वार्षिक प्रतिवेदन

Annual Report

2019-20

आई सी एम आर – राष्ट्रीय पोषण संस्थान
ICMR - NATIONAL INSTITUTE OF NUTRITION
भारतीय आयुर्विज्ञान अनुसंधान परिषद
Indian Council of Medical Research
हैदराबाद, तेलंगाना, भारत
Hyderabad, Telangana, INDIA

Annual Report
2019-20

आई सी एम आर – राष्ट्रीय पोषण संस्थान

ICMR - NATIONAL INSTITUTE OF NUTRITION

भारतीय आयुर्विज्ञान अनुसंधान परिषद

Indian Council of Medical Research

हैदराबाद, तेलंगाना, भारत

Hyderabad, Telangana, INDIA

Contents

Scientific Staff

DIRECTOR
DR. HEMALATHA R

SCIENTIST 'G'

Shri. Thingnganing Longvah
Shri. K. Venkaiah
Dr. A. Laxmaiah
Dr. P. Uday Kumar
Dr. Vijayalakshmi Venkatesan
Dr. B. Dinesh Kumar
Dr. A. L. Khandare
Dr. Y. Venkataramana
Dr. G. Bhanuprakash Reddy

SCIENTIST 'F'

Dr. Bharati Kulkarni
Dr. J. J. Babu Geddam
Dr. N. Arlappa
Dr. S. N. Sinha
Dr. S. Ahmed Ibrahim
Dr. Sudhip Ghosh
Dr. P. Suryanarayana
Dr. R. Harikumar
Dr. J. Padmaja
Dr. C. Suresh

SCIENTIST 'E'

Dr. I. I. Meshram
Dr. M. Maheshwar
Dr. G. Maruthi Subba Rao
Dr. Ayesha Ismail
Dr. P. Raghu
Dr. N. Harishankar
Dr. Sanjay Basak
Dr. M. S. Radhika
Dr. K. Rajender Rao

SCIENTIST 'E'

Dr. Shobi Veleri
Mr. J. Sreenivasa Rao
Dr. M. Satish Kumar
Dr. B. Ramalingam

SCIENTIST 'D'

Dr. P. Amrutha Rao
Dr. Sylvia Fernandez Rao
Dr. M. V. Surekha
Dr. Ajumeera Rajanna
Dr. R. Ananthan
Dr. S. Devindra
Dr. Gargi Meur

SCIENTIST 'C'

Dr. P. Sujatha
Dr. Santosh Kumar Banjara
Dr. Paras Sharma
Dr. Devraj J. Prasannanavar
Dr. Raja Sriswan Mamidi
Dr. Vakdevi V
Dr. Mahesh Kumar Mummadi
Dr. Naveen Kumar Boiroju
Mr. V. Raju Naik

SCIENTIST 'B'

Dr. Sudhakar Reddy Vadde
Dr. Ravindranadh Palika
Dr. Dasi Teena
Dr. P. Raghavendra
Dr. Anwar Basha
Dr. D. Subrahamanyam

Administrative Staff
(Ministerial & Secretarial)

Senior Administrative Officer

Vacant

Administrative Officers

Mrs. D. V. Lakshmi Rani
Mrs. Latha Kumaraswamy
Mrs. R. C. Padmini Mohan
Mr. S. Lakshmanan

Accounts Officer

Mr. M. Rajagopala Chary

Group "B" Staff
Section Officers

Mr. P. Dhanasekharan
Mr. K. Sivarami Reddy
Mr. V. Elisha
Mrs. Alice Mary
Mr. D. Sunil
Mr. M. Babu

Assistants

Mrs. Shakila Banu
Mr. G. R. Srinivas
Mrs. P. Prabhavathi
Mr. Mohammed Ilyas
Mr. Shaik Jamaluddin
Mrs. Mini Pramod
Mr. C. Prabhu
Mr. Y. Bala Narayana
Ms. T. Anuradha Jayalaxmi
Mr. A. Narsing Rao
Ms. G. S. Gautami
Mr. A. Satyanarayana Prasad

Group "C" Staff
Upper Division Clerks

Mr. M. Raghuram
Mrs. M. Rekha
Mr. U. Somayya
Mr. A. Venkata Ramana
Mr. D. Ramanjaneyulu
Mrs. G. Y. Anita

Lower Division Clerk

Mr. Akram Khan
Mr. K. Venkatesh

Group "B" Staff
Private Secretaries

Mrs. G. Prashanthi
Mrs. V. Swayam Prabha
Mrs. K. Sailaja
Mr. G. Durga Prasad

Personal Assistant

Mr. G. Mahesh Kumar

Group "A" Staff
Technical Officer-C
(Library Information Officer)

Mr. B. Narayana

Group "B" Staff
Sr. Technician (3) (Assistant Library Information Officer)

Mr. Prakash Kulkarni

Technical Staff

Group "A" Staff

Principal Technical Officer

Dr. D. Vijaya Bhaskara Rao

Senior Technical Officer

Dr. M. Satyavani
Mr. S. Ananda Rao
Dr. S. Hemalatha

Technical Officer - C

Mr. K. Srinivasa Rao
Mr. R. Radha Krishna Sarma
Dr. Amulya Rao
Mr. P. Ajey Kumar
Mr. R. Raghunadh Babu
Mr. P. Krishnaswamy
Mr. Korra Mangthya
Mr. Virendra Vasant Panpatil
Mr. R. Naveen Kumar
Mrs. B. R. Annapurna
Mrs. K. Sharada
Mrs. P. Sailaja
Mr. M. Krupadanam
Mr. V. Bhasker
Mrs. S. A. Brinda

Technical Officer -C (Engg. Suppt. Staff)

Mr. N. Satyanarayana
Mrs. L. Vijaya Durga

Technical Officer-B

Dr. K. Uma Kailash
Mr. B. Venkateswara Rao
Mr. M. Seshacharuyulu
Mr. K. Vasudev
Mrs. P. Anitha Chauhan
Mr. S. Laxman
Mr. Ch. Ranga Rao
Mr. P. Madhusudhana Chary
Mr. C. Sai Babu
Mr. Narottam Pradhan

Technical Officer-B (Engg. Suppt. Staff)

Mr. M. Asaithurai

Group "B" Staff

Technical Officer - A

Mrs. P. S. Prashanthi
Mr. S. P. V. Prasad
Mr. Sree Ramakrishna
Mr. B. Giri Babu
Mr. M. Srinivas
Mr. Surender Jatavath
Mr. P. S. Rama Rao
Mr. K. Suryam Reddy
Mr. K. Subhash
Mrs. B. Tulja
Dr. Sreenu Pagidoju
Mr. Sunu P. V
Mr. Srinivasu Kurella
Mr. N. Samyukthakumar Reddy
Dr. Shashibhushan Kumar
Mrs. G. Neeraja
Mr. D. Rakesh Naik
Mrs. K. Usha Rani
Mr. Vanka Janardhan
Dr. Kiran Kumar Angadi

Technical Officer-A (Engg. Support Staff)

Mr. A. Santosh Kumar
Mr. V. Bhuvaneswaran

Technical Assistant (Tech. Staff)

Dr. Jagdish Buwade
Mr. Sreedhar Mudavath
Mr. Hrusikesh Panda
Dr. K. Prathap Reddy
Mr. V. Sai Santhosh
Mr. Golajapu Mahendra
Mr. Jamalpur Ravi Prakash

Technical Assistant (eng. & Suppt.)

Ms. Niharika

Sr. Technician (3)

Mr. K. Vinod Reddy
Mr. G. Janardhan
Mr. G. L. Stephen

Sr. Technician (3) (Engg. & Suppt.)

