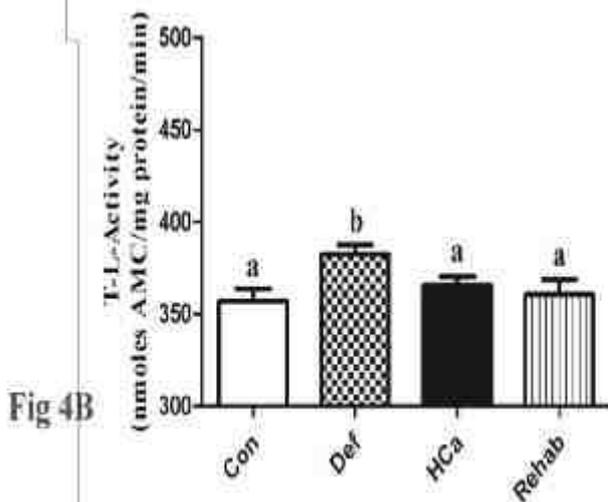
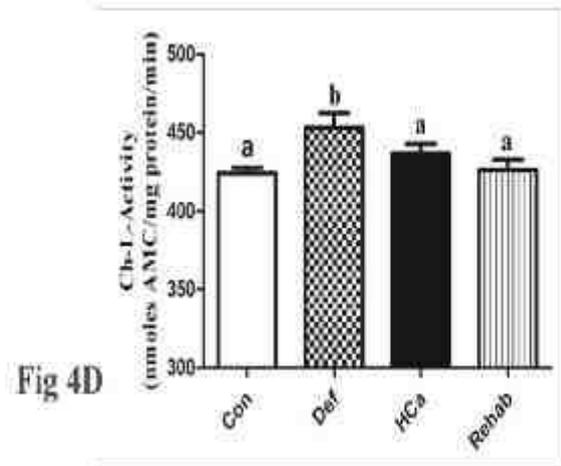
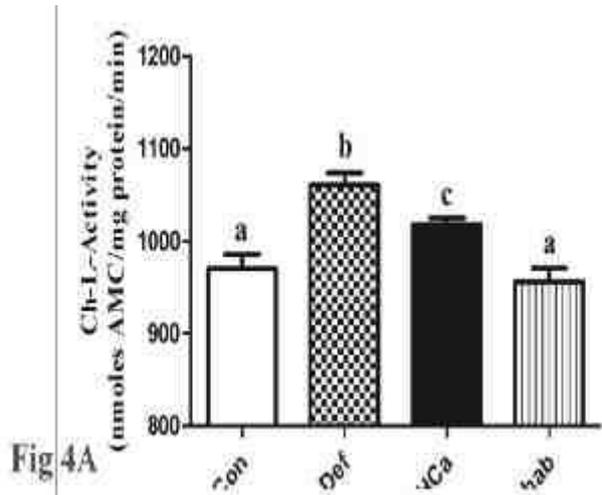
Total protein degradation was measured in one epitrochlearis muscle, whereas the contralateral muscle was used for the measurement of protein synthesis. Total proteolysis as measured by tyrosine released is shown in Fig 2A, whereas total protein synthesis measured by  $C^{14}$ -tyrosine uptake is shown in Fig 2C. Fig 2B depicts the urinary 3-MH excretion in the different groups of rats. Data is shown as mean  $\pm$  SEM of measurements from six rats in each group. Data with different superscripts are significantly different from each other at  $P \leq 0.05$ . (Control group—Con; Deficient group - Def; High calcium group-HCa; Supplemented with D group - SD).

**Fig 3. Vitamin D deficiency alters expression of genes in the muscle**



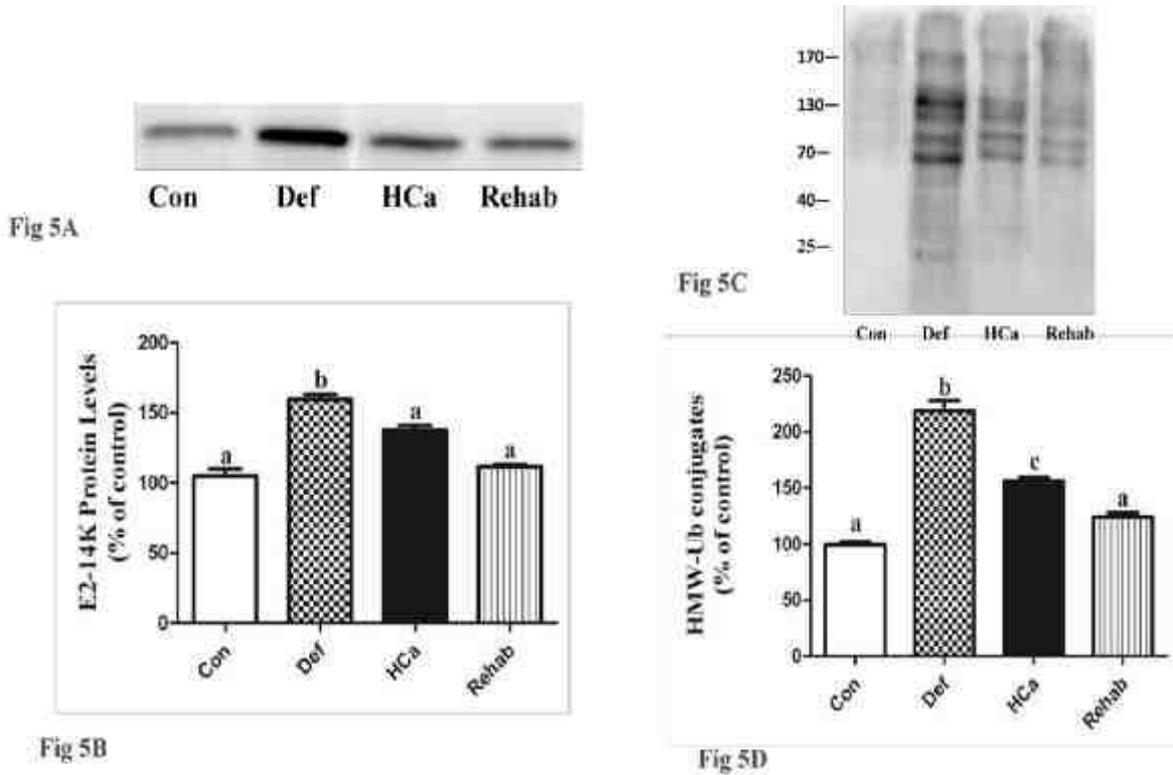
RNA was isolated from gastrocnemius muscle followed by preparation of cDNA from different groups. Semi-quantitative PCR was done (n=6) using 18S rRNA as a housekeeping gene. Fig 3A depicts expression of atrophy marker genes (Atrogin-1 & MuRF1) and proteasomal subunit genes PSC2 & PSC8. Fig 3B depicts expression of myogenic genes (MyoD, MyoG, Myf5) and Mstn. Fig 3C depicts expression of lysosomal enzyme genes (cathepsin B & L) and calpain enzyme genes (m&u calpain) respectively. Data is shown as mean  $\pm$  SEM of measurements from six rats in each group. Data with different superscripts are significantly different from each other at  $P \leq 0.05$ . (Control group—Con; Deficient group - Def; High calcium group-HCa; Supplemented with D group - SD)

**Fig 4. Vitamin D deficiency increases muscle 20S proteasomal enzyme activities**



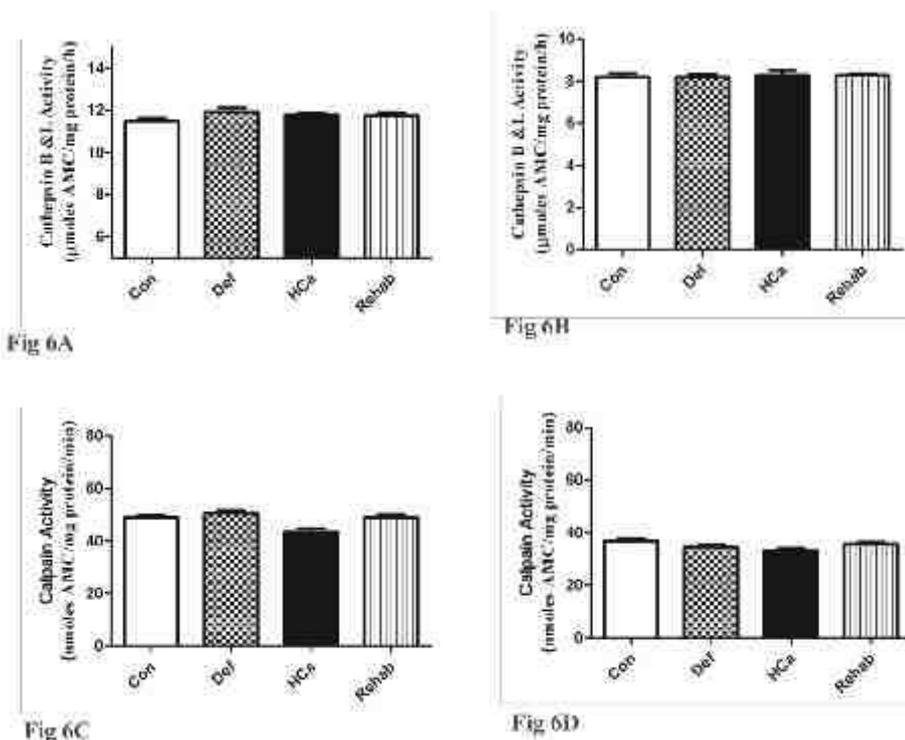
Gastrocnemius and soleus muscle extracts were prepared from 21 week old rats from the different groups. 20S proteasomal enzyme activities namely Ch-L, T-L and Cp-L were measured using specific substrates as described in methods section. Figures 4A-4C depicts data from GM, whereas figs 4D-4F show data from soleus muscle respectively. Ch-L activity (figs 4A & 4D), T-L activity (figs 4B & 4E) and Cp-L activity (figs 4C & 4F) respectively. (Control group– Con; Deficient group - Def; High calcium group-HCa; Supplemented with D group - SD).

**Fig 5. Vitamin D deficiency increases expression of the E2- ubiquitin conjugating enzyme and high molecular weight (HMW) ubiquitin (Ub) conjugates**



Cytosolic and myofibrillar fractions were prepared from mixed fibre epitrochlearis muscle of 21 week old rats of different groups. The expression of E2 Ub conjugating enzyme and HMW Ub conjugates was examined by western blot using specific antibodies. Fig 5A & 5B shows E2 WB while Fig 5C & 5D depicts Ub conjugates. Data is shown as mean  $\pm$  SEM of measurements from four rats in each group. Data with different superscripts are significantly different from each other at  $P \leq 0.05$ . (Control group– Con; Deficient group - Def; High calcium group-HCa; Supplemented with D group - SD).

**Fig 6. Vitamin D deficiency does not alter either lysosomal or calpain activities in muscle**



Gastrocnemius and soleus muscle extracts were prepared from 21 week old rats from the different groups. Lysosomal and calpain activities were measured as described. Fig 6A and 6B depict lysosomal activity in GM and soleus; whereas Fig 6C and 6D show calpain activity in GM and soleus respectively. Data is shown as mean  $\pm$  SEM of measurements from six rats in each group. Data with different superscripts are significantly different from each other at  $P \leq 0.05$ . (Control group– Con; Deficient group - Def; High calcium group-HCa; Supplemented with D group - SD)

## CONCLUSION

The study showed that, muscle wasting in vitamin D deficient rats results due to increased muscle protein breakdown despite food intake being similar to vitamin D sufficient rats. The coordinated up regulation of enzyme activities, gene and protein expression of various components of the UPP suggests a major role for this pathway in vitamin D deficiency induced muscle wasting. The study also demonstrates that, calcium alone in the absence of vitamin D, can partially correct most of the muscle changes. The data presented in this study appears to be important from a clinical perspective in that it highlights the significance of maintaining optimum levels of vitamin D and calcium for good musculoskeletal health.

## 6. ISOLATION AND CHARACTERIZATION OF PROCYANIDINE-B2 AS A NOVEL ANTIGLYCATING AGENT FROM CINNAMON

Modification of cellular proteins, lipids, and nucleic acids by nonenzymatic glycation to form advanced glycation end products (AGEs) is an age-related process. Non-enzymatic glycation is a complex series of covalent chemical reactions between the carbonyl group of reducing sugars and the amino group of proteins. The initial reversible reaction between carbonyl and the amino groups yields the Schiff's base, which undergoes a spontaneous rearrangement to form a more stable Amadori product. The latter, over a period of time, undergoes a series of irreversible reactions involving multiple dehydration, fragmentation and oxidative modifications through highly reactive dicarbonyl intermediates (such as glyoxal, methylglyoxal, and 3-deoxyglucosone) to form stable heterogeneous adducts called AGE. Glycation-modified molecules bind to multiligand receptors (RAGE) able to recognize AGEs and to neutralize their function, like the soluble receptor for AGEs (sRAGE), or to initiate various signaling pathways, resulting in enhanced oxidative stress and transcriptional activation when bound to the membrane-associated RAGE.

Although AGE formation takes place during the normal aging process, it is accelerated in hyperglycemic conditions. An overwhelming body of evidence indicates that non-enzymatic glycation of proteins is implicated in a number of biochemical abnormalities associated with aging and diabetes such as atherosclerosis, nephropathy and cataract.

For example, glycation of lens proteins has been considered to be one of the mechanisms responsible for both age-related and diabetic cataract, which is the leading cause of blindness. Similarly AGE are thought to play a role in the pathogenesis of diabetic nephropathy. The rate of AGE accumulation is related to the severity of disease. Thus inhibition of AGE is considered to be one of the promising approaches for the prevention and treatment of diabetic complications. Hence, efforts are being made in identifying natural sources of antiglycating agents that can be tested for their therapeutic value against AGE-mediated pathologies.

In the course of identifying and testing new antiglycating agents, we have evaluated a number of traditional and very common dietary sources and found that some spice principles, fruits and vegetable have the potential to inhibit AGE formation under *in vitro* conditions. Among them, aqueous extract of cinnamon has significantly prevented AGE formation under *in vitro* conditions. Cinnamon is one of the commonly found spices in India. Proanthocyanidins of cinnamon bark have been shown to act as carbonyl scavengers. However, the potential of active components responsible for inhibition of AGE is not known. Therefore, in this study we employed a set of complimentary methods; spectroscopic, electrophoretic, chromatographic and immunochemical, to evaluate antiglycating potential of cinnamon and to characterize its active principle against eye lens protein glycation.

## METHODOLOGY

**Extraction and isolation of active principle from cinnamon:** In brief, the bark of cinnamon was grounded into fine powder and extracted with absolute ethanol. The ethanol-extract was centrifuged, filtered and concentrated. The dried ethanol-extract was partitioned with ethyl acetate to give an ethyl acetate-soluble fraction. The ethyl acetate-soluble fraction was dissolved in 95% ethanol and loaded onto a Sephadex LH-20 column. The bound fractions were eluted with 95% ethanol and followed by 50% acetone. The fractions were tested for inhibition of protein glycation *in vitro* using goat lens total soluble proteins (TSP).

**Mass Spectrometry:** Mass spectrometry analysis was done by using 4000-QTRAP triplequadrupole hybrid mass spectrometer equipped with a liquid chromatography and operated in the positive turbo ion spray (ESI) mode. The analysis was carried out in the multiple reaction monitoring (MRM) positive ESI mode with high resolution. A full scan of the mass spectra was recorded in order to select the most abundant mass to charge ratio (m/z) ion (Q1), using continuous infusion in the positive ionization mode of ESI. The most abundant product ion for each compound was then selected for MRM analysis.

**Qualitative and quantitative HPLC:** F2-fraction along with standard procyanidines was subjected to HPLC analysis for both comparative chromatogram and quantity respectively. HPLC analysis was carried out with a RP-C18 column. Elution was carried out using solvent A (0.1 % HCOOH) and solvent B (100% methanol) in presence of gradient by solvent B from 0 min 15%, 0-10 min 15-50 %, 10-20 min 50-100%, 20-23 min 100% over a period of 60 min run time with a flow rate of 3.5 ml/min. Detection was at 280 nm.

**In vitro glycation of proteins: Eye lens total soluble proteins (TSP) were obtained from six month old goat lenses as reported previously.** A 10% homogenate of goat lenses was prepared in phosphate buffer saline, pH 7.4 and centrifuged at 10,000xg for 30 min at 4°C. Supernatant (referred as TSP henceforth) was used for *in vitro* glycation. Each 1 ml reaction mixture contained 10 mg of TSP, 0.2 M phosphate buffer, pH 7.4, 0.2 M fructose, 50 µg of penicillin and streptomycin and 0.01% sodium azide. Reaction tubes were incubated in dark at 37°C for 3 weeks. At the end of the incubation, unbound sugars were removed by dialysis against the same buffer.

**Inhibition studies with cinnamon and its fractions:** For inhibition studies, concentrated stocks of cinnamon and its fractions on LH-20 column were prepared in water. Various concentrations of cinnamon and its fractions were added to *in vitro* protein glycation assay and incubated in dark at 37°C for 3 weeks as described above. At the end of the incubation, unbound reactants were removed by dialysis and protein concentration was determined. The extent of protein glycation in the absence and presence of cinnamon and its fractions along with standard procyanidines were evaluated by monitoring protein cross-linking on SDS-PAGE, AGE related non-tryptophan fluorescence, protein carbonyl content, and immunoblotting as described below.

**Fluorescence measurements:** Fluorescence measurements were performed using a spectrofluorimeter. For all the measurements, 0.15 mg/ml protein in 20 mM sodium phosphate buffer (pH 7.4) was used. AGE-related non-tryptophan fluorescence of glycated and control proteins was monitored by exciting at 370 nm and emission was recorded between 400 and 500 nm.

**Glyco-oxidative damage:** Glyco-oxidative damage of proteins was monitored by estimating total protein carbonyls, according to previously reported method.

**Immunodetection of AGE:** Formation of specific AGE was detected by immunoblotting using anti-CML-KLH, anti-AGE-BSA and anti-MGO-BSA antibodies. Glycated proteins were resolved on a 12% SDS-PAGE and transferred onto nitrocellulose membrane. The membrane was incubated for 2 h in blocking buffer containing 5% skimmed milk powder. Subsequently, it was incubated overnight with respective primary antibodies separately. Membrane was then incubated with HRP-conjugated goat anti-rabbit antibody for 2 hr and using the substrate buffer containing diaminobenzidine and hydrogen peroxide, detection was performed.

**Hemoglobin- -gluconolactone ( -Glu) assay:** The assay involves the determination of glycated hemoglobin (HbA1c) in blood after incubation with δ-gluconolactone for 22 h using cation-exchange

system. Samples were prepared by mixing 200  $\mu$ L of whole blood (collected from healthy human volunteers after overnight fasting) with 50 mmol/L  $\delta$ -gluconolactone in PBS in the absence and presence of varying concentrations of cinnamon fractions. Reaction contents were incubated at 37°C for 22 h with occasional brief vortex mixing. The percentage of HbA1c was determined using an ion-exchange system. The percentage HbA1c was calculated as per the manufacturer's protocol.

## RESULTS

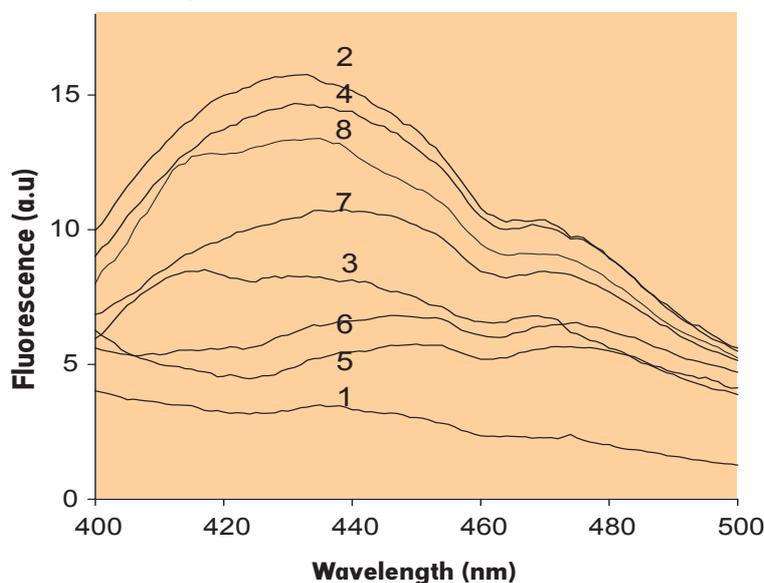
**Inhibition of lens TSP glycation by cinnamon and its fractions:** Antiglycating effect of cinnamon and its fractions was evaluated by incubating them with TSP of goat lens and fructose for 21 days. AGE-related non-tryptophan fluorescence, which represents cumulative heterogeneous AGE fluorescence in a non-specific manner, was monitored to assess the effect of cinnamon and its fractions (Fig 1). While different fractions of cinnamon have inhibited *in vitro* AGE formation to varied extent, F2-fraction has shown maximum inhibition of AGE-fluorescence. In case of eye lens TSP glycation, F2 inhibited the AGE-fluorescence in a dose-dependent manner (Fig 2). Hence, subsequent analysis was carried out with F2-fraction.

**Characterization of active component from cinnamon responsible for AGE inhibition:** Cinnamon bark was extracted as described above and subjected to bioassay guided fractionation on Sephadex® LH-20 column. From these fractions (F1-F5) F2-fraction was identified with highest activity using an *in vitro* TSP glycation assay. Further characterization of F2-fraction was accomplished including accurate mass in LC-MS analysis, which indicated to be procyanidin-B2 (Fig 3). MS/MS analysis on ion m/z 578 confirmed this ion to be procyanidin-B2 based on the spectrum of standard procyanidin-B2 (Fig 3).

The F2-fraction was further analyzed for purity of procyanidin-B2 using reverse phase HPLC which gave an estimate of 80% enrichment of F2-fraction with procyanidin-B2 when compared with standard procyanidin-B2 (Fig 4). Inhibition of AGE-fluorescence by standard procyanidin-B2 (Fig 2) further substantiates that F2-fraction is enriched with procyanidin-B2.

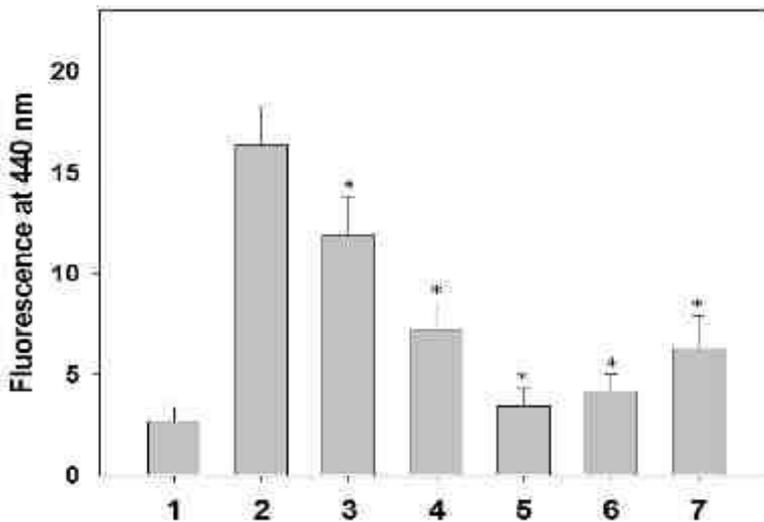
During TSP glycation, protein bound metals are released from the glycated TSP leading to formation of free radicals through Fenton reaction. Metal catalyzed oxidation may cause covalent modification of proteins by introducing carbonyl groups into amino acid residues of proteins. Trapping the reactive carbonyl compounds may be a valuable strategy for inhibiting or delaying the progressive glycation reactions. Hence, total protein carbonyls were estimated in the absence and presence of procyanidin-B2 enriched F2-fraction. Increased carbonyl content (four fold) of lens proteins upon fructose modification is an indicator of glyco-oxidative damage. F2-fraction was effective in lowering the carbonyl content of TSP during *in vitro* glycation in a dose dependent manner (Fig 5). Hence, F2 was an efficient scavenger of dicarbonyls.

**Fig 1. Inhibition of AGE formation by cinnamon extracts and its fractions**



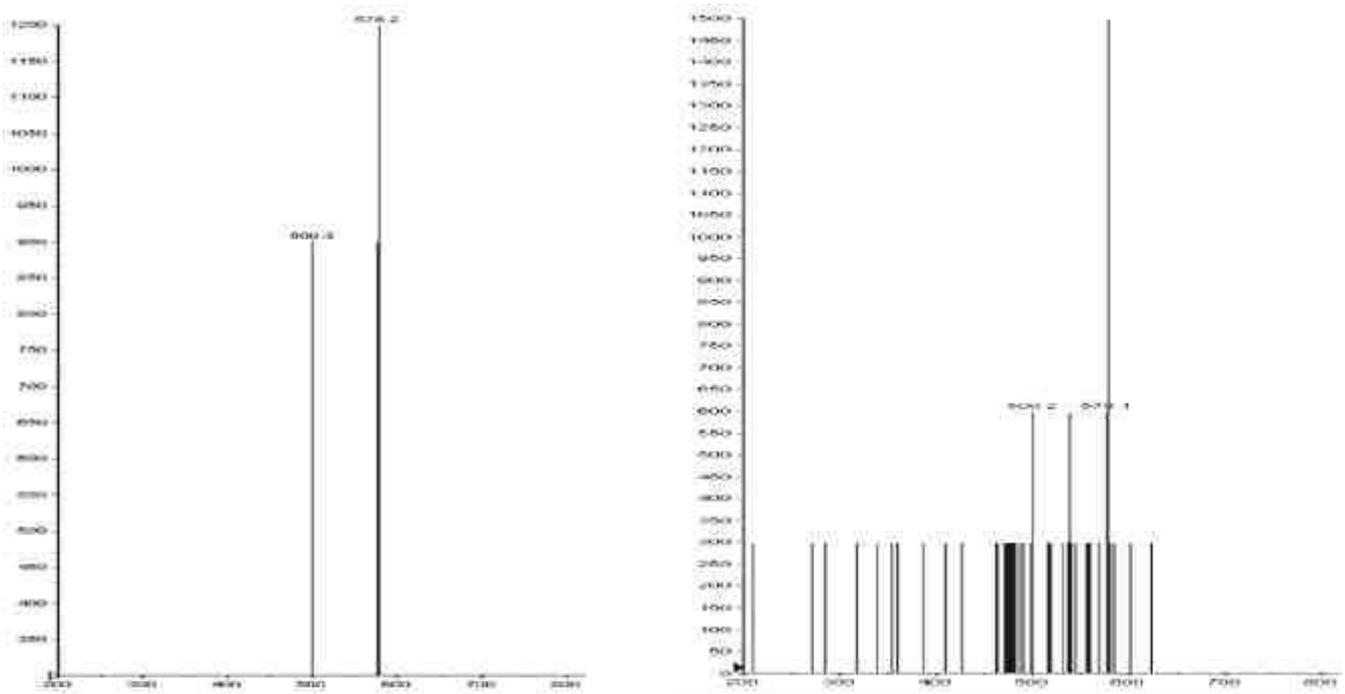
Trace 1, protein alone; trace 2, protein + 200 mM fructose (F); trace 3, protein + F + 1 mg ethanol extract of cinnamon; trace 4, protein + F + 1 mg F1, trace 5, protein + F + 1 mg F2, trace 6, protein + F + 1 mg F3, trace 7, protein + F + 1 mg F4, trace 8, protein + F + 1 mg F5. a.u., arbitrary units.

**Fig 2: Inhibition of AGE formation by F2-fraction of cinnamon**



AGE fluorescence in the absence and presence of various concentrations of F2-fraction. *Bar 1*, protein alone; *bar 2*, protein + 200 mM fructose (F); *bar 3*, protein + F + 100 µg F2; *bar 4*, protein + F + 500 µg F2; *bar 5*, protein + F + 1000 µg F2; *bar 6*, protein + 1000 µg F2; *bar 7*, protein + F + 500 µg procyanidin-B2. Data are mean SE (n=3) and superscript '\*' denotes significantly different from bar 2 (p<0.05).

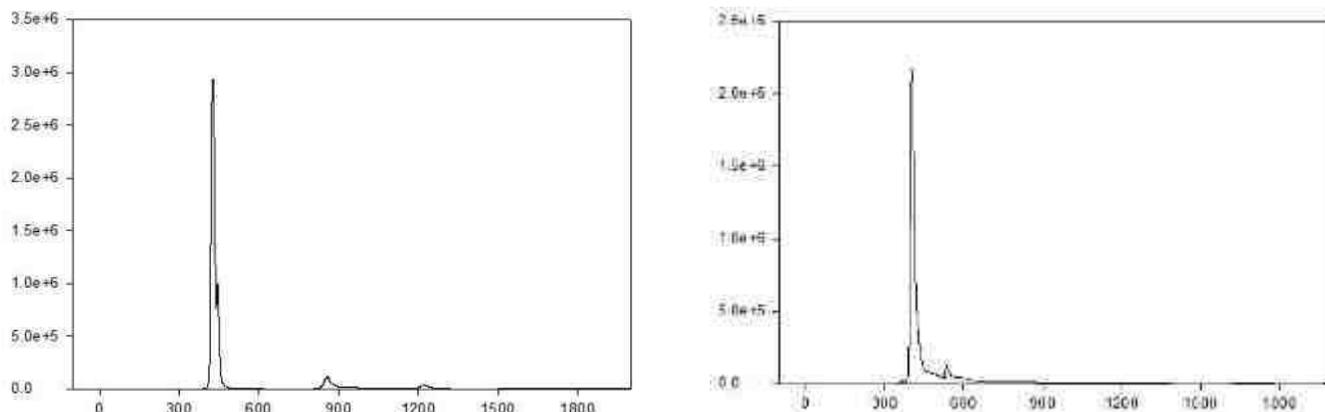
**Fig 3. LC-MS analysis of F2-fraction of cinnamon (A) and standard procyanidin-B2 (B)**



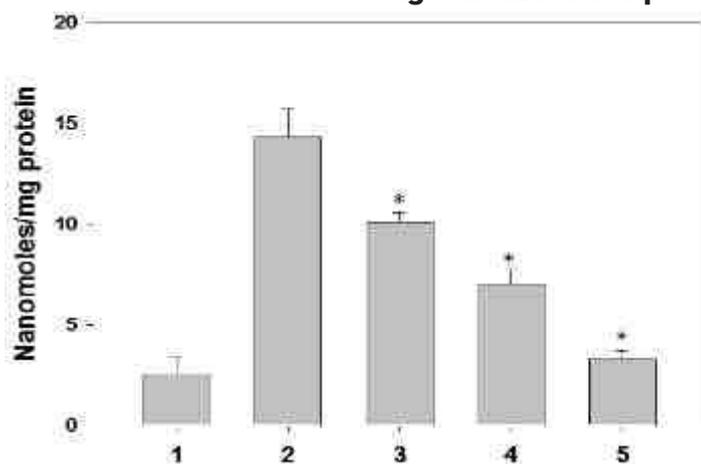
AGE is a collective term referred to a heterogeneous group of chemical structures that range from CML to diverse structures such as pentosidine, argyropyrimidine and vasperlysine. Thus, the effective F2-fraction in reducing some of these AGE such as CML and MGO using antibodies raised against CML-KLH and MGO-BSA was evaluated. Data obtained with immunoblotting demonstrated the presence of diverse antigenic determinants on the surface of protein. For example, anti-CML-KLH has detected some cross-linked species of approximately 22 and 54 kDa along with HMW aggregates above 97 to 200 kDa (Fig 6A). Anti-MGO-BSA demonstrated the presence of cross-linked aggregates near to 22 and 54 kDa and along with HMW aggregates near to 200 kDa (Fig 6B). Interestingly, F2-fraction reduced the formation of all the above-mentioned antigenic AGE-structures on lens proteins in a dose dependent manner (Fig 6). Since these AGE are the main protagonists of diabetic complications, the antiglycating ability and the potential to ameliorate the diabetic complications makes procyanidin-B2 a suitable compound for the treatment of AGE-associated pathologies.

The significance of antiglycating potential of F2-fraction and standard procyanidine-B2 was further assessed by estimating the formation of HbA1c under *ex vivo* conditions. HbA1c is an Amadori product formed from the reaction between glucose and the amino-terminal of valine residue of Hb  $\beta$ -chain. Human blood incubated with  $\delta$ -gluconolactone showed high levels of HbA1c as compared with control blood. Interestingly, F2-fraction and standard procyanidine-B2 inhibited the HbA1c formation in human blood indicating that the F2-fraction acts at early stages of glycation.

**Fig 4. HPLC analysis of F2-fraction of cinnamon (A) and standard procyanidin-B2 (B)**

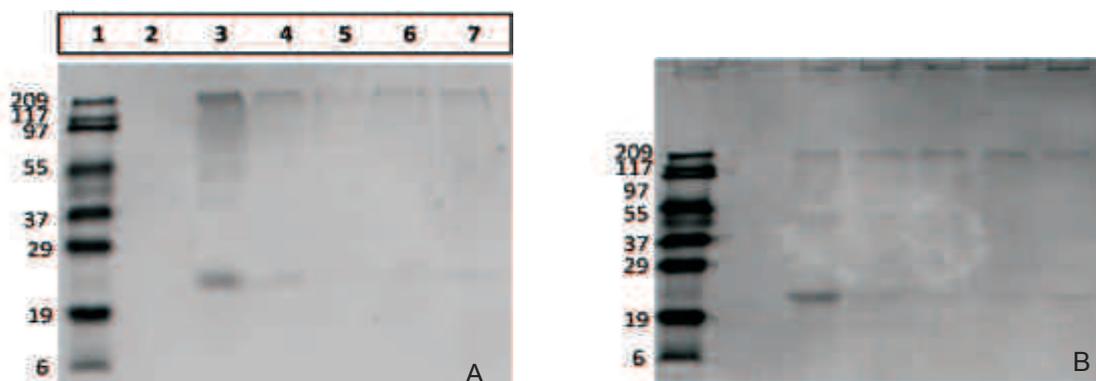


**Fig 5. Inhibition of protein carbonyl content**



Protein carbonyl content of lens protein upon *in vitro* glycation in the absence and presence of LH20 fraction F2. Bar 1, protein alone; bar 2, protein+200 mM fructose (F), bar 3, protein+F+100  $\mu$ g fraction F2; bar 4, protein+F+500  $\mu$ g fraction F2, bar 5, protein+F+1000  $\mu$ g fraction F2. Data are mean SE (n=3) and superscript '\*' denotes significantly different from bar 2 (p<0.05).

**Fig 6. Immunodetection of AGE in lens protein**



Blots of lens protein upon *in vitro* glycation in the absence and presence of F2- fraction and procyanidineB2 were probed with anti-CML-KLH (A) and anti-MGO-BSA (B) antibodies. Lane 1, molecular mass markers; lane 2, protein alone; lane 3, protein+200 mM fructose (F); lane 4, protein +F+ 100  $\mu$ g F2- fraction; lane 5, protein+F+500  $\mu$ g F2- fraction; lane 6, protein+F+1000  $\mu$ g F2-fraction; lane 7, protein+F+100  $\mu$ M procyanidine B2 standard.

## CONCLUSION

These data confirm our initial studies, which showed the presence of antiglycating activity in the crude extracts from cinnamon. The present study describes the characterization of procyanidin-B2 from cinnamon as a new antiglycating agent and its mechanism of action. In conclusion, natural products such as procyanidin-B2 are attractive as therapeutic leads in the treatment of diabetic complications.

## 7. AMELIORATION OF DIABETIC NEPHROPATHY IN RATS BY PROCYANIDIN- B2 FROM CINNAMON THROUGH INHIBITION OF AGE FORMATION

*Diabetes mellitus* is the most common metabolic disease and a leading cause of end-stage renal disease (ESRD). Considerable evidence suggests that chronic hyperglycemia is the major culprit for microvascular complications of diabetes including diabetic nephropathy (DN) that develops in 20-40% of patients with type 1 and type 2 diabetes. During nephropathy, glomerulus exhibits both morphological and functional changes in all elements that constitute the glomerular filtration barrier (GFB) of the kidney: endothelium, glomerular basement membrane, and glomerular podocytes. Alterations in glomerular filtration barrier results in reduced glomerular filtration rate with poor renal outcome ranging from microalbuminuria to overt proteinuria. The clinical manifestations of DN include thickening of glomerular basement membrane (GBM), glomerular hypertrophy, mesangial cell expansion and loss of podocytes. Decreased expression of podocyte proteins such as nephrin and podocin was reported in experimental models of nephropathy. While several mechanisms that may either manifest in podocyte damage or cause glomerular changes in diabetes have been discussed, the precise molecular mechanism that mediates hyperglycemia-mediated podocyte loss remains largely unknown.

Among several biochemical and molecular events that manifest during diabetes, formation of advanced glycation end-products (AGE) has been suggested as a major mechanism in pathogenesis of DN. N-carboxymethyl-lysine (CML), pentosidine and methylglyoxal derivatives are examples of a few well-characterized AGE. The characteristic structural changes of DN such as thickening of GBM and mesangial expansion are accompanied by accumulation of AGE, leading to glomerulosclerosis and interstitial fibrosis. Experimental and human studies suggest that AGE promote inflammation and aggravate diabetic complications by inducing secretion of cytokines and growth factors including MCP-1, TGF- $\beta$  and VEGF. Alternatively, pathological effects of AGE are mediated by their interaction with intracellular receptors for AGE (RAGE). Activation of RAGE by AGE has been implicated in promoting renal damage and development of DN via activating an array of cellular signaling cascades. In rodents, either administration of RAGE neutralizing antibodies, or genetic deficiency in RAGE protected against renal injury in diabetes. Hence, identification and testing of novel anti-AGE agents with greater efficacy is much warranted.

In the earlier work, aqueous extract of cinnamon (*C. zeylanicum*) has significant inhibitory potential against the AGE formation under *in vitro* conditions, was demonstrated. Subsequently it was characterized procyanidin-B2 (PCB2) as the active component of cinnamon extract that is involved in AGE inhibition using bioassay-guided fractionation with eye lens proteins under *in vitro* conditions. In the present study, the effect of cinnamon and PCB2 on the AGE formation and development of early renal manifestation (albuminuria, creatinuria) in experimental diabetic rats was investigated.

## METHODOLOGY

**Experimental design and dietary regimen:** Two-month-old male WNIN rats with an average bodyweight of  $220 \pm 17$  g were used in the study. All the animals were fed with AIN-93 diet *ad libitum*. The control (Group I; n=9) rats received vehicle that consists of 0.1 M citrate buffer, pH 4.5 while the experimental rats received a single ip injection of streptozotocin (STZ) (32 mg/kg) in citrate buffer. About 72 hrs post-injection with STZ, fasting blood glucose levels were monitored. Animals having blood glucose levels  $< 150$  mg/dl were excluded from the experiment and the rest were distributed into three groups (Groups II-IV). Animals in group II received AIN-93 diet alone, group III animals received the AIN-93 diet supplemented with 3% cinnamon powder while group IV animals received AIN-93 diet enriched with 0.002% PCB2. Approximately 3g cinnamon bark powder yields 0.002g PCB2. Based on pilot study, we fed diabetic animals either with 3% cinnamon or 0.002% PCB2 in the diet. Animal care and experimental protocols were in accordance with institutional animal ethical committee. Animals were housed in individual cages in a temperature and humidity-controlled room with a 12 h light/dark cycle. All the animals had free access to water. Food intake (daily) and body weights (weekly) were monitored.

**Biochemical estimations:** Serum glucose was measured by the glucose oxidase-peroxidase (GOD-POD) method using a commercially available kit. HbA1c (RBC), albumin, creatinine (urine) and urea (plasma) were also estimated using commercially available kits.

**Blood, kidney collection and processing:** Blood was collected once a week from the retro-orbital plexus for estimation of glucose. 24 hr urine was collected from experimental animals by placing them in metabolic cages. At the end of 12 weeks duration of diabetes, the animals were sacrificed by CO<sub>2</sub> asphyxiation. Kidneys were perfused via abdominal aorta with 100 ml of normal saline. The left renal vein was punctured to permit the perfusate to drain and the kidney was removed immediately and placed in 4% paraformaldehyde for subsequent histologic studies. The remaining kidney was snap frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for isolation of either RNA or protein for further studies.

**Analysis of RBC-IgG cross-linking:** The amount of IgG bound to RBC was quantified using ELISA as described earlier.

**Quantitative Real-Time PCR:** Total RNA was extracted using tri-reagent. Isolated RNA was further purified by RNeasy Mini Kit and quantified. Four  $\mu\text{g}$  of total RNA was reverse transcribed using high capacity cDNA reverse transcription kit. Real-time PCR was performed in triplicates with 25 ng cDNA templates using SYBR green RT-PCR kit with gene specific primers. Normalization and validation of data were carried using  $\beta$ -actin as an internal control and data were compared between control and diabetic samples according to comparative C<sub>T</sub> ( $2^{-\Delta\Delta\text{Ct}}$ ) method.

**Immunohistochemistry:** Kidneys were collected at the end of animal experiment and fixed in 4% paraformaldehyde in sodium phosphate buffer (pH 7.2), followed by embedding and sectioning using standard protocols. Immunolocalization of nephrin, podocin, CML, PKC- $\alpha$  and VEGF were carried out on 4  $\mu\text{M}$  thick paraffin sections of kidneys taken on chrome alum gelatin coated slides. The kidney sections were deparaffinized and antigen retrieval was done by heating the slides in 0.1 M sodium citrate, pH 6.0 for 10 min at  $60^{\circ}\text{C}$  in microwave oven. The endogenous peroxidase activity was quenched by incubating the slides in 3% H<sub>2</sub>O<sub>2</sub> for 30 min. To prevent non-specific binding of the antibody, blocking was done by incubating the slides in 10% normal goat serum in PBS at room temperature for 1 hour. The slides were incubated overnight at  $4^{\circ}\text{C}$  with primary antibody in 1% normal goat serum in PBS. Subsequently incubated with biotinylated secondary antibody for 30 min and followed by incubation of slides for 30 min with Vectastain elite ABC reagent. The proteins were localized by brown staining in the sections by addition of DAB solution containing H<sub>2</sub>O<sub>2</sub>. Slides were observed under an epifluorescence microscope and images were captured.