Mr. B. Om Prakash
Mr. Michael Fernandez
Mr. A. Anjaiah

Group "C" Staff
Sr. Technician (2)

Mr. P. Satish Babu
Mr. E. Srinivas
Mr. G. Venkataraji Reddy

Sr. Technician (2) (Eng. & Suppt.)

Mr. Mohd. Younus
Mr. YVL Narasimha Rao
Mr. S. Devendran
Mr. Sriramulu Naidu
Mr. R. Sahadeva
Mr. K. Srinivasa Raju
Mr. Purna Chandra Beshra

Sr. Technician (1)

Mr. M. Sripal Redy

Sr. Technician (1) (Eng. & Suppt.)

Mr. P. Narender Kumar
Mr. Ramavath Ramsingh
Mr. Polishetty Naidu

Technician (2) (Eng. & Suppt.)

Mr. B. Bal Reddy
Mr. Srinivas Dheeravath
Mr. K. Satyanarayana

Technician-1 (Eng. Suppt.)

Mr. Shrinath Madiyalkar
Mr. N. Om Prakash
Mr. K. B. Srinivasa Rao
Mr. Satram Mahesh Babu
Mr. Naga Saeeswar Kurmala
Mr. Mahendr Sing Jadav

Sr. Technician (2) (Driver Grade – I)

Mr. K. Krishna
Mr. V. Kondaiah

Sr. Technician (1) (Driver Grade – I & II)

Mr. Syed Mohd. Ali
Mr. D. Amruthanathan
Mr. K. Jangaiah

Sr. Technician (3) (Junior Staff Nurse)

Mrs. B. V. Nancharamma

Sr. Technician (2) (Junior Staff Nurse)

Mrs. K. Venkataramana
Mrs. S. Rojamani

Sr. Technician (2) (Auxiliary Nurse Midwife)

Mrs. Ch. Anitha
Mrs. D. Rani
Mrs. G. Tulasi Bai

Sr. Technician (1) (Auxiliary Nurse Midwife)

Mrs. G. Vijayalakshmi
Mrs. V. Aruna Reddy
Mrs. E. Sheela

Auxiliary Nurse Midwife

Mr. Dadigiri Narasimhulu
Mrs. N. Jhanshi

Technician "C" (Eng. & Suppt.)

Mr. Potta Dasaratha

Technician-1 (Nursing Attendant)

Ms. R. Rajyalakshmi
Ms. Govada Bhavani
Ms. Valentina Teriscova
Ms. Swarupa D

Lab Assistant -1

Mr. Jalalappa
Mr. B. Nageswara Rao
Mr. Manga Narasaiah
Mr. K. Rajaiah
Mr. Mohd. Yaseen
Mr. Mohammed Chand
Mr. P. V. Poulous
Mr. P. Shiva Shanker
Mr. Y. Agreepa Raju
Mr. K. Balraj
Mr. Mohd. Maulana
Mr. Mukkera Krishna
Mr. D. Dasaratha
Mr. K. Kasipathi
Mr. S. Chandraiah
Mr. Syed Asif Ali
Mr. Manchikanti Krishna
Mr. Mabbu Ramulu
Mr. K. Gopal
Mr. K. Harinarayana
Mr. Gandamalla Narasimha
Mr. J. Nageswara Rao
Mr. C. Chandramouli
Mr. B. K. Mahadevaiah
Mr. Dhanavath Saida
Mr. V. Dasatham
Mr. Mohammed Abdul Sattar
Mr. P. Srihari
Mr. Mohd. Habeeb Uddin
Mr. A.venugopal
Mr. T. Govind
Mr. Shaik Mukthar
Mr. V. Rajkumar
Mr. A. Chandraprakash
Mr. M. Narasimha
Mrs. M. Satyamma
Mr. E. Krishna
Mr. P. Nagulu
Mrs. C. Sivaleela
Mr. G. Satyapaul
Mr. Srihari Ram
Mr. D. Venkatesh
Mr. M. Seenu
Mr. Mohd. Sabeer
Mr. M. Kishan
Mr. M. Somaiah
Mr. A. Narsing Rao
Mr. Majeed Shareef
Mr. V. Somaiah
Mr. R. Punna Reddy
Mr. K. Srinu
Mr. M. Upender
Mr. Y. Ramulu
Mr. K. Narender

Lab. Assistant - 1 (Eng. & Suppt.)

Mr. N. Narasimha
Mr. J. Bhujender
Mr. K. Parthasarathy
Mr. D. Ravinder
Mr. N. Ramesh Kumar
Mr. Ningala Yadagiri
Mr. G. Venkatesh

Lab Attendant - 2

Mr. A. Shanker
Mr. P. Ravinder
Mr. M. Narsing Rao
Mr. D. Madhava Reddy
Mr. B. V. Sudershan Babu
Mr. I. Poshetty
Mr. Gattu Yadagiri
Mr. N. Bhasker
Mr. A. Jangaiah
Mr. Puppala Dasarath
Mr. S. Narahari
Mr. K. Venkatesh
Mr. P. Narasimha
Mr. E. Kondal Reddy
Mr. K. Venkat Reddy
Mr. Ch. Shanker
Mr. M. Komura Reddy
Mr. G. Upender
Mrs. G. Saraswathi
Mr. P. Balarjun

Lab Attendant - 2 (Eng. & Suppt.)

Mr. Bommaka Srinu
Mr. Neelakanta

Laboratory Attendant-1

Mr. Ch. Guruswamy

Multi Tasking Staff (MTS) (Technical Staff)

Ms. Ambati Rajitha
Ms. D. Saritha
Ms. Dooseti Sai Rupa
Mr. Guguloth Lalu
Mr. Gundu Satyanarayana
Mr. Badeger Mahesh Kumar
Mr. Arra Naresh
Mr. G. Tatanand
Mr. Burla Govardhan
Ms. Ganne Radha
Ms. Vadde Gowthami
Ms. Emandi Bhavani Eswari
Mr. K. Krishna
Mr. J. Sai Prasad
Mr. N. Durga Prasad
Mr. Avuladas Srinivas Goud
Mr. S. Naresh Kumar
Mr. A. Ganesh
Mr. N. Ashok Kumar
Mr. S. Sandeep Goud
Mr. Mohammed Rasheed Uddin

Multi Tasking Staff (MTS) (Engineering Suppt. Staff)

Mr. Guguloth Suresh
Mr. Arpit Arzare
Mr. Orsu Rajkumar
Mr. K. Venkatesh Babu
Ms. M. Mounika

Trainee

Mr. Shaik Abdul Quadeer

Research Highlights

Prevalence and predictors of vitamin B12 deficiency - Genetic associations for low vitamin B12 levels – A multi-center pan India study:

Iron – folic acid fortification without considering B_{12} level in the individuals may cause adverse events and this could perhaps be one of the rate-limiting factors in national iron folic acid supplementation programme. The current study was taken up to map the B12 deficiency in India, to estimate the contribution of B12 deficiency to the burden of anemia.

Improving Health and Nutritional Status of Vulnerable Segment of population by implementing multi-component Health and Nutrition Education intervention as a sustainable model of Intervention – Andhra Pradesh, Gujarat, Jharkhand and Telangana:

Nutrition status data were collected from 14 districts in 4 states, and based on the baseline, formative research findings district specific multi-component health and nutrition education intervention strategies were developed and implemented in 3 phases in 7 Districts of Gujarat, 5 districts of Jharkhand, one district each in Andhra Pradesh and Telangana. Education materials such as posters, flip charts, table calendars, banners, which were developed and finalized after pre-testing were used for creating nutrition awareness to the adolescent girls, pregnant women and lactating mothers. The mode of education was person-to-person, focus group meetings and counselling. The project staff contact with each of the beneficiary in the target areas was about 7-10 for counselling. There was significant improvement in all nutrition indicators and IYCF practices. The number of children with stunting, wasting and undernutrition decreased in all districts except one.

A community-based intervention on maternal and new-born care among the migrant urban poor living in non-notified slums through Janani Suraksha Yojana (JSY) and Home-based New Born Scheme in Hyderabad city:

The survey was done as a case control study with those mothers who delivered under JSY program compared to those who were under the new KCR-kit program. It was observed that KCR-kit increased the awareness of the government services leading to increased uptake and improved maternal and child health indicators.

Comprehensive National Nutrition and Health Survey (CNHS) - district level survey (Pilot study):

This survey was carried out in the district of Nalgonda in Telangana to explore the possibility of conducting a comprehensive data collection, including essential biomarkers on health and nutritional status of the population by establishing a field lab. It is feasible to conduct similar trials and will be cost-effective if done simultaneously in many districts, and more so, if periodic monitoring of health and nutrition status has to be done to study the effectiveness of national programs and reduction in disease burden, as well as to suggest mid-course corrections in program implementation.

Correlation of Prakruti (ayurgenomics) with dietary patterns, HLA-DRB1 genes and disease severity in rheumatoid arthritis (RA) patients:

Vata prakriti was associated with Rheumatoid arthritis patients whereas Pitta and Pitta *kapha prakriti* were protective for RA patients. Vata prakriti subjects had more severity in terms of anti CCP titres, disease duration and DAS sores than other prakriti subjects. HLA-DRB1 *04 gene was associated whereas HLA-DRB1*07 and 14 genes were protective for RA patients. High consumption of red and organ meat, plain rice and less consumption of green leafy vegetables, fruits in RA cases were observed. Consumption of a healthy balanced diet has beneficial effect on development as well as progression of RA.

Relative telomere length and mitochondrial DNA copy number variation with age: Association with plasma folate and vitamin B12:

Telomere attrition and mitochondrial DNA variations are implicated in the biological aging process and genomic stability can be influenced by nutritional factors. In this study we assessed the relative telomere length (rTL) and mitochondrial DNA copy number (mtCN) in aged individuals and their association with plasma folate and vitamin-B12 (B12) levels. The subjects in the ≥60 years age group have significantly shorter telomeres and lower mtCN compared to the <60 years age group. A significant positive correlation was observed between the rTL and mtCN, and both of them were positively associated with plasma folate and vitamin B12 levels. The study revealed a decline of rTL and mtCN with age in the Indian population and their association suggests that they may co-regulate each other with age. Futher, folate and B12 may delay aging by preventing the reduction in rTL length and mtCN.

Supplementation vitamin B12 ameliorated retinal lesions in diabetic rats:

Diabetic retinopathy (DR) is a most common complication of diabetes involving microvasculature and neuronal alterations in the retina. Previously we have reported that vitamin-B12 (B12) deficiency could be an independent risk factor for DR in humans. In this study, we investigated the impact of dietary supplementation of B12 on retinal changes in diabetic rats. B12 supplementation to diabetic rats showed to be beneficial by preventing retinal hypoxia, VEGF overexpression, and ER stress-mediated cell death in the retina. Considering the general prevalence of micronutrient deficiency and its contribution to many metabolic and age-related disorders, such as diabetes, and cardiovascular diseases in India, ameliorative effects of B12 on DR merits attention.

To study the effect of legume prebiotic milk on gut microbiota, adiposity and inflammation in obese rat:

Prebiotics have been shown to alter the composition of gut microbiota and metabolic functions through gastrointestinal pathways. Legumes are important food crops, of which carbohydrates constitute the main fraction with smaller but significant amounts of α-galactosides. Our study was proposed to assess the effect of legume prebiotic milk on the prevention of overweight and obesity in obese rat. Blood glucose and lipid profile of raffinose, red gram, green gram, black gram and bengal gram oligosaccharide fed obese rat showed promising results when compared to control obese rat. The serum Cholesterol content in all legume oligosaccharide fed group were decreased when compared to control group. The triglycerides, LDL and VLDL cholesterol were decreased and HDL cholesterol level increased in legume oligosaccharide fed animals when compared to control group. Gain in body weight was significantly lower in legume prebiotic fed group (352.6±90.82) when compared to raffinose fed group (591.9±171.97) and basal diet fed group (635.7±115.32). The body fat percent was much lower in legume fed group (33.96±7.3) when compared to raffinose fed group (53.3±1.92) and basal fed group (57.9±5.4).The ceacum content analyzed for gut bacteria by PCR based detection using genus specific primers (16s rDNA sequences) showed significant increase in *Bifidobacteria* in green gram prebiotic fed group followed by bengal gram prebiotics and red gram prebiotic fed groups. The results indicate that legume prebiotic milk is promising since there was a decrease in blood glucose level, improved lipid profile, and improved body mass composition.

Effect of non-digestible carbohydrate of kidney beans on diet induced metabolic syndrome in a rat model:

The prevalence of metabolic diseases associated with dynamic changes in dietary macro nutrient intake has been studied during the past decades. The present study was undertaken to determine the prebiotic potential of Kidney Beans (*Phaseolus vulgaris* L.) non digestible carbohydrates and also to study its effects on some biochemical and hematological parameters in

obesity and diabetes induced rats. Blood glucose and lipid profile values showed promising results in kidney bean prebiotic fed rat group when compared to high fat and high sucrose fed animals. Weight gain was much lower in kidney bean fed group (277.3±6.1) when compared to raffinose (324.07±26.76) and high fat group (390.17±9.17). Similar results were observed for high sucrose fed animals, kidney bean group showed average weight of 274.43±8.09, raffinose group 359.20±11.76 and high sucrose fed groups 434.77±19.88.

The fasting blood glucose was significantly lower in kidney bean fed group (84.7±3.1) when compared to raffinose fed group (86±4.6), high fat group (99.7±9.3) and similar results were observed for high sucrose fed group animals. The body fat percent was lower in kidney bean group (12.20±3.05) when compared to raffinose group (13.70±2.62) and high fat fed group (25.03±0.75). The ceacum content was analyzed for gut bacteria by PCR based detection using genus specific primers (16s rDNA sequences) showed increase for *Lactobacillus* is significant in high sucrose raffinose fed group followed by high sucrose kidney bean and high fat raffinose group when compared to control, whereas significant fold increase was shown for *Bifidobacteria* in high fat raffinose, followed by high fat kidney bean fed groups.

Studies on nitrate and nitrite in Indian foods:

Nitrate and nitrite ions are ubiquitous in the environment and naturally found in plant foods as a part of the nitrogen cycle. There are several studies related to the beneficial effect of nitrate, nitrite and nitric oxide consumption, and nitrites are also known to cause methemoglobinemia in infants and cancer, hypotension in adults and therefore its consumption is restricted. The World Health Organization (WHO) recommended the upper limit of concentration of daily nitrate and nitrite uptake to be 3.7 mg/kg and 0.06-0.07 mg/kg, respectively. Due to the growing concern of N-nitroso compounds, accurate and robust methods are necessary for long-term monitoring of nitrate and nitrite concentrations in foods for susceptible populations. The study indicates that green leafy vegetables were high in nitrite and nitrate content as compared to the other food samples analyzed. Among the non-vegetarian foods analyzed chicken was found to have more nitrates as compared to fish. It was observed that in most of the samples in cooked form the nitrite and nitrate content reduced significantly except for a few, which could be due to the addition of other ingredients. The nitrite and nitrate content in green leafy vegetables ranges from 0.36 to 6.63 mg/kg (spring onion and amaranth) and 78.96 to 388 mg/kg (spring onion and sorrel), vegetables 0.04 to 14.43 (cabbage round head and French bean) and 2.35 to 132.73 mg/kg (capsicum green and cabbage round head), roots 0.00 to 0.91mg/kg (beetroot and potato), 33.94 to 149.46 mg/kg (potato and carrot) non-vegetable foods ranges from 0.00 to 16.82 (chicken salami and chicken sausage) and 12.52 to 36.48 (chicken sausage and chicken fries).