**Western Blot Analysis:** Tissue lysates were prepared in homogenization buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.5 % sodium deoxycholate, 0.1 % SDS) containing protease inhibitor, 1 mM sodium orthovanadate and 50 mM NaF. Lysates were centrifuged at 12,000xg and aliquots of the supernatants were separated by 12 % SDS-PAGE and transferred to nitrocellulose membrane. After probing with

corresponding primary antibodies, antigen-antibody complexes were detected with horseradish peroxidase-labeled secondary antibodies, respectively, and visualized using enhanced chemiluminescence reagents.

**Statistical analysis:** Data were expressed as mean±SEM unless otherwise stated. One-way analysis of variance (ANOVA) with pair wise comparisons according to the Tukey method was used in this study. Differences were considered significant if the p-value was less than 0.05.

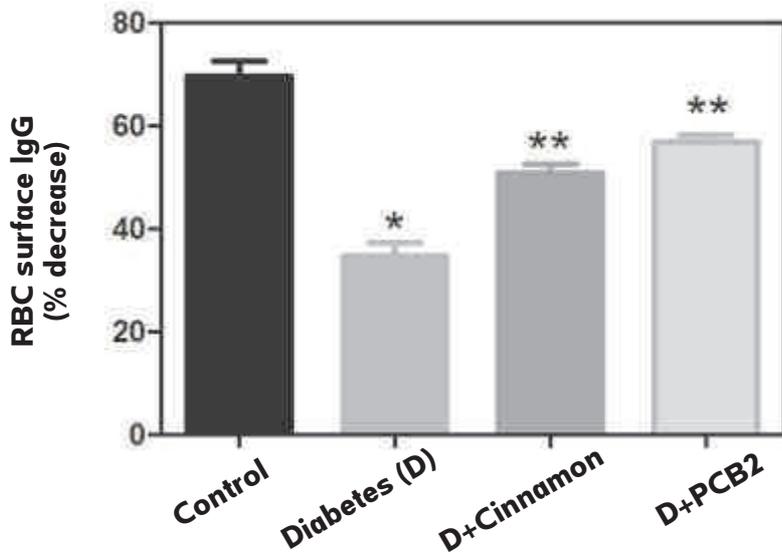
## RESULTS

- 1. Animal characteristics:** Diabetic rats showed increase in food intake compared with the control animals. Despite increased food intake, the bodyweight of diabetic animals decreased significantly compared with non-diabetic control animals (Table 1). Fasting blood glucose levels were elevated in diabetic rats and persisted for 12 weeks of the study compared with control animals. While, feeding of cinnamon and PCB2 to diabetic rats had no effect on the altered body weights, the blood glucose levels were marginally lowered (but statistically significant) (Table 1). The toxic effects of feeding cinnamon or PCB2 to control rats was not noticed and they were similar to untreated control animals.
- 2. Diabetes induced protein glycation was inhibited by cinnamon and PCB2:** Glycated hemoglobin (HbA1c) levels in untreated diabetic rats were significantly higher compared with control animals (Table 1). While feeding of PCB2 ameliorated HbA1c accumulation in experimental diabetic rats, cinnamon had no significant effect in preventing HbA1c accumulation in diabetic rats (Table 1).
3. Further, the ability of cinnamon and PCB2 to prevent cross-linking of IgG on red blood cell surface (RBC-IgG) was investigated. During diabetic conditions, there is a considerable increase in RBC-IgG cross-linking that provide an index of AGE mediated protein cross-linking. Dietary supplementation with cinnamon and PCB2 significantly decreased RBC-IgG cross-linking by 45% and 40% respectively compared to that of diabetic rats (70%) (Fig. 1A).
4. Further, whether cinnamon and PCB2 prevent the accumulation of CML, an AGE that is abundant in renal tissues of diabetic rats was investigated. Immuno-histochemical examination revealed that cinnamon and PCB2 prevented the CML formation in glomeruli of diabetic rats (Fig.1B). Immunoblotting also substantiate immunehisto-chemical findings that cinnamon and PCB2 prevented the CML formation in kidney of diabetic rats (Fig.2).
5. The cytotoxicity of AGE in cells is mediated via cell-surface receptor for AGE (RAGE). Therefore, expression of RAGE in these experimental animals was measured. Increased expression of RAGE was observed in diabetic rat kidney compared with control rats. Interestingly, cinnamon and PCB2 prevented hyperglycemia induced expression of RAGE in diabetic rat kidney (Fig.2).
- 6. Diabetes induced PKC expression is prevented by dietary cinnamon and PCB2 intervention:** The activation of protein kinase C-α (PKC-α) in the kidney from diabetic animal is well known and

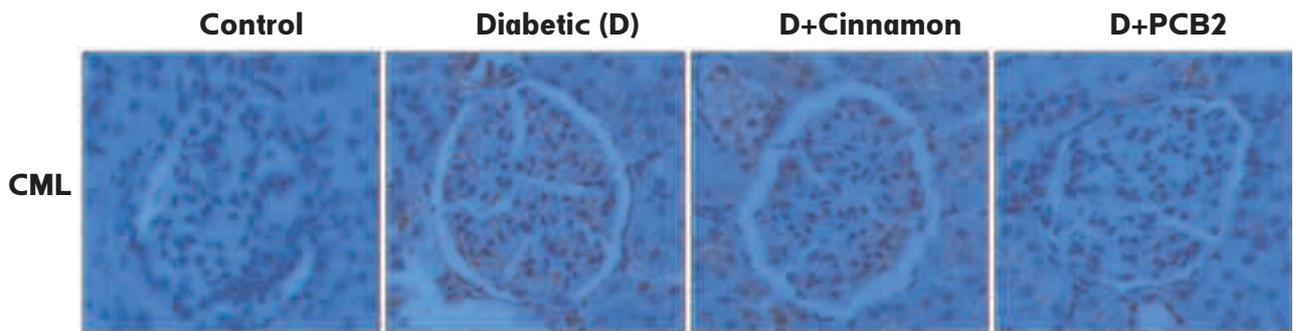
**Table 1. Effect of cinnamon and PCB2 on body weight, blood glucose, glycosylated haemoglobin, plasma urea, and albumin to creatinine ratio in diabetic rats.** These parameters were measured at the end of 12 weeks of chronic hyperglycemia.

Parameter	Control	Diabetic (D)	D+Cinnamon	D+PCB2
Body weight (g)	264±5.0	217±4.9*	234±4.9	241±6.2
Blood glucose (mg/dL)	89±2.0	365±13.0*	329±9.2*	311±10.2*
Glycosylated Hb (%)	6.72±0.54	11.9±0.72*	10.8±0.61	9.00±0.36**
Urea in plasma (mg/dL)	35.3±6	82±5*	71±7	44 ±4**
Albumin/creatinine(µg/mg)	1760±135	5870±225*	3920±236**	1710±214**

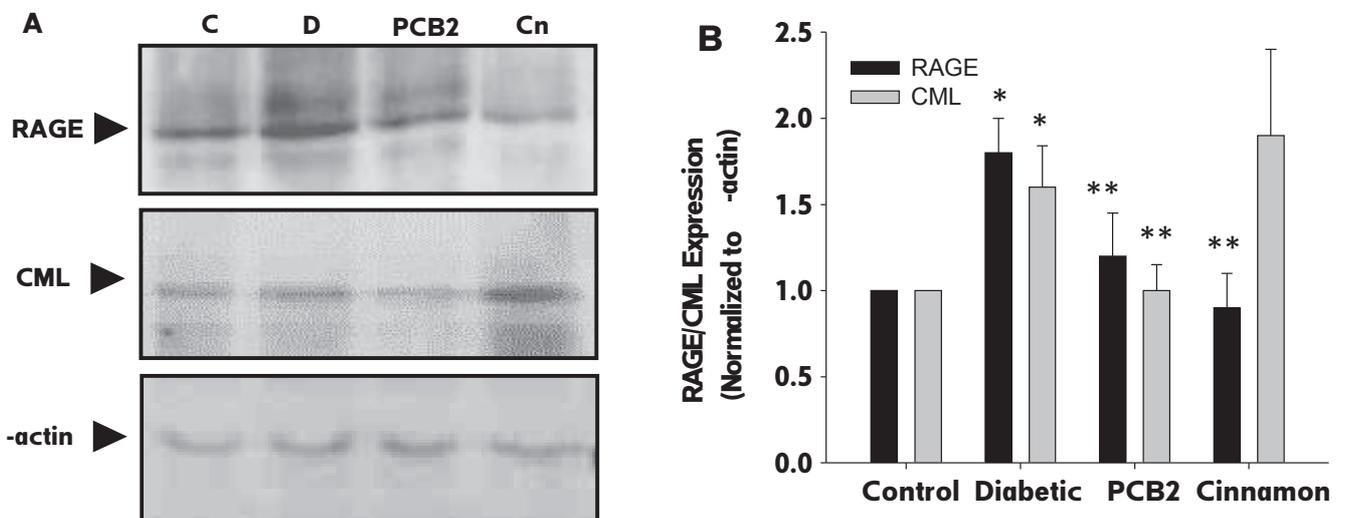
Data are mean±SE (n=6) and superscript (\*) denotes significantly different from control and superscript (\*\*) denotes significantly different from diabetes (p<0.05).



**Fig 1. Cinnamon and PCB2 inhibit protein glycation and AGE formation in diabetic rats.** AGE mediated RBC-IgG cross-linking (A), immunohistochemical analysis of glomerular accumulation of CML (B). Data in panel A were presented as mean±SE (n=6). (\*p<0.01, vs control rats; \*\*p<0.01, vs diabetic rats) and data in panel B are representative of three independent analyses. The images in panel B were taken at 400X magnification.



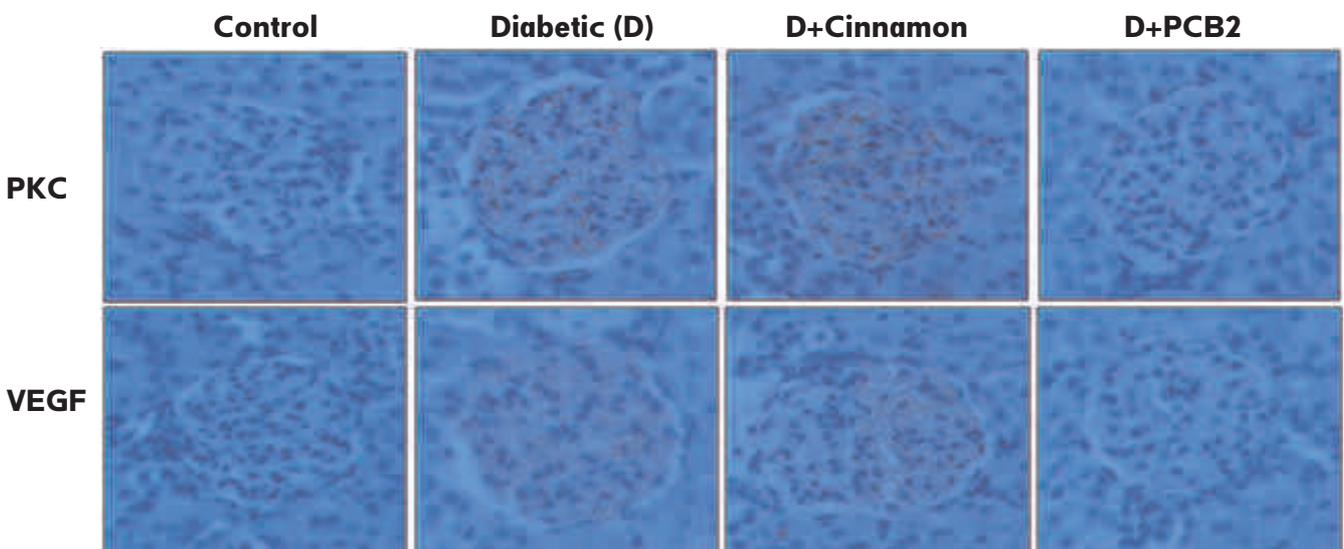
**Fig 2. Panel A- Immunoblotting analysis of CML and RAGE from control, diabetic and diabetic rats treated with cinnamon or PCB2. Panel B-Densitometric quantification of expression of CML and RAGE normalized to  $\beta$ -actin.** Data in panel B were presented as mean±SEM, n=6. (\*p<0.01, vs control rats; \*\*p<0.01, vs diabetic rats).



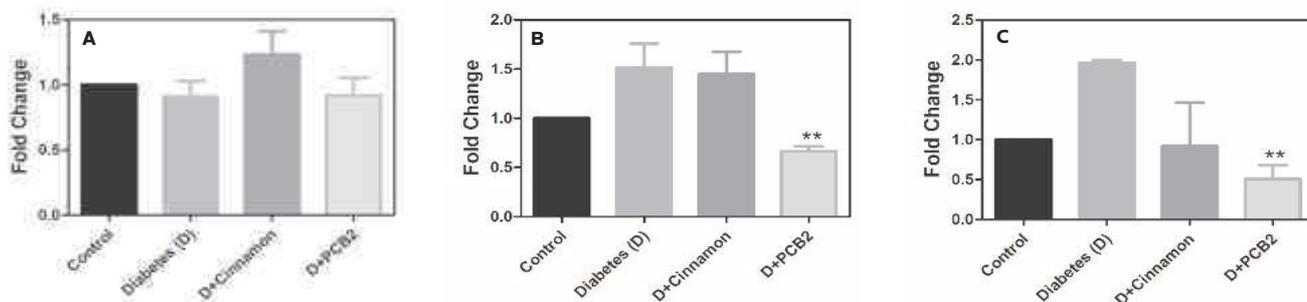
increased expression in podocytes in renal biopsies of patients with DN has been reported. It is evident from earlier studies that AGE induces activation of PKC- $\alpha$ . Therefore, PKC- $\alpha$  in experimental rats were measured. In accordance with earlier studies, immunohistochemical staining revealed that PKC- $\alpha$  expression is increased in glomeruli from diabetic rat. Interestingly, cinnamon and PCB2 feeding prevented the hyperglycemia mediated expression of PKC- $\alpha$  in diabetic rat kidney (Fig.3).

7. To elucidate possible mediators of PKC- $\alpha$  in renal damage under hyperglycemic conditions, we analyzed glomerular expression of VEGF. In untreated diabetic rats, increase in VEGF expression was observed (Fig.3). In contrast, this increase in VEGF expression is significantly reduced in diabetic rats fed with PCB2, but not completely prevented in cinnamon fed rats (Fig.3).
8. **Anti-AGE effect of cinnamon and PCB2 prevented diabetes induced expression of cell adhesion and inflammatory molecules:** Cell adhesion molecules promote endothelial dysfunction through perturbations in coagulation, permeability, vasomotor function and cell adhesion, leading to the development of macro and microvascular renal lesions. The expression of ICAM was not induced during diabetes (Fig. 4). Alternatively vascular cell adhesion molecule-1 (VCAM) in diabetic rats was also measured. There was an increased expression of VCAM in untreated diabetic rats (Fig. 4). Though, dietary supplementation of cinnamon had meager effect on VCAM expression in diabetic rats, PCB2 significantly prevented the hyperglycemia induced VCAM expression (Fig. 4). Monocyte chemoattractant protein-1 (MCP-1), an inflammatory cytokine, levels were elevated in response to AGE and activates the macrophage infiltration into renal tissue. Elevated expression of MCP-1 in untreated diabetic rats is prevented with dietary supplementation of cinnamon and PCB2 (Fig. 4).
9. **Cinnamon and PCB2 prevented depletion of podocyte slit-diaphragm proteins:** Expression of nephrin and podocin in diabetic rats was analyzed. It was found that, mRNA expression of both nephrin and podocin were decreased by 40% in diabetic rat glomerulus (Fig.5). Dietary supplementation of cinnamon and its active component PCB2 attenuated the hyperglycemia mediated loss of glomerular nephrin and podocin expression. In concurrence with RT-PCR analysis, immunohistochemical staining and immunoblotting analysis found diminished expression of nephrin and podocin in untreated diabetic rats whereas cinnamon and PCB2 supplementation mitigated the loss of glomerular nephrin and podocin expression (Fig. 6).

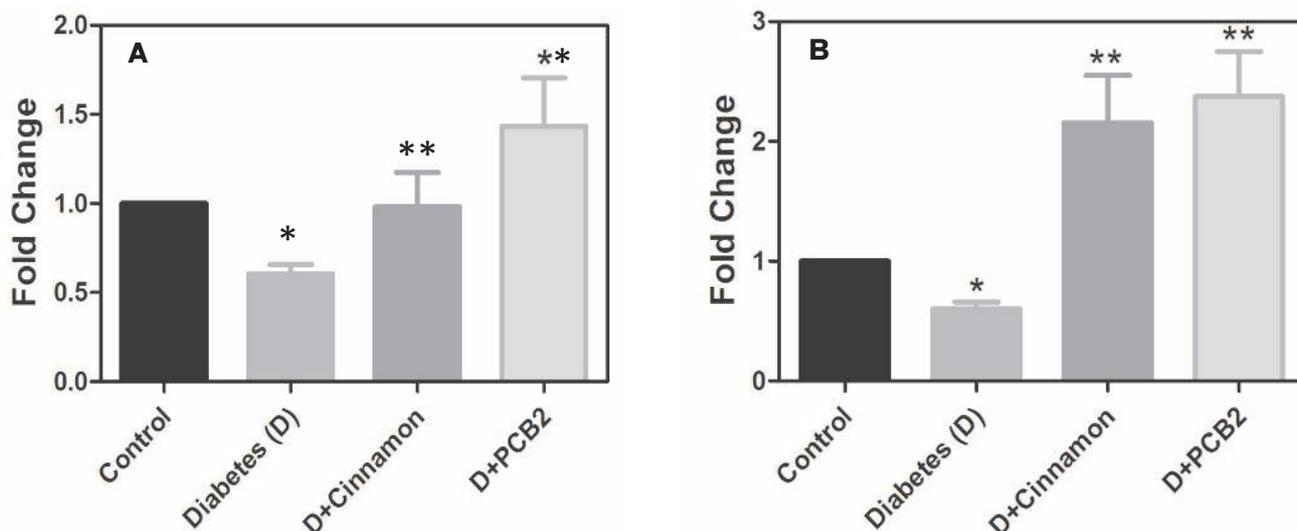
**Fig 3. Cinnamon and PCB2 mitigate the glomerular PKC- and VEGF expression in diabetic rats:** Immunohistochemical staining using anti-PKC- $\alpha$  upper panel) and anti-VEGF antibodies (lower panel) on paraffin sections of kidney cortex of control, diabetic, diabetic rats treated with cinnamon and PCB2. Original images were taken at 400X magnification. Data are representative of three independent analyses



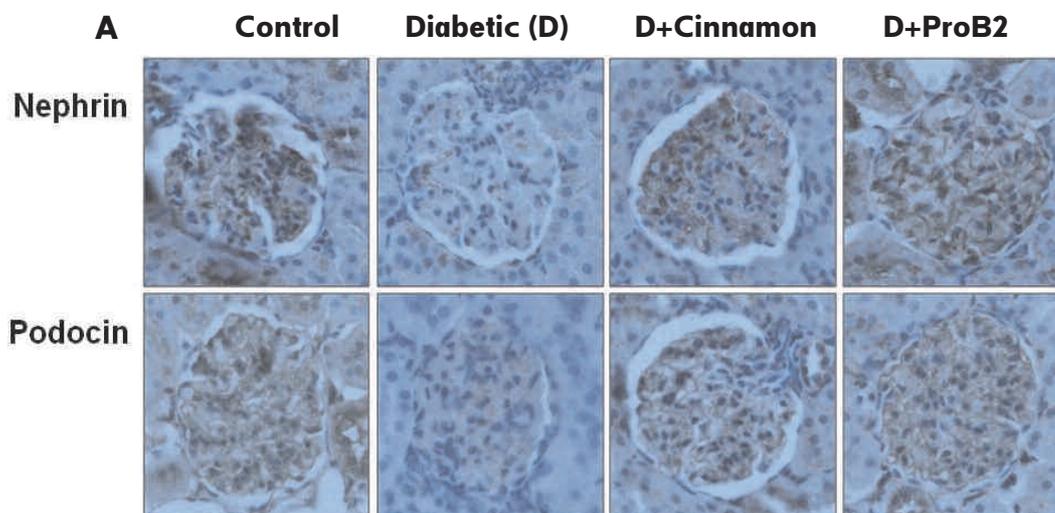
**Fig 4. Cinnamon and PCB2 prevented renal expression of cell adhesion and inflammatory molecules in diabetic rats:** Quantitative RT-PCR analysis of steady state expression of ICAM (A), VCAM (B) and MCP-1 (C) in control, diabetic and diabetic rats treated with either cinnamon or PCB2. Data are mean  $\pm$  SEM, n=6. (\* $p$ <0.01, vs control rats; \*\* $p$ <0.01, vs diabetic rats).

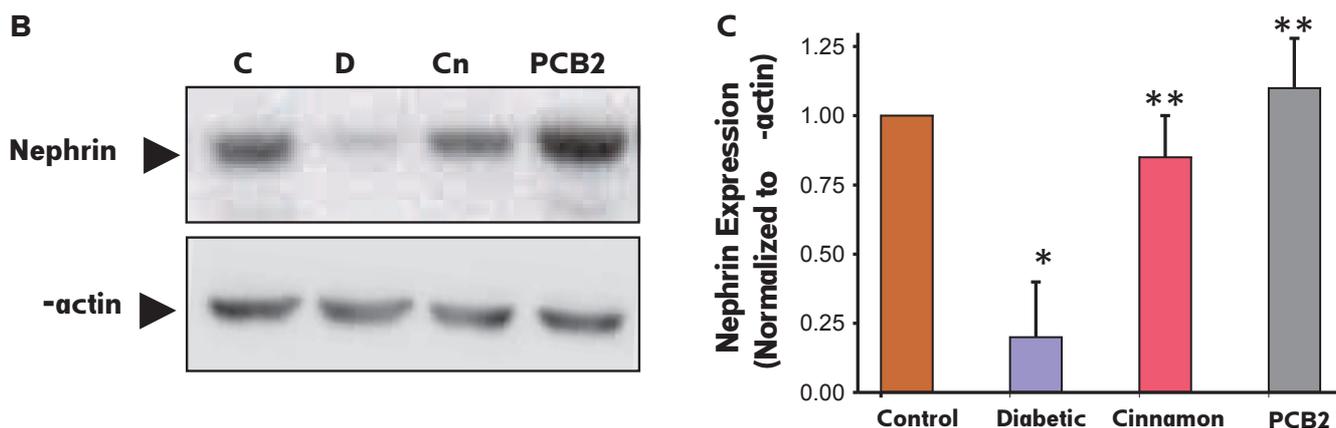


**Fig 5. Inhibition of AGE by cinnamon and PCB2 preserve nephrin (A) and podocin (B) expression in glomeruli from diabetic rats:** Data are mean  $\pm$  SEM, n=6. (\* $p$ <0.01, vs control rats; \*\* $p$ <0.01, vs diabetic rats).



**Fig 6. Panel A-** Immunohistochemical analysis of glomerular expression of nephrin (upper panel) and podocin (bottom panel); Immunoblotting analysis of nephrin (B) and its densitometric quantification of expression (C). Data are mean  $\pm$  SEM, n=6. (\* $p$ <0.01, vs control rats; \*\* $p$ <0.01, vs diabetic rats).





10. **Cinnamon and PCB2 ameliorate proteinuria in diabetic rats:** Diabetic rats excreted twice the amount of albumin compared to control rats. Urinary albumin content is significantly decreased in rats fed with cinnamon and PCB2 (Table 1). Diabetic rats excreted greater amounts of creatinine compared with non-diabetic control rats whereas feeding diabetic rats with cinnamon and PCB2 resulted in the diminished excretion of creatinine (Table 1). Noticeably, PCB2 is more effective in preventing creatinine excretion in diabetic rats than cinnamon. Furthermore, we estimated urea content in the plasma. Diabetic animals displayed  $82 \pm 5$  mg/ml urea in plasma against  $35 \pm 6$  mg/ml in control rats. Plasma urea content was restricted to  $71 \pm 7$  and  $44 \pm 4$  mg/ml in cinnamon and PCB2 fed rats, respectively (Table.1).

## CONCLUSION

The results obtained in the present study suggest that cinnamon and particularly its active principle PCB2 ameliorate diabetes-induced proteinuria and podocyte injury in rats. This effect, at least in part, could be attributed to suppressing renal AGE-RAGE stimulated MCP-1 and PKC- $\alpha$  expression, thereby modulating slit diaphragm proteins nephrin and podocin expression. Furthermore, this study emphasizes the pivotal role that AGE inhibitors in the prevention of DN and offers an important nutraceutical based therapeutic approach to combat diabetic kidney diseases.

## 8. EFFECT OF SOLUBLE LUTEIN AND SOLUBLE CURCUMIN AGAINST DIABETIC CATARACT

Diabetes is one of the most occurring non-communicable, heterogeneous, metabolic disorders characterized by hyperglycemia resulting from defective insulin production, resistance to insulin action or both. According to the latest WHO estimation currently there are about 366 million diabetic people in the world, it is expected to increase to 552 million by 2030 and India has about 62 million diabetics. Prolonged exposure to chronic hyperglycemia can lead to various complications including vascular and non-vascular complications. The insulin independent tissues such as retina, kidney, peripheral nerves and lens are most affected tissues in long term complications of the diabetes, which results in the development of diabetic retinopathy, nephropathy, neuropathy and cataract respectively. Cataract is characterized by opacity of the eye lens, and the leading cause of blindness worldwide. The development of cataract in diabetes is 2-5 times more when compared with the non-diabetic counterparts. Furthermore, patients with diabetes mellitus have higher complication rates from cataract surgery. Both diabetes and cataract pose

an enormous health and economic burden, particularly in developing countries, where diabetes treatment is insufficient and cataract surgery often inaccessible. Though the etiology of cataract is not fully understood, oxidative damage to the constituents of the eye lens is considered to be a major mechanism in the initiation and progression of various types of cataracts, including diabetic cataract. This research has prompted the use of dietary antioxidants in an attempt to slow the cataract progression.

Lutein and zeaxanthin are xanthophylls that belong to the class of organic compounds called carotenoids. These are present in macula of the human eye which are very useful to protect the eye from the high frequency light and reduce the risk of age related macular degeneration and cataract development. Lutein and zeaxanthin are synthesized by plants, found in high quantities in green leafy vegetables and fruits such as kale, spinach, collards, Broccoli, yellow carrots, green peas, corn, oranges, and eggs. Previous studies reported that rats treated with combination of insulin and lutein showed delayed development and maturation of cataract, than when treated with lutein or insulin alone, and also could prevent the diabetes-induced decrease of glutathione content (3). Earlier studies conducted in the laboratory showed that lutein delayed diabetic cataract in rats at 1% in the diet but not at (0.1%).

Further, lutein (1%) only delayed but could not completely prevent diabetic cataract. Previously we have also shown that curcumin at 0.01% in the diet delayed diabetic cataract in rats. In this background, the main objective of the study is to investigate the effect of soluble lutein and soluble curcumin in comparison to regular lutein and curcumin in preventing or delaying diabetic cataract in streptozotocin (STZ) induced diabetic rats. The assumption is that increasing bioavailability of lutein and curcumin with soluble forms will improve their therapeutic efficacy. Omni Active Health technologies developed the technology named Ultra SOL Dry Nutrient System (DNS) through which lutein formulated into a more bioavailable product with trademark Lutemax-2020™. Safety assessment studies of Lutemax 2020™ confirmed its safety in both acute and sub chronic toxicity studies in Wistar rats. The purpose of this study is to evaluate the potential protective effects of soluble lutein and soluble curcumin over their corresponding regular formulations against diabetes-induced cataract in rats.

## METHODOLOGY

Male WNIN rats (2 months old; average bw 213±14 g) were acclimatized in experimental room for two weeks. Diabetes was induced in overnight fasted animals by a 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 the control (Group I; n=12). Fasting blood glucose levels were measured 72 h after STZ injection. Animals having blood glucose levels >150 mg/dL were considered diabetic and were divided into five groups (Group II- VI). A group of control rats (n=6) were fed with 0.01% soluble curcumin (Group VII) and soluble % lutein alone (Group VIII). All the animals were housed in individual cages maintained on their respective diets for 12 weeks and drinking water was provided ad libitum throughout the study period.

**Animal Care:** Institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the IAEC (institutional animal ethical committee) of National Institute of Nutrition. Animals were housed in individual cages in a temperature and humidity-controlled room with a 12-h light/dark cycle. All the animals had free access to water. Food intake (daily) and body weights (weekly) were monitored. During the course of the study, 3 animals in Group II and 2 animals each from Groups III-VI had expired due to hyperglycemia.

### Experimental groups and diets

	Group	Number	Diet
I	Control	12	AIN 93
II	Diabetic	14	AIN 93
III	Diabetic + SC	12	AIN 93 with soluble curcumin 0.01 %
IV	Diabetic + RC	12	AIN 93 with regular curcumin 0.01 %
V	Diabetic + SL	12	AIN 93 with soluble lutein 0.5 %
VI	Diabetic + RL	12	AIN 93 with regular lutein 0.5 %
VII	Control + SC	6	AIN 93 with soluble curcumin 0.01 %
VIII	Control + SL	6	AIN 93 with soluble lutein 0.5 %

**Slit lamp examination and cataract grading:** Eyes were examined every week using a slit lamp biomicroscope on dilated pupils. Initiation and progression of lens opacity was graded into five categories (0-4) as described by us earlier.

**Blood/ lens collection and processing:** Blood was drawn once every week from retro orbital plexus for glucose and insulin estimation. At the end of 12 weeks, animals were sacrificed by CO<sub>2</sub> asphyxiation and lenses were dissected by posterior approach and stored at -70°C until further analysis. A 10% homogenate was prepared from 3-5 pooled lenses in 50 mM phosphate buffer, pH 7.4. All the biochemical parameters were analyzed in the soluble fraction of the lens homogenate (15,000x g at 4°C) except for lens malondialdehyde (MDA), which was determined in the total homogenate.

**Biochemical estimations:** Lens MDA, as thiobarbituric acid reacting substances (TBARS), protein carbonyl content were determined according to the methods described previously. Total, soluble and insoluble protein was assayed by Lowry method using BSA as standard.

**Statistical analysis:** One-way ANOVA was used for testing statistical significance between groups of data and individual pair difference was tested by means of Duncan's multiple-range test. Heterogeneity of variance was tested by the non-parametric Mann Whitney test where p<0.05 was considered as significant.

## RESULTS

**1. Fasting blood glucose:** The plasma glucose concentrations of the untreated diabetic rats were significantly higher than that of the control rats throughout the experiment (Fig 1). Although, there was a marginally lower mean fasting plasma glucose levels in groups treated with soluble curcumin (SC) and soluble lutein (SL), no significant effect of treatment on plasma glucose in diabetic rats was observed (Fig 1).

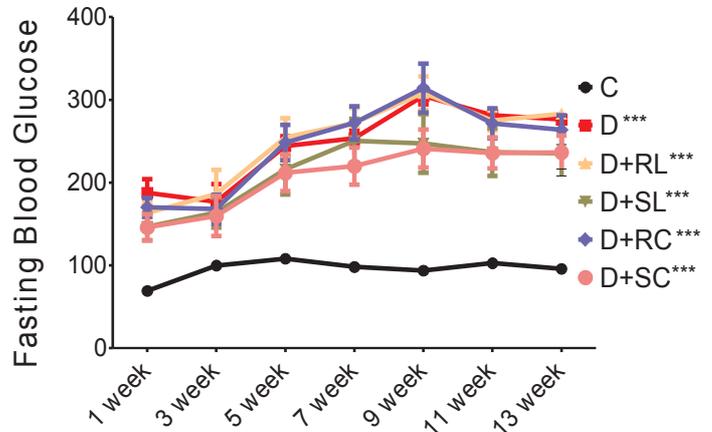
### 2. Cataract development and progression:

The onset of cataract due to hyperglycemia was observed in diabetic animals after 3-4 weeks of diabetes induction. The average incidence of cataract was calculated and presented in Fig 2. Though there was no delay in the onset there is a clear delay in the progression and maturation of cataract in soluble curcumin (SC) and soluble lutein (SL) treated groups when compared to untreated diabetic group (D). Group-D animals showed lens opacification (stage-IV) by the end of 10<sup>th</sup> week while the treatment groups showed stage-2.5 to 3. The data clearly indicates there is a significant delay in the progression and maturation of cataract in intervention groups from sixth week onwards when compared to group-D. At the end of ten weeks, the severity of cataracts was significantly lower in groups D+RL (stage 3.1), D+SL (stage 2.7), D+RC (stage 3.0) and D+SC (stage 3.2) than in Group-D (Stage 4), indicating that intervention delayed the maturation of diabetic cataract due to slow progression, Further SL seems to be more effective than RL but SC has not shown any superiority in efficacy over RC in progression of cataract. All the lenses in control group during the entire experimental period appeared to be normal, clear and free of opacities.

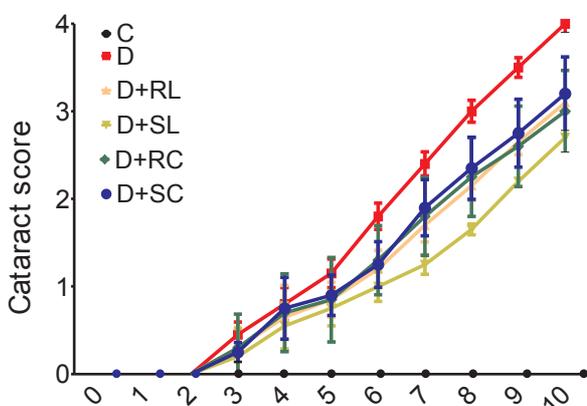
**3. Glycated haemoglobin levels (HbA1c):** Glycated haemoglobin (HbA1c) is the marker of chronic glycemic status. The data obtained suggested that there was a significant higher HbA1c levels in diabetic animals (>6.5%) (Fig 3). Feeding of diabetic rats with soluble curcumin (SC) and soluble lutein did not decrease the HbA1c levels compared to untreated diabetic group. However, SC decreased the

**Fig 1. Effects of soluble curcumin and soluble lutein on fasting plasma glucose in diabetic rats.**

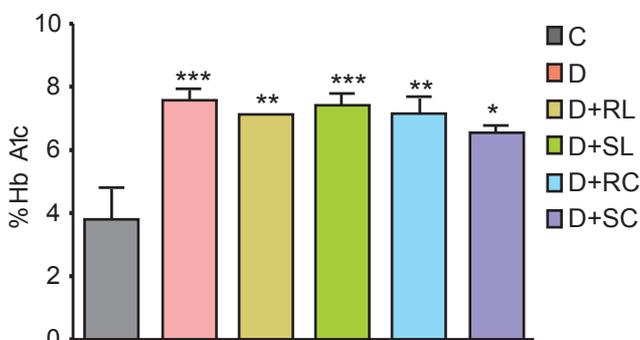
Data were expressed as mean ± SEM. Control (control rats); D (diabetic rats); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein); D+RC (diabetic+regular curcumin); D+SC (diabetic + soluble curcumin). \*\*\*=p< 0.001



**Fig 2. Delay of diabetic cataract in rats by soluble curcumin and soluble lutein.** Data were expressed as mean  $\pm$  SEM. Control (control rats); D (diabetic rats); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein); D+RC (diabetic+ regular curcumin); D+SC (diabetic + soluble curcumin).



**Fig 3. Effects of soluble curcumin and soluble lutein on glycated haemoglobin levels.** Data were expressed as mean  $\pm$  SEM. Control (control rats); D (diabetic rats); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein); D+RC (diabetic+ regular curcumin); D+SC (diabetic+soluble curcumin). \*\*\*P<0.001; \*\*P<0.01 and \*P<0.05.



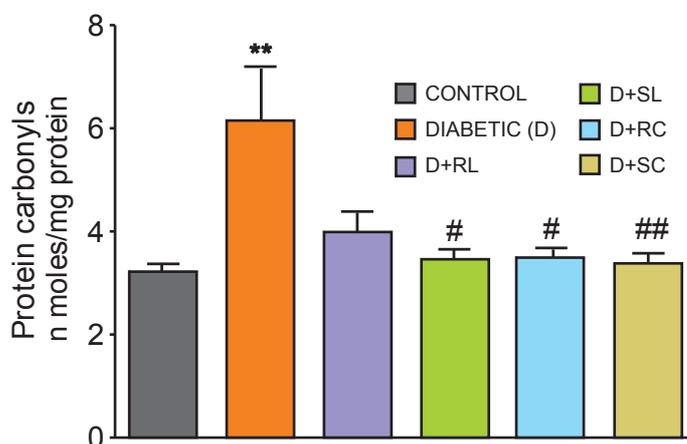
HbA1c percentage moderately not statistically significant. These results were in agreement with fasting blood glucose pattern between groups.

**4. Protein content and protein solubility:** The total and soluble protein content in the lenses of all the experimental groups was analyzed. There was a significant decrease in both total and soluble protein in untreated diabetic group compared with the control group (Table 1). This could be due to a partial leakage of proteins into the aqueous humor or aggregation of proteins and insolubilization. Among the treatment groups, SL and RC significantly prevented loss of soluble protein compared to group-D, whereas, SL alone had shown significant difference against group D in percentage of soluble protein. Though SC and RL had shown partial beneficial effect in preventing insolubilization of lens proteins but were not significant statistically (Table 1).

**5. Protein carbonyls:** Protein carbonylation is a consequence of oxidative stress. The untreated diabetic group showed significant elevation in protein carbonylation compared to control group (Fig 4). Among the treatment groups, except RL-group, all the intervention groups (SL, RC and SC) showed significantly lower carbonyls than untreated diabetic group (Fig 4). The results indicated the beneficial effect of SL over RL. However, both RC and SC demonstrated significant inhibition of protein carbonyl formation (Fig 4).

**6. Lipid peroxidation:** Besides protein carbonylation, lipid peroxidation is another important biomarker for oxidative stress. In order to know the status of oxidative stress in the lens we measured levels of TBARS as an indication of lipid peroxidation. The levels of TBARS in untreated diabetic group are higher than control group (Fig 5). TBARS levels of groups RL and RC are not statistically significant with untreated diabetic group whereas group-SL and SC showed significantly lower TBARS level when compared to untreated diabetic group (Fig 5). This suggest the efficacy of

**Fig 4. Protein carbonyl content in lens proteins.** Data were expressed as mean  $\pm$  SEM. Control (control rats); D (diabetic rats); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein); D+RC (diabetic+regular curcumin); D+SC (diabetic+soluble curcumin).\*\*\*p< 0.001, \*\* P<0.01 and \*P<0.05 vs C; ## =P<0.01 and # P< 0.05 vs D.



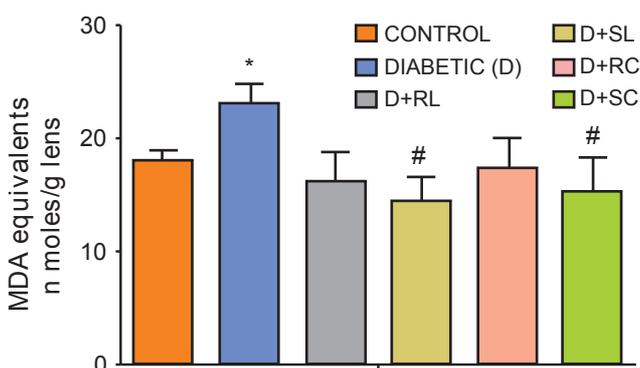
both SL and SC over RL and RC, respectively in preventing lipid peroxidation in lens under diabetic conditions.

**7. Sorbitol levels:** There were significantly elevated levels of sorbitol in untreated diabetic group when compared with control group (Fig 6). Among the intervention groups, except SC, remaining treatments have not lowered sorbitol accumulation compared to untreated diabetic group. Group-SC showed significantly lower sorbitol levels when compared to untreated diabetic group (Fig 6). This might be attributed to the additional pharmacological action of curcumin as aldose reductase inhibition.

**Table 1. Protein content in total and soluble fraction of lens homogenate.** Data were expressed as mean ± SEM (n=6). Control (control rats); D (diabetic rats); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein); D+RC (diabetic+regular curcumin); D+SC (diabetic + soluble curcumin). \*\*\*p<0.001, \*\*P<0.01 and \*P<0.05 vs C; ##P<0.01 and #P<0.05 vs D.

Group	Total protein (mg/g lens)	Soluble protein (mg/g lens)	Percentage soluble protein
Control (C)	516.5±9.3	388.77±8.2	75.19±2.2
Diabetic (D)	281.3±31.0***	128.8±11.0***	46.8±2.5***
D+RL	311.7±7.8	156.5±4.7	50.35±1.9
D+SL	376.5±20.9	228.7±14.3##	60.08±1.8#
D+RC	368.5±19.2	202.6±21.6#	54.43±3.8
D+SC	351.8±41.5	178.9±25.9	50.16±1.3

**Fig 5. Effects of soluble curcumin and soluble lutein on lipid peroxidation.** Data were expressed as mean ± SEM. Control (control rats); D (diabetic rats); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein); D+RC (diabetic+regular curcumin); D+SC (diabetic+soluble curcumin). \*P<0.05 vs C; # =P<0.05 vs D



**Fig 6. Effects of soluble curcumin and soluble lutein on sorbitol levels.** Data were expressed as mean ± SEM. Control (control rats); D (diabetic rats); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein); D+RC (diabetic+regular curcumin); D+SC (diabetic+soluble curcumin). \*P<0.05 vs C; # P<0.05 vs D.