Development of e-learning modules on nutrition and health under *Poshan Abhiyaan* initiative of Government of India:

Twelve Nutrition and Health Education (NHE) e-learning modules were developed and uploaded on ICMR-NIN website www.nin.res.in by providing cross-link to ICMR-DHR, MWCD and SWAYAM portals in order to provide access to the community from different parts of the country. The e-learning modules on various nutritional themes are expected to educate general public and girls & boys in adolescent age group and master trainers (paramedics, *Anganwadi* workers, and others). The e-learning module is currently available in Hindi and has had over 60000 registrations and 5.9 lakh certificates have been generated online

Impact of Salmonella killing lytic bacteriophages on probiotic microflora was initiated in January 2019, in which no spots and inhibition zone were observed both in the test assay and the agar well diffusion assays while results of turbidometric assay showed that even after incubation up to 24h the growth of probiotic microflora remained unaffected.

Ameliorative potential of tamarind fruit extract on the NaF - induced alterations in the bone related parameters in Saos-2 cell line:

Tamarind fruit extract treatment showed ameliorative potential and prevented NaF induced alterations in bone related parameters in Saos-2 cell-line.

Prevalence of fluorosis in the community of selected districts of India (Prakasam district from Andhra Pradesh) and development of an appropriate intervention model for prevention and control of fluorosis:

Dental fluorosis was 5% among 5-18 years age in category I villages (8 villages; <1.00 ppm fluoride in drinking water), 13.1% in category II (7 villages; 1.5-3.0 ppm fluoride in drinking water) and 16.2% in category III (9 villages; >3.00 ppm fluoride in drinking water) in Prakasam district, Andhra Pradesh. g). The fluoride levels in the food samples were higher in category II & III compared to category I.

As expected, the urinary fluoride was significantly higher in category III (>3.00 ppm fluoride) as compared to category I (<1.00 ppm fluoride) and category II (1.5-3.0 ppm fluoride in drinking water). The T3 levels significantly increased in the category III as compared to category I and category II. The TSH levels were significantly decreased in the category III and category II compared to category I.

Toxicokinetics of common organophosphate compounds in acute poisoning cases:

Toxicokinetic study of pesticide showed that monocrotophos and dimethoate were more absorbed and toxic than other pesticides. A negative correlation was obtained between pesticide concentration and the acetylcholinesterase enzyme. The time for treatment to the survival of patients ingested monocrotophos and dimethoate was observed to be less than 12h. However, for pesticides like chlorpyrifos, propanil, triazophos and acephate treatment time was between 36 and 72h.

I. PUBLIC HEALTH NUTRITION

1. PREVALENCE OF VITAMIN B12 DEFICIENCY (A MULTI-CENTERIC STUDY)

Vitamin B12 has great relevance in the Indian context given the fact that a significant proportion of Indian population is vegetarian. Vitamin B12 is associated with several disease conditions like megaloblastic anemia, impaired immune defence, gastrointestinal and neurological disorders, metabolic diseases etc. The data on vitamin B12 deficiency is available only in the regions in and around Delhi (northern India), Pune (Maharashtra), Bangalore and Hyderabad (Southern India) and Varanasi (Eastern India). Region-wise data is not available in India. Therefore, the present study was undertaken to map vitamin B 12 deficiency in five regions of India.

Objectives: To estimate the extent of vitamin B12 deficiency across the country in different age, gender and socioeconomic groups.

Methodology

It was a community based cross-sectional study carried out in 8 states (Madhya Pradesh, Odisha, West Bengal, Assam, Meghalaya, Gujarat, Andhra Pradesh and Tamil Nadu) in India by adopting multistage random sampling procedure. Districts were categorized into 3 to 4 groups based on human development Index (HDI) rank. In each state, 2 districts, one each from low and high HDI scores were selected. From each district, 4 urban wards, 4 urban slums and 4 villages were selected and from each ward/slum/village, 8 adolescents (4 boys & 4 Girls), 8 adults (4 men & 4 women) and 8 elderly (4 men & 4 women) were covered randomly for the present study (8 x 3 age groups=24 subjects). Thus 300 samples were covered from each district. Socio-economic and demographic particulars, blood pressure measurements and anthropometric measurements were collected from all the subjects. However, 24 hour dietary recall was carried out on 50% of the subjects to assess the food and nutrient consumption. Fasting blood samples were collected for estimation of fasting blood glucose, lipid profile, hemogram, B12, folate, homocysteine, ferritin, holo-TC levels.

Results

A total of 4619 adolescents, adults and elderly people were covered from 8 States and majority (81%) of the subjects selected were literates. The overall prevalence of B12 deficiency was 21% (men: 22.9%; women: 18.8%) and it was 21% in adolescents (Boys: 23.9%; girls: 17.3%), 22% in adults (Men: 23.9%; Women: 20.5%) and 19% in elderly (Men: 20.7%; women: 17.9%) people. The B12 deficiency was higher in Gujarat (36.7%), Telangana and Odisha (25% each) and lowest in Assam (3%). The prevalence of folate deficiency was 25% (men: 27.6%; women: 23%) and it was highest in Telangana (39.7%), Madhya Pradesh (33%), and lowest in Assam (11%). About 25% (boys: 26.3%; girls: 24.5%) of adolescent, adults (men: 28.2%; women: 21.9%) and in elderly (men:28.5; women:23.9%) also had folate deficient. About 46% had Homocystine deficiency and it was highest in Tamil Nadu (63.5%) and Assam (55%), and lowest in Gujarat (29%). The overall prevalence of Anaemia was 35% (Men: 27%; Women: 43%) and it was higher in Assam (71%),

Odisha (55%) and MP (45%) and was lower (17%) in Telangana. The prevalence was 34%, 31% and 41% among adolescent, adults and elderly, respectively (Fig 1). The prevalence of Homocystine deficiency was 50%, 45% and 44% in adolescents, adults and the elderly, respectively. Ferritin deficiency was observed in 22% people and it was highest in Tamil Nadu (32%), Telangana (26%), Gujarat (26.7%), while lowest in Assam (15%).

The prevalence of HTN was 34% & 31% among men and women, and was highest in West Bengal (44% & 47% respectively). The prevalence of diabetes was 15% and 13% among men and women, and was highest in the Gujarat (25% & 23% respectively). The prevalence of overweight/obesity (BMI≥25) among adults (18-59 years) was 27% and 39% among men and women, and was highest in Tamil Nadu (43% & 63% respectively) Fig 2 & 3.

Fig 1. Prevalence of vitamin B12, folate and anemia and ferritin deficiency among population in 8 select states in India

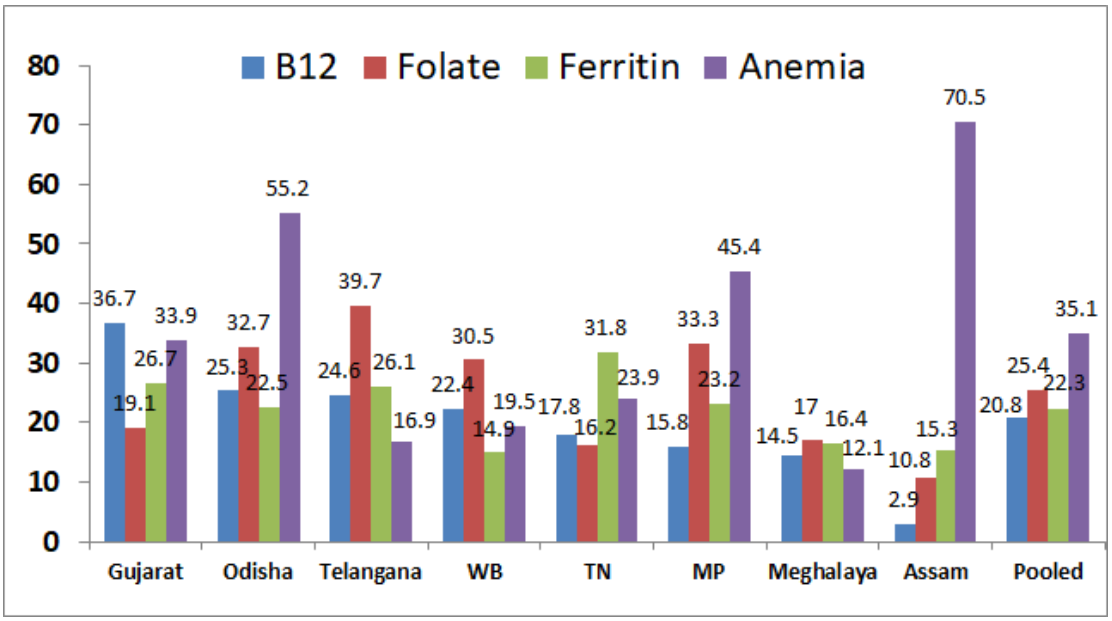


Fig 2. Prevalence (%) of Overweight/obesity, HTN & diabetes among adult men (18-59 yrs)

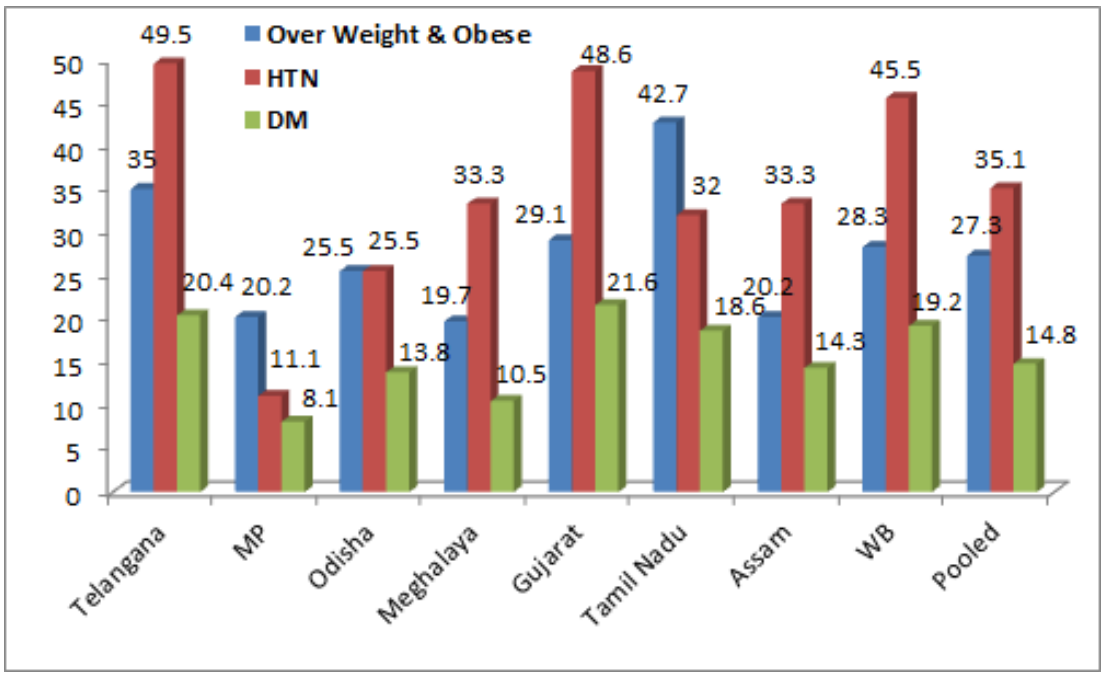
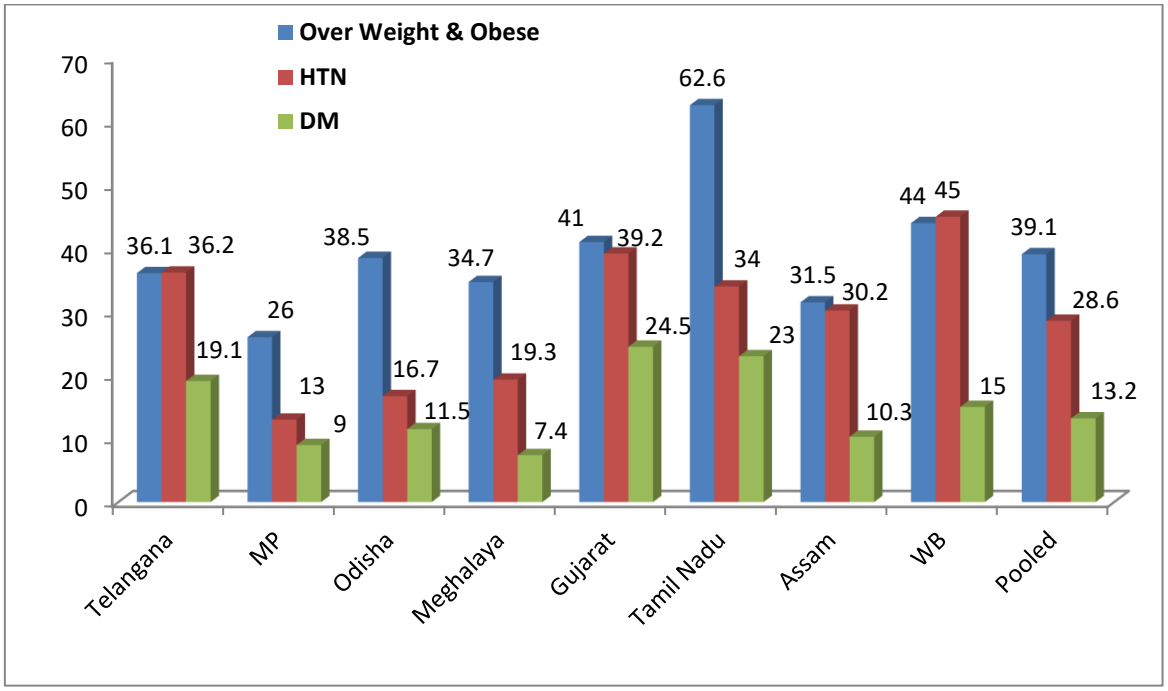


Fig 3. Prevalence (%) of Overweight/obesity, HTN & diabetes among adult women (18-59 yrs)



Inference & Conclusion

The prevalence of Anemia was highest in Assam in-spite of low prevalence of vitamin B12 deficiency (3%), and folate deficiency (11%). The prevalence of NCDs was also very high in all the states. There is a need to take up a risk reduction intervention studies to prevent and control dual burden of malnutrition (undernutrition and overnutrition).