## CONCLUSION

In conclusion, soluble lutein is more effective in delaying diabetic cataract compared to regular lutein at dose of 0.5% in the diet which is reflected in molecular analysis related to catarctogenesis. Increased bioavailability of soluble lutein might explain the observed biological effects of soluble lutein compared to regular lutein. However, the efficacy of soluble curcumin was almost comparable with regular curcumin, and the reasons needs to be investigated further.

## 9. EVALUATION OF WNIN/Gr-Ob RAT AS MODEL FOR OBESITY ASSOCIATED TYPE 2 DIABETIC COMPLICATIONS: EFFECT OF GINGER ON TYPE 2 DIABETIC CATARACT

Type 2 diabetes (T2D) is most common form of diabetes which accounts for nearly 90-95% of the diabetic people in the world. There are several factors which can influence the development of T2D in humans. Obesity has been recognized as an important risk factor for T2D. There are several epidemiological and clinical evidences that support the association as well as increased risk of T2D in obese people. In recent years it has been reported that the prevalence of obesity particularly abdominal obesity and T2D has been increasing in India. This increased prevalence of obesity has a direct correlation with the increasing prevalence of T2D in India. Chronic diabetes can lead to various complications such as cataract, retinopathy, nephropathy, neuropathy and cardiovascular problems. Further, obesity is associated with several degenerative diseases, including coronary heart disease, type 2 diabetes, hypertension, certain types of cancers and some ocular disorders. The ocular complications of obesity include diabetic retinopathy, cataracts, and macular degeneration, and exophthalmos. However, there are no experimental studies to explain the underlying molecular basis of obesity associated T2D complications. Although, there are some experimental animal models such as ob/ob and db/db mouse models, Zucker and Koletsky rats to study obesity associated type 2 diabetes, molecular basis of obesity and T2D associated complication has not been investigated. Further, these models have a defined genetic background, the etiology of obesity and T2D is multifactorial. Hence, there is a need to evaluate suitable models to understand mechanisms involved in obesity associated T2D induced complications.

At the National Center for Laboratory Animal Sciences (National Institute of Nutrition), a spontaneously developed obese rat was isolated from the existing WNIN stock of rats, and a colony of WNIN-Obese (WNIN/Ob) rats was established by selective breeding. Subsequently, it bifurcated into another strain with impaired glucose tolerance (IGT)- WNIN/GR-Ob (1,2). The phenotype and associated biochemical, histological and pathophysiological characteristics of WNIN/Ob and WNIN/GR-Ob rats have been reported in detail elsewhere (2,3). In essence, starting from 35-40 days of their age, WNIN/Ob and WNIN/GR-Ob phenotype are different from its respective lean littermate and there is a progressive increase in body weight until the age of 6-9 months. In addition to IGT, WNIN/GR-Ob rats show hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia. Further, these rats also develop a few degenerative conditions such as retinal degenerations as early as 4 months of their age. An increased susceptibility for development of cataract due to diabetes in WNIN/GR-Ob was reported.

Neonatal streptozotocin (nSTZ)-induced rodent model is well recognized model for T2D. Earlier this nSTZ model were used for studying, variety of T2D associated complications. Hence, in the present study we evaluated WNIN/GR-Ob rats for type-2 diabetic cataract by injecting STZ to two day old rat pups. Since ginger showed hypoglycemic, anti-lipidemic and anti-obesity activity, we studied protective effect of ginger against obesity associated diabetic cataract and the associated biochemical alterations.

### METHODOLOGY

**Experimental design and dietary regimen:** Two-day old WNIN/GR-Ob rat pups (45) were injected with STZ at a dose of 90 mg/kg body weight dissolved in 0.1M citrate buffer, pH 4.5. Control pups (18) received only vehicle. After weaning, obese pups were identified from non-obese (lean) after 40 days and maintained in individual cages. All STZ injected obese rats developed mild hyperglycemia by one month but not lean rats. Further, obese diabetic rats were subdivided into two groups; one group maintained on AIN93G/M diet served as diabetic control and another group maintained AIN93M/G with 3% ginger. The freeze-dried ginger was powdered and mixed with AIN-93M diet in required dose. Though the leans rats did not develop hyperglycemia, they were maintained along with control obese rats on AIN93M/G diet throughout the experimental period. During this experimental period regular food intake, weekly body

weights and fasting blood glucose levels were monitored and the experiment was conducted for a period of five months. Animal care and protocols were in accordance with and approved by the Institutional Animal Ethics Committee (IAEC).

**Homeostasis model assessment (HOMA):** Insulin resistance was assessed at the age of two months and at the end of 5 months by homeostasis model assessment (HOMA)-IR as described earlier for rats using the following equation:  $HOMA-IR = [\text{fasting plasma glucose (mg/dl)} \times \text{fasting plasma insulin } (\mu\text{U/ml})] / 2,430$ .

**Lens examination and cataract progression:** Eyes were examined every week using a slit lamp biomicroscope on dilated pupils to observe changes in the lens transparency. Onset, progression and maturation of cataract were classified as we reported previously.

**Clinical parameters:** Plasma glucose and insulin were measured by the glucose oxidase-peroxidase (GOD-POD) kit and RIA kit methods respectively.

**Immunohistochemistry (IHC) of pancreas:** Pancreas tissues were paraffin embedded and blocks were prepared. Sections were deparaffinized in xylene and dehydrated with decreasing grades of alcohol (90%, 70% and 50%). Antigen retrieval was done by boiling the slides in 0.1M citrate buffer, pH 6 for 20 min. The endogenous peroxidase activity was quenched by incubating the slides in 3% H<sub>2</sub>O<sub>2</sub> for 30 min followed by two washes with PBS. To prevent non-specific binding of the antibody, blocking was done by incubating the slides in 10% normal goat serum in PBS at room temperature for 1 h. Slides were incubated overnight at 4°C with anti-rat polyclonal insulin primary antibody raised in rabbit in PBS containing 1% normal goat serum. Slides were washed with PBS and incubated further with biotinylated anti-rabbit secondary antibody for 30 min followed by incubation with VECTASTAIN Elite ABC reagent. Sections were developed using 3% H<sub>2</sub>O<sub>2</sub> and DAB and observed under microscope. Results were expressed in the form of average percent of insulin positive cells in the islets.

**Lens processing and protein content:** At the end of the experiment, animals were sacrificed by CO<sub>2</sub> asphyxiation and lenses were dissected by posterior approach and stored at -80°C until further analysis. A 10% homogenate was made from 4-5 pooled lenses in 50mM phosphate buffer, pH 7.4. Before centrifugation, a set of aliquots were made for estimation of total protein, MDA and sorbitol. The remaining total homogenate was centrifuged at 10,000× g for 30 min at 4°C. The supernatant was referred to as the soluble fraction. Total and soluble protein content was estimated by Lowry method and the percentage of soluble protein was calculated.

**Crystallin distribution and protein cross links:** Crystallin distribution in the soluble protein fraction was performed by size-exclusion chromatography on a 600 x 7.5 mm column (TSK-G3000 SW;) in an HPLC system. The column was equilibrated with 0.1 M sodium phosphate buffer (pH 6.7) containing 0.1 M sodium chloride at a flow rate of 1 mL/min. Soluble protein samples (1 mg/mL) of individual groups were loaded onto the column and separated with a flow rate of 1 ml/min. Protein peaks were detected at 280 nm using UV detector.

**Polyol pathway intermediates:** The status of the polyol pathway in the eye lens was assessed by analyzing the activity of aldose reductase (AR) and sorbitol levels. AR activity and sorbitol levels were assayed in lens total soluble fraction by spectrophotometer, according to the reported methods.

**Oxidative stress and antioxidant status:** Oxidative stress status in these lenses was assessed as thiobarbituric acid reacting substances (TBARS) and protein carbonyl content as described earlier.

**Statistical Analysis:** One-way ANOVA was used for testing statistical significance between groups of data and individual pair difference was tested by means of Duncan's multiple-range test. Heterogeneity of variance was tested by the non-parametric Mann Whitney test. A p<0.05 was considered significant.

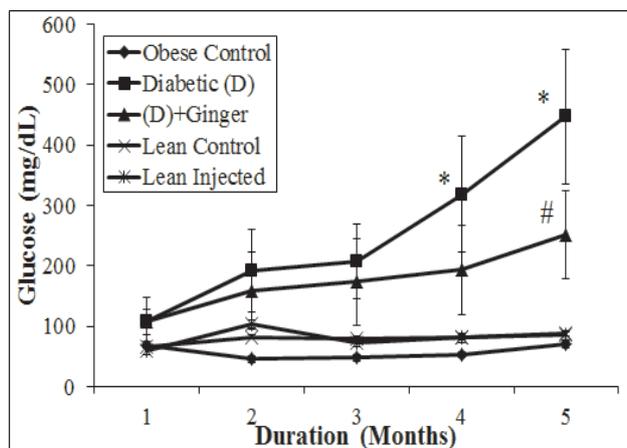
## RESULTS

1. Though there was no difference in average daily food intake between control (19.46±2.81 g) and nSTZ injected lean (20.20±2.48 g) rats, there was an increase in food intake (22.00±3.94 g) of control obese rats when compared to their respective lean. Whereas, a significant increase in food intake

(33.84±3.79 g) was observed with diabetic obese rats when compared to control obese rats (22.00±3.94 g). However, feeding of ginger did not affect the food intake (32.79±4.96 g).

- As expected, the body weights of obese rats are higher (650±67 g) than lean (423±26 g) at the end of experiment. Despite the increased food intake, body weights of the diabetic obese animals are decreased (615±47 g) when compared to control obese animals. However, feeding of ginger to diabetic rats did not improve the body weights to a significant extent.
- There was no difference in fasting blood glucose levels between control lean and obese animals during the experimental period (Fig 1). Interestingly, injection of STZ to the neonatal obese rats resulted in hyperglycemia but not the lean animals. Obese animals are known to have the characteristics of insulin resistance and impaired glucose tolerance. This could be the reason why only obese animals developed hyperglycemia. Further, there was a gradual increase of glucose levels in nSTZ obese rats from first month to severe hyperglycemia by the age of five months. Interestingly feeding of dietary ginger has shown hypoglycemic property by reducing fasting glucose levels when compared to untreated diabetic obese rats (Fig 1).
- As expected, insulin resistance index (HOMA-IR) was significantly higher in control obese rats when compared to lean, indicating that these animals exhibited insulin resistance (Table 1). However, we did not calculate HOMA-IR in diabetic animals since they already developed mild hyperglycemia at the age of two months.
- There was about 20% decrease in insulin levels in obese diabetic rats (untreated and treated with ginger) compared to control obese at two months of age. However, insulin levels in these animals increased to that of control obese animals by the age of five months (Table 1). It is interesting to note that the diabetic obese animals exhibited both hyperglycemia and high insulin levels (insulin resistance) which are commonly observed in T2D.
- Lenses were examined for opacity from the age of one month to till the end of the experiment. The onset of cataract was observed only in obese rats having hyperglycemia at the age of two months and cataract was matured by five months (Fig 2). Feeding of ginger at 3% level in the diet has delayed onset, progression and

**Fig 1. Fasting blood glucose levels.** Glucose levels (mg/dL) were plotted as a function of duration in months. Data are the mean ± SD (n=8-10). \* and # represent the values are significantly different from lean control and obese rats respectively. P<0.05 were considered significant.



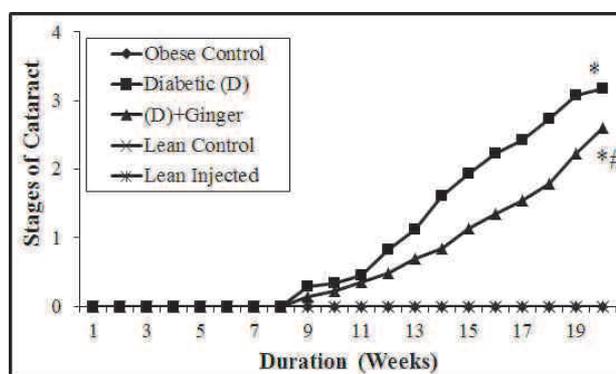
**Table 1. Fasting insulin and HOMA-IR index at two and five months.** Fasting glucose and insulin were estimated at two and five months of age. HOMA-IR was calculated using fasting glucose and insulin levels. The data are mean±SD (n=8-10). \* and £ represent the values are significantly different from lean control and obese rats respectively. P<0.05

Groups/Duration	Two months		Five months	
	Fasting insulin (µU/ml)	HOMA-IR	Fasting insulin (µU/ml)	HOMA-IR
Control Lean	26±9.59	1.18	24.14±6.42	1.21
Control Obese	121±32.12*	2.22	127± 11.49*	4.95*
Diabetic Obese (D)	87±17.10*£	N/A	105± 32.53*	N/A
D + Ginger	91±13.66*	N/A	122± 35.80*	N/A

maturation of cataract in these animals (Fig 2). Interestingly, STZ injected lean rats have not developed any opacification. Control obese and leans rat lenses are clear throughout the experiment.

7. To investigate the possible mechanisms by which ginger delayed the onset and progression of neonatal STZ-induced diabetic cataract in obese animals, we have studied various biochemical mechanisms related to cataractogenesis. There was a significant decrease in protein (total, soluble and percent soluble protein) content in the diabetic obese rat lens when compared to the control obese rat indicating increased insolubilization of lens proteins. Interestingly, feeding of ginger significantly prevented these changes in the lens (Table 2). The prevention of loss in lens soluble protein in diabetic obese rats was well correlated with the delay of maturation of cataract in this group (Fig 2).
8. The possible alterations or modifications in the crystallin profile by size-exclusion chromatography was studied. The distribution profiles of lens crystallins showed a decrease in  $\beta$ - and  $\gamma$ -crystallin peaks and an increase in the  $\alpha$ -crystallin-associated high molecular weight (HMW) aggregate peak in untreated diabetic obese lens when compared to control obese lens (Fig 3). The decrease in  $\beta$ - and  $\gamma$ -crystallins suggests protein degradation in obese diabetic cataract lens. The formation of HMW aggregates may be due to either cross-linking of degraded products or some other changes. It is interesting to note that changes in the crystallins due to diabetes were minimized by feeding with ginger (Fig 3).
9. Activation of a polyol pathway has been linked to several diabetic complications. AR, a key enzyme of polyol pathway, converts excess glucose to sorbitol, the accumulation of which is associated with many secondary complications including cataract and retinopathy. In the present study, there was a significant accumulation of sorbitol due to increased AR activity in the lens of untreated diabetic obese rats lens when compared to control obese animals indicating activation of polyol pathway (Table-3). Feeding of ginger has shown marginal inhibition of AR activity as well as sorbitol accumulation in diabetic obese rats (Table 3).

**Fig 2. Onset, progression and maturation of cataract.** Cataract formation was monitored weekly by slit-lamp microscope. Stages of cataract in each group were averaged at the given time and the average stage of cataract (n=8-10) was plotted as a function of time. Cataract formation or lens opacity was determined (score 0 to 4). \* and # represent the values are significantly different from lean control and obese rats respectively. P<0.05 were considered significant.

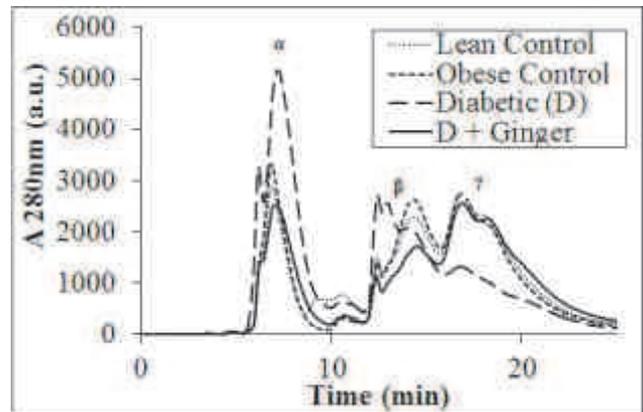


**Table 2. Total, soluble and percent soluble protein content in the lens.** Total, soluble protein content was measured and percent soluble content was calculated based on the soluble protein. The data are mean  $\pm$  SD (n=8-10). \* and £ represent the values are significantly different from lean control and obese rats respectively. # represents the values are significantly different from obese diabetic rats P<0.05.

Parameters/Groups	Lean control	Obese control	Diabetic obese (D)	(D) + Ginger
Total Protein (mg/g lens)	496 $\pm$ 34.40	566 $\pm$ 4.6*	396 $\pm$ 65.1*£	493 $\pm$ 88.6£#
Total soluble protein (mg/g lens)	331 $\pm$ 16.38	365 $\pm$ 19.8	193 $\pm$ 64.3	286 $\pm$ 46.6
% Soluble Protein	66.7	64.5	48.8*£	58.4£#

10. Oxidative stress is known to play an important role in the development of various complications of diabetes and obesity. Thus, we measured oxidative stress and antioxidant defense status in these animals. There was an increased lipid peroxidation as indicated by increased levels of MDA as well as protein oxidation as there was an increase of protein carbonyls in the lenses of diabetic untreated obese animals when compared to control rats (Table 4). Interestingly, feeding of dietary ginger marginally prevented oxidative stress in the lens by inhibiting MDA and protein carbonyls (Table 4).

**Fig 3. Distribution profile of lens crystallins.** The distribution profile of crystallins in the soluble fraction of lens of different groups was monitored by size exclusion chromatography



**Table 3: Polyol pathway intermediates in the lens.** AR activity was expressed as  $\mu\text{moles NADPH oxidized/h/100 mg protein}$ . Sorbitol was expressed as  $\mu\text{moles/g lens}$ . The data are the mean  $\pm$  SD (n=8-10). \* and £ represent the values are significantly different from lean control and obese rats respectively.

Parameter/Groups	Lean control	Obese control	Diabetic obese (D)	(D) + Ginger
Aldose reductase	49.02 $\pm$ 5.84	53.47 $\pm$ 5.14	64.98 $\pm$ 3.79*£	59.42 $\pm$ 12.75*£
Sorbitol	0.25 $\pm$ 0.09	0.46 $\pm$ 0.21*	4.93 $\pm$ 2.03*£	3.76 $\pm$ 2.02*

**Table 4. Lipid peroxidation and protein carbonyls in the lens.** \*and £ represent the values are significantly different from lean control and obese rats respectively. The data are the mean  $\pm$  SD (n=8-10). #represents the values are significantly different from obese diabetic P<0.05.

Parameter/Groups	Lean control	Obese control	Diabetic (D)	(D) + Ginger
MDA	4.14 $\pm$ 1.53	5.60 $\pm$ 2.81	8.02 $\pm$ 1.59*£	6.16 $\pm$ 2.18*#
Protein carbonyls	3.42 $\pm$ 0.09	3.50 $\pm$ 0.33	4.75 $\pm$ 0.37*£	3.60 $\pm$ 0.27*#

## CONCLUSION

STZ injection to two day old rat pups resulted in development of hyperglycemia only in obese but not in lean rats indicating obesity is susceptible for development of diabetes. WNIN-GR/Ob diabetic animals develop mild hyperglycemia by one month and progresses to sever hyperglycemia over a period of five months. These animals also developed diabetic complications particularly cataract. Based on these experiments, nSTZ- WNIN-GR/Ob rat appears to be a suitable model for studies on T2D induced complications, particularly diabetic cataract, and also for dietary intervention studies.

## 10. DEVELOPMENT OF RAPID ASSAYS FOR THE DETECTION OF GENETICALLY MODIFIED (GM) FOODS

GM foods are derived through recombinant DNA technology, promise far more potential in terms of yield and quality of the end products than conventional methods. The technology involves insertion of genes from different species, which have particular desired characteristic's, into a plant where such characteristic's are required. The most widely grown GM crops include cotton, soybean, maize and canola in various countries. The need to verify presence of biomarkers in GM foods has created a demand for analytical testing to comply with labeling requirements, which define a threshold level, to check unapproved GM varieties that inadvertently enter the food supply and so to safeguard consumers, import and export sectors. Therefore, there is a need to monitor and verify the presence and amount of GMOs in agricultural crops and in products derived thereof.

Therefore, this project was initiated based on a public-private partnership (PPP) model between Agilent and National Institute Nutrition, as per guidelines of ICMR.

### OBJECTIVES

To develop rapid PCR-based assay methods to detect commercially available genetically modified crops.

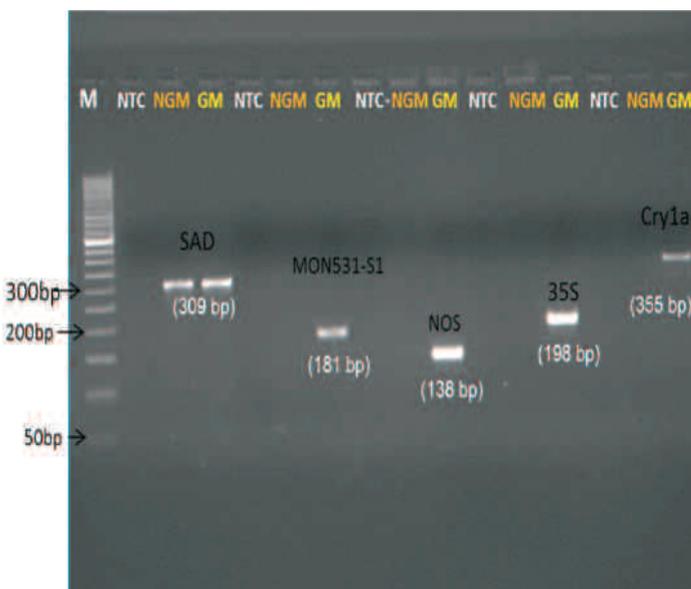
#### Details of the work done

Genetically modified (GM) foods are derived through recombinant DNA technology that promises better yield and quality of the end products than conventional breeding methods. At present, the most widely grown commercially approved GM crops include soybean, maize, cotton, and canola. While the application of novel biotech crops on food production for a better nutrition is debatable, but developing a detection method to verify presence of transgenic biomarkers in a food sample is an important prerequisite for biosafety and regulation. In this project we report development of PCR based singleplex and multiplex detection method of some crops using specific primers for the transgenes as well as events.

#### 1. Detection of GM cotton (Event MON531)

Primers were designed to detect transgenes like CaMV35S promoter, cry1Ac, nopaline synthase (NOS) and an MON531. In addition, a cotton specific endogenous gene sad1 (stearoyl-acyl carrier protein desaturase), was used as an internal control. Genomic DNA was extracted and purified from the test and control materials using DNeasy Kit (Qiagen). PCR was performed for 35 cycles using the specific primers and the amplicons were resolved in a 2% agarose gel. It has been possible to detect each of the transgene specifically in GM test samples that failed to amplify in isogenic-non GM as well as in no template control (NTC), indicating specificity of the assay (Fig. 1). Using these primers together in a mixture it has been possible to detect the cotton events as well as transgenes in a single tube reaction (Fig. 2). The specificity of the transgenes (NOS, cry1AC and CaMV promoter) was also examined by performing realtime PCR

**Fig 1. Singleplex PCR-based detection of cotton events (MON-531) and transgenes.** (GM-Genetically modified cotton; NGM-control isogenic sample that is not genetically modified; NTC-no template control, M-marker)

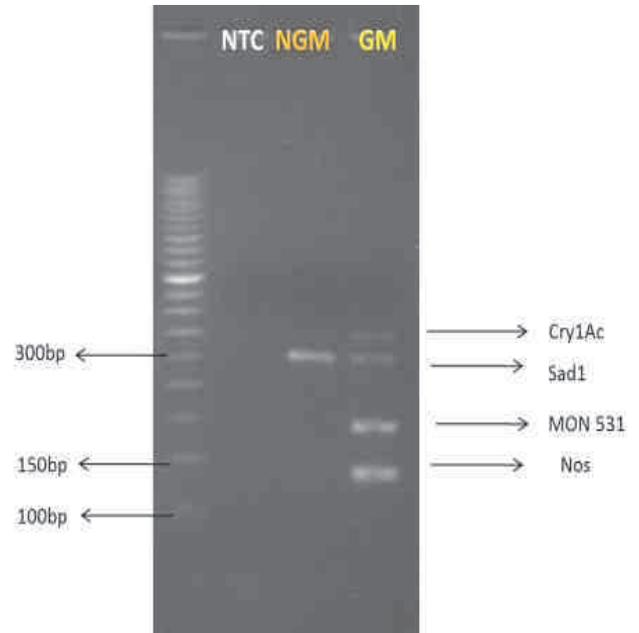


(Fig. 3). It was found that the all designed primers could specifically amplify the designated genes efficiently. The endogenous reference gene *sad1* was detectable in both GM as well as non GM samples whereas the GM-specific transgenes could be detected only on GM cotton samples. No amplification was detectable in no template control (NTC).

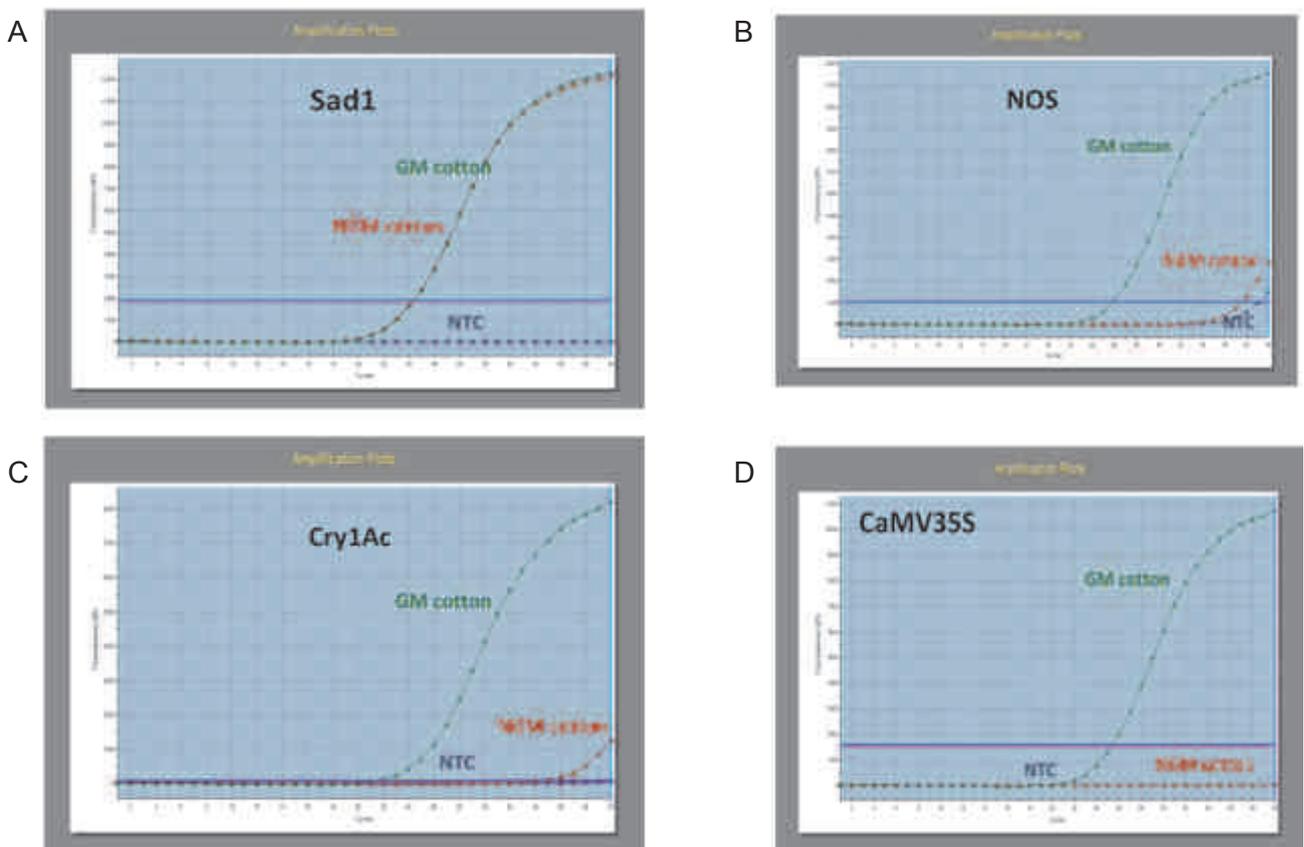
## 2. Detection of GM maize

Genomic DNA (gDNA) was extracted and purified from the test and control materials using DNeasy Kit. The quality and quantity of DNA was determined by a spectrophotometer. PCR was performed for 35 cycles using the specific primers and the amplicons were resolved in a 2% agarose gel. It has been possible to detect each of the transgene in maize (GA-21 event) specifically in GM test samples that failed to amplify in isogenic non GM as well as in no template control (NTC), indicating specificity of the assay (Fig. 4). The primers designed for the maize event (GA-21) as well as transgene (NOS) were also detected efficiently in realtime PCR (Fig. 5).

**Fig 2. Multiplex PCR-based detection of cotton events (MON-531) and transgenes.** (GM-Genetically modified cotton; NGM-control isogenic sample that is not genetically modified; NTC-no template control).



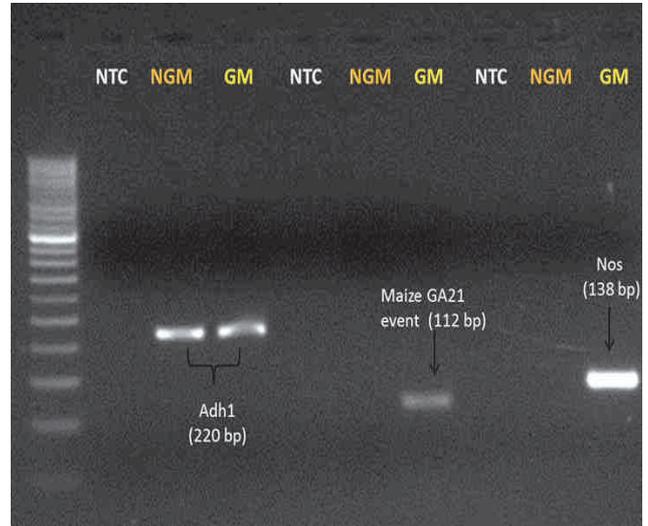
**Fig 3. Detection of transgenes in GM cotton** by realtime PCR (A) endogenous reference gene *sad1* (B) NOS transgene (C) *cry1Ac* transgene and (D) CaMV 35S promoter. NTC is no template control. NGM is non-GM cotton



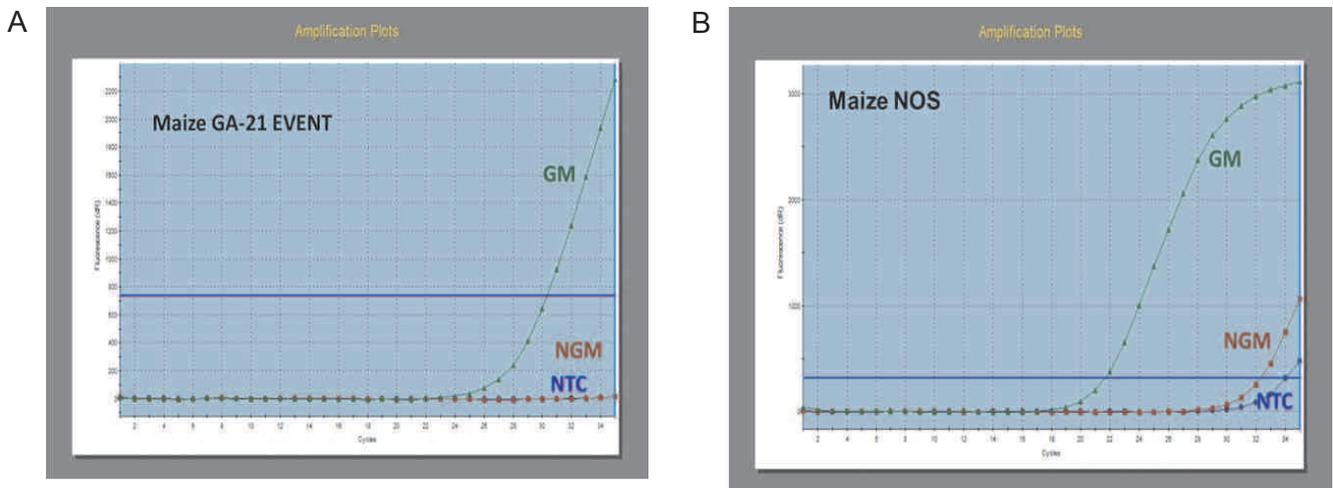
### 3. Detection of GM potato

Genomic DNA (gDNA) was extracted and purified from the test and control materials using DNeasy Kit from the dried tubers of GM potato (event EH 92-527-1) and non-GM controls. PCR was performed for 35 cycles using the specific primers designed and the amplicons were resolved in a 2% agarose gel. It has been possible to detect each of the transgene in potato (NOS) as well as the specific event (EH 92-527-1) in GM test samples that failed to amplify in isogenic-non GM as well as in no template control (NTC), indicating specificity of the assay. The enzyme granule-bound starch synthase (GBSS) was used as an endogenous reference control which was amplified in both non-GM as well as GM samples as expected (Fig 6). The Potato transgene (NOS) was also detected using realtime PCR. The presence of a single peak in the thermal denaturation curve (melt curve) indicate specificity of the amplification using designed primers (Fig 7).

**Fig 4. Singleplex PCR-based detection of Maize events (GA-21) and transgenes.** (GM- Genetically modified maize; NGM-control isogenic sample that is not genetically modified; NTC-no template control, M-marker); Adh1-Alcohol dehydrogenase used as internal control; NOS-nopalinn synthase.



**Fig 5. Detection of transgenes and event (GA21) in maize by realtime PCR** (A) Maize GA-21 event (B) NOS transgene. NTC is no template control. NGM is non-GM maize.



### 4. Detection of GM rice

Genomic DNA (gDNA) was extracted and purified from the test and control materials using DNeasy Kit from the dried leaves of GM rice (event LLrice62) and non-GM controls. PCR was performed for 35 cycles using the specific primers designed and the amplicons were resolved in a 2% agarose gel. It has been possible to detect each of the transgene in rice (NOS) as well as the specific event (LLrice62) in GM test samples that failed to amplify in non-GM controls as well as in no template control (NTC), indicating specificity of the assay. The enzyme Sucrose Phosphate Synthase (SPS) was used as an endogenous reference control which was amplified in both non-GM as well as GM samples as expected (Fig 8).

**Fig 6. Singleplex PCR-based detection of Potato event (EH-92-527-1) and transgene.**

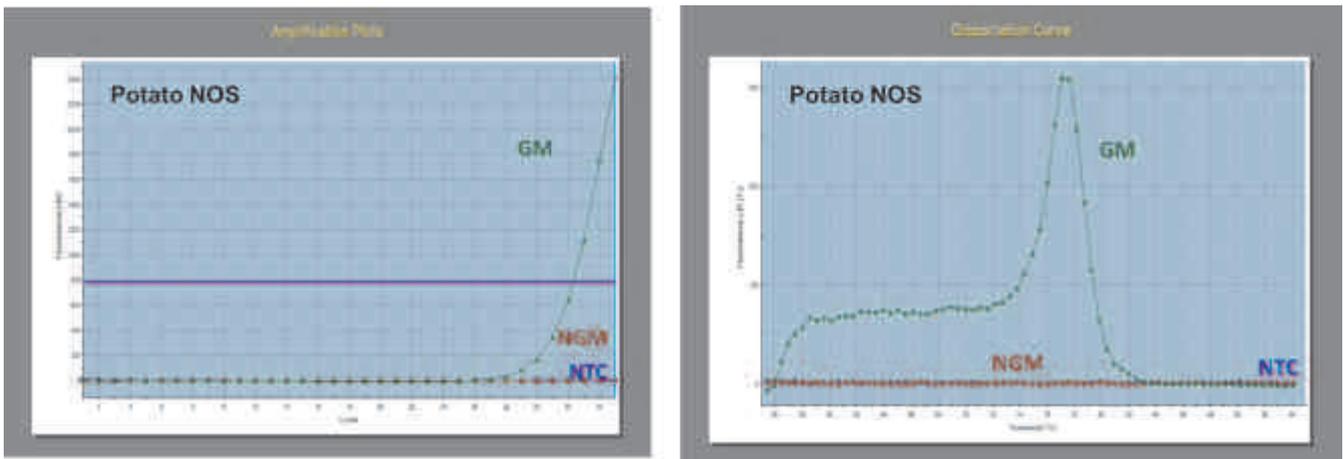
GM-Genetically modified potato; NGM-control isogenic sample that is not genetically modified; NTC-no template control; NOS- nopalín synthase, GBSS-Granule-bound starch synthase, used as an internal control.



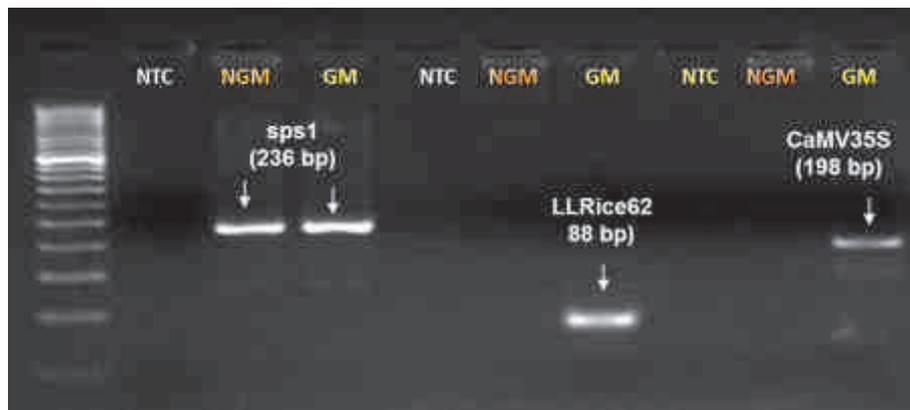
**5. Multiplex-PCR to detect multiple GM crops simultaneously**

When an unknown sample is presented as a whole grain, it's easy to identify from the morphological features of the grain which crop needs to be analysed like soybean or wheat. But in processed food materials e.g. flour it will be difficult to detect by appearance whether it's soy flour or wheat flour or rice flour. In food processing industries like flour mills there could be inadvertent mixing up of flours while handling/processing or there could be deliberate adulteration. In such a scenario, multiplexing kits with different endogenous reference gene can identify whether the flour presented is soybean flour or wheat flour or rice flour or any mixture of these. Also such admixture situations may arise in food matrices where more than one GM crop could be used.

**Fig 7. Detection of transgenes in potato (EH-92-527-1) by realtime PCR.** The fluorescent trace of the NOS transgene amplification (left panel) and the melt curve of the same gene (right panel). NTC-no template control.



**Fig 8. Singleplex PCR-based detection of rice event (LLRice62) and transgene (NOS).** GM-Genetically modified rice; NGM-control isogenic sample that is not genetically modified; NTC-no template control; NOS- nopalín synthase, SPS- sucrose phosphate synthase, used as an internal control.

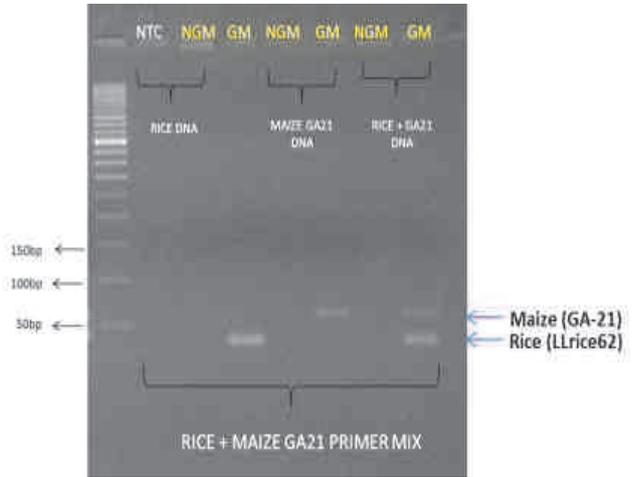


In such cases GMO detection is a significant challenge and will need multiplexing of events as well as endogenous reference genes to identify nature of the matrix as well as whether there is contamination with GMO or not. Therefore, we also developed multiplex PCR methods that can detect GM in an admixture of multiple crops.

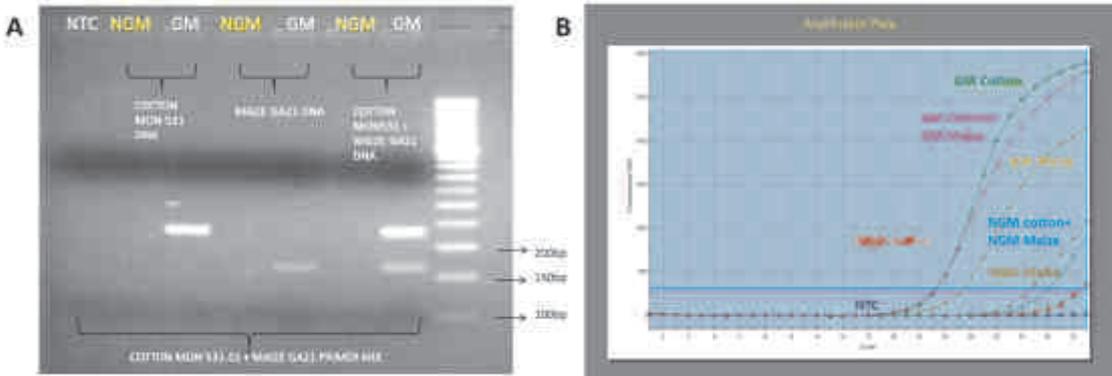
**A. Detection of GMOs in a double mixtures using multiplex PCR:**

In order to develop a method that can detect multiple transgenic events when these events are mixed in a sample, multiplex PCR was performed. This was done by preparing a co-mixture of genomic DNAs (GM and non-GM) in two different combinations of transgenic events and was amplified with their respective primer mixtures. Accordingly, different combinations of GM crops like rice and maize (Fig.9), maize and cotton (Fig.10), maize and potato (Fig. 11) were prepared and we were able to detect each of the GM-specific events in the admixture using their respective primer mixtures. Event specific amplicons were detected both in agarose gel electrophoresis as well as realtime PCR.

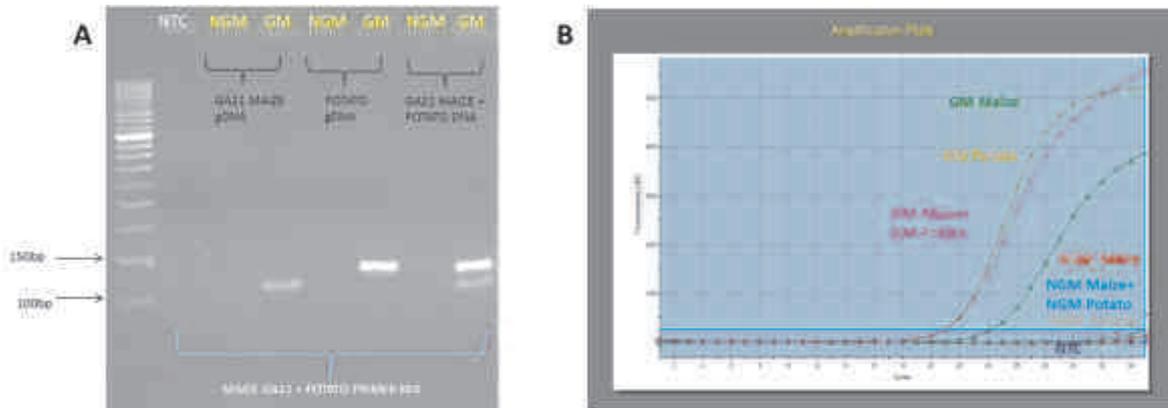
**Fig 9. Detection of Rice (LLrice62) and Maize (GA-21) in a mixture in a multiplex PCR using event-specific primer mixture. NTC-no template control**



**Fig 10. Detection of cotton (MON531) and Maize (GA-21) in a mixture using event-specific primer mixture (A). Similar detection was performed using realtime PCR (B). NTC-no template control; GM-genetically modified. NGM-non-genetically modified.**



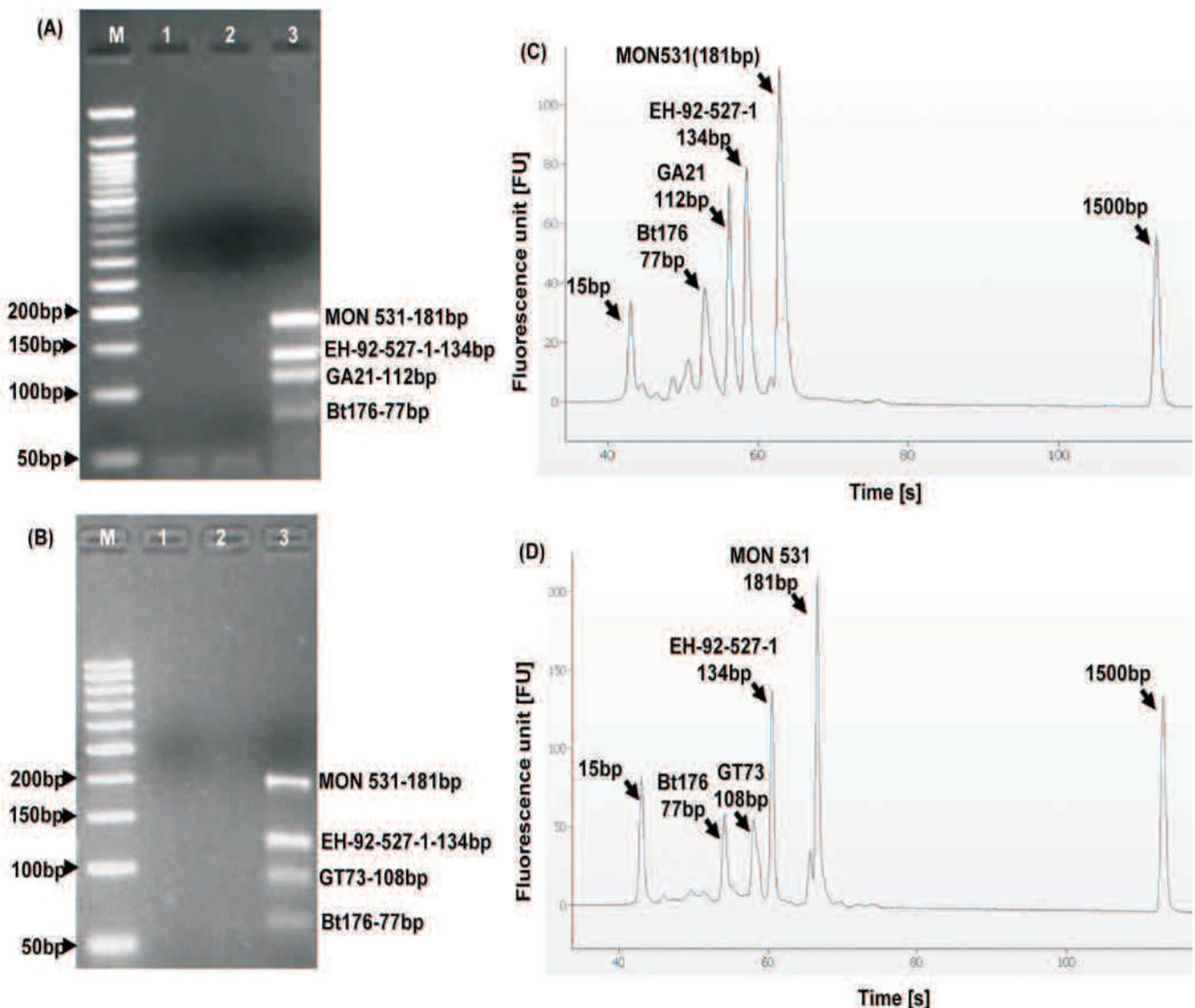
**Fig 11. Detection of maize (GA-21) and potato (EH 92-527-1) in a mixture using event-specific primer mixture (A). Similar detection was performed using realtime PCR (B). NTC-no template control; GM-genetically modified. NGM-non-genetically modified.**



## 6. Multiplex-PCR of multiple events in mixture

We also developed PCR methods that can detect multiple transgenic events where more than two events are mixed in a sample using multiplex PCR. This was done by preparing a co-mixture of genomic DNAs (GM and non-GM) in two different combinations of transgenic events (A) MON531+ EH 92-527-1+GA21+ Bt176 and (B) MON531+ EH 92-527-1+ GT73 + Bt176 and amplified with their respective primer pair mixture. The genomic DNA of all the events was combined in equimolar concentration (10ng of each DNA) and used as template. PCR was carried out to amplify these DNAs and amplicons were analyzed by 2% agarose gel electrophoresis as well as in an Agilent 2100 bioanalyzer. In combination of (A), four transgenic events such as MON531 (181bp), EH 92-527-1(134bp), GA21 (112bp), Bt176 (77bp) were detected simultaneously (Fig. 12A, Lane 3 and Fig.12C). In another combination of (B), multiplex PCR amplified four bands or four peaks correspond to MON531 (181bp), EH 92-527-1 (134bp), GT73 (108bp), Bt176 (77bp) (Fig.12B, Lane 3, Fig.12D). The non-GM DNA mixture was not amplified by the primer pair mixture (Fig.7A & B, Lane 2).

**Fig 12. Multiplex detection of MON531, EH92-527-1, GA21, Bt176, and GT73 transgenic events by PCR-capillary electrophoresis.** (A) Agarose gel electrophoresis of the amplicons from a mixture of cotton (MON531), potato (EH-92-527-1), maize (GA-21) and cotton (Bt-176). (B) Capillary electrophoretogram of the same samples as described in A. (C) Agarose gel electrophoresis of the amplicons from a mixture of cotton (MON531), potato (EH-92-527-1), canola (GT73) and cotton (Bt-176). (D) Capillary electrophoretogram of the amplicons from a mixture of cotton (MON531), potato (EH-92-527-1), canola (GT73) and cotton (Bt-176).



## CONCLUSION

In conclusion we have been able to develop suitable PCR methods, using specific primers targeting to events, transgenes like NOS, cry1Ac, CaMV35S promoter using singleplex as well as multiplex PCR in GM crops cotton, maize, potato, rice and canola. In addition, we verified the specificity of the designed primers using realtime PCR. Also we developed multiplex PCR methods where we could detect admixture of different GM crops in different combinations which could be useful in detecting inadvertent or otherwise mixture of more than two GM crops during handling, processing as well as complex matrices like processed food and feed.

## 11. ASSESSMENT OF BODY COMPOSITION IN INDIAN FEMALES USING DIFFERENT TECHNIQUES

The fat content of the human body has physiological, nutritional and medical importance; both in health and disease situations and hence, there has been considerable interest in the measurement of human body composition in vivo over the past 4 to 5 decades. Several laboratories based techniques such as hydro densitometry (under water measurement); measurement of naturally occurring  $^{40}\text{K}$  isotope, bio-electrical impedance analysis and isotopic dilution technique using  $\text{D}_2\text{O}$  has been developed. All these techniques work on different principles and have the limitations such as being complex, expensive or time consuming.

The body size and composition of Indian population vary widely and differ markedly from those of Western population. Therefore, the usage of various regression equations to predict body composition that are developed for western population may not give accurate measurement of body composition in our population, since the regression equations would best fit in that population where they have been derived from. This is because those factors are dependent upon age, sex and ethnicity. Hence, the present study is designed to validate the existing methods to assess body composition and to develop regression equations using skin fold measurement and bio-electrical impedance analysis methods to suite Indian population for accurate appraisal of body composition.

Therefore, keeping in view the need of the day, to facilitate accurate and expedient method to measure body composition suitable to Indian female population, the present study is proposed with the following objectives.

### OBJECTIVES

- To examine the existing techniques used in the assessment of body composition and to identify the degree of agreement and suitability of different methods for accurate appraisal of body composition in Indian females.
- To assess the age related changes in body composition in Indian females with reference to ratio of lean body mass to fat mass.
- To compare the body density obtained from different combinations of skin fold thicknesses with that obtained by gold standard method (Densitometry), for assessing the body composition of Indian females.
- To verify the degree of association of fatness as assessed by densitometry with various anthropometric measurements and their derivatives, and to test /redefine the existing cut-off points suitable to Indian females.
- To study the influence of body composition on Resting Metabolic Rate in a subsample of adult women.

## METHODOLOGY

**Study design:** The study design is cross-sectional

### Selection of Subjects

**Sample size:** It is proposed to recruit apparently normal and healthy female subjects (n=265) between the age of 7 and 60 years. The sample size was derived taking the mean difference  $-1.49 \pm 2.28$ , a sample size of 53 in each group was fixed at 95% confidence interval and 90% power with a allowable error of 2 units.

### Anthropometric measurement

The anthropometric measurements such as weight, height, circumferences (neck, chest, waist, hip, thigh and calf), and skinfold measurements (triceps, biceps, sub-scapular and supra-iliac) were measured on all the volunteers by using standard procedures.

### Body Composition Methods used

1. Skin fold measurement method (SKF) - Holtain Calipers (Holtain Ltd, UK)
2. Hydro-densitometry/ Under Water Weighing System (UWW) - Under Water Weighing System (HWS1, Cranlea, UK) with the Novatech Load cell.
3. Air Displacement Plethysmography (ADP) - BodPod (LMI, USA).
4. Bioelectrical Impedance Analysis Method (BIA) - InBody-720 (Biospace, Korea)

### Energy Expenditure

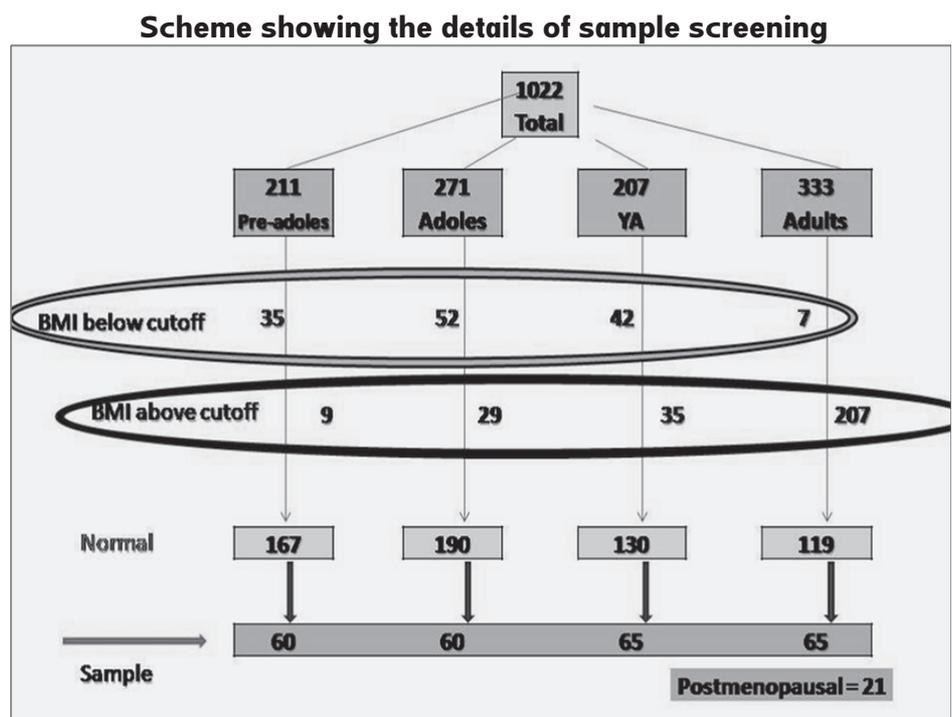
- Resting Metabolic Rate (RMR) was measured by indirect calorimetry using Oxycon Pro (Jaeger, Germany).

## STATISTICAL ANALYSIS

- Descriptive statistics like mean and SD was calculated.
- ANOVA was used to compare the age groups.
- Correlation and Scatter plots was used to check the primary association between the methods.
- Bland Altman plots was plotted to analyze the agreement between the methods.
- Regression analysis was done to check the unit increase in fat percentage with increase in different combinations of skin folds.
- ROC curve analysis was used to analyze the efficiency of various anthropometric indicators to predict fatness.

### Screening of participants

A total of 1022 subjects screened for the inclusion criteria from pre-adolescent, adolescent, young adult and adult groups to reach a sample size of 250 female participants.



## RESULTS

The two compartmental body composition parameters were assessed using the four methods. The lean body mass and fat percentage is given in the following table.

Category	Wt kg	SKF		BIA		ADP		UWW	
		Fat %	LBM kg						
Pre-adolescents	21.3±3.4	21.1±2.6	16.8±2.3	17.8±4.6	17.5±2.4	17±6.3	17.6±2.2	-	-
Adolescents	43.4±10.3	27±3.7	31.4±6.3	29.1±7.4	30.2±5.1	26.5±6.6	31.5±6.1	28.1±5.7	32.3±6.3
Young Adults	51.8±6.0	28.6±3.7	36.9±3.6	33.3±5.0	34.4±3.9	28.6±6.3	36.8±4.1	27.6±4.2	37.1±3.9
Adults	55.1±5.9	34.7±4.9	35.7±2.6	37.9±4.8	34±3.2	34.6±6.7	35.8±3.2	27.8±2.8	38.6±5.9
Post-menopausal	58.4±4.7	39±2.8	35.6±2.7	40.7±3.7	34.5±2.8	39.2±4.3	35.4±2.9	-	-

Values are Mean±SD

The objective wise summary of the results are given below:

### Objective-I

- In Pre-adolescents, there is a good agreement between ADP and BIA when compared to ADP and SKF method.
- In adolescents, Young Adults and Adults there is a good agreement between ADP and SKF method.
- This suggests there is no one specific method that would function as an accurate method in all the age groups when compared to the reference method. The methods used should be determined specifically for the specific population group.

### Objective-II

- There is a significant difference between fat percentage in all the age groups except between adolescents and young adults.
- There is a considerable increase in the fat percentage from young adults to the adult group, and from adults to post menopausal group, which indicates a need for intervention during the transition to the middle age, so as to maintain optimal lean to fat ratio for healthy aging.

### Objective-III

- Regression equations for fifteen different combinations of skinfold thickness were drawn.
- All the equations drawn were significant at 0.001 level.

### Objective-IV

- In young adults and adults the best suited indicators for fatness were identified based on the AUC and the cutoffs were determined at the higher sensitivity and specificity.
- In young adults the best indicators identified were AC, BMI, TC, CC, HC and NC in respective order with moderate accuracy. Whereas the WHR was the poorest of the indicators studied.
- In adults the best indicators identified were AC and Calf Circumference but WHR was the poorest among the indicators studied.

### Objective-V

- RMR was assessed in a subsample (n=30) of adult group. The mean of the 24 hr RMR was found to be 1306.1±220.7 Kcal.

## 12. ERGONOMIC STUDY TO TEST THE EFFICACY/ SUITABILITY OF BICYCLE DRIVEN CHARKHA AMONG WOMEN SPINNERS

Khadi industry is one of the traditional industries in India. It came to focus during the pre-independence era. Mahatma Gandhi initiated Khadi movement in 1920s, using the hand driven charkha for rural self-employment and self-reliance. Even today, the main objective of the Khadi commission is to promote Khadi as a cottage industry, providing scope for employment of women and women empowerment. In this direction, there have been many modules developed in the khadi sector over the past few decades. One among which is the Cycle Charkha designed by an NGO group called SADHANA. The Khadi commission wanted to test its efficacy and viability over the existing hand driven charkha before inducting the same in to the Khadi sector. In this direction, the present study has been initiated to test the efficacy and suitability of bicycle driven charkha in place of traditional hand driven charkha among women spinners in Khadi sector, which is expected to render more comfort and also to help in earning more wages in a given time. Hence, the study was taken-up to compare and understand the efficacy, comfort, effectiveness or usefulness of Bicycle Charkha as against Traditional Hand Charkha through the following objectives.

### OBJECTIVES

- To measure anthropometry and body composition of the subjects.
- To measure Resting Metabolic Rate (RMR) and energy cost of standard activities like sitting, standing and walking.
- To compare the energy cost of spinners working on Bicycle Charkha and on Traditional Charkha.
- To measure functional parameters related to cardio-respiratory system like heart rate,  $O_2$  Pulse, breathing frequency, tidal volume, ventilation( $V_E$ ),  $VO_2$ ,  $VCO_2$ ,  $EqO_2$ ,  $EqCO_2$  etc., during RMR, standard activities and working on bicycle charkha and traditional hand driven charkha.
- To compare work efficiency and energy economy of two modules/units.
- To compare product outcome
- To compare physical comfort
- To compare energy economy and
- To compare wage earnings

### METHODS

#### *Study Design*

With the help of Sadhana team, a field laboratory was established at the study village where the study was conducted. All the available 20 women spinners in the village were contacted and the purpose of the study and procedures involved therein were explained. The women who volunteered to participate in the study were listed for necessary scrutiny based on the inclusion and exclusion criteria. Finally, 15 women were selected after due scrutiny.

**Selection of Subjects:** Of all the available women volunteers listed, 15 apparently healthy, NPNL volunteers were selected based on the age, health status (without any physical deformity) and length of experience in spinning. All the selected volunteers were given ID numbers.

**Physical Parameters:** Anthropometric profile such as height, weight, circumferences (at head, neck, chest, waist, hip, thigh, and calf), sitting height, and leg length were recorded using standard procedures by a well trained investigator. The BMI, BSA, and WHR were derived using appropriate procedures.

**Body Composition:** The body composition of the women spinners was measured using sum of the skin fold thickness at triceps, biceps, sub-scapula and supra-iliac using Harpenden calliper. The fat free mass

and fat mass was assessed using age and gender matched regression models as suggested by Durnin and Womersly (1974).

**Physiological Parameters:** The functional parameters related to cardio-respiratory system like energy cost, heart rate, O<sub>2</sub>-Pulse, breathing frequency (BF), tidal volume, ventilation(VE), VO<sub>2</sub>, VCO<sub>2</sub>, EqO<sub>2</sub>, EqCO<sub>2</sub> etc., were recorded at rest (RMR) and during standard activities (sitting, standing & walking) using indirect calorimetric method (Oxycon Mobile, Cardinal Health care, Germany).

The energy cost of spinning on bicycle driven charkha and traditional hand driven charkha were measured using indirect Calorimetry method. The physiological efficiency, physical work performance and productivity were evaluated based on the functional parameters tested. Every day, three subjects were brought to the field laboratory and were tested for anthropometry, body composition and physiological parameters at rest and at different activities. The comparison between bicycle driven charkha and traditional hand driven charkha was done based on the functional parameters related to cardio-vascular and cardio-respiratory system. Based on these functional parameters physiological efficiency, physical work performance and productivity was assessed between the two charkhas.

**Data analysis:** The information generated was analysed using appropriate statistical tools to compare the differences between the variables studied on bicycle charkha and traditional hand driven charkha using SPSS-16.

## RESULTS

The present investigation was carried out to determine the physiological response and energy expenditure at basal condition as well as during standard activities like sitting, standing and walking of women khadi spinners. About 20 women khadi spinners were identified and recruited for the study, after due screening. A total of 15 khadi spinners were assessed for the following parameters:

- Anthropometric measurements
- Circumferences
- Body composition
- Physiological response at basal condition as well as during standard activities like sitting standing and walking
- Work Efficiency: Comparison between Hand and Cycle Charkha.

**The results are as follows:**

### **Physical Characteristics (Anthropometric Profile and Body Circumferences)**

The physical profile such as anthropometric measurements (age, height, weight, BMI, BSA, WHR, leg length and sitting height) and body circumferences (mid upper arm, waist, hip and calf) of the women spinners are given in Table-1.

The mean age of the women spinners was 36.4 years. Based on the Broca's index, the women spinners were having normal body weight corresponding to their height. Similarly, considering their Body Mass Index (BMI), the nutritional status of these women was found to be normal.

**Table 1. Anthropometric profile and circumferences of women spinners**

Variables	Mean- Sd
Age (yrs)	36.4 ± 10.78
Height (cm)	149.2 ± 6.5
Weight (Kg)	49.7 ± 10.0
BMI (Kg/m <sup>2</sup> )	22.3 ± 4.2
BSA (Kg/sqm)	1.020 ± 0.155
Waist Hip Ratio	0.8 ± 0.01
Leg length (cm)	87.8 ± 4.7
Sitting height (cm)	77.2 ± 4.0
Mid Upper Arm Circumference (cm)	24.2 ± 2.7
Waist circumference (cm)	74.2 ± 9.2
Hip circumference (cm)	89.4 ± 8.0
Thigh circumference (cm)	47.8 ± 5.1
Calf circumference (cm)	29.2 ± 2.9

values are Mean±SD

### Body composition

The values of body composition of women spinners are given in table 2. From the results, it can be observed that these women were having mean fat mass of 14.4kg which amount to 29.5% of fat and mean fat free mass was found to be 33.3kg (70.5%). Based on the percent fat, these women spinners can be categorized as overweight.

**Table 2. Body composition of women spinners**

Variables	Mean±Sd
Triceps (mm)	16.5 ± 6.9
Biceps (mm)	7.8 ± 4.0
Sub-scapular (mm)	20.4 ± 9.5
Supra-iliac (mm)	12.2 ± 7.9
sum (mm)	56.9 ± 25.6
Body density (kg/m <sup>3</sup> )	1.032 ± 0.012
Fat (%)	29.5 ± 5.7
Fat Mass (Kg)	14.4 ± 5.0
Fat Free Mass (%)	70.5 ± 5.7
Fat Free Mass (Kg)	33.3 ± 4.3

### Functional parameters during standard activities

The mean values of various functional parameters related to cardio-respiratory system during standard activities are given in Table 3. The Resting Metabolic Rate (RMR) i.e. energy expenditure at rest was found to be 0.967 k.cal per minute with the basal heart rate of 83 beats per minute, while the energy cost of sitting, standing and walking was found to be 1.063, 1.058 and 2.386 k.cal/min. The energy cost when expressed in terms of BMR factor was 1.099, 1.094 and 2.467 for sitting, standing and walking respectively. The functional parameters at rest were considered as baseline so as to evaluate physiological efficiency and physical work capacity during spinning activities using hand charkha and bicycle driven charkha.

### Functional parameters during spinning activity

From Table 4, it can be observed that the cardio respiratory parameters like Breathing Frequency (BF), Respiratory Exchange Ratio (RER), Ventilatory Equivalent for oxygen and carbondioxide (EQO<sub>2</sub> and EQCO<sub>2</sub>) etc. did not show any significant difference between hand charkha and cycle charkha. However, the energy expended (MET), oxygen consumed (VO<sub>2</sub>) and Heart Rate (HR) were significantly higher in cycle charkha (4.0±0.80 Kcal/min, 652.0±46.0ml/min, 134 ± 14.3 bpm, respectively) as compared to hand charkha (3.237 ± 0.688 Kcal/min, 501.3 ± 86.7 ml/min, 105 ± 20.1 bpm, respectively). The increase in heart rate as against RMR was 26.5% for hand charkha and 61.4% for cycle charkha, whereas from hand charkha to cycle charkha the increase in heart rate was 34.9%. The significant increase in these functional parameters could be due to continuous involvement of larger muscle group (leg muscles) in pedalling cycle charkha. From the work efficiency parameters, it can be seen that there is no significant difference in Oxygen Pulse (O<sub>2</sub>/HR) indicating that oxygen transported per heart beat is comparable for both, Hand and Cycle charkha. On the other hand, the BMR-factor indicates significantly higher work intensity for cycle charkha (4.201 ± 1.012) than Hand charkha (3.392 ± 0.916).

**Table 3. Functional Parameters during Standard Activities**

Parameters	RMR	Sitting	Standing	Walking
VO <sub>2</sub> (ml/min)	160.6 ± 32.4	170.9 - 40.2	175.0 - 39.6	397.8 - 100.9
MET(Kcal/min)	0.967 ± 0.127	1.063 ± 0.174	1.058 ± 0.174	2.386 ± 0.412
HR (bpm)	83 ± 14.2	87 ± 14.3	94 ± 14.3	104 ± 14.6
BF (1/min)	21 ± 3.4	21 ± 0.4	22 ± 3.7	27 ± 4.2
VE (L/min)	6 ± 1.2	7 ± 1.9	7 ± 1.7	13 ± 3.1
RER	0.96 ± 0.10	0.91 ± 0.09	0.91 ± 0.07	0.87 ± 0.06
EQ O <sub>2</sub> (l/l)	24.9 ± 3.4	26.0 ± 4.5	26.3 ± 4.6	25.0 ± 3.1
EQ CO <sub>2</sub> (l/l)	25.8 ± 2.8	28.5 ± 4.0	29.2 ± 4.9	28.5 ± 3.5
O <sub>2</sub> /HR (ml/1)	1.9 ± 0.4	2.0 ± 0.5	1.8 ± 0.4	3.8 ± 0.6

values are Mean±SD

However, the energy cost per gram yield of yarn was lower in cycle charkha ( $1.552 \pm 0.315$  Kcal.min/g) as compared to hand charkha ( $2.698 \pm 0.573$  kcal.min/g). Although, the oxygen consumption (ml/min) and energy cost (Kcal/min) is more, the energy utilised to produce one gram yarn is less in cycle charkha compared to hand charkha. Thus, it can be deduced that cycle charkha has higher Physiological Efficiency and Physical Work Capacity as compared to hand charkha.

**Table 4. Functional parameters and work efficiency during spinning activity (Hand and cycle charkha)**

Parameters	RMR	Charkha			
		Hand	Cycle	t-value	Sig.
VO <sub>2</sub> (ml/min)	160.6 ± 32.4	501.3 ± 86.7	652.0 ± 46.0	4.742	0.001
MET(Kcal/min)	0.967 ± 0.127	3.237 ± 0.688	4.000 ± 0.800	4.212	0.002
HR (bpm)	83 ± 14.2	105 ± 20.1	134 ± 14.3	4.341	0.001
BF (1/min)	21 ± 3.4	28 ± 5.9	31 ± 3.2	0.773	0.457 <sup>NS</sup>
VE (L/min)	6 ± 1.2	17 ± 2.6	22 ± 2.6	3.363	0.007
RER	0.96 ± 0.10	1.04 ± 0.05	1.02 ± 0.1	0.699	0.501 <sup>NS</sup>
EQ O <sub>2</sub> (l/l)	24.9 ± 3.4	27.9 ± 2.9	28.0 ± 3.3	0.129	0.900 <sup>NS</sup>
EQ CO <sub>2</sub> (l/l)	25.8 ± 2.8	26.7 ± 2.2	27.4 ± 2.6	1.004	0.339 <sup>NS</sup>
O <sub>2</sub> /HR(ml/1)	1.9 ± 0.4	5.1 ± 1.2	4.9 ± 0.5	1.145	0.279 <sup>NS</sup>
BMR-factor	1.0	3.392±0.916	4.201±1.012	3.919	0.003
Kcal.min/g	-	2.698±0.573	1.552±0.315	9.130	0.000

values are Mean±SD; NS= Not Significant

### Comparison of Work output v/s Wage earnings between Hand Charkha and Cycle Charkha

It was observed that in Cycle Charkha, the women spinners were spending 1.552 kcal for producing one gram of thread as against 2.698 kcal in Hand Charkha for the same amount of produce which was statistically significant. Further, a comparison was made between work output and wage earnings in two different spinning modules, which are depicted in Table 5.

From Table 5, it can be seen that in Hand Charkha, the spinners could spin 2.25 hanks per hour, whereas, in Cycle Charkha it was increased to 4.99 hanks per hour resulting in 121% increase in their produce. This increase in the produce was achieved only with 23.5% increase in energy expenditure in Cycle Charkha. Thus, by adopting Cycle Charkha the spinners would earn more wages as compared to Hand Charkha.

**Table 5. Comparison of work output v/s wage earnings between hand charkha and cycle charkha**

	Hand Charkha	Cycle Charkha
Hanks/hour	2.25	4.99
Wages/hank (Rs)	3.25	3.25
Wages/hour (Rs)	7.31	16.21
Wages/8 hours (Rs)	58.50	129.74

## RECOMMENDATIONS

### Physiological efficiency

- All the physiological parameters tested to assess the work efficiency of the two modules indicated that though the oxygen consumed (VO<sub>2</sub>/min) and over all energy expended (MET) is more in Cycle Charkha, the energy utilised to produce one gram yarn yield is less in cycle charkha compared to hand charkha. Thus, the cycle charkha has a higher Physiological Efficiency, Physical Work Capacity and energy economy compared to hand charkha.

### Postural difference

- In Hand Charkha, the women spinner moves only her upper body with one hand being put to use to rotate the charkha. This causes angular drift in the upper body due to curvature of one side, which will

lead to postural difference and discomfort. In the long term, it can result in stress on the spinal cord. Whereas, sitting comfortable on a cycle charkha, pedalling and leaving both the hands free, results in lesser stress on the body of the women spinner and is ergonomically feasible.

#### ***Body weight neutralised in cycle charkha***

- In Cycle Charkha, the person is seated comfortably on the cycle and the body weight gets neutralised. But, in Hand Charkha the Body Weight needs to be adjusted since the person has to move the upper body on a constant repetitive manner to rotate the Charkha. This causes added strain to the upper body and uneasiness to the women spinner.

#### ***Thread correction in cycle charkha***

- In Hand Charkha, the women spinners have to use their hands for rotating the charkha and for any thread correction the spinning has to be discontinued. While, In Cycle Charkha only the legs are used for pedalling the charkha and both the hands are free for thread correction. Thus, in cycle charkha, the spinning can be continued uninterrupted, making it more efficient, time saving and ergonomically viable.

#### ***Height of the seat***

- It was observed that in cycle charkha the height of the seat was fixed and could not be adjusted, this resulted in improper pedalling and discomfort. Hence, it is recommended that an adjustable seat is provided to suit individual's leg length.

#### ***Prior training required for cycle charkha***

- The women spinners using Cycle Charkha need to get due acclimatisation, with appropriate saddle adjustment. Therefore, it is recommended that prior training and proper acclimatisation is necessary before handling cycle charkha.

#### ***Financial aspects***

- The work efficiency is greatly improved by using Cycle Charkha, which in turn increases the production. This would facilitate in earning more wages per day, which in turn increases over all income of the women spinners.

#### ***Promote physical fitness and khadi produce***

- The lighter side of the recommendation is the promotion of physical fitness by using Cycle Charkha and at the same time objectives of the Khadi Board can be further elaborated.

### **CONCLUSION**

Based on the results of the study and above observations, the Cycle Charkha was found to be more efficient, convenient and ergonomically viable over the traditional hand driven charkha and hence, opined appropriate measures can be initiated for its induction in the khadi sector.

### 13. ANTI-INFLAMMATORY POTENTIAL OF DIETARY n-3 POLYUNSATURATED FATTY ACIDS IN EXPERIMENTAL ULCERATIVE COLITIS: BIOCHEMICAL AND MOLECULAR MECHANISMS

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease, is the major chronic idiopathic inflammatory condition of the gut. It is characterized by recurrent episodes of inflammation and tissue degeneration. Although the etiology of IBD remains unknown, it has been suggested to be due to complex interaction among genetic, immune and environmental factors. The incidence of IBD is high in western countries whereas in developing countries the incidence has been low. However in recent years the incidence has increased significantly in developing countries including in India. Further, South Asian immigrants adopting western life style and diet are at increased risk of IBD suggesting that dietary factors may play a major role in the development of IBD.

The polyunsaturated fatty acids (PUFA) namely linoleic acid (LA, 18:2n-6) and  $\alpha$ -linolenic acid (ALA, 18:3n-3) are the short chain dietary precursors, converted into biologically active long chain PUFA (LC PUFA) such as arachidonic acid (AA, 20:4 n-6) or eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) respectively. These two classes of PUFA are metabolically and functionally distinct and have opposing physiological function. The PUFA composition of the cell membrane is determined by the dietary levels of n-6 and n-3 PUFA and their ratio. High intake of n-6 PUFA creates proinflammatory environment which in turn may affect the development or progression of several diet related chronic diseases including IBD.

Epidemiological studies revealed that increased consumption of n-6 PUFA is associated with the risk of developing UC whereas, n-3 PUFA has been reported to confer protection. Further, a recent prospective cohort study demonstrated that high levels of adipose tissue AA, which reflects the dietary intake of n-6 PUFA is associated with UC thereby implicating the role of n-6 PUFA, in the pathogenesis of UC. In the present study we have investigated the effects of substitution of n-6 PUFA with n-3 PUFA (ALA/EPA & DHA) on inflammatory response in DSS induced colitis.

#### METHODOLOGY

##### ***Effects of substitution of linoleic acid with $\alpha$ -linolenic acid (precursor of long chain n-3 PUFA) on inflammatory response in DSS induced ulcerative colitis.***

To investigate the effects of substituting linoleic acid (LA, 18:2 n-6) with  $\alpha$ -linolenic acid (ALA, 18:3 n-3), weanling Sprague Dawley rats (n=70) were randomly divided into five groups: a non colitic control group with LA:ALA ratio of 215 (CON-215, n=14) and DSS induced colitic groups with varying LA:ALA ratios of 215 (DSS-215, n=14), 50 (DSS-50, n=14), 10 (DSS-10, n=14) and 2 (DSS-2, n=14). Vegetable oil formulations were made by mixing groundnut oil, palmolein and linseed oil and used as a source of dietary fat. Total PUFA (LA + ALA), SFA and MUFA were similar in all the groups. The LA : ALA ratio was altered by substituting LA with ALA to obtain the ratios of 215, 50, 10 and 2.

##### ***Effects of substitution of LA with long chain n-3 PUFA (fish oil) on inflammatory response in DSS induced ulcerative colitis.***

A separate animal experiment was conducted to investigate the effects of substituting LA with long chain (LC) n-3 PUFA (DHA & EPA) present in fish oil. Weanling Sprague Dawley rats (n=70) were randomly divided into five groups: a non colitic control group with LA: LC n-3 PUFA ratio of 215 (CON-215, n=14) and DSS induced colitic groups with varying LA:LC n-3 PUFA ratios of 215 (DSS-215, n=14), 50 (DSS-50, n=14), 10 (DSS-10, n=14) and 5 (DSS-5, n=14). Vegetable oil formulations were made by mixing groundnut oil, palmolein and fish oil and used as a source of dietary fat. Total PUFA (LA + LC n-3 PUFA), SFA and MUFA were similar in all the groups. The LA : LC n-3 PUFA ratio was altered by substituting LA with LC n-3 PUFA to obtain the ratios of 215, 50, 10 and 5.

### **Induction and assessment of colitis**

All the animals were fed the respective experimental diets for 90 days. After 78 days of pre-feeding with specified experimental diets, ulcerative colitis was induced by oral administration of freshly prepared 4% DSS (mol wt 36-50kDa) in sterilized drinking water for 11 days. The non-colitic group received plain water. All the animals were continued on the respective diets till euthanization. Body weight, stool consistency and presence of fecal occult blood or gross rectal bleeding were recorded daily for each animal. These parameters were used to calculate the average daily Disease Activity Index (DAI).

Food and water intake were measured daily. After 11 days of induction of colitis, all the animals were fasted overnight. Blood was collected from retro-orbital sinus in EDTA tubes and plasma was separated and stored at -80°C for further analysis. Rats were euthanized by CO<sub>2</sub> asphyxiation method. Abdominal cavity was exposed by midline laparotomy and the entire colon from ileocecal junction to the anal verge was removed and the length of the colon was measured. The colon was then flushed with ice-cold saline, opened longitudinally and a portion of distal colon was immediately fixed in 10% neutral buffered formalin and embedded in paraffin for histopathological analysis. Mucosa was gently scraped with a microscopic slide and the collected mucosal samples were snap frozen in liquid nitrogen and stored at -80°C for the biochemical determinations. The following parameters were analyzed.

- ❖ Colon myeloperoxidase and alkaline phosphatase activities
- ❖ Lipid extraction and quantification of colon phospholipid fatty acids
- ❖ Plasma and colonic mucosal nitrite and nitrate (Nox)
- ❖ Colonic mucosal cytokine (TNF- $\alpha$  and IL-1 $\beta$ ) levels
- ❖ Histological assessment of colonic inflammation by H & E staining

The results are summarised as follows;

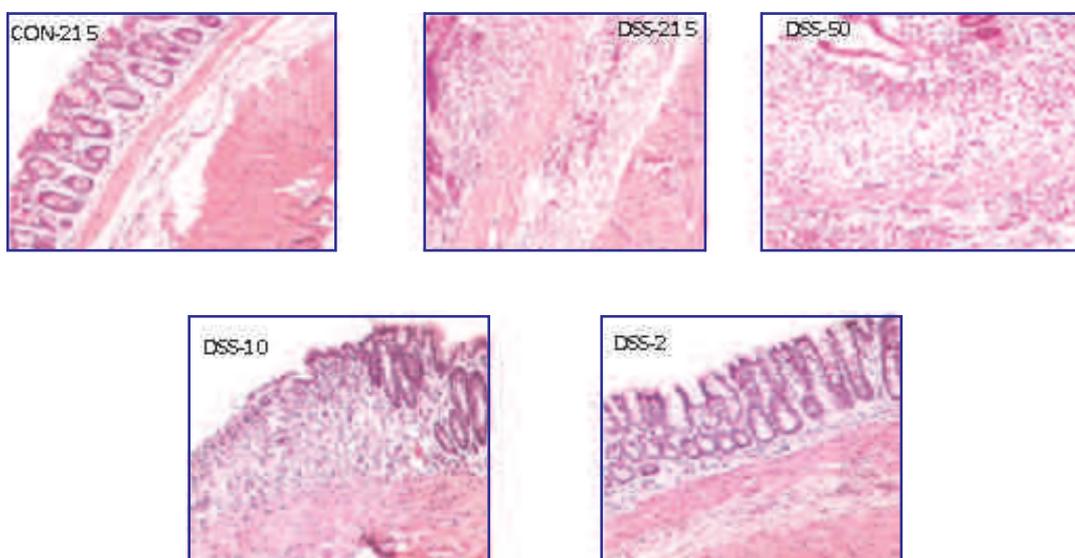
### **Effects of substitution of linoleic acid with $\alpha$ -linolenic acid (precursor of long chain n-3 PUFA) on inflammatory response in DSS induced ulcerative colitis**

- ❖ Substitution of LA with ALA (LA:ALA ratio of 2) increased the food intake without any significant change in body weight. Compared to non-colitic control group, the water consumption was higher in colitic groups. However, there was no difference in the consumption of water, within the colitic groups.
- ❖ Rats given 4% DSS in drinking water for 11 days developed symptoms of colitis without mortality. Based on the clinical symptoms (weight loss, stool consistency and intestinal bleeding), the DAI which assesses the severity of colitis was calculated on daily basis. The DAI became progressive from 2nd day of DSS treatment and peaked between days 9 and 11 depending upon the dietary treatment. Rats fed diet with LA: ALA ratio to 2 (DSS-2) had significantly less severity of the colitis as evidenced by lower DAI score. The decrease in DAI score was apparent on day 9, 10 and 11 compared to other groups. The severity of the colitis was also assessed by measuring the length of the colon which is an indirect marker of inflammation. DSS administration caused shortening of colon compared with non colitic control rats. The decrease in colon length was significantly attenuated in rats fed diet containing LA: ALA ratio of 2.
- ❖ DSS treatment significantly increased colonic MPO activity to a level approximately 4 times higher than the non colitic control group. However substitution of LA with ALA significantly decreased the MPO activity suggesting that ALA exerts antiinflammatory effect by reducing the neutrophil infiltration into the colonic mucosa. In addition to MPO activity, the degree of colonic inflammation was also assessed by measuring colonic ALP activity which has been proposed as a marker of colonic inflammation. DSS induced colitis was associated with significant increase in colonic ALP and increasing ALA in the diet reduced the activity.
- ❖ DSS treatment as such did not have any effect on fatty acid composition. However altering dietary LA:ALA ratio in DSS treated groups showed marked effects on n-6 and n-3 PUFA composition. Decreasing LA:ALA ratio in the diet was associated with decrease in proportion of LC n-6 PUFA (20:4

n-6, 22:4 n-6 and 22:5 n-6) and increase in LA. This was accompanied by a progressive increase in the proportion of ALA and LC n-3 PUFA (20:5 n-3, 22:5 n-3 and 22:6 n-3). Incorporation of EPA was observed in the group fed lowest LA:ALA ratio (highest ALA content).