2. IMPROVING HEALTH AND NUTRITIONAL STATUS OF VULNERABLE SEGMENTS OF POPULATION BY IMPLEMENTING MULTI-COMPONENT HEALTH AND NUTRITION EDUCATION INTERVENTION AS A SUSTAINABLE MODEL OF INTERVENTION IN GUJARAT

Many supplementary feeding programmes, have been taken up to ensure food and nutrition security of population. Despite implementation of these programmes for more than four decades, impact evaluations at different points of time showed limited effects. One of the major recommendations was to develop a model that could promote multi-disciplinary convergence to identify the existing lacunae in implementation of the current programmes, promote effective implementation of the same by instituting an independent supervisory and quality control mechanism, which would be self-sustaining in nature. Current study aimed to develop such an intervention in Gujarat, India.

Objectives, Phase I

General objective

To assess nutritional status of children, women in reproductive age, adolescent girls and formulate an intervention based on formative research and evaluate the same after 15-18 months of intervention.

Specific objectives

1. To conduct rapid assessment of nutritional status of <5 year children in the rural areas of Ahmedabad.
2. To assess the awareness, perceptions and practices of various stakeholders about the existing national nutrition intervention programmes, adopting Community Needs Assessment (CNA) technique.
3. To identify strengths and weaknesses in the implementation of the existing national nutrition intervention programmes.
4. To assess the health seeking behaviour and practices of vulnerable segments of population such as children (care takers), adolescent girls, pregnant women and lactating mothers.
5. To assess awareness, perceptions and practices of environmental, personal hygiene and food safety among mothers of <5 year children.
6. To assess the performance of functionaries working in the areas of health and nutrition.

Phase-II: Based on the formative research in phase-I, appropriate nutrition intervention strategies were developed, piloted, pretested, finalised and implemented for at least 18 months and evaluated for its impact.

Intervention materials

These include posters, banners and flow charts that were explained to all the beneficiaries in house to house visits and group meetings held with pregnant and lactating women, adolescent girls. Health and nutrition education was imparted to them also through posters and banners. Every HH having pregnant women, lactating mother and mother of under 5 child was visited at least 3 times by the project staff to impart health and nutrition education.

Phase –III: In the final phase, impact evaluation was carried out after one and half years of regular implementation of intervention programme.

Methodology

Sampling Design: It was a community based cross sectional study carried out by using systematic random sampling procedure. Seven districts and 2 blocks from each districts were selected. From each block, 5 villages and 50 HH from each village were covered. From each selected HH, one under 5 children was covered for various investigation such as ANC care practices of mothers of <12 month children, IYCF practices, feeding practices, Vitamin A supplementation, IFA supplementation, hand washing practices of mothers and anthropometric measurements of mother and children were carried out.

Results

District	Amreli		Vadodara		Surendranagar		Panchmahal		Dangs	
Particulars	**Base line**	**End line**	**Base line**	**End line**	**Base line**	**End line**	**Base line**	**End line**	**Base line**	**End line**
HH covered	**500**	**506**	**500**	**497**	**500**	**488**	**500**	**503**	591	481
Children for Anthro.	**475**	**500**	**687**	**493**	**489**	**485**	**488**	**486**	598	480
Literate father	77.8	**85.9**	71.7	**77.4**	81.3	**85.6**	90.2	**93.4**	83.3	83.4
Literate mother	59.9	**75.2**	51.0	**59.3**	58.9	**74.3**	74.3	**83.9**	77.5	82.0
Use of tap water	46.5	**73.3**	46.6	**52.7**	58.4	**72.1**	12.0	**8.7**	23.7	26.8
Use of Sanitary latrine	60.1	**61.8**	45.0	**67.4**	33.4	**48.4**	19.1	**24.1**	43.8	79.6
ANC registr. before 12 weeks of gestation	79.4	**89.7**	65.4	**71.7**	74.4	**78.7**	64.4	**77.7**	74.8	73.2
At least 4 ANCs	69.5	**86.6**	51.9	**67.8**	58.0	**74.7**	59.9	**72.0**	52.4	69.0
Consumed >=100 IFA tablets	68.0	**76.3**	**29.3**	**70.4**	64.8	**70.0**	48.6	**56.7**	75.6	72.6
Institutional delivery	79.4	**87.8**	**92.5**	**94.7**	**81.2**	**93.3**	**93.9**	**89.8**	72.1	82.4
LBW (<2.5 kg)	12.2	**16.7**	22.4	**25.0**	17.6	**19.3**	16.4	**12.1**	16.3	16.9
Initiation of breast feeding within 1 hr	38.9	**53.8**	**36.1**	**65.1**	37.5	**38.0**	18.5	**30.6**	47.6	52.8
Excl. breast feeding up to 6 months	40.0	**68.7**	37.2	**78.4**	23.5	**63.5**	35.7	**69.5**	35.9	73.6
Full Immunization (12-23 month)	95.3	**96.0**	81.0	**92.8**	92.5	**88.9**	72.5	**87.0**	84.9	73.9
Nutritional status										
Underweight	38.9	**32.0**	51.5	**51.9**	48.2	**41.5**	46.3	**48.4**	56.9	48.4
Stunting	35.8	**35.2**	51.8	**49.3**	42.0	**43.1**	45.5	**52.1**	52.5	51.8
Wasting	20.2	**13.1**	27.5	**23.3**	25.7	**19.2**	24	**21.1**	28.5	19.9
Anemia										
Pre-school children	92.7	**67.3**	95.4	**80.1**	90.1	**82.8**	90.9	**76.8**	--	--
NPNL women	94.1	**56.0**	95.0	**79.5**	88.6	**80.2**	95.1	**82.0**	--	--
Lactating mothers	90.3	**44.4**	94.5	**73.9**	90.4	**65.2**	93.5	**79.6**	--	--
Adolescent girls	92.9	**44.7**	97.0	**70.5**	86.1	**56.2**	94.5	**67.2**	--	--

After the intervention, there was an improvement in ANC registration before 12 weeks of gestation, and infant and young child feeding practices. The prevalence of undernutrition had declined in four districts except in Panchmahal District. The prevalence of anaemia also declined compared to the baseline.

3. PRE-TESTING OF USEFULNESS OF NCEARD DEVELOPED HEALTH AND NUTRITION EDUCATION MATERIAL (ALGORITHM) FOR PREVENTION AND MANAGEMENT OF MATERNAL MALNUTRITION AT COMMUNITY AND HOSPITAL SETTINGS IN THE STATE OF TELANGANA

In order to strengthen the delivery of maternal nutrition services, the Maternal Health Division, Ministry of Health and Family Welfare has taken several steps in the recent years, including initiating drafting a guidance document for maternal malnutrition prevention and management. Additionally, National Centre of Excellence and Advanced Research on Diets in Department of Food and Nutrition (NCEARD), Lady Irwin College, New Delhi was set up (with UNICEF support) to provide technical and policy guidance support to the Ministry on issues related to Maternal Nutrition. In accordance with the mandate given, the NCEARD has drafted the maternal malnutrition prevention and management guidelines (algorithm, counselling material) for PMSMA sites, which was discussed with subject experts in July 2018. The algorithm is intended to be pretested at various sites across the country and in Telangana, Division of Public Health Nutrition, ICMR – National Institute of Nutrition has been given this responsibility. The pre-test will be done at least in 4 sites viz, a tribal PHC in Adilabad district, a rural PHC in Karimnagar district, a tertiary care maternity hospital and a Medical College (urban set-up) in Secunderabad. The present study was taken up with the following objectives:

1. To assess the operational feasibility and acceptability of the use of the algorithm developed by NCEARD at various health care institutes.
2. To identify difficulties if any in using the algorithm.
3. To obtain feedback observed during the use of algorithm and test the feasibility to scale up the same.