- ❖ DSS induced colitis was associated with increased colonic mucosal TNF- $\alpha$  and IL-1 $\beta$ . Decreasing LA:ALA ratio to 2, significantly suppressed DSS induced increase in TNF- $\alpha$  and IL-1 $\beta$  levels.
- ❖ Neither DSS treatment nor dietary ALA altered the plasma and colon TBARS levels. DSS administration significantly increased plasma and colon NOx levels. This increase in colon NOx was completely prevented by ALA supplementation.
- ❖ No histological abnormalities were observed in non colitic rats. In contrast, DSS administration showed typical changes in colonic architecture with mucosal ulceration, massive infiltration of inflammatory cells into mucosa and submucosa, depletion of goblet cells, erosion and crypt loss. However decreasing LA:ALA ratio to 2 (DSS-2) showed milder inflammatory cell infiltration, evidenced by epithelial regeneration and restitution.

### Histological appearance of representative H and E stained colon tissue sections

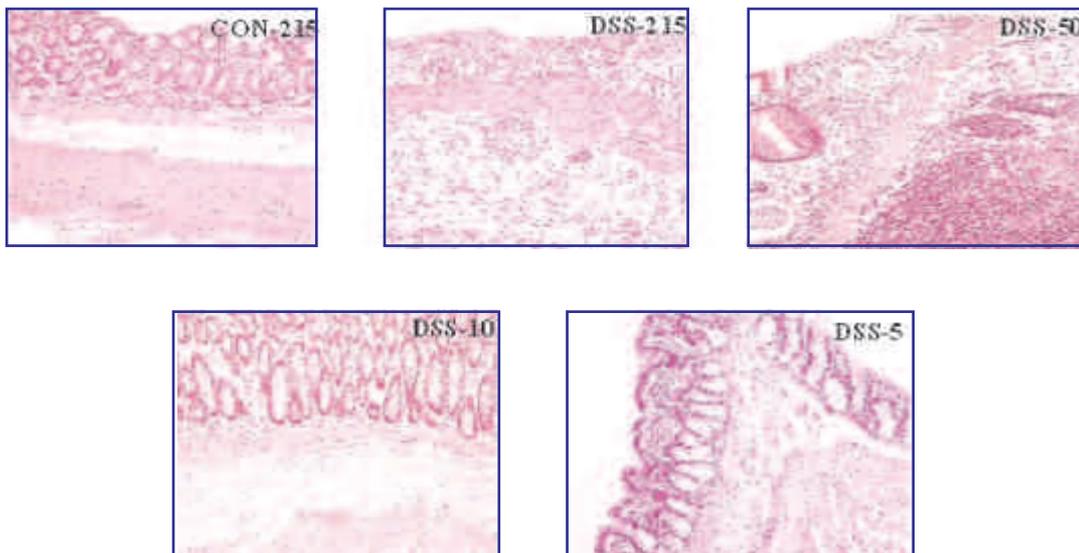


### **Effects of substitution of linoleic acid with long chain n-3 PUFA (fish oil) on inflammatory response in DSS induced ulcerative colitis.**

- Compared to non-colitic control, DSS induced colitis was associated with decrease in food intake and body weight gain. However increasing the LC n-3 PUFA in the diet (DSS-10 and DSS-5) significantly increased the food intake without any changes in body weight gain. Water intake was higher in colitic rats and increasing LC n-3 PUFA did not alter the water intake.
- DSS administration caused significant colonic damage as reflected in the increased DAI. Increasing the LC n-3 PUFA in the diet (DSS-10 and DSS-5) reduced the symptoms of the colitis as evidenced by decrease in DAI index. The colon length which is the marker of inflammation was significantly decreased in DSS induced colitic rats. However, increasing LC n-3 PUFA in the diet (DSS-10 and DSS-5) significantly increased the colon length.
- DSS induced colitis was associated with significant increase in colonic MPO activity. However, substitution of LA with LC n-3 PUFA significantly decreased the MPO activity suggesting that LC n-3 PUFA exerts antiinflammatory effect by reducing the neutrophil infiltration into the colonic mucosa. In addition to MPO activity, the degree of colonic inflammation was also assessed by measuring colonic ALP activity which has been proposed as a marker of colonic inflammation. DSS induced colitis was associated with significant increase in colonic ALP and increasing LC n-3 PUFA in the diet reduced the activity.

- Substituting LA with LC n-3 PUFA in DSS treated groups altered the n-6 and n-3 PUFA composition. Increasing LC n-3 PUFA was associated with decrease in LC n-6 PUFA (20:4 n-6, 22:4 n-6 and 22:5 n-6). This was accompanied by a progressive increase in the proportions of LC n-3 PUFA (20:5 n-3, 22:5 n-3 and 22:6 n-6). Incorporation of EPA (20:5 n-3) was observed only in the group fed with highest levels of LC n-3 PUFA (DSS-5).
- Compared to noncolitic control, DSS induced colitis was associated with significant increase in colonic mucosal proinflammatory cytokines such as TNF $\alpha$  and IL-1 $\beta$ . However, substitution of LA with LC n-3 PUFA (DSS-10 & DSS-5) significantly reduced the cytokine levels.
- DSS administration significantly increased plasma and colon NOx levels. However, substitution of LA with LC n-3 PUFA (DSS-10 and DSS-5) normalized the plasma and colon Nox.
- DSS administration showed typical changes in colonic architecture with mucosal ulceration, massive infiltration of inflammatory cells into mucosa and submucosa, depletion of goblet cells, erosion and crypt loss. However, decreasing LA: LC n-3 PUFA ratio to 10 or 5 (DSS-10 and DSS-5) showed milder inflammatory cell infiltration, evidenced by epithelial regeneration and restitution.

**Histopathological appearance of representative H and E stained colon tissue sections**



**CONCLUSIONS**

- Substitution of LA with ALA (n-6:n-3 ratio of 2) or LC n-3 PUFA (n-6:n-3 ratio of 10) mitigates the DSS induced colitis as evidenced by reduction in neutrophil infiltration, preservation of colonic architecture and reversal of the shortening of the colon length as well as improvements in the symptoms of the colitis.
- The bioequivalence of ALA (precursor of LC n-3 PUFA) from vegetable oils vs LC n-3 PUFA (biologically active product) from fish oil for the prevention of DSS induced colitis would be 5:1.
- The antiinflammatory effects of n-3 PUFA supplementation was associated with decreased production of proinflammatory mediators involved in the inflammatory response of the colon such as nitric oxide and cytokines such as TNF- $\alpha$  and IL-1 $\beta$ .
- The beneficial effects of n-3 PUFA supplementation could be ascribed to the increased levels of LC n-3 PUFA at the expense of LC n-6 PUFA in the structural lipids of colon.

# V. PUBLICATION, EXTENSION AND TRAINING

## A. EXTENSION ACTIVITIES

### 1. PUBLICATIONS

The quarterly periodicals, Nutrition (English) and NIN Monthly Newsletter were published. The publications Recommended Dietary Allowances and Diet and Heart Disease were reprinted on popular demand.

### 2. TRAINING PROGRAMMES

#### *Regular Training Programmes*

This year, a total of twenty four candidates have attended the regular training programmes of the Institute viz. (i) MSc (Applied Nutrition) IV Batch 2013-14 – 16 participants (ii) Post-Graduate Certificate Course in Nutrition - 8 participants including two from Bangladesh.

The Mini-Convocation for the 3<sup>rd</sup> Batch of Msc (AN) was held on March 7, 2014. Certificates were awarded to the successful candidates and also Dr.B.K.Nandi Fellowships and Prize was given to the meritorious students.



### 3. EXTENSION ACTIVITIES

#### *3.1 Exhibitions*

- 11<sup>th</sup> Infra Educa - 2013, a mega exhibition on promotion of Science and Technology, Research & Development, Health and Health Care scheme among young aspirants of Jammu & Kashmir, Jammu (June 28-29).
- Represented NIN in ICMR Pavilion at the II Science and Technology Expo 2013 organised by Sansa Foundation at Panjim, Goa (Sept.28-30).
- Participated on behalf of ICMR in the Mega Science Expo of the 101<sup>st</sup> Indian Science Congress, organized at University of Jammu, Jammu (Feb. 3-7, 2014).



#### *3.2 Popular Lectures/Awareness Camps*

- Delivered an extension lecture on Nutrition and Health to the School Students of



Hyderabad Public School. Also, importance of physical activity for adolescent age group was explained to the school children in order to promote Oorja Clubs (Energy Clubs to promote healthy eating behavior and physical activity among adolescents) in programme organized by Kendriya Vidyalaya (Central Schools) of Hyderabad and Nalgonda (April 8 & 9, 2013).

- An extension lecture on Nutrition and Health was delivered to the members of Agarwal Community, Hyderabad. About 50 members participated in the programme (Apr. 28).
- A nutrition awareness session at Mars High School, Bahadurpura, Hyderabad was held on 8<sup>th</sup> July 2013. About 250 students and their teachers attended.
- Popular talk on Nutrition and dietary guidelines was delivered on 20<sup>th</sup> July, 2013 at Bharat PG & Degree College, Kachiguda, Hyderabad . Over 75 girl students and their teachers took part.
- Conducted a nutrition awareness session for the parents of pre-school children at KIDZEE Play School, Tarnaka on 7<sup>th</sup> September 2013. About 60 parents participated in the interactive session.
- Addressed the students of Madina Public School, Hyderguda on 17<sup>th</sup> September 2013 and delivered a talk on 'Nutrition during adolescence'. Over 300 students participated in the session.
- Delivered a popular talk for mothers of infants/pre-school children from slums of Hyderabad at “Empowering Parents” session on 4<sup>th</sup> October 2013 at the National Conference of Indian Academy of Pediatrics and IYCF-IYCNCN held at Hotel Marigold, Hyderabad.
- Delivered lectures on “Nutrition and Revised Dietary Guidelines” for two batches of Senior Executives of Bharat Electronics Limited as part of awareness programme organized by the company. Importance of Nutrition in day to day life and new dietary guidelines were explained to the participants. In each batch 250 employees participated in the programme (Nov. 14).
- Delivered an extension lecture on “Nutrition and Health” to the ISW Personnel of AP Police Academy, Hyderabad (Nov. 20).
- Delivered a popular talk on the importance of Nutrition and Health to the police officials working with Security Wing of Intelligence Department. About 50 officials working with Chief Minister's security wing participated in the programme (Nov. 20).
- Delivered an extension lecture on Nutrition and Health for the students of Nutrition and the faculty members of PG College, Nashik. About 100 students and faculty members of the college attended the Programme (Nov. 29).
- Delivered a popular talk on 'Dietary Guidelines and Junk Foods' to the school children of 6-10 classes at Iqra Mission High School at Nawab Shah Kunta, Hyderabad on 3<sup>rd</sup> Dec 2013. Over 300 students took part.
- Delivered a popular talk on “Role of Nutrition in Health Research activities: NIN's contributions. About 150 students from Medical, Nursing and Nutrition colleges participated in the event organized at Sri Ramachandra University, Chennai (Dec. 10).
- Delivered an extension lecture on “Nutrition and Health” to the ISW Personnel of AP Police Academy, Hyderabad (Dec.18).
- Delivered 2 extension lectures on “Nutrition and Health” to the Andhra Bank Executives, at Madhapur, Hyderabad (Dec. 20 & Jan, 17).
- Delivered an extension lecture on “Nutrition and Health” for Police Officials, Intelligence wing, Police Headquarters, Somajiguda, Hyderabad. About 100 police officials participated in the programme (Jan. 4, 2014).
- Delivered a talk on Nutrition and Health to the parents in cooking completion programme organized by Sahaja Kids, Hyderabad. About 40 parents participated in the event. (Feb. 23, 14).

- Participated in the Awareness Programme on Diet and Nutrition for the employees from Bharat Dynamics Limited, Hyderabad on 4<sup>th</sup> March 2014. Over 100 employees of all cadres participated.
- Delivered a talk on health and nutrition and inexpensive healthy foods at a slum in the old city, Hyderabad.

### 3.3 Radio Talks and TV Programmes

- Radio talk for AIR, Hyderabad on “Mahilalu, Pillalaku, - Poshakaharam”. (Nov. 27)
- Radio talk for AIR, New Delhi on “Junk Foods” in English and Hindi. (March 29)
- Accessibility to government health care services by the poor migrant population of Hyderabad on ETV (April 25).
- Good / adverse effects of energy drinks on adolescents was aired on TV10 (July 25).
- “Samathulam.. Aarogya Suthram” (Balanced Diet for Complete Health) on Eenadu (Sept. 9).

## 4. SPECIAL EVENTS

### 4.1 World breast feeding week celebration (Aug. 1-7, 2013)

The following programmes were organized in connection with the World Breast Feeding Week celebrations:

- In association with Food and Nutrition Board, Hyderabad an awareness programme was organized for “ICDS workers of Alwal”, at Malkajgiri government boys high school campus, Hyderabad. The primary health centre medical officer, ICDS project officer, supervisor’s, teachers and students participated in the programme. Delivered lecture on Nutrition and Health (Aug. 3, 2013).



- The National Institute of Nutrition and the World Vision of India jointly organized a programme at Tarnaka, Hyderabad for the “World Vision grass root level workers those who were working in the urban areas”. The topics covered were General Nutrition Information for women, Nutritional need during pregnancy and lactation, The importance of exclusive breast feeding, complementary feeding and when it should be started, personal hygiene practices while preparing the food and feeding time were elaborately discussed with the participants. One hundred members participated in this programme (Aug. 5, 2013).
- In collaboration with World Vision an awareness programme was organized at Shad Nagar, Werlcharla village especially for pregnant and lactating women. An informative lecture covering the topics about nutrition during pregnancy, exclusive breast feeding, food requirement of the body during pregnancy, Importance of micro nutrients, personal and environmental hygiene, safe drinking water, disease prevention and immunization, exclusive breast feeding and after sixth month why complementary feeding should be initiated were explained to them (Aug. 7, 2013).
- An awareness programme was organized at Welcharla village, Shad Nagar for the adolescent girls at Government High School. All the topics related to growth and development was explained to them, with emphasis was given on micronutrients in human health.
- DHAN foundation and ICDS workers jointly organized the programme at “Hanuman Nagar, Moula Ali, Hyderabad for pregnant and lactating women. Important Nutrition topics were explained to them with special focus on breast feeding and complementary feeding practices (Aug. 8, 2013).

## 4.2 National Nutrition Week Celebrations (Sept. 1-7, 2013)

In connection with the National Nutrition Week celebrations, the following programmes were organized;

- A one day Symposium on “Food Security for Health and Nutritional Well-being” was organized in association with Food and Nutrition Board (Sept. 3).
- Delivered a lecture on Nutrition with special focus on nutritional need for adolescent girls and infant feeding mothers, in the awareness program organized by ICDS department, Mallapuram. Anganwadi workers, school teachers, pregnant and lactating mothers and school students participated in the programme (Sept. 5).
- Delivered a lecture on nutritional need for adolescent girls in an awareness program organized at Railway Girls High School, Lallaguda for the students of 7<sup>th</sup> to 10<sup>th</sup> standard and to school teachers (Sept. 6).



## B. RESEARCH ACTIVITIES

### 1. NUTRITION EDUCATION FOR ADOLESCENTS: AN INTERVENTIONAL APPROACH TO CREATE AWARENESS ON “EAT RIGHT AND PLAY WITH MIGHT”

Adolescents are most important group of population and the adolescent phase is very critical in the life cycle. They are also vulnerable to adopt faulty eating habits mostly due to peer pressure, which coupled with lack of physical activity results in overweight and obesity among adolescents. Food patterns established during childhood are likely to be maintained for life and may have long-lasting influences on their future health and the health of their families. Furthermore, an inadequate diet during childhood could result in unfavorable physiological consequences that could lead to diet-related chronic diseases. Therefore, the dietary patterns of school children is of concern to health professionals. It is well documented that children have unhealthy eating behaviors, including skipping meals, frequent snacking on energy-dense foods, lack of sufficient physical activity and engaging in unhealthy weight-loss methods. Therefore, a study was conducted to impart education on nutrition, health and physical activity through interactive approach in a school setting.

#### GENERAL OBJECTIVE

- To promote healthy eating practices and regular physical exercises among school going children/ adolescents through *OORJA* (Energy) clubs in schools of Hyderabad, Andhra Pradesh.

#### Specific Objectives

- To develop, evaluate and implement a school-based nutrition education program and physical exercise interventions through *OORJA* (Energy) clubs.

- To organize workshops and training programs for facilitators of the program.
- To promote effective nutritional practices and physical education and communication that supports and encourages healthy behavior.

## **METHODS**

### ***Institutional review board and Ethical clearance***

Before initiating the project, the study protocol was reviewed by the Scientific Advisory Committee of the Institute and approval was obtained. Similarly, Institute's ethical clearance (IEC) was also obtained vide ref. no: 04/2012/II. An informed consent was obtained from the parents of those children who participated in the study and as well as the Principals of the concerned schools.

***Study Design:*** Institution based cross-sectional study

### ***Study Participants***

The participants were from classes 6<sup>th</sup> and 7<sup>th</sup> belonging to urban schools in Hyderabad, Secunderabad and Nalgonda.

### ***Sample Size***

The OORJA (Energy) Clubs: Eat Right - Keep Moving initiative was launched in about 19 Kendriya Vidyalaya schools in Hyderabad region. From each school, 4 students were drawn as trainers at random. About 74 adolescents were given exposure emphasizing the importance of nutrition as well as physical activity in a workshop mode. These adolescents in turn developed IEC material and presented them to the rest of the adolescents through PowerPoint presentations. Improvement in the levels of nutritional knowledge as well as physical activity was assessed at two time points. Initially, the trainers were assessed for their improvement in the knowledge levels and the concept of the programme. The second intervention was undertaken by the adolescent trainers at the behest of the adolescents (500no.) to assess the improvement in the knowledge levels and also the behavioral changes among the adolescents in their respective schools.

## **RESULTS**

About 70% of the students were aware of the importance of breakfast but only 32% reported to be taking it daily. Most of them were aware of the importance of different food groups. Although they knew the ill-effects of carbonated beverages, 85% of them consumed it frequently. About 65% of the adolescents indicated the consumption of fried foods often while 35% indicated consumption of boiled eggs. More than 50% adolescents indicated lack of physical activity after the school hours. About 41% indicated that inadequate physical activity was due to lack of facilities, coupled with the pressure towards academics. However, these adolescents participated in the physical activity without realizing the benefits of the same, as part of the physical education in the schools. About 71% adolescents indicated the influence of TV advertisements on their food behavior while 29% indicated that peer pressure impacted their food choices. In the first intervention, a significant improvement in the knowledge levels of nutrition and also importance of the physical activity was observed among the trainers.

In the second point of intervention, adolescent trainers were made to carry out peer education and trained the rest of the adolescents by imparting knowledge related to nutrition and health, including physical activity. There was significant interaction and discussion among the adolescents about the importance of nutrition and physical activity among the peers. The results indicated that there was significant improvement in the knowledge levels of the adolescents with regard to breakfast, importance of nutrition and regular physical activity. The sustainability of the programme among the adolescent population is expected to achieve remarkable behavior changes among these adolescents which needs to be assessed.

## **CONCLUSIONS**

- An integrated approach on nutrition education combining interactive lecture methods and participatory approaches which engage students in training other peers, proved to be effective in imparting nutrition knowledge and in creating awareness on the importance of physical activity.

- As a part of this project, data on the intervention is being generated from different parts of the country to assess the efficacy of the Oorja clubs on the overall health of the adolescent population in the country.
- A peer education model, wherein, selected adolescents carry out integrated nutrition and health education in the school set up in non-formal context is being developed for use in the schools. Kendriya Vidyalaya Sansthan, Secunderabad which run several schools in AP has come forward to partner with NIN to carry out a pilot study of the model in the selected schools in Hyderabad. It is also proposed to replicate the model in other schools after carrying out needful modifications. Oorja model is expected to affect desirable behavioural changes in the realm of nutrition and physical exercises among adolescent population in the communities.

## **2. NUTRITIONAL AWARENESS AMONG PRIMARY SCHOOL CHILDREN: ROLE OF INTERVENTIONAL APPROACHES**

Childhood is an impressible age and those impressions are with us lifelong. School science books provide information on nutrition and it is important to assess the quality of nutrition knowledge a child possesses. Many groups of students and general public, approximately 5-6 thousands visit National Institute of Nutrition from different states of India to gather information on nutrition and health. Visit to the museum will help the visitors to go through the different charts on all aspects of nutrition like food groups, balanced diet, micronutrient deficiency diseases etc. School children after going through the museum could have a good interactive program with the scientists on various aspects of nutrition, health, good food habits and hygiene. Through the interactive session it was observed that the health and nutrition knowledge in general, among these children is not adequate even though they have nutrition as a part of their science subject. Therefore an attempt was being made to educate the children on the importance of nutritious foods and good eating habits and how they can have a healthy life style in future. It is also observed that the school children show more interest in the session, since it is supported by visuals.

### **OBJECTIVES**

- To assess nutritional awareness status among primary school students.
- To develop various academic activities and games to educate children on nutrition and health.

### **METHODOLOGY**

One government school and one corporate school were included in the study. Children between 8-10 years i.e., 3<sup>rd</sup> class/4<sup>th</sup> class will be recruited as subjects. A pilot study was conducted on 30-40 primary school students. The level of nutritional and health knowledge were assessed. The sample size for the final trial in the experiment will be calculated after the pilot study. The experimental study would be conducted in the schools where pilot study was not conducted.

In the experimental group a baseline study was carried out by using various activities like recognizing foods, fill in the blanks, match the following, differentiating healthy and unhealthy foods, which are based on colourful pictures to make the activities attractive and memorable. The knowledge assessment will be done individually as the students have to recognize the food items and the activity sheets have to be filled up in presence of the investigator.

The intervention would be based on the above mentioned activities using colourful pictures and samples of foods like cereals and pulses. The same activities would be repeated with the students who participated in the study. It is also proposed to conduct an awareness program of the parents of the students at the end of the present study.

### **Expected Outcome**

- Improvement in the knowledge levels in the children
- Children would turn into agents of change as they may influence parents' about the right food choices

### **CONCLUSIONS**

The findings at the end of the study were impressive. As the questionnaire and the intervention material were all based on colourful visuals and samples of cereals and pulses, the children were able to grasp well.

As the initial knowledge on fruits and vegetables was less in the Government school compared to corporate school on fruits and vegetables, the increase in the knowledge in the govt. school was more than the corporate school. For example, in recognizing the fruits, the increase in knowledge was 25.5% in the govt. school were as in the corporate school it was only 0.2%. The increase in knowledge in govt. school on vegetables was 29.8% were as in corporate school it was 14.3%. It was observed that the increase in knowledge on health, nutrition and hygiene in general was 19% in the govt. school and 15.1% in the corporate school. Maximum increase in knowledge was seen on cereals and pulses in both the schools. The increase in knowledge on cereals was 91.4% and 94.7% in government and corporate schools respectively and on pulses was 36% and 50.2% in govt. and corporate schools respectively.

As the results are remarkable the study can be continued at the high school level also.

# VI. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

## 1. PREVALENCE AND SEVERITY OF FLUOROSIS IN DODA DISTRICT, JHAMMU AND KASHMIR (J&K)

Hydrofluorosis/ fluorosis are caused due to prolonged intake of water with excess fluoride (>1.0 ppm). In India, fluorosis is a public health problem and is endemic to 204 districts of 21 states of India including the Doda district of J & K. There are few reports available on prevalence of fluorosis in Doda district. The referred studies suggested that in Doda District, 90% of the population studied were suffering from dental fluorosis, 12% were having skeletal deformities as well as bone pains and 60% of the population above the age of 25 years was suffering from dyspepsia. Recently, a local newspaper (Greater Kashmir) of J & K reported that more than 5000 people in Doda district are affected with fluorosis. On a request basis analysis of water samples from Doda, J & K was carried out at National Institute of Nutrition, Hyderabad, it was found that fluoride content of 6 water samples of 8 were above the permissible level. The preliminary data available is not adequate to know the prevalence of fluorosis (dental and skeletal) in Doda district, J & K. Therefore, at the request of Ministry of Health, J & K, the present study was being undertaken to ascertain the problem of fluorosis in the community of Doda District, J & K.

### GENERAL OBJECTIVE

- To ascertain the problem of fluorosis in Doda District, J & K

### SPECIFIC OBJECTIVES

- Fluoride mapping in the Doda district, J&K.
- Clinical examination and biochemical investigations in water, urine and blood samples

### STUDY DESIGN

It was a cross sectional epidemiological study in randomly selected school going children aged 5-14 years (in both sexes), at randomly selected schools of Doda District, J&K.

### METHODOLOGY

The study was started after approval from Institutional Ethics Committee, National Institute of Nutrition, Hyderabad.

Clinical examination was done for screening of dental symptoms of fluorosis.

### *Dental mottling*

Dental mottling and Community Fluorosis Index (CFI) was measured on the basis of dental fluorosis (Dean's classification) viz., normal, questionable, very mild, mild, moderate, moderately severe, and severe and each of the seven categories were assigned numerical score as 0, 0.5, 1, 1.5, 2, 3 and 4 respectively.

### SAMPLE COLLECTION

**Collection of drinking water samples and estimation of fluoride:** Drinking water samples from affected and non-affected areas of school children were collected in a clean 100ml plastic container.

Water samples were carefully transported to NIN laboratory and estimated for fluoride levels using 'Ion selective fluoride metre, Model: EA 940 (Orion Research), Boston, USA.

**Collection of spot urine samples and estimation of fluoride:** Spot urine samples were collected randomly in clean 100ml plastic bottles, 2 to 4 drops of toluene was added as a preservative. The samples were transported to National Institute of Nutrition and stored at 4°C till further use. Fluoride levels were analyzed using 'Ion selective fluoride metre Model: EA 940 (Orion Research), Boston, USA.

**Collection of blood samples:** Blood samples were collected in plain serum vacutainer (*Vacurette*, Greiner Bio-one, Austria) by a trained technician. Collected samples were kept in ice and transferred to local Govt. Hospital laboratory; serum was separated by centrifugation at 2000rpm for 20 min and stored at -20°C using SLR refrigerator at the hospital. The serum samples were transported to NIN laboratory in cold conditions using a vaccine carrier. Serum samples were kept at -80°C till further use.

### **Biochemical investigations**

Serum samples were analysed for different biochemical parameters like Creatinine, Urea, Calcium, Iron and Alkaline Phosphatase (ALP) using "Alfa Wassermann" Auto analyser, model: Ace Alera, USA.

Serum Osteocalcin was measured for intact Osteocalcin using 'MicroVue' Osteocalcin immune assay kit, supplied by Quidel corporation, USA, Para Thyroid Hormone (PTH), 25-(OH)VitaminD, 1,25 (OH)<sub>2</sub> VitaminD, T3 and T4 were measured by using DiaSorin kits, supplied by DiaSorin Minnesota, USA. TSH-CTK-3 was measured by immunoradiometric assay (IRMA) kit, supplied by DiaSorin Saluggia (VC) Italy.

### **Anthropometric measurements**

Weight's were measured using digital weighing machine. The height was assessed using height rod. BMI was calculated using the formula

$$\text{BMI} = \frac{\text{Weight (in kg)}}{\text{Height (meter)}^2}$$

### **Statistical Analysis**

Descriptive statistics like mean, median, SD and prevalence was carried out for all variables. Comparison of mean values of blood parameters was done using ANOVA / t-test across the gender and groups. Association between clinical examination and biochemical investigations like serum creatinine, Urea, Calcium, Iron and Alkaline Phosphatase (ALP), TSH, Osteocalcin, Para Thyroid Hormone (PTH), 25-(OH)VitaminD, 1,25(OH)<sub>2</sub> VitaminD, T3 and T4 were tested using Chi-square test. Level of significance was considered as 0.05.

## **RESULTS**

Mean drinking water fluoride levels of the children studying in the affected and non-affected village schools are given in the Table 1. The fluoride levels were highest in the Golibagh followed by Malwas village (3.84 and 3.12 ppm respectively), whereas in control area it was 1.13ppm. An average intake of fluoride areas of both affected and non-affected school children through drinking water and tea is given in Table2. It reveals that highest intake of fluoride was in the children of Golibagh village school (5.88 ppm) and lowest was observed in Ghat school (3.53 ppm) of affected area schools. However from non-affected area the fluoride levels of school children were 3.16 ppm. The average fluoride exposure dose (mg/kg/day) was also calculated and given in Table 3, it was found that Ghat school children were exposed minimally 0.05 mg/ kg/ day whereas Golibagh village children shown 0.20 mg/kg/day. The minimum safe level exposure dose is 0.05 mg/kg/day. In control area schools, an average fluoride exposure dose is 0.04 mg/ kg/ day which was lower than minimum safe level exposure dose.

The percentage of fluorosis prevalence and Community Fluorosis Index (CFI) from the selected schools is given in Table 4. There was low community fluorosis index seen in Ghat middle school children (0.18), whereas highest in Malwas School (2.65). The control schools showed CFI 0.21 and 0.32 in Green Model School and Govt. Secondary School of Doda respectively.

**Table1. Water fluoride levels (Mean ± SD) in affected and non-affected area schools of Doda District, J & K**

S. No	Name of the School	Mean Water Fluoride level (ppm)
<b>Affected Schools</b>		
1	Govt. Girls Middle School Ghat	2.05 (1.07 - 2.59)
2	Govt. Boys Middle School Ghat	2.05 (1.07 - 2.59)
3	Royal Academy, Ghat, Doda.	1.48 (0.23 - 2.71)
4	Public High School , Ghat, Doda	1.44 (0.55 - 2.60)
5	Public High School , Arnora	2.04 (1.68 - 2.58)
6	Govt. UPS, Malwas	3.12 (2.97- 3.20)
7	Govt. UPS, Golibagh	3.84 (3.83 - 3.84)
8	Govt. High school , Ganika	1.85 (1.57 - 2.13)
<b>Non affected Schools</b>		
9	Green Model school Doda.	1.13 (0.32 –1.67)
10	Gov.t Hr. sec school, Doda.	1.13 (0.32 – 1.67)

**Table 2. Daily Fluoride intake from Water and Tea (Mean ± SD) in affected and non-affected area School children of Doda district, J & K**

S.N	Name of the School	Daily Fluoride intake form Water& Tea (ppm)
<b>Affected Schools</b>		
1	Govt. Girls Middle School Ghat	5.10 (3.06 - 7.15)
2	Govt. Boys Middle School Ghat	5.10 (3.06 - 7.15)
3	Royal Academy, Ghat, Doda	3.56 (2.01 - 5.03)
4	Public High School, Ghat, Doda	3.53 (1.99 - 5.01)
5	Public High School, Arnora	4.12 (2.38 - 5.78)
6	Govt. UPS, Malwas	5.16 (3.10 - 7.22)
7	Govt. UPS Golibagh	5.88 (3.58 - 8.18)
8	Govt. High school, Ganika	3.96 (2.26 - 5.52)
<b>Non-affected Schools</b>		
9	Green Model school Doda.	3.16 (1.77 - 4.56)
10	Govt. Hr. sec school, Doda.	3.16 (1.77 - 4.56)

If we consider boys and girls for fluorosis prevalence from the affected village schools, CFI was lower in Ghat school boys (0), girls (0.18) whereas, was highest in Malwas school boys (2.78), girls (2.72). In control schools it was 0.18 in boys and 0.24 in girls in Green Model School, Doda, whereas 0.32 in boys of Govt. Higher Secondary School, Doda. An average prevalence of dental fluorosis based on community fluorosis index, was found more in girls (0.94) whereas it was less in boys (0.87). CFI value 0.6, is an optimum index value above which fluorosis is considered to be a public health problem. The villages Golibagh (CFI=1.75) and Malwas (CFI=2.65) have more CFI than normal 0.6. Hence, these villages have public health problem. Percentage of dental fluorosis was calculated by number of children affected with different grades of fluorosis from total number of children in the school. It was revealed from affected area schools like Golibagh and Malwas, were 100% children were affected with fluorosis whereas 20 to 31% children were affected from control areas wherein most of them fall in grade 1 only.

**Table 3. Fluoride Exposure Dose through drinking water for different age groups of affected and non-affected area children in Doda district, J & K**

S.No	Name of the School	Mean Water Fluoride level (ppm)	Fluoride Exposure dose level		
			Min	Max	Average (mg/kg/day)
<b>Affected Schools</b>					
1	Govt. Girls Middle School Ghat	2.05	0.06 - 0.15	0.10	
2	Govt. Boys Middle School Ghat	2.05	0.06 - 0.17	0.10	
3	Royal Academy, Ghat, Doda	1.48	0.05 - 0.15	0.66	
4	Public High School, Ghat , Doda	1.44	0.03 - 0.06	0.05	
5	Public High School, Arnora	2.04	0.05 - 0.12	0.08	
6	Govt. UPS, Malwas	3.12	0.12 - 0.23	0.19	
7	Govt. UPS, Golibagh	3.84	0.13 - 0.29	0.20	
8	Govt. High School, Ganika	1.85	0.06 - 0.14	0.08	
<b>Non-affected Schools</b>					
9	Green Model school Doda.	1.13	0.03 – 0.07	0.04	
10	Gov.t Hr. sec school, Doda.	1.13	0.03 - 0.06	0.04	

**Table 4. Community Fluorosis Index (CFI) and % of dental fluorosis prevalence in affected and non-affected area school children**

Name of the School	Students (Boys & Girls)	Water Fluoride level (ppm)	No. of students classified according to Deans classification							Total no. of Students	CFI	School average CFI	% of Fluorosis incidence
			0	0.5	1	1.5	2	3	4				
<b>Affected Areas</b>													
Govt. Girls Middle School, Ghat	Boys	2.05	14	3	2	2	0	0	0	21	0.31	0.49	34
	Girls		9	3	2	3	1	1	0	19	0.68		52
Govt. Boys Middle School, Ghat	Boys	2.05	10	0	0	0	0	0	0	10	0	01.8	0
	Girls		15	0	1	0	1	0	0	17	0.18		12
Royal Academy, Ghat, Doda.	Boys	1.48	22	2	5	3	5	3	1	41	0.81	0.85	46
	Girls		18	7	3	5	4	4	1	42	0.90		57
Public High School, Ghat , Doda	Boys	1.44	25	2	14	7	4	2	0	54	0.80	0.84	54
	Girls		22	5	11	9	7	1	0	55	0.89		60
Public High School, Arnora	Boys	2.04	4	2	6	3	1	0	0	16	0.97	0.88	75
	Girls		6	0	4	2	2	0	0	14	0.79		57
Govt. UPS, Malwas	Boys	3.12	0	0	1	1	0	3	1	6	2.58	2.65	100
	Girls		1	0	2	0	2	0	6	11	2.72		90
Govt. UPS, Golibagh	Boys	3.84	0	1	3	2	2	4	0	12	1.25	1.75	100
	Girls		0	0	2	0	1	1	0	4	1.75		100
Govt. High school, Ganika	Boys	1.85	26	0	3	2	0	0	1	32	0.37	0.34	19
	Girls		21	0	2	0	1	0	1	25	0.32		16
<b>Non-Affected Areas</b>													
Green Model school, Doda.	Boys	1.13	16	27	13	5	2	0	0	207	0.18	0.21	22
	Girls		99	24	16	4	1	0	0	144	0.24		31
Gov.t Hr. sec school, Doda	Boys	1.13	60	18	7	6	3	0	0	94	0.32	0.32	36

Dental fluorosis grading combine (boys and girls), but only boys and only girls at different levels of fluoride is given in the graphs (Fig.1-3). Urinary fluoride was significantly higher ( $p<0.05$ ) in affected children as compared to the non-affected area school children, whereas there was no difference in serum urea and creatinine (Table 5) of Doda district school children, J&K. Serum total ALP, PTH, 25 - OH vitamin D, 1, 25(OH)<sub>2</sub> vitamin D and T4 were significantly higher in affected school children ( $p<0.01$ ) as compared to non-affected school children of Doda, whereas there was no significant difference in Osteocalcin, T3 and TSH among the two groups (Table 6).

Fluorosis risk was assessed based on some of the biochemical investigations, kidney related (Creatinine, urea), bone related (ALP, Osteocalcin, PTH, 25OH vitamin D, and 1, 25 (OH) vitamin D, liver related (AST and ALP), thyroid related (T3, T4 and TSH) and anemic (iron) parameter. These results revealed that urea and creatinine was above normal range in affected village children and they were at risk by 15% and 35% for urea and Creatinine respectively. It may not be true since creatinine may rise based on their non-veg consumption during last 12 hrs. The bone related parameters like ALP, Osteocalcin and PTH was above range and the children from affected area were at risk by 71%, 5% and 18% respectively. 25-OH vitamin D was lower (below range) 31% in affected school children whereas only 14% in control area village school children. 1, 25(OH) vitamin D 3% were at risk in affected area whereas there was no risk found in control area school children. 30% children from affected area were anemic where as 65% from control areas were anemic (Table 7).

The team has covered 10 schools in Doda district of J&K out of which 8 are rural and 2 urban. The schools were divided in to 2 categories affected and non-affected based on their drinking water fluoride levels. Out of ten schools 8 belong to rural, affected, whereas two were urban and non-affected. In those 8 schools their average drinking water fluoride level was 1.43 – 3.84ppm. Out of a total of 371 students checked, 48% (171 were affected and in different dental fluorosis grades), and boys and girls were equally affected.

**Table 5. Drinking Water and Urinary Fluoride, Urea and Creatinine (Mean ± SD) levels of Serum in affected and non-affected area School children**

S. No	Category	Age of the children	Water fluoride (<1 ppm)	Urine fluoride (<1.5 ppm)	Serum Urea (10.0- 50.0 mg/dL)	Serum Creatinine (0.5-1.2mg/dL)
1	Affected (N=60)	13.1±1.52	1.83±0.93**	3.65±3.26*	23.73±10.79	0.86±0.76
2	Non- affected (N=20)	13.0±1.68	1.03±0.19	1.84±0.88	14.60±9.20	0.43±0.16

**Table 6. Serum Total ALP, Osteocalcin, PTH, 25-OH Vitamin D, 1, 25(OH)<sub>2</sub> Vitamin D, T3, T4 and TSH (Mean ± SD) of affected and non-affected school children of Doda district, J & K**

S. No	Category	Total ALP (98- 279 IU/L)	Osteocalcin (9.0 - 42.0 ng/ml)	PTH (13 -54 pg/ml)	25-OH vitD (9.0- 37.6 ng/ ml)	1,25(OH) <sub>2</sub> vitD (25.1- 66.1 pg/mL)	T3 (0.8- 2.0 ng/ml)	T4 (6.1- 11.8 µg/dL)	TSH (0.3- 4.0 mU/L)
1	Affected (N=60)	534.67± 379.29**	17.22± 11.26	41.54± 31.64*	20.03± 19.26*	99.18± 42.64*	2.05± 1.76	7.09± 6.04*	3.71± 1.43
2	Non-affected (N=20)	314.80± 111.50	16.87± 6.88	31.30± 8.65	9.10± 5.67	146.10± 25.17	2.03± 2.03	16.12± 3.49	4.30± 1.24

\*

Out of 8 affected schools, 2 schools belongs to Malwas and Golibagh having high drinking water fluoride (>3 ppm). In these village schools more than 95% students were affected with different grades of dental fluorosis (out of 33 students 32 were affected, 12 in grade I, 4 in grade II, 8 in grade III and 8 were in grade IV shown in fig 1-2.). Under control schools two schools named, Green Model School and Govt. Higher Secondary Schools had average drinking water fluoride level of 1.13 ppm. (0.32 ppm -1.18 ppm). 445 students were examined for clinical signs and symptoms of dental fluorosis only 56 were affected (12.5%). Most of them are in grade I, 6 students were in grade II (Table 8) & (Fig 3 - 5).

Nutritional status of the boys from affected and non-affected schools was assessed by measuring their height and weight (BMI). It was registered that the BMI ranged from 14.59 to 18.70 for the age groups of 6 to 15 years in affected area boys, whereas it was 15.87 to 18.71 for same age groups from control area school boys. On comparison with WHO reference range all age group (6 to 15 years) from affected and control area boys had less BMI than WHO reference age group. However age 10-15 years affected area boys aged 11 and 12 years had BMI from 16.15 & 16.28, which was low when compared to non-affected area boys ( BMI=17.03 & 17.49) (Table 9). Likewise nutritional status of the girls from affected and non-affected schools was assessed by measuring their height and weight (BMI). It was revealed that the BMI was ranged from 15.40 to 20.47 for the age groups 6 to 15 years in affected areas girls, whereas 15.37 to 19.74 for same age groups from non-affected area school girls. On comparison with WHO reference range all age group (6-15 years) girls had less BMI than WHO reference. However (age 10-15 years) affected area girls aged 12 years BMI (18.53), which was low as compared to non-affected area girls (18.66) (Table 10) & (Fig 4 and 5).

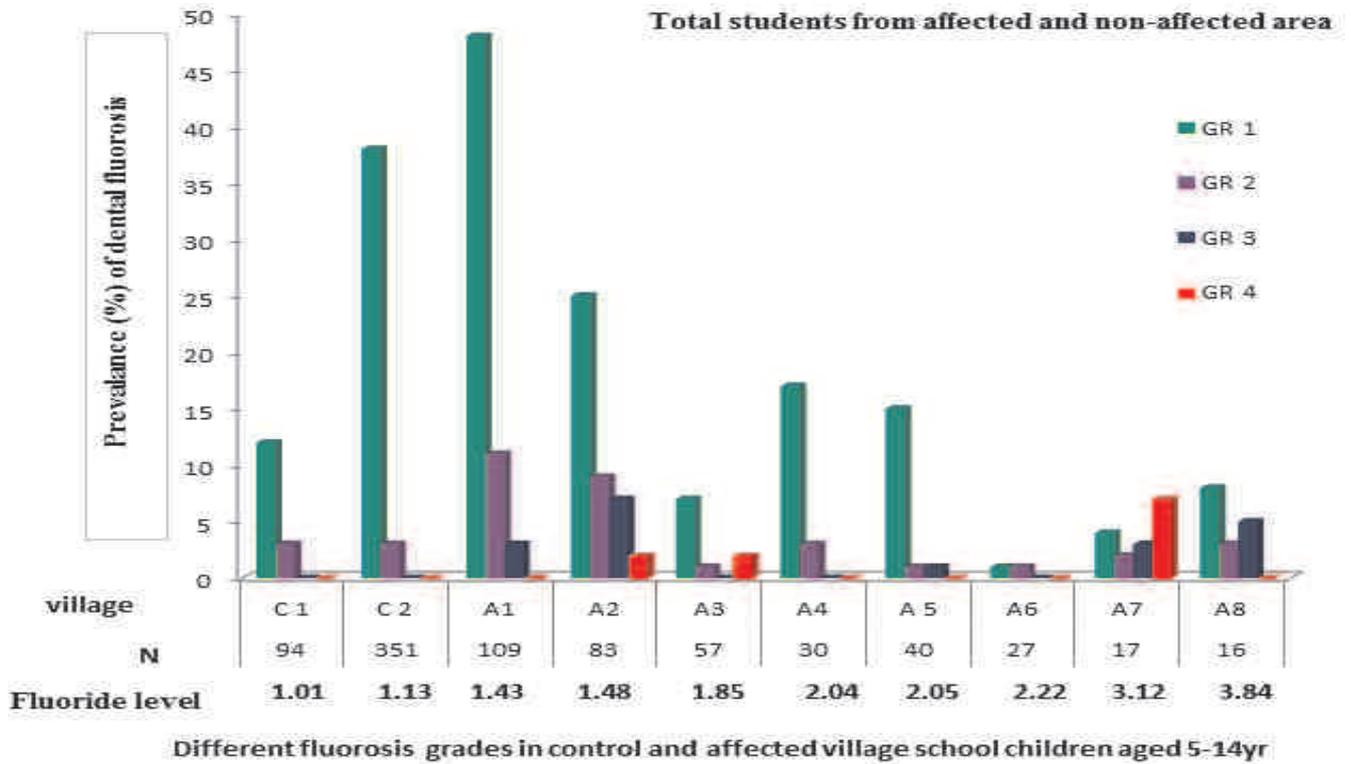
**Table 7. Biochemical parameters of serum of affected and non-affected area school children in Doda district, J & K**

Sl. No		Affected area school children (n=60)			Control area school children (n=20)		
		Below Range (%)	Normal Range (%)	Above Range (%)	Below Range (%)	Normal Range (%)	Above Range (%)
1	Creatinine	15 (25)	36 (60)	9 (15)	0	13 (65)	7 (35)
2	Urea	4 (6.7)	54 (90)	2 (3.3)	6 (30)	14 (70)	0
3	ALP	2 (3.3)	15 (25)	43 (71.7)	0	6 (30)	14 (70)
4	Osteocalcin	6 (10)	51 (85)	3 (5)	0	20 (100)	0
5	PTH	4 (6.7)	45 (85)	11 (18.3)	0	20 (100)	0
6	25 OH Vitamin D	19 (31.7)	32 (75)	9 (18.3)	14 (70)	6 (30)	0
7	1,25 (OH) <sub>2</sub> VitaminD	2 (3.3)	9 (15)	49 (81.7)	0	0	20 (100)
8	T3	16 (26.7)	21 (35)	23 (38)	7 (35)	6 (30)	7 (35)
9	T4	30 (50)	16 (26.7)	14 (23.3)	1 (5)	1 (5)	18 (80)
10	TSH	0	37 (61.7)	23 (38.3)	0	9 (45)	11 (55)
11	AST	0	45 (76.3)	14 (23.7)	0	17(89.5)	2 (10.3)
12	ALT	4 (6.7)	56 (93.7)	0	3 (15)	16 (80)	1 (5)
13	Ca	39 (65)	9 (15)	12 (20)	19 (95)	0	1 (5)
14	Iron	18 (30)	42 (70)	0	13 (65)	7 (35)	0

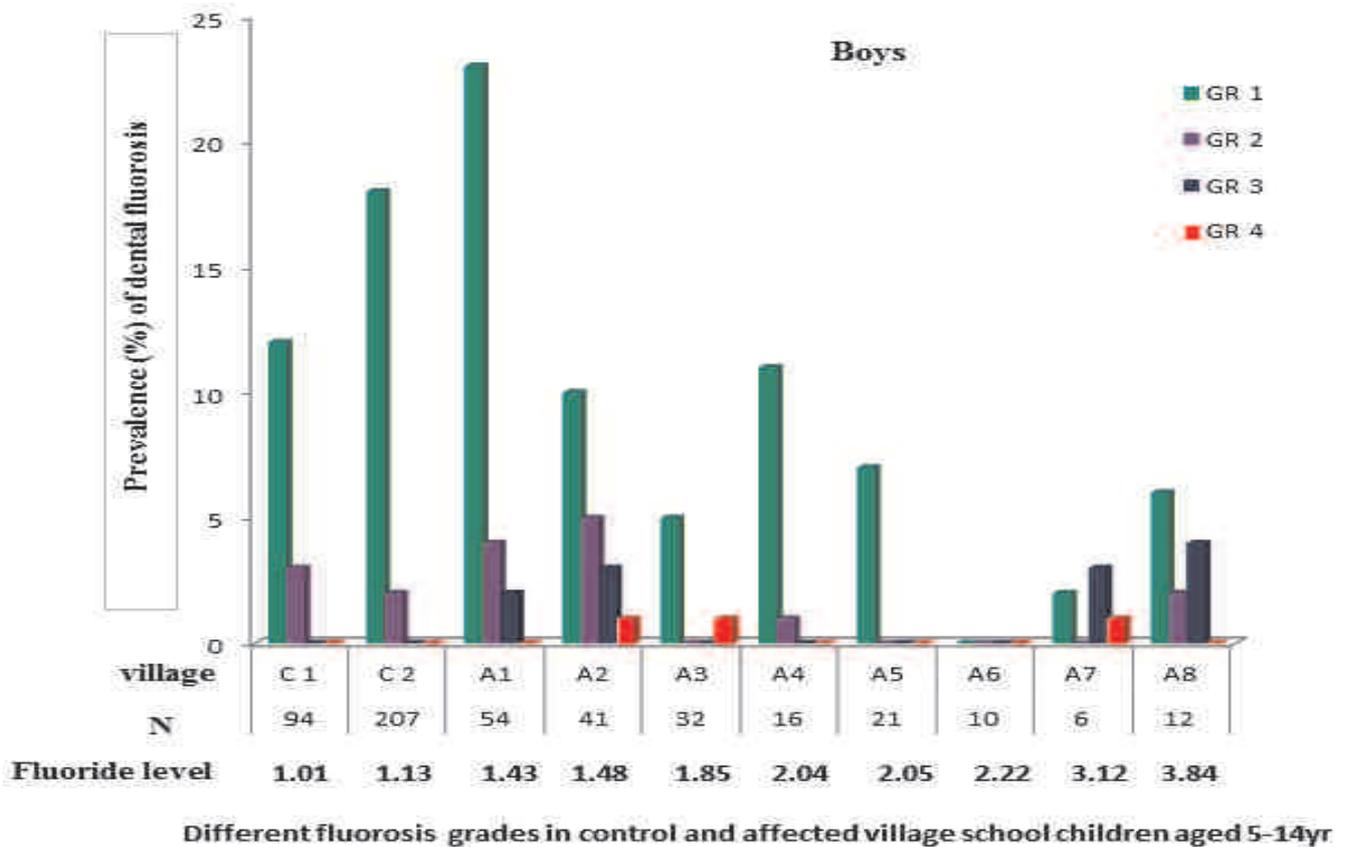
**Table 8. Dental fluorosis grades, water fluoride, percentage of students affected and gender wise affected and non-affected area school children.**

S. No	Name of the School	Average Drinking water F <sup>-</sup> (ppm)	No. of Boys	No. of Girls	No. of Affected Boys (%)	No. of affected Girls (%)	No. of Boys				No. of Girls				
							Dental Fluorosis Grade				Dental Fluorosis Grade				
								I	II	III	IV	I	II	III	IV
Non-affected area schools															
1.	Green Model Hr. Sec School (N=351)	1.13	207	144	20 (9.6)	21 (14.5)	18	2	0	0	20	1	0	0	
2.	Govt. Boys Hr. sec school (N=94)	1.13	94	0	15 (15.9)	0 (0.0)	12	3	0	0	0	0	0	0	0
	Total (N=445)		301 (67.6)	144 (32.3)	35 (62.5)	21 (37.5)	30	5	0	0	20	1	0	0	
Affected area schools															
1.	Govt. Girls Middle School, Ghat (N=40)	2.05	21	19	7 (33.3)	10 (52.6)	7	0	0	0	8	1	1	0	
2.	Govt. Boys Middle school, Ghat (N=27)	2.05	10	17	0 (0.0)	2 (11.7)	0	0	0	0	1	1	0	0	
3.	Royal academy, Ghat (N=83)	1.48	41	42	19 (46.4)	24 (57.1)	10	5	3	1	15	4	4	1	
4.	Govt.Hr. Sec.School Ghat (N=109)	1.43	54	55	29 (53.7)	33 (60.0)	23	4	2	0	25	7	1	0	
5.	Public High School Arnora (N=30)	2.04	16	14	12 (75.0)	8 (57.1)	11	1	0	0	6	2	0	0	
6.	Govt. UPS Malwas (N=17)	3.12	6	11	6 (100.0)	10 (90.9)	2	0	3	1	2	2	0	6	
7.	Govt. UPS Golibagh (N=16)	3.84	12	4	12 (100.0)	4 (100.0)	6	2	4	0	2	1	1	0	
8.	Govt. High School Ganika (N=57)	1.85	32	25	6 (18.7)	4 (18.1)	5	0	0	1	2	1	0	1	
	Total 379		193 (50.9)	186 (49.1)	91 (48.9)	95 (51.1)	64	12	12	3	61	19	7	8	

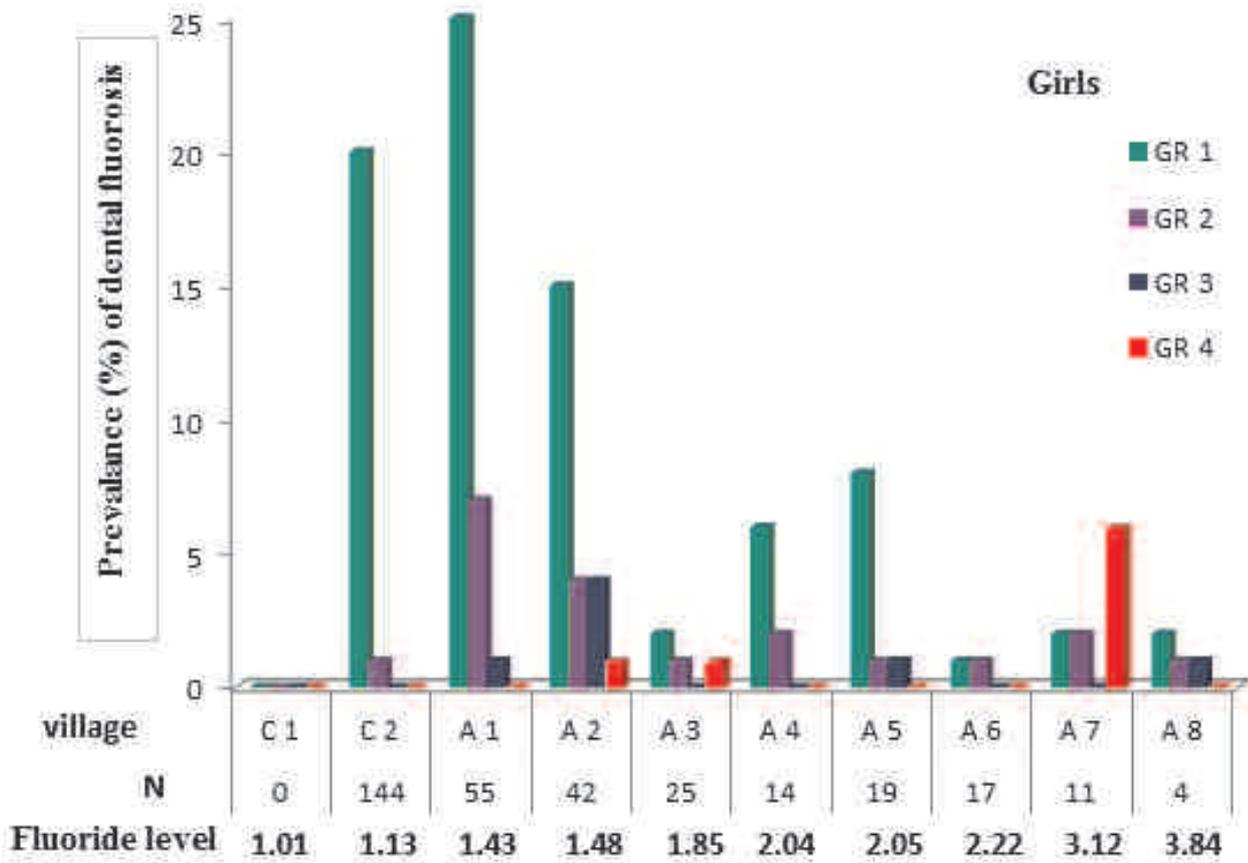
**Fig 1. Water fluoride level and grading of dental fluorosis in affected and non-affected area school children (boys and girls) of doda**



**Fig 2. Water fluoride level and grading of dental fluorosis among boys in affected and non-affected area of doda**

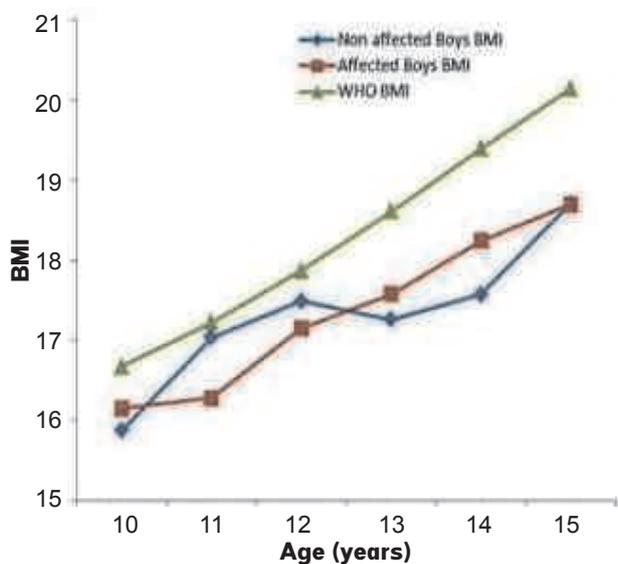


**Fig 3. Water fluoride level and grading of dental fluorosis among girls in affected and non-affected area school children of doda**

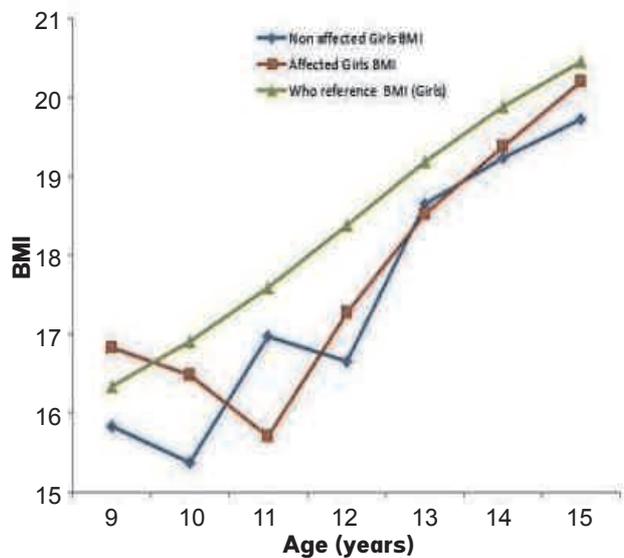


Different fluorosis grades in control and affected village school children aged 5-14yr

**Fig 4. Graphical representation of boys BMI, comparison with same age WHO boys reference BMI**



**Fig 5. Graphical representation of girls BMI, comparison with same age WHO girls reference BMI**



**Table 9. Dental fluorosis grades, water fluoride, percentage of students affected and gender wise affected and non-affected area school children**

Sl. No	Age	BMI (Mean $\pm$ SD)		WHO Reference BMI(Boys)
		Non-affected Boys	Affected Boys	
1.	6	-	16.00 $\pm$ 1.39 (n=3)	15.38
2	7	-	14.59 $\pm$ 0.87 (n=3)	15.60
3	8	-	16.93 $\pm$ 2.38 (n=9)	15.88
4	9	-	17.02 $\pm$ 2.66 (n=3)	16.23
5	10	15.87 $\pm$ 1.76 (n=11)	16.15 $\pm$ 0.93 (n=9)	16.67
6	11	17.03 $\pm$ 1.95 (n=21)	16.28 $\pm$ 0.88 (n=15)	17.22
7	12	17.49 $\pm$ 2.25 (n=26)	17.15 $\pm$ 2.34 (n=33)	17.87
8	13	17.26 $\pm$ 1.84 (n=59)	17.58 $\pm$ 1.81 (n=48)	18.61
9	14	17.57 $\pm$ 1.66 (n=65)	18.24 $\pm$ 1.44 (n=38)	19.39
10	15	18.71 $\pm$ 2.83 (n=71)	18.70 $\pm$ 2.01 (n=17)	20.14

**Table 10. Nutritional status (BMI) of the girls from affected and non-affected area village schools compared with WHO BMI of same age group girls**

Sl. No	Age	BMI (Mean $\pm$ SD)		WHO reference BMI (Girls)
		Non-affected Girls	Affected Girls	
1.	6	-	16.39 $\pm$ 1.66 (n=4)	15.32
2	7	-	15.40 $\pm$ 1.79 (n=4)	15.52
3	8	-	16.17 $\pm$ 1.02 (n=8)	15.87
4	9	15.83 $\pm$ 3.62 (n=3)	16.83 $\pm$ 1.53 (n=6)	16.34
5	10	15.37 $\pm$ 1.04 (n= 8)	16.48 $\pm$ 1.51 (n=6)	16.91
6	11	16.98 $\pm$ 4.06 (n=17)	15.70 $\pm$ 1.44 (n=10)	17.60
7	12	16.65 $\pm$ 2.49 (n=24)	17.28 $\pm$ 2.26 (n=23)	18.39
8	13	18.66 $\pm$ 2.95 (n=41)	18.53 $\pm$ 2.12 (n=41)	19.19
9	14	19.24 $\pm$ 2.24 (n=11)	19.38 $\pm$ 2.59 (n=47)	19.90
10	15	19.74 $\pm$ 3.39 (n=33)	20.21 $\pm$ 2.51 (n=29)	20.47

## 2. ASSESSING THE THERMAL STABILITY OXYTOCIN IN MILK AND DIGESTIVE STABILITY OF OXYTOCIN *IN VITRO* AND *IN VIVO*

Oxytocin (OT), a neuropeptide, is first synthesized in the hypothalamus of the brain as 125 amino acid precursor, and is transferred to the posterior pituitary after proteolytic processing and disulfide bond assembly. OT is secreted into the blood stream in response to various physiological stimuli. Apart from brain, OT is also synthesized in various other tissues and organs, including the uterine epithelium, ovary, testis, vascular endothelium and heart. It is now known that OT elicits its biological actions by binding to G-protein coupled receptor. In structural terms, OT is a nona-peptide wherein the first cysteine residue is disulfide bonded to the 6<sup>th</sup> cysteine, thus creating partial cyclic peptide. The disulfide bridge in OT is essential for its interaction with the receptor and thus, biological activity. OT has variety of biological actions that include modulation of lactation, memory, sexual arousal and social behaviour.

It has been demonstrated that the plasma OT concentrations increase in response to nipple suckling by infants to induce milk let down. In mice lacking oxytocin, milk ejection was specifically blocked and is reversed by oxytocin injections. Therefore, synthetic OT is a drug of choice for facilitating lactation after the child birth. Similarly, injection of oxytocin in dairy animals is reported to induce milk let down. It has been demonstrated that long term OT injections over a period of 305 days increased milk yield without apparent changes in milk composition or to animal health. On the other hand short term administration of

OT appears to enhance milk ejection reflex, without effects on mammary gland metabolism. Since, OT administration rapidly induces milk letdown, it is being used indiscriminately by dairy farmers in India. Despite lack of scientific evidence, there is a public perception on the speculated adverse health consequence of milk produced by oxytocin injections, which needs to be addressed through scientific methods. Nevertheless, there is paucity of information on the OT content of milk, its modulation by OT injection, and the fate of milk derived OT during milk boiling and gastro-intestinal digestion.

To address some of these concerns, the objectives of the present study were therefore to record the OT content of milk samples produced with and without OT injections and also to assess its stability during boiling and gastro-intestinal digestion using *in silico*, *in vitro* and *in vivo* methods.

## OBJECTIVES

- To assess the oxytocin concentration in pituitary extract (used as a source of oxytocin) and in milk, commercially sold in and around Hyderabad.
- To assess the digestive stability of oxytocin using simulated *in vitro* digestion representing infant and adult digestive conditions.
- To assess the proteolytic digestion of oxytocin in the presence of specific proteases *in vitro*.
- To assess the digestive stability and absorption of orally fed oxytocin in adult rats.

## METHODS

Standard oxytocin (Sigma Cat#O3251) and all other chemicals were procured from Sigma Chemical Co., Bangalore, India, unless otherwise specified.

**Estimation of oxytocin content in milk samples:** Initial survey conducted in several areas of Hyderabad City, India revealed that majority of the farmers (who supply milk to local households) are using OT injections for milk let down. In the absence of reported data on oxytocin content in dairy cattle given oxytocin injections, the Hyderabad city was divided in to 4 zones (South, North, East & West) and from each zone 30 samples of each control and treated was assigned, which is considered as large sample size theoretically.

A total of 221 milk samples (50 ml each) were randomly collected from milch buffaloes (*Murrah buffaloes*) during mid lactation period (between 3-5 months) from 43 different farmers in and around the twin cities of Hyderabad and Secunderabad. Among these 121 samples were collected from buffaloes administered exogenous oxytocin injections and the remaining were collected without oxytocin injections (n=120). The samples were immediately placed on ice and brought to the laboratory and kept frozen at -20 °C until analysis. On the day of the assay, 10 ml milk samples were centrifuged in 15 ml tubes at 10000 g for 30 min at 4°C to separate the fat and whey fractions. The whey fractions were collected by puncturing the tubes at the bottom, of which 50 µl aliquots were used directly for OT measurement using competitive enzyme immunoassay (EIA) as described by the manufacturer (Phoenix Pharmaceuticals, Burlingame CA, USA).

**In silico studies:** OT amino acid sequence was fed to the MS-Digest software (<http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msdigest>) and digested *in silico* with various gastric and intestinal proteases to identify the possible proteolytic sites.

**Reverse phase (RP) - high performance liquid chromatography analysis of oxytocin:** The HPLC (Agilent Technologies, Model#1100, Palo Alto, CA, USA) system equipped with an auto-sampler injector and a ultraviolet-visible detector controlled by Chemstation software (Agilent, USA) was used. Separation was carried out using a analytical scale C-18 reversed-phase column (Thermo-Hypersil ODS, 5µ, 250 X 4.6mm) protected by a C-18 guard column (20 mm X 3.9 mm) at ambient temperature and at a flow rate of 1.0 mL/min. OT (50 µl injections) was eluted from the column using a gradient of 80% solvent A (water with 0.1% (v/v) trifluoroacetic acid) for 8 minutes, followed by 20 - 40% solvent B (100% acetonitrile containing 0.1% (v/v) trifluoroacetic acid) for 8 to 30 minutes, and 80% A from 30 to 40 minutes. OT was identified by the retention time and quantified by comparing the peak areas with pure standard.

**Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry:** To further confirm the identity and integrity of disulfide bond of OT, the HPLC peak fractions were subjected to MALDI-TOF analysis before and after reduction with dithiothreitol (DTT). Briefly, the major HPLC peak fraction was collected and aliquoted in two portions. One of these aliquots was boiled in the presence of 10 mM DTT to achieve reduction of the disulfide bond. The peptide was then desalted and enriched by C-18 zip-tips (Millipore, India) as described by the manufacturer. The samples were mixed with equal volume of 50% acetonitrile, 0.1% TFA containing 4 mg/ml  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA), spotted on a stainless steel target plate and dried in air. The MALDI mass spectrometer was an ABI 5800 TOF-TOF (Applied Biosystems, Foster City, CA, USA). The instrument was equipped with a nitrogen laser and operated in a positive-ion delayed extraction reflector mode. External calibration was performed by using a standard peptide/protein mixture. Usually, 250 individual spectra of each spot were averaged to produce a mass spectrum.

**Thermal stability:** Ten ml of milk samples (spiked with 500  $\mu$ g of standard OT) taken in 15 ml conical tubes were boiled for a period of 60 min. At 0, 10, 30, and 60 min time points, 1ml samples were withdrawn, cooled immediately on ice. The samples were subjected to ultrafiltration through 3 kDa cutoff centrifugal filters (Amicon Ultra, Millipore, India) at 5000 g for 30 min at 4°C. The filtrate containing low molecular mass components (>3kDa) were collected and 50  $\mu$ L aliquots of these samples were immediately analyzed by HPLC as described above.

**Digestion with simulated gastric fluid:** Standard protocol was followed with minor modifications and is in accordance with the *United States Pharmacopia*. Simulated gastric fluid (SGF) consists of 3.2 mg/ml pepsin in 0.03 M NaCl at pH 2. Aliquots of SGF (200  $\mu$ L) were placed in 1.5 ml tubes to which equal volumes of OT (50  $\mu$ g) either in water or in milk (spiked with OT and boiled for 20 min to simulate household boiling) was added and incubated in a shaking water bath at 37°C for a period of 2h. At the beginning (0 h) and at the end of incubation (2 h), 50  $\mu$ L aliquots of the samples were analyzed by HPLC as described above. To study the effect of disulfide bond integrity on pepsin digestion, OT peptide was first reduced with DTT and alkylated with iodoacetamide as described previously<sup>21</sup> and then subjected to peptic digestion exactly as above.

**Digestion with simulated intestinal fluid:** Simulated intestinal fluid (SIF) was prepared consisting of 10 mg/mL pancreatin in 0.05 M NaH<sub>2</sub>PO<sub>4</sub> buffer pH 7.5 (PB). Aliquots (65  $\mu$ L) of SIF were diluted to 200  $\mu$ L with PB and mixed with equal volumes of OT (50  $\mu$ g) exactly as above for 2h. All the incubations were carried out in triplicates. At 0, 60 and 120 min time points, 50  $\mu$ L aliquots were analyzed by HPLC as described above. Digestion reactions were also carried out similarly with pancreatin solutions pretreated with 10 mM phenylmethylsulfonyl fluoride (PMSF), a specific inhibitor of serine proteases.

**Oxytocin digestion in vivo in rats:** The experimental protocols were approved by the Institutional Animal Ethics Committee of National Institute of Nutrition, Hyderabad. Adult WNIN male rats (n= 18) weighing about 140 g (supplied by the National Center for Laboratory Animal Sciences, Hyderabad) were housed in individual plastic cages and provided with laboratory chow and water ad-libetum. Diet was withheld for 24hr prior to the experiment. Standard Oxytocin (0.2 mg/ml) either in saline or in milk were administered by oral gavage to rats in such a way as to obtain gastric and intestinal contents at 0, 1hr & 3hr. At the end of the feeding period rats were killed by keeping them in CO<sub>2</sub> chamber. Gastric contents were collected from the cardiac sphincter end of the stomach while entire intestinal contents were collected by purging with 0.5 ml of saline. The gastric and intestinal contents were transferred into 15ml centrifuge tubes containing 100  $\mu$ L of protease inhibitor cocktail. The contents were centrifuged at 12,000 rpm for 5 min at 4°C and stored frozen at -20°C. The experiments were performed in triplicate for each batch. The gastric and intestinal contents were diluted 10 fold with saline, and the oxytocin content was estimated by ELISA as describe above. Further, the oxytocin content was also estimated by RP-HPLC method as described above after the ultrafiltration of samples through 3kDa cutoff filters.

**Statistics:** Each analysis was carried out in triplicates and the experiments were repeated at least twice to generate 6 observations. The % OT was computed assuming the peak area of OT in control (without heat, pepsin or pancreatin treatment) sample as 100%. Data are presented as mean $\pm$ S.D. Means between

treatments were compared by one-way analysis of variance followed by post-hoc Least significant differences (LSD) test (SPSS software, version 11.0).  $P < 0.05$  was considered significant.

## RESULTS

**Oxytocin content of milk samples:** The OT content of milk samples varied from 0.015 ng/ml to 0.17 ng/ml with a mean OT content of  $0.06 \pm 0.03$  ng/ml ( $n=241$ ). The mean OT content of milk samples obtained without OT injections ( $0.06 \pm 0.031$  ng/ml,  $n=120$ ) and with OT injections ( $0.06 \pm 0.028$  ng/ml,  $n=121$ ) remained similar ( $p=0.73$ , Fig 1).

**In silico digestion of oxytocin:** *In silico* digestion analysis revealed that pepsin (gastric protease), chymotrypsin and elastase (intestinal proteases) possess specific proteolytic cleavage sites in OT amino acid sequence while trypsin (intestinal protease) has no such sites.

**HPLC analysis of oxytocin and its characterization by MALDI-MS:** HPLC analysis of OT either in its pure form or when it is spiked in to milk revealed several minor peaks at about 4 to 6 min and a major peak at 10.6 min, which could be OT. To confirm this, peak fraction using MALDI-TOF MS spectroscopy which demonstrated peaks at 1007, representing molecular weight of OT with internal disulfide bond, and two other peaks at 1029 and 1045 amu, representing the Na (22 amu) and K (38 amu) adducts of OT were analyzed. Similarly, reduced OT showed 3 peaks similar to that of native OT, except that all the peaks are shifted to a 2 amu higher mass, consistent with the addition of 2 hydrogen atoms during reduction. The reduced and alkylated OT also produced a similar chromatographic profile, except that it was eluted 0.9 min later compared to native OT.

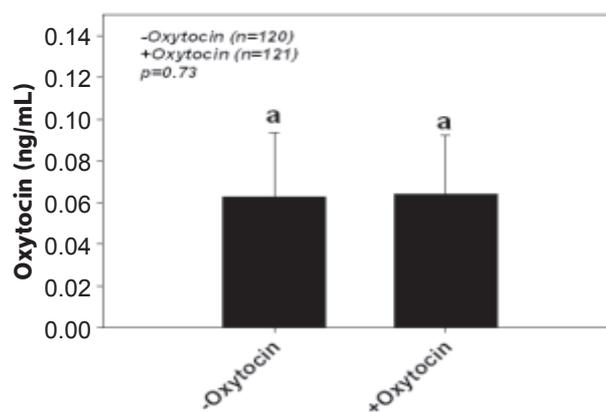
**Stability of oxytocin during boiling:** The mean % OT remained similar during milk boiling, over a period of 0 ( $100 \pm 4.7$ ) to 60 min ( $96.4 \pm 7.4$ ).

**Stability of oxytocin in simulated gastric fluid:** The mean % OT remained similar, after 0 hr ( $100 \pm 5.2$  and  $100 \pm 9$ ) or 2 hr ( $97.8 \pm 10.2$  and  $101 \pm 10.1$ ) digestion with gastric pepsin at pH 2, either in the absence (Fig. 2A) or presence of milk (Fig. 2B), respectively. In contrast, incubation of reduced OT with gastric pepsin for a period of 2h led to  $99.6 \pm 0.2$  and  $99.3 \pm 0.4$  reduction to the initial peptide concentration (100%), either in the absence or presence of milk, respectively (Fig 2).

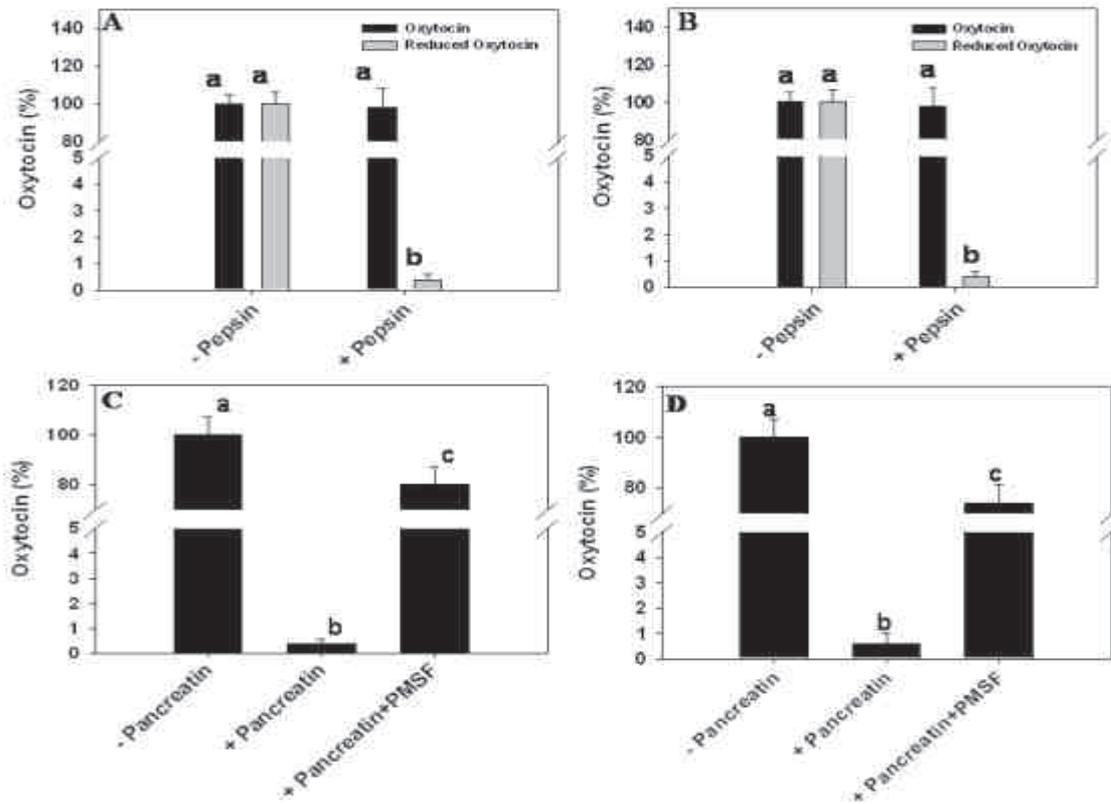
**Stability of oxytocin in simulated intestinal fluid:** The mean % OT content significantly reduced upon incubation with pancreatin for a period of 1h ( $0.37 \pm 0.2$ ) compared to 0 h ( $100 \pm 7.2$ ) time point in the absence (Fig. 2C) or presence of milk (Fig. 2D), respectively. Further, time course studies revealed that as little as 5 min incubation with pancreatin is sufficient to digest more than 90% OT (data not shown). Pretreatment of pancreatin with PMSF, a serine protease inhibitor, abrogated the pancreatic digestion of OT as evidenced revealing higher mean % OT remaining after digestion (Fig 2). Similarly, pancreatin also digested reduced OT completely (data not shown).

**Digestive stability of oxytocin in rat models:** As shown in Fig 3, oxytocin content of OT in gastric contents collected from rats remained similar at 0 ( $125 \pm 27$   $\mu\text{g/mL}$ ) and 1h ( $112 \pm 32$   $\mu\text{g/mL}$ ) time points as estimated by EIA method. Further, the peak area of oxytocin at 0 and 1h time points also remained similar when analyzed by HPLC method. Nevertheless, we could not carry out the estimation of oxytocin in gastric sample at 3 h time point, as there was no collectable gastric fluid obtained at that time. In contrast, the oxytocin was not detectable either by EIA or by HPLC method in intestinal contents.

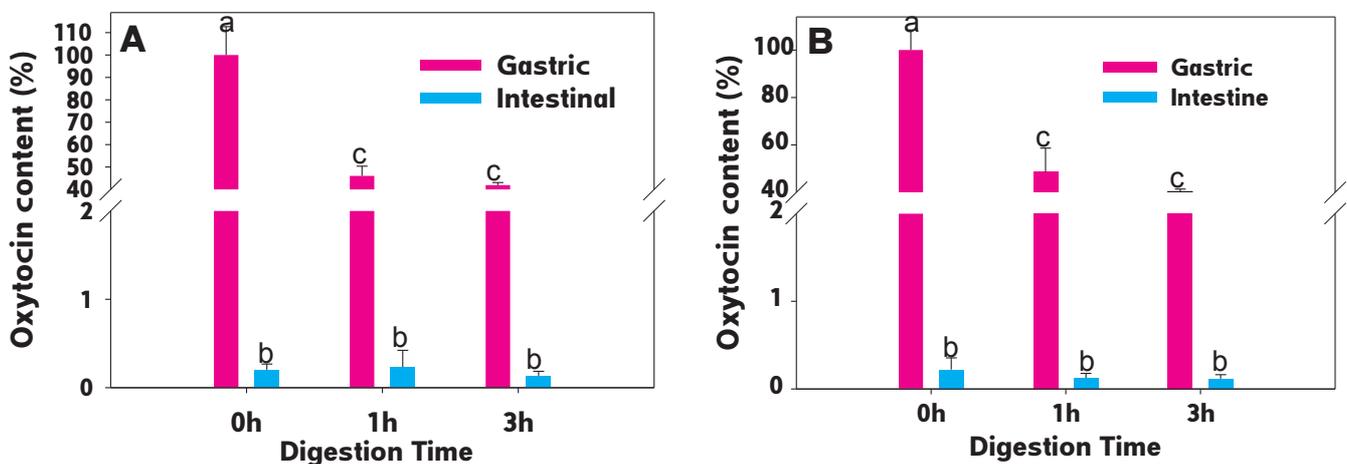
**Fig 1. Oxytocin content of milk samples:** Whole milk samples (50 ml) produced without ( $n=120$ ) and with ( $n=121$ ) oxytocin injection were collected from local dairy farmers. The oxytocin content of milk samples was measured using a competitive enzyme immunoassay kit. The bars represent mean+SD and bars with common superscript are statistically similar ( $p > 0.05$ ).



**Fig 2. Stability of oxytocin during gastric and intestinal phase of digestion:** Native or reduced oxytocin in the absence (A) or presence of milk (B), was incubated from 0 to 2hr with gastric fluid at 37°C. Oxytocin in the absence (C) or presence of milk (D) was incubated with intestinal fluid with and without 10 mM PMSF for 0 to 1hr. Aliquots (100 µL) of these samples were analyzed by HPLC. The % oxytocin was calculated assuming the peak area of oxytocin at 0 h as 100%. The bars represent mean±SD (n=6) and bars that do not share common superscript differ significantly (p<0.05).



**Fig 3. Digestive stability of oxytocin in vivo in rats:** Oxytocin peptide (25 µg/mL) either in saline (A, n=9) or in presence of milk (B, n=9) was administered to fasted rats by oral gavage and the gastric and intestinal contents were collected immediately after administration (0h) and after 1 and 3h time points (n=3 for each time point). The oxytocin content of gastric contents was estimated by EIA method. The % oxytocin in various samples was computed considering the oxytocin content in gastric juice at 0h time point as 100%. The bars represent mean±SD (n=6) and bars that do not share common superscript differ significantly (p<0.05). It should be noted that the oxytocin content of intestinal juice at all time points tested is >0.3%, which can be considered negligible.



## CONCLUSIONS

Together, these results demonstrate that OT content of milk samples is similar regardless of OT injections used. Further, OT is found to be stable to heat treatment and gastric pepsin digestion, while it is completely digested during the simulated intestinal digestion. Interestingly, reduced OT is digested by pepsin, implying that internal disulfide bridge of OT renders the peptide resistant to peptic digestion. On the other hand phenylmethylsulfonyl fluoride (PMSF), a serine protease inhibitor, abrogated the pancreatin induced digestion of OT. The studies in rats further confirms the intestinal digestion of OT *in vivo*. These studies strongly suggest that exogenous OT injections do not influence its content in milk and OT administered orally is rapidly digested during intestinal digestion.

## 3. FISH EGG PROTEIN HYDROLYSATES AS NUTRACEUTICAL/ HEALTH FOOD IN PROMOTION OF IMMUNO - MODULATORY ACTIVITIES

The fish egg (roes) is an abundant and underutilized aquatic by-product, reported to have good amount of protein. However there is limited information on their role in promoting immunomodulatory effects. Our earlier studies confirm the presence of 70 % protein in locally available fish viz., *Cirrhinus mrigala*.

In India *Labeo rohita* is an abundantly consumed fish with specific reference to its muscle, while discarding the viscera (10-20 %) and eggs (25-30 %). In view of this the present investigation is planned to utilize these wasted fish eggs as resources, in a sustainable way by preparing fish egg peptides. In addition, evaluation of therapeutic potentials of such peptides is important so as to promote them as nutraceutical/ functional foods.

### AIMS AND OBJECTIVES

- Preparation of protein hydrolysates (peptides) from fish eggs and peptide characterization.
- Determination of chemical composition and analysis of amino acid profile of fish egg protein hydrolysates (peptides).
- Evaluation of bioactive properties of protein hydrolysates viz., immunomodulatory and antioxidant activities.

### Work done during the year

### METHODOLOGY

Immunomodulatory effects of rohu fish egg protein hydrolysates were investigated in BALB/c mice model which is standardized at our centre. The experimental data has been evaluated with oral administration of peptides at 0.25, 0.5 and 1 g/kg BW daily for 45 days in mice induced with immunosuppressant drugs.