Methodology

The study was planned as a pre-, and post- test institutional cross sectional study. The study subjects were pregnant women who attend the designated hospitals for ANC check-up. The health and nutrition education material was pre-tested for its feasibility and usefulness at the following health care facilities:

1. Tribal PHC, Utnoor, Adilabad
2. Rural PHC, Saidapur, Karimnagar
3. Urban Hospital, Gandhi Medical College, Secunderabad

The algorithm for service package at community level was also tested by applying it on pregnant women attending antenatal clinics on the Village Health and Nutrition Day by the trained ANM. Generally, 2 VHNDs are conducted in a village every month. In view of time and resources available, we could cover at least 300 pregnant women at each health care facility and at least 100 pregnant women at community setting (i.e., VHND) to implement the draft algorithm by re-orientation of the health functionaries.

The designated staff were initially trained in the modules, guidelines and counselling procedures at respective sites for 2 days and requisite material were printed and provided to health care facility. From each facility, one medical officer and one ANM was trained at tribal and rural PHC. However, at urban hospital, two Medical Officers and two ANMs were trained in the methodology. After completion of training, the staff proceeded to implement the draft Algorithm in their respective health facility (at ante-natal clinics) and community (Village Health and Nutrition day meetings) using respective modules under the close supervision of NIN scientific staff. The study was carried out for 3 months from February to May 2019.

Results

Positive leads for implementation of algorithm

1) *Streamlining use of MUAC measurement for assessing maternal nutrition*: Algorithm stressed on inclusion of MUAC measurement for assessing the nutritional status as part of ANC care. Adult MUAC as an indicator for maternal nutrition was introduced in entire Telangana and few ICDS projects of Karnataka for regular monitoring of nutritional status of pregnant women at Angawanwadi centers. Implementation of algorithm further strengthened its usage as part of ANC care at facility (PHC/Sub center). MUAC measurement was not part of ANC care in any facility before algorithm implementation.

2) *Strengthening of deworming service as a part of ANC:* It was observed that albendazole tablets for deworming were not routinely given in second trimester, as part of ANC care. With implementation of the algorithm, its usage as part of regular antenatal care was strengthened in field sites.

3) *Improving the utility of regular anthropometric data at ANC:* Anthropometric measurements (Height and Weight) were being carried in all facilities. However, no meaningful interpretation was done based on this information. Weight was being measured regularly only for monitoring weight gain. Introduction of BMI helped in easy categorization of women as per their nutritional status to deliver the necessary services under ANC care. Also, use of BMI charts helped in easy visual classification of the nutritional status of the mother.

Following are some of the observations

1. Women not at nutritional risk: 4.7%
2. Women at nutritional risk but no medical risk: 71.8%
3. Women at severe nutritional risk and/or medical risk-23.5%

Inferences and Conclusion

After implementing the algorithm in these hospitals, it was observed that the algorithm was feasible to be scaled up. The challenges experienced were communicated to the Government along with recommendations to overcome the same. These interventions may be scaled up in all other hospitals for the control of maternal nutrition and to improve the birth outcome.

4. NATIONAL SERO-SURVEILLANCE TO MONITOR THE TREND OF SARS-COV-2 INFECTION TRANSMISSION IN TELANGANA: COMMUNITY-BASED SERO-SURVEILLANCE

The National Sero-surveillance study was conducted to monitor the trends of SARS-CoV-2 infection transmission in 3 select districts of Telangana has been carried out during 15-17 May 2020, with the following objectives:

Objectives

1. To estimate and monitor the trends of sero-prevalence for SARS-CoV-2 infection in the general population, and
2. To determine the socio-demographic risk factors for SARS-CoV-2 infection and delineate the geographical spread of the infection in the general population.

Methodology

This sero-surveillance was designed as a repeat cross-sectional survey of adults aged 18 year or more. The districts were categorized into four strata according to the reported COVID-19 cases per million population (zero, low: 0.1-4.7, medium: 4.8-10 and high: >10), as per data from the ICMR testing laboratory reporting portal. Fifteen districts from each stratum were selected randomly for a total of 60 districts in India. Out sixty districts, 3 districts (Jangaon, Nalgonda and Kamareddy) were selected from the Telangana State.

For the purpose, we have enrolled randomly about 400 individuals in each district (10 villages/ clusters X 4 random starting points X 10 households from each point X 1 adult from each house). A brief interview was conducted about his/her personal profile, few questions in relation to Covid-19 infection and 3-5 ml of venous blood samples were collected for SARS Cov-2 antibody testing by ELISA method. After collection of the blood samples, the same were processed at district head-quarter hospitals for separation of serum and the serum samples were sent to ICMR-NIRT, Chennai for Antibody testing within couple of days.

The results were shared to DG, ICMR for preparation of National Report. The results were released officially on 12th June 2020 by the Secretary DHR, GoI and DG-ICMR, New Delhi recently in the press conference at ICMR, New Delhi.

A total of 1212 individuals (18 years and above) were covered for the study from the 3 districts viz., Jangaon, Nalgonda and Kamareddy. As per the protocol, antibody testing of sera samples collected from 3 districts and the result of lgG positivity by district is given in Tables 1-3.

Table 1. Number of subjects covered for SARS-CoV-2 Antibody Test and Number observed Positive and Negative in the District of Jangaon, Telangana state

Village/Cluster	Negative cases	Positive cases	Total
Hanmanthpur	40	0	40
Jangaon (M) WARD NO.-0002	40	0	40
Kallem	40	0	40
Kanchanpalle	40	0	40
Kodavatoor	41	0	41
Lakshmakkapalle	41	1	42
Madhapuram	40	0	40
Manchuppula	42	0	42
Raghavapur	40	0	40
Zaffergadh	39	1	40
Total	**403 (99.01%)**	**2 (0.49%)**	**405 (100.0%)**

One sero-positive case was observed in each of the villages Lakshmakkapalle and Zaffergadh in the district of Jangaon and the prevalence of sero-positivity in the district was about 0.5% (Table 1).

Table 2 Number of subjects covered for SARS Antibody Test and Number observed Positive and Negative in the District of Kamareddy, Telangana state

Village/Cluster	Negative cases	Positive cases	Total
Bhavanipet	40	0	40
Bhiknoor	41	0	41
Chinna Edgi	39	1	40
Dharmaram	40	0	40
Jalalpur	43	0	43
Kamareddy (M) WARD NO.-0004	40	0	40
Neral	40	0	40
Pedda Kodapgal	40	0	40
Talamadla	40	0	40
Yerrapahad	40	0	40
Total	**403 (99.75%)**	**1 (0.25%)**	**404 (100.0%)**

Only one sero-positive case was also observed in the district of Nalgonda i.e., in Chenna Edgi village and the prevalence of sero-positivity in the district was about 0.25% only (Table 2).

Table 3 Number of subjects covered for SARS Antibody Test and Number observed Positive and Negative in the District of Nalgonda, Telangana state

Village/Cluster	Negative cases	Positive cases	Total
AKKENE PALLE	40	0	40
Chetla Chennaram	40	0	40
Gundla Palle	41	0	41
Indugula	40	0	40
Kattangoor	40	0	40
Nalgonda (M) WARD NO.-0004	40	0	40
Nerallapalle	40	1	41
Peddavoora	40	0	40
Thanedar Palle	41	0	41
Thimmapuram	40	0	40
Total	**402 (99.75%)**	**1 (0.25%)**	**403 (100.0%)**

Only one sero-positive case was observed in the district of Kamareddy district and the prevalence of sero-positivity in the district was 0.25% only (Table 3).