1. Lymphocyte proliferation activity
2. Peritoneal macrophage phagocytosis capacity
3. Natural killer cell activity
4. CD4<sup>+</sup> and CD8<sup>+</sup> cell count
5. Mucosal immunity
6. Allergenicity potential

## RESULTS

- REPHs has increased splenic lymphocyte proliferation with maximum at 0.5 g/kg BW, as compared to Alcalase hydrolysate (1 g/kg BW).
- A significant increase ( $p < 0.05$ ) effect with REPHs on phagocytosis capacity of peritoneal macrophages.
- NK cell activity was increased by 77% ( $P < 0.05$ ), in mice treated with pepsin hydrolysate at 1 g/kg BW compared with control group effect of REPHs on natural killer (NK) cell activity.
- *Effect of REPHs on splenic T lymphocyte subpopulations ( $CD4^+$  &  $CD8^+$ ):* There was a significant increase ( $p < 0.05$ ) in T-helper ( $CD4^+$ ) and T-cytotoxic ( $CD8^+$ ) cells population in the mice treated with trypsin hydrolysate 0.25 g/kg BW.
- *Effect of REPHs on small intestine mucosal immunity (secretory-IgA):* A significant increase ( $p < 0.05$ ) was observed in the content of secretory-IgA (S-IgA) in the small intestine lumen of mice that received pepsin hydrolysate 0.25 g/kg BW and Alcalase hydrolysate 1 g/kg BW.
- *Estimation of allergic response (IgE):* In comparison with the control group, immunoglobulin E was found to be normal in mice treated with REPHs. No significant increase or decrease of IgE level was observed in sera of mice treated with REPHs, when compared with that of control group.

## CONCLUSIONS

- Chemical composition revealed the presence of high protein content with all essential amino acids, minerals and fatty acids, especially, n-3 fatty acids in protein hydrolysate prepared out of fish eggs.
- Molecular mass distribution of the hydrolysates showed presence of low mass peptides mostly below 10 kDa.
- Hydrolysates showed dose dependent antioxidant activity in various *in vitro* models.
- The rohu egg protein hydrolysates stimulated both humoral and cell mediated immune responses in BALB/c mice after 45 days of administration.

## 4. EVALUATION OF THE IMPACT OF GENETIC POLYMORPHISM ON PHARMACODYNAMIC ACTIVITY OF COMMONLY PRESCRIBED ANTIHYPERTENSIVE DRUGS (THIAZIDE DIURETICS, ACE INHIBITORS, CCBS AND $\beta$ -BLOCKERS)

Hypertension is a growing concern all over the world and is expected to increase up to 29% by the year 2025. The guidelines are updated for treatment of hypertension both by national and international agencies as per the indigenous needs and which is based on therapeutic outcome.

In the recent past, a new dimension to determine the altered therapeutic compliance of many drugs like anti-epileptic, anticancer, have emerged on the scene. One of the major causes is due to genetic polymorphism. The preliminary information suggests the role of genetic polymorphism in altering the therapeutic outcome with antiepileptic, anticancer and some other categories of drugs. In developing countries like India with added constraints of altered nutritional status, therapeutic outcome of drugs for chronic diseases are still under investigations especially in relation to nutri-pharmacogenomic profile. Therefore, the study is planned with the following objectives-

## OBJECTIVES

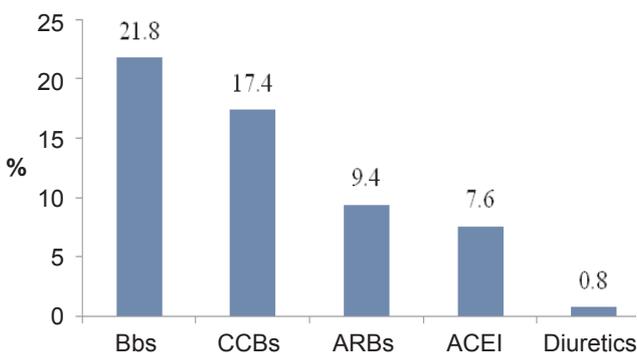
1. To monitor the pharmacodynamic activity of primary antihypertensive drugs (Thiazide diuretics, ACE inhibitors, CCBs and Bbs).
2. To assess the impact of genetic polymorphism on pharmacodynamic response among population receiving the antihypertensive drugs.
3. To determine the relationship, if any, between genetic polymorphism, nutritional, ethnic, socio-cultural profile and therapeutic compliance of antihypertensive drugs.

## WORK DONE DURING THE YEAR

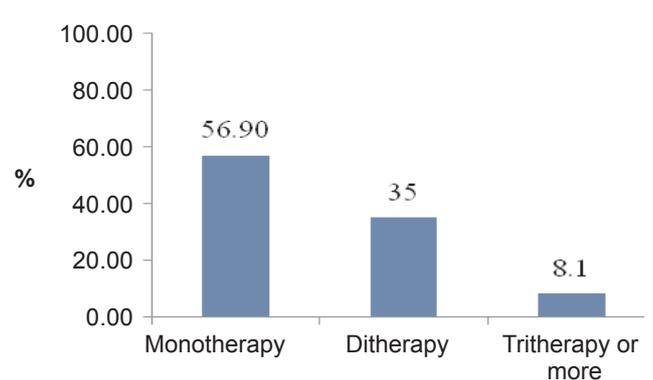
To obtain the first objective and in continuation with the survey to evaluate the consumption profile of antihypertensive drugs, carried out at Gandhi Hospital, a pretested schedule was incorporated. A total of 1169 individuals were initially screened. Of these, 382 were hypertensives among whom 106 patients were uncooperative and noncompliant and so were excluded. Besides, 28 patients who were treated with other classes of antihypertensive agents (blockers, vasodilators and other classes of antihypertensive) due to their fewer numbers, were also not included for the purpose of the study.

- Of the 276 hypertensive cases considered in the study, 159 (57.6%) were males and 117 (42.4%) were females. The mean age of the population was found to be  $53.2 \pm 11.97$  ( $P=0.468$ ).
- Among all antihypertensive drug visits, BBs (21.8%) and CCBs (17.4%) were the most commonly prescribed drugs, followed by ARBs (9.4%), ACEIs (7.6%), thiazide diuretics (0.8%), and combination therapy (43.1%) (Fig 1). Among all the prescriptions, in BBs atenolol (21.4%) and metoprolol (0.4%) constituted the majority while for CCBs, the commonest drug was Amlodipine (14.1%) followed by nifedipine (2.2%).
- The mean  $\pm$ SD SBP and DBP recorded was  $145.97 \pm 23.314$  mm Hg and  $92.87 \pm 13.773$  mm Hg respectively. The majority of patients confirmed with hypertension were in the age group of 40-60, almost equally distributed among both the sexes.
- The majority of patients were on monotherapy (56.9%), followed by two class combination therapy (35.0%). The proportion of subjects prescribed at least 3 classes of antihypertensive drugs was therefore low (8.1%) (Fig 2). The most common prescription for two-drug therapy consists of BBs-CCBs (13.4%), followed by Diuretics +ARB (6.5%).

**Fig 1. Percentage of classes of drugs prescribed**



**Fig 2. Percentage of drugs prescribed (Monotherapy vs. Combination therapy)**



# VII. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

## A. SERVICES ACTIVITIES

### 1. *Breeding and supply of animals*

During the period a total 28,396 animals were bred out of which 23,034 animals were supplied to various institutions outside and 2,402 animals were utilized for our institutional research. An amount of Rs.52,65,934 (Rupees fifty two lakhs sixty five thousand nine hundred and thirty four only) was generated.

### 2. *Supply of animal feed*

#### a. *Stock animal feed*

The stock feed of 64,515 Kgs (Rat & Mouse feed 56,350 Kgs + Guinea pigs & Rabbit feed 8,165 Kgs) was prepared during the period. Out of this, a total of 20,693 Kgs feed (Rat & Mouse feed 17,138 Kgs + Guinea Pigs & Rabbit feed 3,555 Kgs) was supplied to other institutions there by generating an amount of Rs.24,57,125/- (Rupees Twenty four lakh fifty seven thousand one hundred and twenty five only). An additional 43,456 Kgs of feed (Rat & Mouse feed 38,930 Kgs + Guinea Pigs & Rabbit feed 4,526 Kgs) was also utilized within the institute.

#### b. *Experimental Animal Feed*

In addition, the Centre also prepared 861 Kgs of custom made experimental animal feed and supplied to 7 institutions outside and to 2 Scientists within the Institute. An amount of Rs. 2,34,775/- (Rupees Two lakh Thirty four thousand seven hundred and seventy five only) was generated. 28% special feed of high protein diet was prepared (our institutional research) for internal consumption.

### 3. *Blood and blood products*

During the period, a total of 832 ml of Blood and blood products have been supplied to 9 different institutions on 18 different occasions and an amount of Rs.1,32,590/- (Rupees one lakh Thirty two thousand five hundred and ninety only) has been earned.

### 4. *Human Resource Development*

During this period in the junior level Laboratory Animal Technicians Training Course (LATTC), there were 12 participants who underwent training in Laboratory Animal Sciences. In the Senior level Laboratory Animal Supervisors Training Course (LASTC) 2 candidates were trained. In the Ad-hoc training course 27 candidates were trained for a period varying from one week to 4 weeks. In addition, on invitation centres faculty participated in the two day workshop & training program organized by Periyar University, Salem, Tamil Nadu and Pinnamaneni Siddhartha Medical College Vijayawada and trained 58 M.Sc. & Ph.D. students on laboratory animal experimentation.

The Centre celebrated World Laboratory Animal Day on 24th April 2013 in association with ICMR and Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA). On this day, a one day orientation program was conducted to nominees of CPCSEA. There were about 250 members representing private and government organisation, who have participated including CPCSEA nominees of IAEC from various institutions. During these celebrations some of the retired staff of the NCLAS were also felicitated.

## B. RESEARCH ACTIVITIES

### 1. STUDIES ON ANTI OBESITY PROPERTIES OF GARCINIA SPECIES

*Garcinia indica* is well spread in tropical rain forest of Western ghats of India. Its common name is 'Kokum' and belongs to family *clusiacea*. Fruit rind of this sp. has been found to contain anti-obesity properties. Hydroxycitric acid which is the principal acid present in the fruit rinds was shown to be responsible for this. HCA suppresses fatty acid synthesis and lipogenesis by inhibiting the ATP citrate lyase which catalyzes the extra mitochondrial cleavage of citrate to Oxaloacetate and acetyl CoA, and thereby limiting the availability of acetyl CoA units required for fatty acid synthesis. In the study, the ability of *G.indica* containing HCA to suppress body fat accumulation in WNIN/GR-ob rats were investigated. The earlier studies revealed that, *Garcinia indica* at higher doses decreased food intake and which in turn reduced animals body weight and visceral fat significantly. Additionally, the supplemented dose showed toxic effect on the testis. Though, there is a decrement in the body fat, correspondingly the LBM of the animals also decreased. Hence, in the present study the animals were supplemented with low dose (3 and 5%) of *G.Indica* powder.

#### STUDY DESIGN

About 45 days old WNIN/GR-ob rats were taken (n=18) and divided into 3 groups of six each. Animal were allowed to acclimatize to experimental conditions by housing them for one week prior to experimental conditions. All the animals were housed in individual ventilated cages and were maintained under standard environmental conditions (22-28°C, 60%relative humidity, 12 hrs L:D cycle) and fed with diet and water *ad libitum*. The experimental protocol was approved by the Institutional animal ethics committee and was as per CPCSEA guidelines.

In the commercial *Garcinia indica* powder, the percentage estimation of HCA was found to be around 20 %. The *G.Indica* powder was mixed with standard rodent chow and daily 25 gms was weighed and given. Animals were randomly divided in to 3 groups and each group contained 6 animals. Group 1 received standard powdered diet. Group 2 received 25 gms of powdered diet containing 3% *G.indica* (180 mg HCA). Group 3 received 25 gms powdered diet containing 5 % *G.indica* (300 mg HCA). The diet supplementation was continued for 12 weeks and after 6 weeks of supplementation of the experimental diet, animals were fasted for 17 hrs and blood was collected from retro orbital sinus and plasma separated to estimate mid way blood glucose and lipid profile contents. Body composition of the animals was done by Total body electrical conductivity (TOBEC) at the end of the experiment.

OGTT was performed by giving oral dose of Dextrose 250 mg/100gms body weight to overnight fasted animals and blood was drawn at 3 points o hr, 1hr and 2 hr. Plasma was separated and stored for glucose estimation. At the end of the experiment animals were euthanized and assessed for the gross necropsy changes and major organs like liver, adipose, testis & muscle were retrieved for histopathology.

#### RESULTS

- Histopathology results revealed that there is no toxicity observed in the vital organs at both levels.
- Wet weights of epididymal fat pads, liver and retroperitoneal fat depots were significantly decreased in obese rats compared to control animals.
- OGTT showed that the plasma glucose levels come to normalcy within 2 hours, where as in controls, it is not observed.
- Plasma cholesterol and triglycerides levels were significantly reduced in experimental animals compared to controls.
- There is no significant reduction in the physical parameters like food intake and body weights.

## CONCLUSION

The maximum activity against hyperlipidemia was exhibited at high dose level of *Garcinia.indica* (5%), which reduced body fat significantly without any toxicity. Since this compound is antiglycating, it helps in correcting insulin resistance. The present study can be explored further with respect to its mechanism of action to develop good anti-hyperlipidemic and anti-glycating agent.

## 2. DOSE AND TIME DEPENDENT EFFECTS OF MUCUNA PRURIENS LINN ETHANOLIC SEED EXTRACT ON STRESS RELATED PARAMETERS IN WNIN/Gr-Ob RATS

Stress is a potential variable in animal experiments and measurable parameters, considered linked to stress include unstable blood chemistry, abnormal organ size (particularly adrenal glands) and any animal's incidence of aggressive behaviour. Housing environment has an impact on biological mechanisms underlying animal behaviour and slight changes during an experiment can alter responses. In addition to stress, increased levels of glucocorticoids (either cortisol or corticosterone), a hallmark of stress has been shown to produce learning deficits. It was seen that, Environmental Enrichment (EE) decreases stress levels, and allows welfare improvement exhibited through improved behaviour such as hiding, climbing and foraging.

### AIMS AND OBJECTIVES

In the present study we have planned to administer, exogenous supplementation of *Mucuna pruriens* ethanolic seed extract as a source of L-DOPA, and, whether this could help the mutants to gain normal dopamine levels with reduction in their glucocorticoids levels, leading to reduced stress.

### Work done during the year

About 35 days old obese mutants (18 males + 18 females) belonging to WNIN/Ob groups were taken and they were divided in to three groups and these animals housed under standard experimental housing conditions. I group animals were control, and II group animals were given low dose (3.5%) of *M.pruriens* powder which was mixed in stock diet. III group animals were supplemented with 7.0% of test compound. *Mucuna pruriens* seeds were purchased from Munnalal Dawasaz, Charminar, Hyderabad. These seeds were cleaned; processed and powdered. *M.pruriens* seeds powder was added to 20% protein diet at two dose concentrations (normal and high) and was supplemented to group II and III animals. Physical parameters like growth, food intake, feed efficiency ratio and activity was monitored for the experimental period of 45 days. Physiological parameters like body composition and bone mineral contents were measured. Biochemical parameters like cholesterol, triglycerides and stress markers like serum cortisol and L-dopamine were analyzed.

### CONCLUSION

Cortisol has many functions. It helps the body use sugar (glucose) and fat for energy (metabolism), and it helps the body to manage stress. By supplementing *Mucuna pruriens* which contains dopamine and by improving environmental conditions, the animals exhibited a lower level of stress.

## 3. EVALUATION OF PROMISING PLANT EXTRACTS AND ACTIVE CONSTITUENTS FOR ANTI-OBESE, ANTI-DIABETIC AND HEPATO-PROTECTIVE PROPERTIES IN WNIN/Gr-Ob RATS

On a global scale, obesity has reached epidemic proportions and is a major contributor to the global burden of chronic disease and disability. Currently, more than one billion adults worldwide are overweight

and at least 300 million of them are clinically obese. Two different types of obesity-treatment drugs are currently available in the market. One of these is orlistat (Xenical), which reduces intestinal fat absorption through inhibition of pancreatic lipase. The other is sibutramine (Reductil), which is an anorectic. Both drugs have side-effects, including increased blood pressure, drying of mouth, constipation, headache, and insomnia. At present, because of dissatisfaction with high costs and potentially hazardous side-effects, we have taken plant extracts that promise body weight reduction and are helpful in treating various complications of obesity.

## AIMS AND OBJECTIVES

The aim of the present study is to evaluate the anti diabetic, anti obese and hepatoprotective activity of the promising plant extracts on WNIN GR/Ob rats. The main objective is to explore the potential of natural products for treating obesity and associated complications and which could be an excellent alternative strategy for developing in future, effective and safe drugs. To evaluate anti diabetic, anti obese and hepato-protective activities of the plant extracts, we have taken 36 WNIN GR/Ob rats and divided them randomly in 6 groups. Animals were acclimatized for one week and placed in individual cages maintained at 22-28 degrees centigrade and which 60 % relative humidity. The experimental protocol was reviewed and approved by Institutional animal ethics committee and as per CECSEA guide lines.

- GROUP 1 : Control Animals
- GROUP 2 : Animals treated with *Acalypha indica*
- GROUP 3 : Animals treated with *Pergularia demia*
- GROUP 4 : Animals treated with *Tinospora cardifolia*
- GROUP 5 : Animals treated with *Aegles marmelos*
- GROUP 6 : Animals treated with *Chloroxylon swetienna*

### General use of the above plant extracts:

1. *Acalypha indica* Linn leaves possess laxative properties, anti-fungal, anti-inflammatory, anti-bacterial and is known to control mutagenicity.
2. *Pergularia demia* leaves contain antidiabetic, anti-inflammatory and antipyretic properties.
3. *Tinospora cordifolia*; common name Guduchi  
Uses; antidiabetic, antiarthritic, antimicrobial, anticancer and is an hepatoprotective agent.
4. *Aegles marmelos* common name is Bilwa patra used to combat dysentery and diabetes
5. *Chloroxylon swetienna*-common name is Indian satinwood and is used for its mosquitocidal activity.

### Work done

From the above plants, leaves were collected dried under shade and extracted with methanol. The methanolic extracts were flash evaporated and was used for the experiment. Extracts were mixed with powdered standard diet and handmade pellets were prepared. Every day in the morning this handmade pellet (containing test compound) was fed to animals and after one hour 15gms of pelleted diet was weighed and given to each animal. The treatment schedule was continued for 30 days. Physical parameters like food intake, growth and physiological parameters like body composition, was measured using total body electrical conductivity (TOBEC) and bone mineral concentration and density was measured by dual X-ray absorptiometry (DXA), in the animals treated with above extractions. Blood was collected from retro orbital plexus after 15 days and 30 days of treatment respectively for the determination of glucose response and lipid contents. After the experiment was over, animals were euthanized and major organs like liver, kidneys, pancreas, adipose tissue and testis were collected for histopathological study.

## RESULTS

1. There was a significant reduction in the body weights and food intake of animals treated with the plant extracts (Table 1).
2. Body composition estimation by TOBEC revealed that, there was a significant increase in the lean

**Table 1. Physical and body composition parameters in animals treated with plant extracts**

Parameters	Control		A.indica		P.demia		A.mermelos		T.cardifolia		C.swetina	
	15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D
<b>Body wt.(g)</b>	613.33	33.26*	473.33	28.41	532.67	7.02	606.67	27.12	543.33	15.26	371.33	22.41
<b>Food Intake(g)</b>	261.42	9.16**	202.8	16.57	198.66	18.92	222.91	29.11	215.16	15.07	217.9	9.38
<b>LBM (g)</b>	234.58	28.42**	288.60	37.99	302.90	19.19	261.34	8.9	271.73	25.43	261.53	26.33
<b>FAT (g)</b>	284.41	16.61*	322.72	29.51	339.42	10.2	303.32	19.8	321.92	33.97	307.46	18.47
<b>FAT%</b>	55.8	0.79*	52.86	1.04	52.86	1.04	53.68	1.45	54.20	0.78	54.08	1.18
<b>BMC (gm)</b>	15.29	0.61	12.49	0.75	11.51	1.01*	13.97	1.86	13.71	1.19	11.77	1.67
<b>BMD (gm/Sq/cm)</b>	0.18	0.001	0.15	0.009	0.15	0.011	0.17	0.001	0.16	0.002	0.16	0.01

\* Values were significantly different from experimental group animals. ( $P < 0.05$ )

\*\* Values were significantly different from experimental group animals. ( $P < 0.001$ )

**Table 2. Glucose and lipid levels in animals treated with plant extract**

Parameters	Control		A.indica		P.demia		A.mermelos		T.cardifolia		C.swetina	
	15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D
<b>GLUCOSE (mg/dl)</b>	331.05	345.32	270.16	228.65	280.45	292.36	291.25	302.45	278.65	242.65	261.22	242.33
	+28.25	+29.82	+14.23	+12.45	+14.25	+12.52	+28.66	+32.65	+12.25	+18.66	+18.66	+12.66
<b>CHOLESTEROL (mg/dl)</b>	153.33	112.38	115.77	89.16	182.61	120.41	189.62	237.27	167.00	158.93	142.05	135.88
	+18.84	+12.58	+25.72	+20.06	+37.25	+41.07	+4.73	+44.8	+26.73	+47.8	+38.12	+18.42
<b>TRIGLYCERIDE (mg/dl)</b>	371.1	314.19	256.06	205.03	482.9	351.84	410.3	280.28	339.77	180.60	515.2	160.41
	+44.58	+45.84	+11.28	+9.74	+14.5	+15.09	+21.28	+12.2	+16.24	+17.2	+11.3	+26.25

\*-Values were significantly different from experimental group animals. ( $P < 0.05$ )

\*\*-. Values were significantly different from experimental group animals. ( $P < 0.001$ )

body mass and reduction in the total body fat content in treated animals compared to controls. However, no significant changes were seen in bone mineral content and density.

- Hypoglycemia was prominent in *A.indica* extract treated animals compared to other experimental animals. Lipid contents like cholesterol and triglycerides were decreased significantly in all the treated animals (Table 2).
- Histopathology results indicated that, there was no toxicity observed in the liver of treated animals with plant extracts. Vacuolation decreased in the animals treated with all the plant extract compared to controls (Table 3).
- There was a varying degrees of degeneration of kidney tubules in all the experimental animals observed, except in controls and animals treated with *A.indica*.

**Table 3. Organ to body weight ratio in animals treated with different plant extracts**

Plant Extract	Body weights	Liver		Kidney		Spleen		Testis		Brain	
		Weight	%	Weight	%	Weight	%	Weight	%	Weight	%
Control	613.33 ±33.26	17.40 ± 1.13	2.84 ±0.44	4.03 ±0.32	0.66 ±0.04	1.53 ±0.20	0.25 ±0.01	3.18 ±0.20	0.52 ±0.04	1.95 ±0.15	0.32 ±0.02
<i>A.indica</i>	473.33** ±28.41	12.40** ±0.62	2.61 ±0.46	2.60** ±0.26	0.55 ±0.02	0.94* ±0.15	0.12 ±0.02	3.43** ± 0.22	0.72 ±0.06	2.18* ±0.12	0.46 ±0.04
<i>P.demia</i>	532.67 ±7.02	12.23 ±0.60	2.30 ±0.38	2.80 ±0.26	0.52 ±0.04	0.97 ±0.20	0.18 ±0.02	3.08 ±0.18	0.58 ±0.04	1.96 ±0.08	0.37 ±0.02
<i>A.mermelos</i>	606.67 ±27.12	13.06 ± 0.66	2.15 ±0.66	3.00 ±0.55	0.49 ±0.04	1.40 ±0.20	0.23 ±0.04	2.98 ±0.12	0.49 ±0.4	1.98 ±0.08	0.33 ±0.02
<i>T.cardifolia</i>	543.33 ±15.26	13.63 ±0.51	2.50 ±0.28	2.70* ±0.32	0.50 ±0.02	1.00 ±0.26	0.18 ±0.02	3.01 ±0.16	0.55 ±0.04	1.88 ±0.06	0.35 ±0.02
<i>C.swetina</i>	371.33* ±22.41	12.2* ±0.88	3.28 ±0.42	3.06 ±0.15	0.82 ±0.08	1.03 ±0.23	0.28 ±0.06	3.04 ±0.16	0.82 ±0.06	2.16 ±0.10	0.58 ±0.04

## CONCLUSION

From the above observations it can be concluded that, *A.indica* showed promising results in terms of reducing the body weights, circulatory glucose levels and lipid levels. There was also a significant reduction in liver and kidney weights, which reflects the anti obesity nature of the compound. *A.indica* also contributed to increase in the weight of brain. These preliminary results were encouraging, and a long term study could be planned to identify the mechanism behind these interesting observations.

## 4. EFFECT OF BIOACTIVE COMPOUNDS ISOLATED FROM *PIPER NIGRUM* ON HIGH FAT DIET INDUCED OBESITY IN SD RATS – A MRI STUDY

Obesity is a complex trait influenced by diet, developmental stage, age, physical activity and genes. Despite the urgent need for safe and efficient therapeutics, the current status for the development of such drugs is still unsatisfactory. Today, there are only very few FDA-approved drugs (Orlistat and Sibutramine) with known mechanism of action in the market for the long-term treatment of obesity. The new anti-obesity drug development continues to focus on either central or peripheral acting inhibitors of food intake. Therefore there is an urgent need to identify breakthrough drugs with paradigm shifting in pharmacodynamics for the treatment of obesity. Some edible medicinal plants have been used as dietary supplements for body-weight management and to control obesity in many countries.

From the beginning of the last century, evidences on the cholesterol-lowering properties of medicinal plants have been accumulating. The importance of such investigations has been confirmed in the treatment of several ailments including obesity, diabetes and atherosclerosis. A number of plant species such as *Garcinia cambogia*, *Zingiber officinale*, *Piper longum*, *Gymnema sylvestre*, resin of *Commiphora mukul* and *Bauhinia variegata* have been reported to exert their anti-lipidemic effects. Anti-obesity effect of *Nelumbo nucifera* leaf extracts has also been well researched in mice and rats. On the anti-obesity and anti-diabetic effects of *Acacia* polyphenols in obese diabetic KKAY mice, fed with high-fat diet has also been well studied. In the present study efforts will be made to isolate the anti-lipidemic factors from the indigenous medicinal plant *Piper nigrum*. It is popularly known as black pepper or king of pepper, finds extensive use in Ayurvedic system of medicine. A number of piperidine and pyrrolidine alkaloids are known to occur in *P. nigrum*, the most important being piperine, known to possess a variety of biological properties like CNS stimulant, analgesic, anti-pyretic and anti-feedant activities.

Isolation of bioactive factors from *Piper nigrum*, studies on the patho-physiological alterations during induced obesity and on treatment with plant extracts were being taken up. Further, studies on the expression of certain transcriptional factors associated with adipogenesis and lipid metabolism during induced obesity and on treatment with plant extracts were also recorded. Animal experimentation, induction of obesity through the supplementation of high fat diet, determination of physical, physiological and biochemical parameters were also studied.

## OBJECTIVES

The supplementation of bio-active test compound isolated from *P.nigrum* having an effect in controlling the body fat, as seen in high fat diet induced obese animal model. The study is aimed to measure the fat distribution (visceral, subcutaneous and femoral) in the animals treated with the test compound by MRI techniques.

### Work done during the year

#### Experimental design

The experimental duration is for 6 months period. 12 weeks old SD rats (48) were inducted for the study and divided into eight groups (6 rats in each group) and these animals were housed under standard experimental housing conditions and acclimatized for one week. All the rats were fed with high fat diet for 24 weeks and after 16 weeks of initial feeding except controls other groups received test compound (*Piper nigrum* extract) which was mixed in the high fat diet for 12 weeks and one group which was kept as control received standard diet, second group received high fat diet control. 3rd group received high fat fed but treated with standard drug, and other groups with different extracts of *piper nigrum* for 12 weeks.

- Group 1 Control animals
- Group 2 Animals treated high fat diet
- Group 3 Animals treated with standard drug
- Group 4 Animals treated with *Piper nigrum* extract
- Group 5 to 7 : Animals treated with different doses of *Piper nigrum* extract

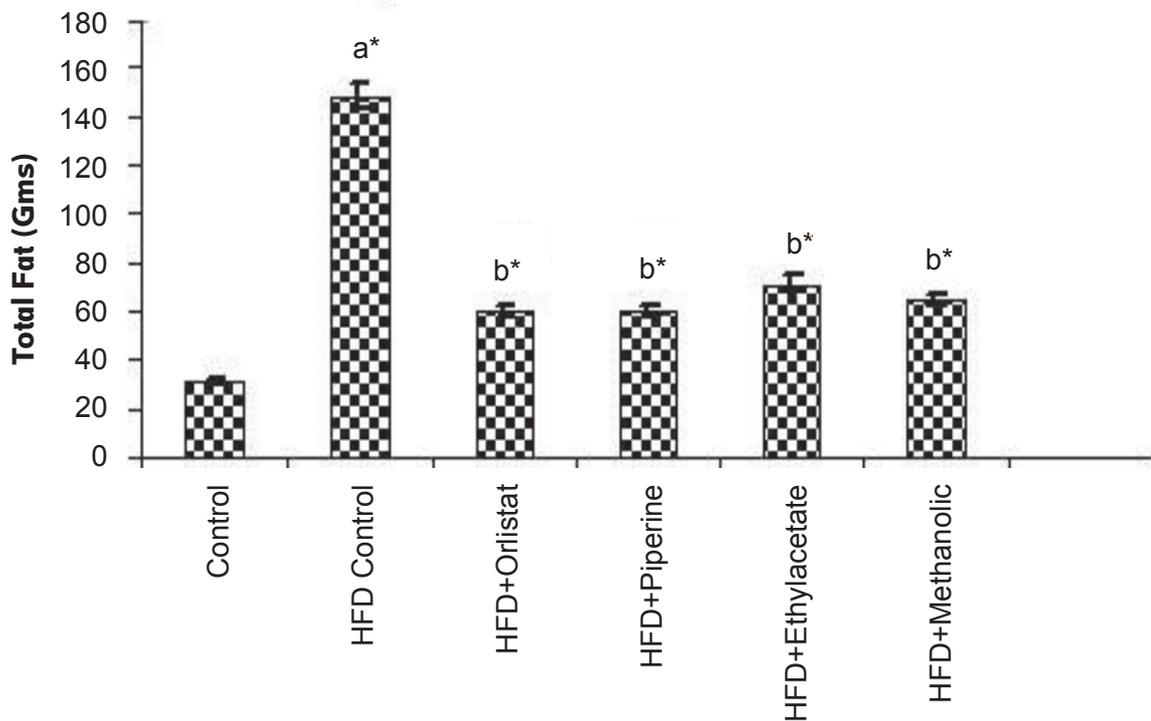
During the experiment physical parameters like daily food intake, weekly body weights, feed efficiency ratio was being calculated. Body composition was measured by TOBEC. Bone mineral parameters like BMC, BMD and fat distribution (ROI) was measured by DXA. Adipose tissue volumes were measured by magnetic resonance imaging (MRI). MRI was carried out using Bruker Biospec 7.0 Tesla horizontal small animal imaging system at Mata Amruta Institute of Medical Sciences, Kochi. T1 weighted MRI of animals treated with *P.nigrum* measured and compared with controls. The difference between the densely packed adipocytes and loosely packed ones in treated animals were measured. Additionally the ratio between visceral, subcutaneous fat content were measured by MRI.

**Biochemical profile:** The blood was collected twice after overnight fasting after 16 weeks of high fat feeding and after the supplementation of *Piper*. Plasma glucose, lipid profile, liver function enzymes SGOT, SGPT, kidney function parameters like bilirubin and alkaline phosphatase were measured in the whole blood differential count.

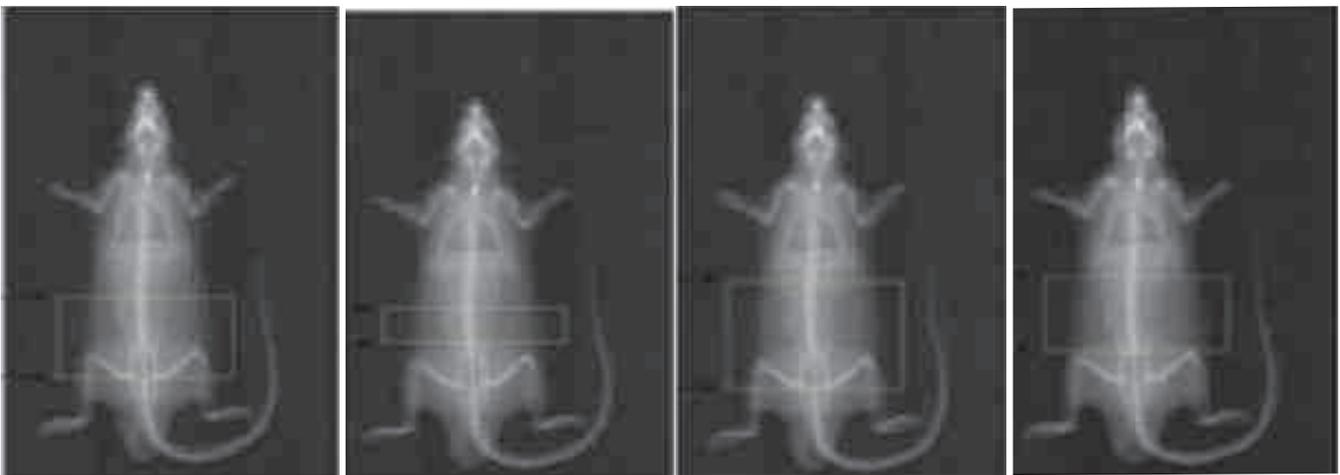
## RESULTS

Rats fed with high fat diet when treated with *piper nigrum* showed a decrease in physical parameters like food intake, growth. Significant increase in body composition parameters like lean body mass and decrease in total body fat and % fat was observed in the HFD rats treated with piperine extracts (Fig 1). DXA analysis revealed that there is a significant increase in bone mineral content and bone mineral density. Region wise fat distribution analysis of the body, revealed that there is predominant deposition of fat at retroperitoneal region (Fig 2). The MRI analysis (Fig 3 & 4) revealed that, there is a significant reduction in the adipose tissue volumes in the whole body and regional adipose tissue volumes i.e., subcutaneous, thoracic, and retro peritoneal. This study of effect of piper nigrum on obesity can be explored further with respect to its mechanism of action to translation research.

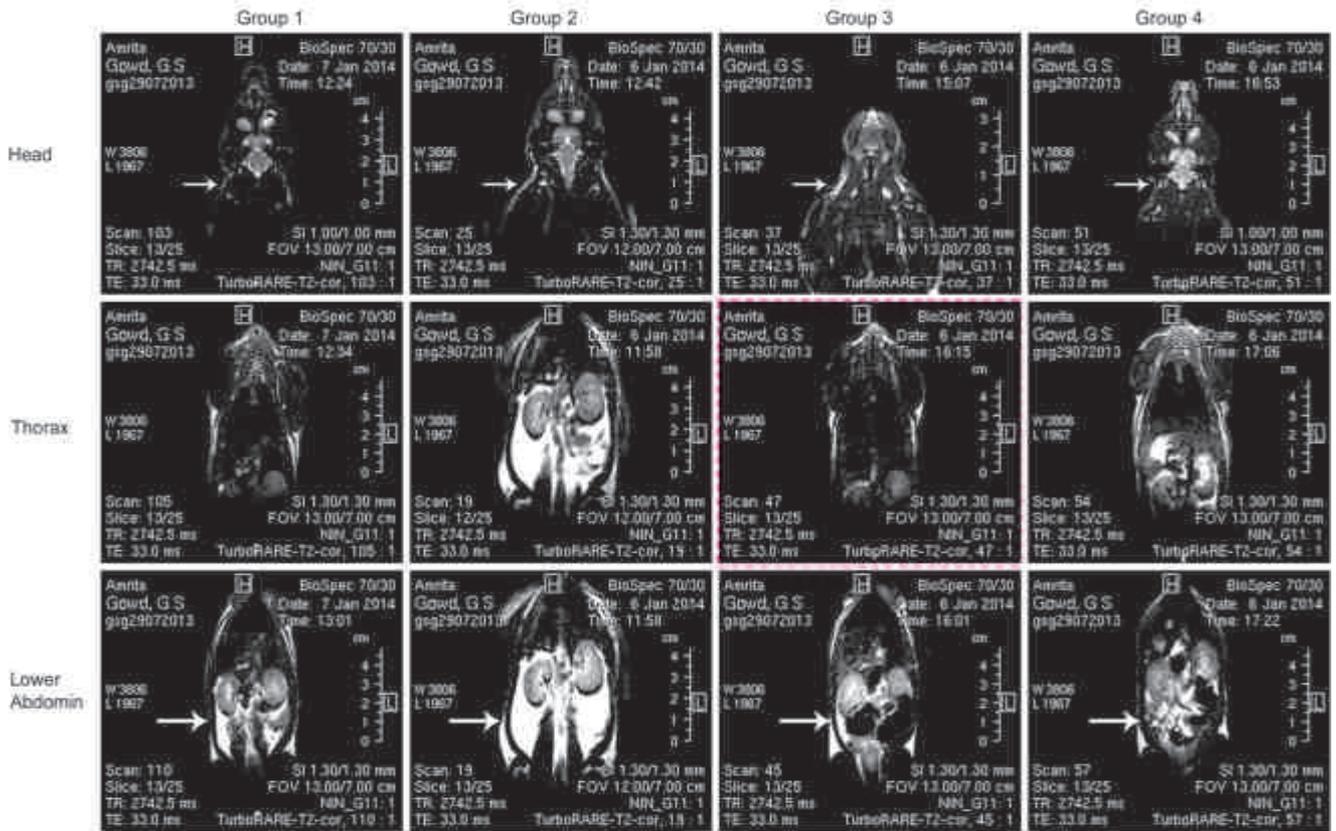
**Fig 1. Body composition analysis (TOBEC) of rats treated with P.nigrum extracts**



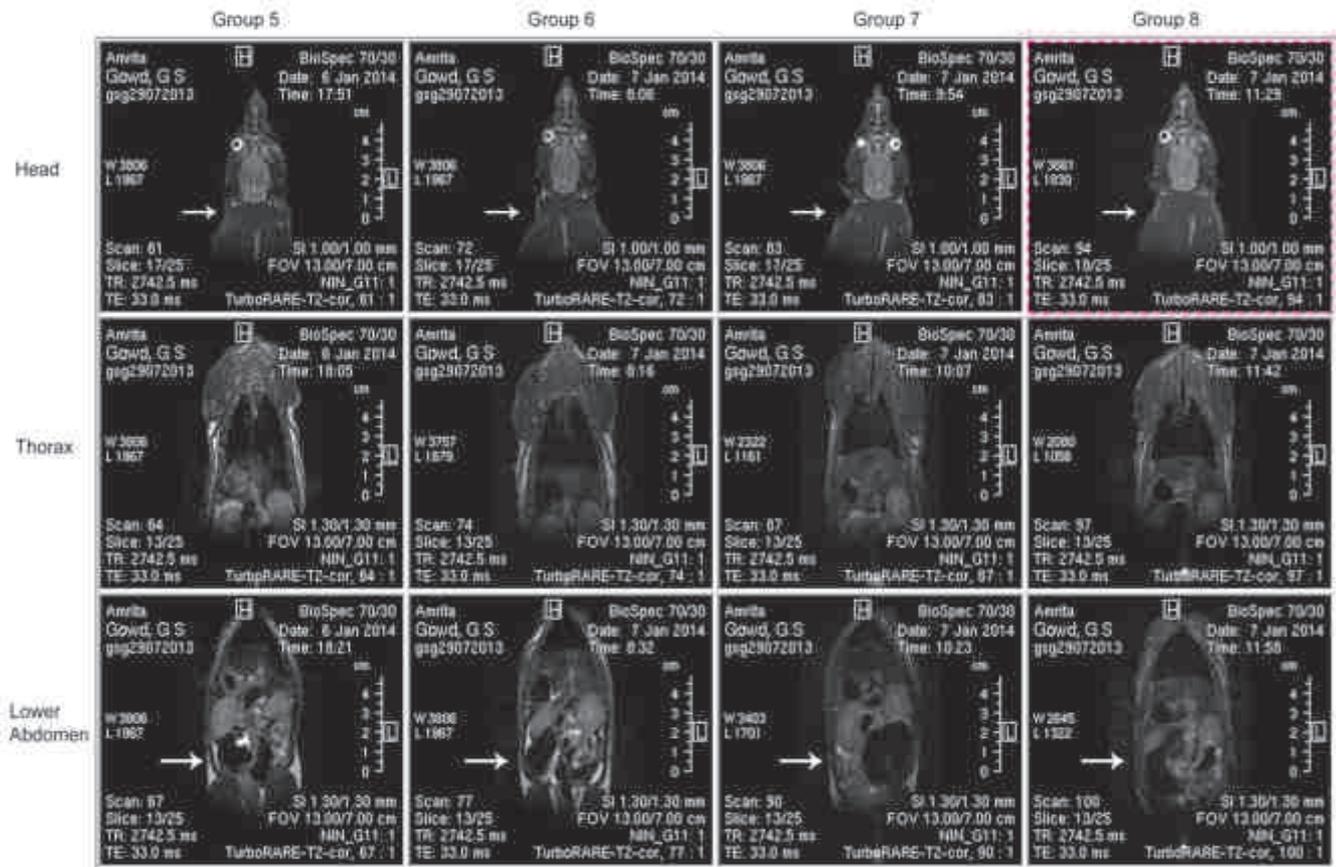
**Fig 2. Regional wise fat distribution in HFD rats treated with P.nigrum extracts**



**Fig 3. 7T MRI scans of HFD rats treated with P.nigrum extracts**



**Fig 4. 7T MRI scans of HFD rats treated with P.nigrum extracts**



# VIII. PRE-CLINICAL TOXICOLOGY RESEARCH CENTRE

## 1. ACUTE TOXICITY STUDY IN MICE AND SUB CHRONIC TOXICITY STUDY IN RATS, *IN-VITRO* ALLERGENICITY TEST OF SEED AND LEAF OF TRANSGENIC MUSTARD CONTAINING HYBRID DMH-11

Transgenic *Brassica juncea* containing barnase, barstar and bar genes has been developed by Centre for Genetic Manipulation of Crop Plants (CGMCP), University of Delhi South Campus (UDSC). As per the Guidelines for safety assessment of genetically engineered plants, 2008, prescribed by ICMR and the accompanying protocols by DBT, the edible plant parts which have the expression of the inserted genes, need to be tested for their safety profile. Therefore, the following investigations has been undertaken to assess the preclinical (pre-market) safety profile of leaf and seed of transgenic *B. juncea*.

- Compositional analysis of key components in leaves and seeds of two events of transgenic *Brassica juncea*.
- Assess potential allergenic Cross-Reactivity by Bioinformatic tools.
- Pepsin digestibility and Thermal stability assay of recombinant Bar, Barnase and Barstar proteins.
- Acute oral toxicity of Recombinant Bar, Barnase and Barstar protein in *Swiss Albino Mice*.
- Sub Chronic oral toxicity study of leaves & Seeds from transgenic *Brassica juncea* in *Sprague Dawley rats*.

### METHODOLOGY

**Compositional analysis:** The leaf and seed samples of transgenic and non-transgenic *B. juncea* have been collected randomly from three different places and homogenized for analysis of i. Proximates, ii. Fibre iii. Secondary Metabolites and Phytosterols, iv. Minerals v. Vitamins vi. Amino Acids vii. Fatty acids by standard procedures of AOAC.

**Bioinformatics:** The databases PubMed, Allergen Online version 12.0 Allergen Database and NCBI Entrez Protein Database were used to accomplish the bioinformatics searches.

**Pepsin digestibility** of the test proteins in SGF at pH 1.2, analyze digested proteins by SDS–PAGE and densitometry.

**Thermal Stability** has been evaluated for test protein and were subjected to different temperatures (0, 25, 37, 55, 75 and 95°C for up to 30 minutes) followed by testing for the enzyme activity.

**Acute Toxicity Study:** Acute toxicity test (14 days) in *Swiss albino* mice, was conducted by exposing test proteins [Bar, Barnase, Barstar] in a concentration of 2000 mg/kg. The vehicle control group of animals received Phosphate buffer. The maximum volume of 2 ml was administered over 24 hours (0.5 ml every 6 hours). This was followed by observing the activity and lethality in addition to bi-weekly monitoring of live phase, cage side and physical observations till 15th day of post exposure. All the animals were euthanized after test compound exposure.

**Sub Chronic Toxicity Study:** This study has been conducted by feeding lyophilized samples of *B. juncea* leaf (2g/kg) and Seeds (100g/kg) to 240 *Sprague dawley* rats, aged 6-8 weeks, weighing 180 -200g. The animals were divided randomly into six groups (i. Control ii. Varuna (NT) iii. EH2 (NT) iv. Varuna barnase v. EH2 barstar vi. DMH-11) to receive the seed, and the other three groups to receive the leaf daily for

90days. This was followed by monitoring the animal's bi-weekly for live phase, cage side, physical and neurological parameters. The clinical chemistry profile, haematology, serum total IgG2a, IgM, IgA and IgE levels, gross necropsy and histopathological observations of all organs were carried out after 48hrs of post exposure to normal diet and test material.

## RESULTS

- Substantial equivalence in composition between transgenic and non transgenic Brassica was observed with changes due to agro-climatic /demographic changes.
- No significant amino acid identity matches between Bar, Barnase, and Barstar proteins with known allergens.
- The results of the pepsin digestibility assay showed that the Bar, Barnase and Barstar test proteins were rapidly degraded, and 90% digested.
- The Bar recombinant protein showed a rapid decrease of activity from 130 units to -32.0 units/min/mg at temperatures from 55C to 95C.
- The enzyme activity assay of Barnase (RNAse) and Barstar (RNAse inhibitor) recombinant proteins indicated that due to heat stability of these proteins, no change in the activity of these enzymes could be observed with heat treatment.
- There were pre-terminal death in mice which received buffer [(2%) on 14<sup>th</sup> day of post exposure]. In surviving animals there were no differences in body weight gain, live phase and physical activity throughout the study period.
- There was no mortality in any group of animals fed with normal diet, transgenic and non-transgenic leaf and seed till end of the experimental period. There was no significant reduction in body weight gain in test groups non transgenic and transgenic. No changes in live phase, physical activity and neurological activity between the control and test groups were recorded throughout the study period. Clinical chemistry and hematology profile in blood / serum samples collected after 48hrs of post exposure, were in normal range, though there were significant changes observed. The total serum IgG2a, IgM, IgA and IgE levels of all the tested groups were comparable to control group. At necropsy, no gross lesions were found in any organ in all groups. No histopathology changes were recorded in organs of animals between control and test groups.

## CONCLUSION

The compositional equivalence recorded, leaf and seed samples of transgenic and non-transgenic *Brassica juncea*. There was no allergency potential observed in the experimental phase. The daily feeding of transgenic Brassica did not induce any adverse events and was found comparable to non-transgenic crop.

## 2. PRE CLINICAL BIOAVAILABILITY AND SAFETY EVALUATION OF TEMOZOLAMIDE CO-CRYSTAL

The role of Temozolomide in treatment of leukaemia, lymphoma and solid tumours, such as melanoma, sarcoma, lung carcinoma, glioblastomas and astrocytomas at clinical level is well established. In addition to good oral bioavailability, it also succeeds extensive tissue penetration property in central nervous tissues treatment, In view of this Temozolomide has potential to treat brain cancer as a concomitant therapy along with radiation. Since the stability of the product is short, Crystalin Research Private Limited has developed TMZ Cocystal using Co-crystallization method to modify physicochemical characteristics of active pharmaceutical ingredients with an intention to increase the shelf life of the product. Therefore, as per regulatory needs, any modification in approved products, the information on its comparative Bioavailability and safety profile is necessary as per the guidelines of Schedule Y of DCGI.

## METHODOLOGY

- The Pharmacokinetic studies was conducted in Sprague Dawley rats using cross over design. Samples were collected immediately before and 30, 60, 90, 120, 180, 240, 480, 720 and 1440 min, after the Temozolomide and its co-crystal were administered in an equivalent dose. The plasma samples using liquid-liquid extraction and were analysed by high performance liquid chromatography (HPLC) with Photo Diode Array Detector was used. The hematology and biochemistry for toxicokinetic study have been monitored.
- The acute toxicity test (14 days) has been conducted with 10, 20 times of Intended Therapeutic Dose in healthy *Swiss albino Mice* & 20 times of Intended Therapeutic Dose in *Sprague Dawley Rats*. This is followed by observation for lethality, bi-weekly monitoring of live phase, cage side and physical observations till 15th day of post exposure.
- The Sub chronic Toxicity toxicity test (28 days) has been conducted in 120 healthy *Sprague Dawley Rats* aged six weeks old, weighing (150-180g). The animals were randomly divided into five groups viz., (i) Control 12(6M+6F), (ii) TMZ 1XTD 12(6M+6F), (iii) TMZ SA 1XTD 12(6M+6F), (iv) TMZ SA 2XTD 12(6M+6F) and (v) TMZ SA 4XTD 12(6M+6F). As a part of routine examination all animals were subjected to qualitative urine analysis. The Temozolomide concentrations in test compound (Temozolomide-cocrystal) were calculated based on the molecular weight (253.94mg). This was followed by monitoring the animals bi-weekly for live phase, cage side, physical and neurological parameters. The clinical chemistry profile, haematology, gross necropsy and histopathological observations of all organs were carried out after 48hrs of post exposure to Test compound.
- The availability of drug in brain tissues has been undertaken in *Sprague Dawley* rats in sub-chronic study, approximately six weeks old, weighing (150-180g) exposed to 1XTD dose of standard and test drug. Necropsy was done and samples are collected on last day immediately after 2hrs of drug administration (Temozolomide and its cocrystal dose). Brain tissue was isolated from sacrificed rat, rinsed with water to avoid additional blood presence and was immediately frozen using liquid nitrogen and stored at - 80°C until analysis. Temozolomide and its cocrystal were extracted from the brain tissues using liquid-liquid extraction and were analysed by high performance liquid chromatography (HPLC) with Photo Diode Array Detector.

## RESULTS

The test and reference standard drugs are bioequivalent.

- The C<sub>max</sub>, AUC and t<sub>max</sub> values between test drug and reference standard drug are not statistically different.
- There were pre-terminal death in mice (70%) received more than 20 times of therapeutic dose on 7<sup>th</sup>, 9<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup> and 13<sup>th</sup> days of post exposure to test compound & (20%) received more than 20 times of therapeutic dose on 11<sup>th</sup> and 14<sup>th</sup> days of post exposure to test compound. There were no abnormal findings with reference to gain in body weights. No gross necropsy changes were observed. The histopathology examinations of autopsy samples are awaited.
- There were pre-terminal deaths, recorded in rats received 4XTD (100%) and 2X TD (17%). Except for lacrimation in 2XTD group, all live phase, physical activity and neurological activity were normal throughout the study period. Clinical chemistry and hematology profile was also in normal range and comparative to standard drug. There were no abnormal findings with reference to gain in body weights. No gross necropsy changes were observed.
- The concentrations with Temozolomide and test compound TMZ SA in brain tissue were comparable. No Temozolomide and Test compound cocrystal was detectable in brain tissues after 15 days of post exposure.

## CONCLUSION

The bioavailability of *Temozolomide-Succinic acid* cocrystal was comparable to the standard drug both in Blood and Brain tissues. The mortality with ten times of the therapeutic dose single exposure was found to be less than 30% in mice and rats. The sub-chronic toxicity results demonstrate the safety levels up to 2XTD as evaluated by live phase activity, clinical chemistry.

# LIBRARY AND DOCUMENTATION SERVICES



Library continued to cater to the documentation and information needs of the Institute and other Research Organizations, Home Science and Medical Colleges. The library has played a key role in reference activities by offering information dissemination services like MEDLINE Searches, Proquest Medical Library Full Text Database of journals and other online retrieval activities using the LAN Network of the Institute. Library continued to participate in exchange of data, journals and information using the URL<[http://Groups.yahoo.com/group/ICMR Librarians](http://Groups.yahoo.com/group/ICMR_Librarians)>.

Resource Sharing and User Education Programmes etc are continuously being undertaken by the Library. Institute's Scientific papers going in for publication in Scientific Journals etc., are being routed through the Library and a data-base of the published papers is also made accessible through on-line services using NIN Website ([www.ninindia.org](http://www.ninindia.org)). The Library services are being further strengthened by continuously receiving support from Indian Council of Medical Research for accessing E-journals from JCCC@ICMR and J-Gate database. The Library is also a member of ERMED Consortia of National Medical Library, New Delhi provided by ICMR for accessing E-journals Online Subscription of 4 Core Journals such as LANCET, NATURE, NEJM, SCIENCE has been renewed by ICMR, is also accessible.

The Library has continued to provide an excellent Photostat support to the Scientists, technical as well as to the administrative staff.

The following library services were expanded as detailed below:

## 1. New Additions

Books	....	15
Reports	....	133
Thesis / Dissertations	....	8
CDROMS	....	18
PC Quest CD's	....	12
General CD's	...	6

## 2. Other Activities

Journals Bound	.....	743
Visitors using the Library	.....	1,019
Circulation of Books/Journals etc	.....	959
No. of E-mails sent outside	.....	2,010
No. of E-mails received	.....	22,150
Photocopying (No. of pages)	.....	3,06,139
Number of Annual Reports mailed	.....	468
No. of INTERNET Searches provided	.....	98
No. of Reprints sent	.....	80

## 3. Total Library Collections

Books	.....	18,097
E-Books	.....	36
Journals (Bound Volumes)	.....	38,286
E-Journals subscribed for 2013	.....	4
Journals subscribed for 2013	.....	383
Journals received (Gratis/Exchange)	.....	320
E-Journals received (Gratis) for 2013	.....	63
Microforms (Microfiche)	.....	1,080
Slides	.....	280
Reports	.....	13,504
Theses & Dissertations	.....	402
MEDLINE CDROMS Discs	.....	383
Current Contents on Diskettes with abstracts	.....	664
Proquest (Full Text E-Journals) on CD ROMS	.....	495
General CD's	.....	254

# Ph.D PROGRAMMES

## Ph.D AWARDEES

S. No	Research Scholar	Title of the Thesis	Award Year	University
1	Mr.P.Muthenna	Characterization of active principles from dietary sources and mechanisms of inhibition of aldose reductase and protein glycation	2013	Osmania
2	Mr.SSS Vara Prasad	Role of 11Beta-Hydroxysteroid dehydrogenase type 1 in the development of obesity in WNIN/Ob obese rats and its regulation by vitamin A and n-6 poly unsaturated fatty acids	2013	Osmania
3	Ms.Little Flower Augustine	Studies on the association between micronutrient status, psychological stress and allostatic load among higher secondary adolescent students	2013	Osmania

## RESEARCH SCHOLARS REGISTERED FOR Ph.D

S. No	Research Scholar (Year of joining)	Title of the Thesis	Supervisor/ Guide	University
1	Jitendra K. Sinha (2009)	Accelerated ageing/reduced longevity of WNIN/Ob obese rats: Role of altered neurochemical profile, oxidative damage & trophic support in the brain	Dr. M. Raghunath	Osmania
2	Anju E. Thomas (2010)	Fetal programming for neuro-muscular skeletal development in the rat offspring – Role of antenatal and perinatal Mg deficiency	Dr. M. Raghunath	Osmania
3	K. Nagabhushan Reddy (2011)	Dietary phytate –Zinc ons: “Role in suppressing colon cancer”	Dr. M. Raghunath	Osmania
4	Shampa Ghosh (2011)	Biochemical molecular and epigenetic changes associated with maternal vit-B12 restrictions induced alterations in C57BL/6 mice offspring.	Dr. M. Raghunath	Osmania
5	Mehraj (2009)	Vitamin D deficiency induced muscle atrophy & adiposity changes: Biochemical & molecular mechanisms.	Dr. Ayesha Ismail	Osmania
6	Bindu (2010)	Studies on the anticancer properties of Murraya koenigii leaves: Role of proteasome inhibition	Dr. Ayesha Ismail	Osmania
7	N. Pallavi (2010)	Regulatory role of zinc in hepcidin mediated iron metabolism	Dr.K.Madhavan Nair	Osmania
8	Y.Sravanthy (2010)	Effects of prenatal iron supplementation on iron-zinc homeostasis and placental zinc transporters: Studies in pregnant women and in BeWo cell lines	Dr.K.Madhavan Nair	Osmania

S. No	Research Scholar (Year of joining)	Title of the Thesis	Supervisor/ Guide	University
9	A. Kiran Kumar (2012)	Metabolic response of zinc depletion and excess in contrasting cells: Studies in osteoblasts, myocytes and enterocytes.	Dr. K. Madhavan Nair	Osmania
10	P. Ravindranath (2011)	Purification, characterization and primary structure elucidation of human milk factor that enhances iron absorption	Dr. P. Raghu	Osmania
11	Purna Chandra (2012)	Manipulation of dietary fat to enhance carotenoid bioavailability and bioconversion to vitamin A: Development of mechanism based strategies.	Dr. P. Raghu	Osmania
12	N. Himaja (2012)	Effects of FoS coated probiotics on fetal immune-programming and other health benefits.	Dr. R. Hemalatha	Dr. NTRUHS
13	Daniella Chyne (2012)	Studies on the biodiversity of food resources in Meghalaya.	Dr. R. Ananthan	Osmania
14	Naga muralidhar (2011)	Genetic and epigenetic approach towards obesogenicity in a rat	Dr. K. Rajender Rao	Osmania
15	M. Ankulu (2011)	Effect of excess nitric oxide in the patho physiology of motor neuron degeneration in neuroalzheimerism.	Dr. Arjun L.Khandare	Osmania
16	S.Alekhya (2012)	Identifying microbiological and hygienic factors affecting safety of stress foods and addressing them through vendor education.	Dr. V.Sudershan Rao	Osmania
17	Sowmya Sharma (2011)	Modeling the developmental origins of health diseases in the mouse embryonic stem cells (mESCs) – Cellular, molecular/epigenetic approaches	Dr. Vijayalakshmi Venkatesan	Osmania
18	Anupama Tyagi (2009)	Anti inflammable potential & n-3 PVFA in experimental biochemical and molecular mechanism	S. Ahmed Ibrahim	Osmania
19	Anil Sakumari (2010)	Modulation of adipose tissue inflammation and function of dietary n-3 pvfa: potential role in metabolic syndrome.	S. Ahmed Ibrahim	Osmania
20	J. Sugeetha (2012)	Impact and dietary n-3 and n-6 pufa on the progression of non alcoholic fatty	S. Ahmed Ibrahim	Osmania
21	Golla Venkateswarlu (2010)	Role of dietary fatty acids in inducing endoplasmic reticulum stress in stromal vascular cells : implications in the development of obesity associated insulin resistance	Dr.Sudip Ghosh	Osmania
22	K.Sandeep Kumar (2011)	Role of miRNA in the development of obesity and diabetes	Dr.Sudip Ghosh	–
23	J. Vahini (2011)	Studies on assessment, identification and modification of glycemic index in dietes commonly consumed by people.	Dr.K.Bhaskarachary	Osmania
24	V.Varsha (2011)	Evaluation if the impact of genetic polymorphism on the pharamacodynanamic activity of commonly prescribed anti hypertensive drugs	Dr. Dinesh Kumar	JNTU(H)

S. No	Research Scholar (Year of joining)	Title of the Thesis	Supervisor/ Guide	University
25	V.Sudhakar Reddy (2009)	Role of small heat shock proteins in diabetic complications: modulation by nutritional and dietary factors.	Dr. Bhanuprakash Reddy	Osmania
26	Sarin Sara Jose (2010)	Studies on nutritional assessment of diabetic complications	Dr. Bhanuprakash Reddy	Osmania
27	S. Vishwaraj (2012)	Proteomics of diabetic complications.	Dr. Bhanuprakash Reddy	Osmania
28	Sneha Jakhota (2012)	Role of RBP4 in the pathophysiology of diabetes	Dr. Bhanuprakash Reddy	Osmania
29	K. Shruthi (2012)	Role of CTRPs in diabetes and retinal degeneration	Dr. Bhanuprakash Reddy	Osmania
30	T. Shalini (2012)	Assessment of health and nutritional status of urban geriatric population	Dr. Bhanuprakash Reddy	Osmania
31	Anuradha Challa (2011)	Impact of dietary facts rich in n-6 and n-3 poly unsaturated fatty acids on adiposity and insulin resistance in diet induction obese rat model: a missing molecular link with vit a metabolism	Dr. A. Vajreswari	Osmania
32	Anantha Krishna Vemuri (2011)	Impact of nutritionally superior high oleic acid varieties of mustard oil on lipid metabolism	Dr. A. Vajreswari	Osmania
33	M.Srujana (2012)	Effect of pesticide exposure among the farm children and their mothers on the various biochemical parameters associated with reproduction, neurotoxic enzymes, oxidative stress and impact on the micronutrient status	Dr.J.Padmaja Rambabu	Osmania
34	B.Venkat Reddy (2012)	Monitoring of organophosphate pesticide metabolite in commonly used fruits, juices and vegetables and urine samples of urban children and its toxic effect.	Dr.S.N.Sinha	Osmania
35	K.Siva Kesava Rao (2013)	Effect of long term pre-diabetes on risk of renal, retinal and lens abnormalities: Biochemical mechanism and role of dietary agents	Dr.P.Suryanarayana	Andhra
36	M.Siva Prasad (2013)	Status of micronutrients and its influence on molecular mechanisms in diabetic nephropathy	Dr.Bhanupraksash Reddy	Osmania
37	Nivedita Dubey (2013)	Nutritional composition bioavailability and allelgenecity profile of nutritionally enriched GM food crops	Dr.B.Dinesh Kumar	Osmania
38	SM Krishna Prasad (2013)	Biochemical and molecular studies on diet induced obesity rat as a model organism	Dr.K.Rajender Rao	Osmania
39	M.Raja Gopal Reddy (2013)	Role of vitamin A metabolic pathway and the development of non-alcoholic fatty liver diseases quotation a study nutrient/nutrient-nutrient interactions	Dr.A.Vajreswari	Osmania

S. No	Research Scholar (Year of joining)	Title of the Thesis	Supervisor/ Guide	University
40	Dripta Roy Choudhry (2013)	Introduction of vitamin C rich fruit in supplementary nutrition programme(snp) for improving micronutrient status, gut health, growth and development: a randomized control trial among ICDS pre-school; beneficiaries	Dr.K.Madhavan Nair	Osmania
41	J.Rishika (2013)	Effect of isoflavones isolated from a naturally available cow pie as a source for the treatment of osteoporosis in MG-63 Human osteo-sarcoma cell and to access with synergetic role with vitamin D in bone formation	Dr.C.Suresh	Not registered
42	V.Srinivas (2013)	Role of maternal fatty acids on antigenic factors in the first trimester placenta and their invasive properties: implication to foeto-placental growth and development	Dr.Sanjay Basak	Osmania
43	D.M.Dinesh Yadav (2013)	Studies on identification of candidate gene(s) associated with obesity in WNIN/ob rat	Dr.K.Rajender Rao	Osmania
44	Padmanav Behera (2013)	Studies on the potential of islet like-cell aggregates (ICAS) generated from mesenchymal stem cell of human placenta for treating Type I diabetics in NOO mice.	Dr.V.Vijayalakshmi	Dr.NTRUHS
45	K.Archana (2013)	Target nutrition communication for promoting consumption of micronutrient rich foods among rural household by developing dietary diversity scores	Dr.K.Madhavan Nair	Osmania
46	Noor Ahmed (2013)	Different food sources ( Meat, Fish, Chicken, Eggs, Milk and milk products) for human consumption are most likely the major contributors of PCBs which is served as an index of precautionary measures for dwellers in metro city of Hyderabad	Dr.S.N.Sinha	Osmania
47	G. Srividya (2013)	Anticancer and proteasome inhibitory potential of cinnamon and its active components: <i>in vitro</i> and <i>in vivo</i> studies.	Dr. Ayesha Ismail	Not registered
48	V.Sai Santosh (2014)	Effect of dietary N-6 and N-3 polyunsaturated fatty acids on the progression of non alcoholic fatty liver disease (NAFLD) in diet-induced model of hepatic steatosis	Dr. Ahmed Ibrahim	Osmania
49	SGD.Naga Lakshmi (2014)	Development and validation of a comprehensive index for assessing food safety at household level	Dr.V.Sudershan Rao	Osmania
50	A.S.Neelima (2014)	Intracellular mechanism of naturally available Neuroprotective compounds in mitigating the combined toxicity generated by the lead (pb <sup>2+</sup> ) in combination with Amyloid peptides in human brain cells.	Dr. C. Suresh	Osmania

## INSTITUTIONAL STAFF REGISTERED FOR Ph.D

S. No	Name of the Staff	Title of the Thesis	Supervisor	University
1.	Dr.A.Laxmaiah (2008)	Assessment of prevalence of overweight/obesity, hypertension and type II diabetes among 20-60 year Urban population in Hyderabad	Dr.B.Sesikeran	Dr.NTR University of Health Sciences
2.	Ajumera Rajanna (2011)	Embryonic stem cells as model system to study the developmental origin of health in micronutrients- obesity/type 2 diabetes	Dr. Vijayalakshmi Venkatesan	Osmania
3.	K.Mangthya Naik (2012)	Studies gastro protective effects of Naga King chili	Dr. R. Ananthan	Osmania

# AWARDS/ HONOURS

## AWARDS/ HONOURS CONFERRED ON SCIENTISTS

Name of the Scientist	Awards/ Honour conferred
<b>Dr. K. Madhavan Nair</b>	Best Poster Award in the 45 <sup>th</sup> National Conference of the Nutrition Society of India held at National Institute of Nutrition, Hyderabad, for Paper titled "Risk factors of anaemia among infants (6-12 months) from rural India. Haveli Ram Pasricha Memorial Prize under Community Nutrition" (Nov. 20-22)
<b>Sylvia Fernandez Rao</b>	Best Poster Award in the 45 <sup>th</sup> National Conference of the Nutrition Society of India held at National Institute of Nutrition, Hyderabad, for Paper titled "Household food insecurity relates to maternal well-being in a rural Indian community. NSI Prize under Community Nutrition" (Nov. 20-22)

## AWARDS/ HONOURS CONFERRED ON RESEARCH FELLOWS/ STUDENTS

Name of the student	Award/ Honour received
Dr. J. Yashovanthi (ICMR PDF)	Best Oral presentation for paper entitled , "Relationship between inflammatory markers, bone turn over and BMD in pre and post menopausal women" at the 9 <sup>th</sup> Annual Conference of the Indian Society for Bone and Mineral Research, held at SKIMS, Jammu & Kashmir Sept 7-8, 2013
Mr. V. Sudhakar Reddy (UGC-SRF)	Awarded Certificate of Merit for his presentation at ASIA-ARVO held at New Delhi during 28-31 <sup>st</sup> October 2013.
The following students were awarded Best Poster Award in the 45 <sup>th</sup> National Conference of the Nutrition Society of India held at National Institute of Nutrition, Hyderabad, (Nov. 20-22)	
Y.Sravanthy (SRF)	Non-transferrin mediated iron uptake in BeWo-juman placental trophoblastic cell lines. Dr.K.Seetharam Bhat Memorial Prize under Experimental Nutrition
SGDN Lakshmi Reddi (SRF)	Foodborne pathogens in street vended juices and their association with food hygiene practices of fruit juice vendors in Hyderabad under Food Science and Nutrition
Aiswarya Ravichandran (MSc (AN) student)	Young Scientist (Junior) Award in Community Nutrition for her paper titled "Perception and Practices Related to Consumption of Energy Drinks among Regular and Non-Regular Young Consumers"

# PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS/ WORKSHOPS/ CONFERENCES/ TRAINING PROGRAMMES

Name of the Scientist	Conference/ Workshop/ Seminar
<b>2013</b>	
<b>Dr. G. M. Subba Rao</b>	International Association of Media and Communication Research (IAMCR) - 2013 Conference, at Dublin, Ireland. Presented a Paper titled "Are perceptions of obesity and its risk factors different among overweight and normal adolescents? - Findings from focus groups in South India" in Health Communication Working Group. Invited as a Panel Speaker to present on "Integrating Entertainment-Education for Nutrition Communication in Pedagogical Settings – Case Studies from India". Chaired a Technical Session in Health and HIV/AIDS Communication working group (June 25-29).
<b>Mr. Mehrajuddin Bhat (ICMR-SRF)</b>	Presented a Poster on "The Ubiquitin Proteasome pathway is upregulated during vitamin D deficiency induced muscle atrophy", at the 5th Annual Ubiquitin Drug Discovery and Diagnostics Conference, held in Philadelphia, PA, USA.(July 22-24)
The following scientists participated in the IUNS 20 <sup>th</sup> International Congress of Nutrition, Granada, Spain and presented the papers mentioned against their names (Sept. 15-20)	
<b>Dr. P. Uday Kumar</b>	Aldose Reductase (AR) activity in RBCs & AR activity and expression in tumours of human cancer subjects (Oral) Role of type of dietary fat in the etiopathogenesis of Carcinogen-induced breast neoplasm in female fischer rats (Poster)
<b>Dr. A. Laxmaiah</b>	Socio-demographic patterns of hypertension, knowledge, behaviours and practices of adult tribal (Adivasis) population in India: A first national nutrition monitoring survey (Oral) Time trends in diet and nutritional status of rural and tribal population in India: A longitudinal national nutrition surveys of NNMB (Poster)
<b>Dr. D. Raghunatha Rao</b>	Nutrition education for adolescents: An interventional approach to create awareness on "Eat right and play with might" (Poster)
<b>Dr. V. Sudershan Rao</b>	Mapping priorities for promoting food safety in Indian households through development and validation of integrated scores based on key determinants (Poster) and Knowledge and use of food label information among Urban consumers in India (Poster)
<b>Dr. N. Harishankar</b>	Oxidative damage, ageing and degenerative diseases in WNIN obese rats (Poster)
<b>Dr. R. Ravinder Naik</b>	Effect of andrographolide on streptozotocin-induced diabetic cataract in Sprague-Dawley rats (Poster)

# WORKSHOP/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN



**1** ICMR National Task Force Project on Migrant's Health Care Stakeholders meeting on the theme "Improving the Access to Government Health Care Services by the Poor Migrant Population of Hyderabad", organized in association with Health Systems Research Division, New Delhi (April 16).



**2** Training of Trainers for "National Programme for Prevention and Control of Fluorosis", was organized at NIN in association with Directorate General of Health Services, Ministry of Health, Government of India, New Delhi (May 21-22).



**3** In connection with the National Nutrition Week Celebrations, a one day Symposium on "Food Security for Health and Nutritional Well-being" was organized in association with Food and Nutrition Board (Sept. 3).

**4** Fifth Batch of two year MSc (Applied Nutrition) for the year 2013-2014 commenced in the month of October. Sixteen candidates were admitted into the programme.

**5** Pre-Conference Workshops on "Current Priorities in Nutrition Research Methodology and Report Writing" and "Prevention of Lifestyle Diseases – Role of Food Science and Nutrition" (Nov. 20).



**6** 45<sup>th</sup> National Conference of the Nutrition Society of India on "Inter-sectoral Approach to Promote Food and Nutrition Security" held at National Institute of Nutrition, Hyderabad (Nov. 21-22).



**7** Training of Doctors on Fluorosis Mitigation under Hogenakkal Water Supply & Fluorosis Mitigation project in collaboration with Tamil Nadu Water Supply & Drainage Board (Nov. 25-29).



8 International Conference on “Emerging issues on health effects of pesticide residues in food and environment-Unmet challenges & research opportunities”, organized by Food & Drug Toxicology Research Centre, National Institute of Nutrition, Hyderabad (Dec.12).

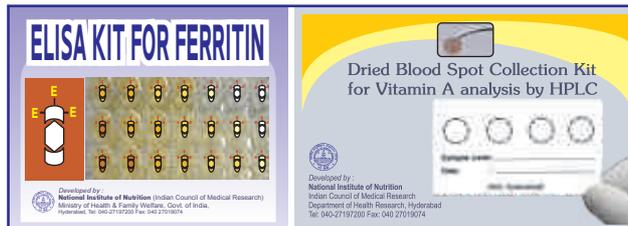


9 A one-week Workshop on “Proteomics for Biomedical Research” under the DHR Human Resource Development Program (Jan. 6-10).



10 Training of Technicians for “National Programme for Prevention and Control of Fluorosis” was organized in association with Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India (Jan.20-24).

11 51<sup>st</sup> Post Graduate Certificate Course in Nutrition (Feb. 3-April 11).



12 Hon'ble Union Health Minister for Health and Family Welfare, Shri Ghulam Nabi Azad launched the following Kits developed by NIN in a function organised by ICMR, at India Habitat Centre, New Delhi on 20<sup>th</sup> February 2014:

- Kits for Rapid Detection of Food Borne Pathogens (developed in association with Bioserve International)
- ELISA kit for the Detection of Ferritin
- Dried Blood Spot (DBS) for diagnosing Vitamin-A deficiency

13 Division of Community Studies, NIN organized the following training programmes:



Re-orientation training programme on Health and Nutrition for the officials of Food & Nutrition Board, Ministry of DWCD, GoI, New Delhi (Feb.3-7).

Training programme on estimation of haemoglobin levels in finger prick dried blood samples by cymethaemoglobin method and collection of finger prick dry blood samples for estimation of serum vitamin A levels for the staff of M.S.Swaminathan Research Foundation (MSSRF), Chennai (Feb.10-14).



Nutritional methodology training programme to assess nutritional status of under five year children in the state of Tamil Nadu for the functionaries of ICDS, Department of ICDS, Government of Tamilnadu (Feb.17-28).



14 Mini-Convocation was conducted for 3<sup>rd</sup> Batch of MSc Applied Nutrition (2011-2013). Ms. Neha John was awarded Biplab K Nandi Fellowship and Gold Medal for being the topper in the batch. Prof. Ravi Raju, Vice-Chancellor of Dr. NTR University of Health Sciences delivered the convocation address (March 7).



15 “National Level Workshop on Biostatistics and it’s Applications in Biomedical Research”, organized by Biomedical Informatics Centre, National Institute of Nutrition (March 24-26).



16 Awareness Workshop on “Biosafety Procedures for Recombinants and Genetically Modified Crops”, organized by Centre for Advanced Research for Preclinical Toxicology, FDTRC, NIN (March 26).

# SERVICES RENDERED TOWARDS INCOME GENERATION

## 1. PATHOLOGY SERVICES

- During the year, a total income of Rs. 1,15,950/- was generated from various projects of Institute's preclinical toxicology and surgical pathology and cytology samples analysis.

## 2. TRAINING PROGRAMMES

- An amount of Rs. 6,40,000/- was generated from the tuition fee collected from the first and second year participants of 2 year MSc (Applied Nutrition) course (1<sup>st</sup> year – 16 and 2<sup>nd</sup> year – 16 candidates).
- An amount of Rs. 2,10,000/- was generated from eight private candidates admitted to the regular training programme viz., Post Graduate Certificate Course in Nutrition including two participants from Bangladesh.

# INSTRUMENTATION SERVICES

## LIST OF EQUIPMENTS PROCURED AND INSTALLED DURING THE YEAR 2013-2014

No.	Name of the Instrument	Make
1	UV-Visible Spectrophotometer	Shimadzu
2	Image Analyzer Gel Doc. System	Preotein Simple USA
3	Multi Mode Reader	Biotek
4	Table Top Centrifuge	Sorvall
5	RT-PCR	Applied Biosystems
6	Shaking water bath	MRC Ltd., Israel
7	Refrigerated Table top Centrifuge	Thermo Fisher
8	LN2 Container	Thermo Scientific
9	LN2 Container	Thermo Scientific
10	Balance 1.5kg(5 No)	Essae Teraoka
11	Balance 6kg(5 No)	Essae Teraoka
12	1Kva online UPS	Emerson
13	Electrophoresis	Hoefer
14	3Kva online UPS	APC
15	Gas Chromatograph Gas Generators	Perkin
16	Spectrophotometer Nano drop	Thermo Scientific
17	Name of the Equipment	Make
18	Balance 6kg	Essae Teraoka
19	Tissue Homogenizer	Kinematica
20	Rotary Evaporator	Buchi
21	Incubator	Thermo Fisher
22	CO2 Incubator	Eppendorf
23	Water purification system	Elga
24	Densitometer Molecular Imager	Bio-Rad
25	Table Top Centrifuge	Hermle

# SCIENTIFIC PUBLICATIONS

## A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Anand Kumar K, Lalitha A, Pavithra D, Padmavathi IJN, Manisha Ganeshan, Rajender Rao K, Venu L, Balakrishna N, Harishanker N, Umakar Reddy S, Chandak GR, Sengupta S, Raghunath M: Maternal dietary folate and/or vitamin B12 restrictions alter body composition (adiposity) and lipid metabolism in Wistar rat offspring. *J Nutr Biochem*. 24:25-31,2013.
2. Ankulu M, Aparna N, Raju Naik V, Amol Shirfule, Khandare AL: Putative ligand-target docking studies of human AMPA selective ionotropic glutamate receptors reveal that  $\beta$ -ODAP has high binding affinity compared to tyrosine and glutamate. *Int Res J Biochem Bioinformatics*. 3:130-139,2013.
3. Anil Kumar P, Bhanuprakash Reddy G: Nutrition & metabolic syndrome. *Rev Endocr Metab Disord*. 14:217, 2013.
4. Anil Kumar P, Swathi Chitra P, Bhanuprakash Reddy G: Metabolic syndrome and associated chronic kidney diseases: nutritional interventions. *Rev Endocr Metab Disord*. 14:273-286,2013.
5. Arlappa N: Vitamin A deficiency control measures: importance of vitamin A supplementation as a public health policy in the Indian context. *J Pub Health Policy*. 34:538-548,2013.
6. Arlappa N, Aatif Qureshi I, Srinivas R: Fluorosis in India; an overview. *Int J Res Dev Health*. 1:97-102,2013.
7. Bailey PK, Charles RV Tomson, Sanjay Kinra, Shah Ebrahim, Radha Krishna KV, Hannah Kuper, Dorothea Nitsch, Yoav Ben-Shlomo: Differences in estimation of creatinine generation between renal function estimating equations in an Indian population: cross-sectional data from the Hyderabad arm of the Indian migration study. *BMC Nephrology* 14:30, 2013.
8. Bailey PK, Charles RV Tomson, Sanjay Kinra, Shah Ebrahim, Radhakrishna KV, Hannah Kuper, Dorothea Nitsch, Yoav Ben-Shlomo: The effect of rural-to-urban migration on renal function in an Indian population: cross-sectional data from the Hyderabad arm of the Indian migration study *BMC Nephrology*. 14:240, 2013.
9. Balakumar M, Saravanan N, Prabhu D, Bhaskaran Regin, Bhanuprakash Reddy G, Viswanathan Mohan, Mohan Rema, Balasubramanyam M: Benefits of early glycemic control by insulin on sensory neuropathy and cataract in diabetic rats. *Indian J Exp Biol*.51:56-64,2013.
10. Bamji MS, Murthy PVVS, Vishnuvardhana Rao M, Tara MS: Development of rural adolescent girls through scientific and social engineering and skill development. *Ind. J. Nutr. Dietet*. 50:102-110, 2013.
11. Bhat M, Kalam R, Qadri SS, Madabushi S, Ismail A: Vitamin D deficiency induced muscle wasting occurs through the ubiquitin proteasome pathway and is partially corrected by calcium in male rats. *Endocrinology*. 154:4018-4029,2013.
12. Bindu N, Rajanna Ajumeera, Anitha Chauhan, Balakrishna N, Raghunath M, Ayesha Ismail. Murraya koenigii leaf extract inhibits proteasome activity and induces cell death in breast cancer cells. *BMC Complement Altern Med*. 13:7,2013.
13. Chalamaiah M, Jyothirmayi T, Bhaskarachary K, Vajreswari A, Hemalatha R, Dinesh Kumar B: Chemical composition, molecular mass distribution and antioxidant capacity of rohu (*Labeo rohita*) roe (egg) protein hydrolysates prepared by gastrointestinal proteases. *Food Res. Int*. 52:221-229, 2013.

14. Debolina Sarkhel, Manoj Kumar, Sudarshan Reddy V, Durgaprasad L, Hanumanth Reddy Ch, Hemalatha Rajkumar: Effects of active components of lactobacillus strains on cancer cell proliferation and inflammatory markers in vitro. *Int J Cell Sci. Biotechnol.* 1:111-115,2013.
15. Dinesh Kumar B, Uday Kumar P, Prasanna Krishna T, Kalyanasundaram S, Suresh P, Jagadeesan V, Hariharan S, Nadamuni Naidu A, Kamala Krishnaswamy: DNA vaccines: getting closer to becoming a reality: purified vero cell-derived inactivated rabies virus vaccine-safety evaluation-toxicology. *Indian J Med Res.* 137:1027-1028,2013.
16. Dinesh Kumar B, Uday Kumar P, Prasanna Krishna T, Kalyanasundaram S, Suresh P, Jagadeesan V, Hariharan S, Nadamuni Naidu A, Kamala Krishnaswamy: Pre-clinical toxicity & immunobiological evaluation of DNA rabies vaccine & combination rabies vaccine in rhesus monkeys (*Macaca mulatta*). *IJMR.* 137:1072-1088, 2013.
17. Durgaprasad L, Manoj kumar Hemalatha R, Ramakrishna Sistla, Naidu VGM, VenuT, Vinod Verma, Ravinder kaur N: Anti-Inflammatory treatments for Chronic Diseases - A Review. *Inflamm Allergy-Drug Targets.* 12:349-361,2013.
18. Filippo Molinari, Vipin Gupta, Poornima Prabhakaran, Kristen M Meiburger, Luca Saba, U. Rajendra Acharya, Giuseppe Ledda, Radhakrishna KV, Gagandeep Kaur Walia, Sanjay Kinra, Andrew Nicolaides, Shah Ebrahim and Jasjit S Suri : Automated IMT estimation and BMI correlation using a low-quality carotid ultrasound image database from India. *Conference proceedings IEEE Eng Med Bio Soc.* 33:43-46, 2013.
19. Gangadhararao A, Dipankar Das, veerasami M, Ramachandran Lakshmikanthan, Senthilkumar, Munishkumar D, Ramalakshmi B, Vijay Bahekar, Falguni Mukherjee, Dev Chandran, Uday Kumarj P, Sesikeran B, Alwar Srinivasan V: Antemortem and postmortem examinations of the cattle calf naturally infected with myhco9bacterium avium subsp. Paratuberculosis. *Eur J Microbiol Immunol.* 3:241-251,2013.
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21. Grama CN, Suryanarayana P, Paatil MA, Raghu G, Balakrishna N, Ravi Kumar MNV, Bhanuprakash Reddy G: Efficacy of biodegradable curcumin nanoparticles in delaying cataract in diabetic rat model. *PLOS one.* 8:e78217, 2013.
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Development of Genetics, Indian Institute of Science  
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V.Ramalingaswami Bhawan  
Ansari Nagar, Post Box. 4911, New Delhi – 110 029

Dr. G.S.Toteja  
Scientist 'G' & Head  
Nutrition  
Indian Council of Medical Research  
V.Ramalingaswami Bhawan  
Ansari Nagar, Post Box. 4911  
New Delhi – 110 029

Shri T.S. Jawahar, IAS  
Sr. Deputy Director General (Administration)  
Indian Council of Medical Research  
V.Ramalingaswami Bhawan  
Ansari Nagar, Post Box. 4911  
New Delhi – 110 029

Smt. Dharitri Panda, ICAS  
Sr. Financial Advisor  
Indian Council of Medical Research  
V.Ramalingaswami Bhawan  
Ansari Nagar, Post Box. 4911  
New Delhi – 110 029