Care may be taken in the interpretation of these results as the IgG positivity is generally detected only after 10-15 days of infection and it does not always indicate active infection. That means the positive individuals in this study might have contracted infection be infected in the first week of May 2020 or before. All the positive IgG subjects might be asymptomatic, not active positive cases at the time of survey.

The above given results indicates that the spread of Covid-19 infection level among general population was only 0.25% to 0.45% in the 1st week of May 2020, but it will not reflect the current situation.

II. MATERNAL AND CHILD NUTRITION

EVALUATION OF THE ROLE AND IMPACT OF PARTICIPATION IN HOMESTEAD FOOD PRODUCTION ON THE NUTRITIONAL STATUS OF CHILDREN

Cereal based habitual diets with minimum dietary diversity could be one of the major reasons for coexistence of triple burden of malnutrition in India. Among the various interventions, Homestead Food Production (HFP) is continually promoted as a strategy to improve dietary diversity among the population in various countries. These studies have shown that HFP enhances the quality of the diet of the population by providing direct access to diverse foods and also, that provide other intermediate outcomes among participants such as improving nutrition knowledge, empowering women, etc. (Ruel et al., 2018). However, there is limited evidence on the benefits of HFP for the improvement of the nutritional status among children in Indian setting. Hence, this study was conducted to bridge the knowledge gap related to the role of homestead food production in improving the nutritional status of children <5 years of age by evaluating the impact of an intervention promoting HFP on the nutritional status of children.

Aims and Objectives

1. To examine the impact of an integrated intervention that promotes Homestead Food Production (HFP) along with health and nutrition education on the nutritional status of children <5 years of age from rural areas in Jangaon District, Telangana.
2. To examine the impact of the integrated intervention on the knowledge, attitude, and practice on child care, nutrition, and health-seeking behavior among mothers in both intervention and control groups.
3. To understand the uptake and sustainability of Homestead Food Production as a strategy to improve child nutritional status.

Methods

This cluster randomized study was conducted in Jangaon district, Telangana. Jangaon district is an agrarian economy, with more than 20% of the total population dependent on agriculture-based livelihoods (Census, 2011). However, the region fares poorly on nutritional indicators among children, with less than 10% of children consuming the recommended minimum adequate diet required for optimum growth and development (NFHS, 2016; WHO-IYCF guidelines, 2010).

Our study aimed to increase the percentage of children consuming minimum acceptable diets in the region to 25% by promoting an integrated homestead food production intervention along with nutrition education. With 95% CI, 80% power, and accounting for 20% dropout and a design effect of 2, the sample size was estimated to be 180 children (<5 yrs of age) per group.

Prior to the study, due permission was taken from the Department of Women and Child Development to conduct the study. The study was registered with the Clinical trials registry of India (Ref: CTRI/2017/07/009053), and ethical approval was obtained from the Institutional Ethics

Committee of National Institute of Nutrition. Eight villages within Lingalaghanpur mandal, Jangaon district, were randomly selected for the study. Out of these, four villages were randomly selected for the intervention, while four were control villages. While both groups received nutrition education, intervention villages, in addition, received a nutritionally dense seed kit (developed by NIN in collaboration with a local NGO) and training on optimum garden practices and organic pest management.

Data collection: The study was conducted in three phases- at baseline (post-summer), midline (post-winter), and end line (post-monsoon seasons) to understand the effect of seasonality on (i) maintaining the garden and (ii) dietary intake, thereby the nutritional status of children and mothers. To ensure participation, we regularly monitored and evaluated the intervention and garden practices were regularly monitored and evaluated. Trained supervisors were available by mobile for participants to clarify issues related to maintaining gardens such as infestation, poor outputs, etc. A qualitative study using focus group discussions and in-depth interviews was conducted among key stakeholders at the household (mother, father, grandmother) and community (local government representatives, shopkeepers, frontline health workers, etc.) level, respectively, to understand existing HFP practices, perceived enablers and barriers to maintain HFP.

Household-level surveys were conducted, and data on the socio-demographic details, dietary purchases, and intake (monthly food purchases, food frequency, and 24 hr diet recall), utilization of outputs from HFP, Knowledge, attitude, and practice of mothers on health and nutrition were collected using a standard questionnaire. We also collected anthropometric data on height, weight, and mid-upper arm circumferences (only children) measured using standard protocols. Dry blood spots were collected to estimate Vitamin A (only child) and hemoglobin (mother and child) levels.

Participant selection: Households with a young child (6-59months of age) in the village were enumerated with the help of the local Anganwadi staff, and interested households were selected to participate in the study.

Exclusion criteria: households with an index child who is a twin or with chronic/congenital diseases were excluded from the study.

Results

The data collected to understand the impact of an intervention is currently being analyzed. The qualitative study among the stakeholders revealed that HFP was commonly practiced in the region. The participants perceived maintaining HFP to be beneficial as it would act as a buffer to fluctuating market availability and prices, add variety to regular diets, and provide access to pesticide-free clean food. Among those who maintain home gardens, crops preferred for cultivation belonged to gourds, beans and other vegetables such as brinjal as they require limited resources such as time and water and not affected by animals. The participants also highlighted structural barriers such as lack of space, water, and penetration of predators (monkeys) that restrict their garden practices.

Inference and conclusion

The findings of the study, so far, highlight the need for agriculture-based interventions to be nutrition-sensitive and context-specific to enable uptake and sustainability. Overall the study will add key evidence to existing knowledge for future studies, aiding them to design and promote an efficient agriculture-based intervention to improve nutritional status among the population.

III. CLINICAL EPIDEMIOLOGY

1. COMPREHENSIVE NATIONAL NUTRITION AND HEALTH SURVEY (CNNHS) REPORT OF NALGONDA DISTRICT, TELANGANA

A Comprehensive National Nutrition and Health Survey (CNNHS) was carried out in Nalgonda District, Telangana State, India on a pilot basis with an aim to assess the current diet and nutritional status among representative samples of infants, pre-school children, school going children, adolescents and adults. Considering the emerging diet and lifestyle related diseases globally and lack of nationally representative data, the prevalence of overweight/ obesity, hypertension, diabetes mellitus, dyslipidaemia among adolescents and adults were also assessed.

Objective

Primary Objective

To conduct comprehensive nutrition surveys among both urban and rural communities simultaneously, by using a standard and uniform methodology at district level initially in three states in three select district on a pilot basis to study the feasibility of repeating the same in all states periodically.

Secondary Objectives

1. To assess food and nutrient intakes among different age/gender/physiological/physical activity groups by 3 days 24 hour recall method of diet surveys.
2. To assess nutritional status in terms of anthropometry, clinical examination, biomarkers (blood and urine) and current and chronic morbidity of all the available individuals in the selected households.
3. Infant and young child feeding (IYCF) practices of rural and urban mothers of under 5 years children.
4. To assess the prevalence and determinants of obesity, hypertension and diabetes including cardio-metabolic risk factors among school age (6-11years), adolescents (12-17years), adults (18-59years) and geriatric population (≥60 years).
5. To assess nutrition knowledge and practices especially on healthy and balanced diet, 24 hour physical activity, and risk behaviours among adolescents and men & women.
6. To assess heavy metals and trace elements in the drinking water, and
7. To assess selected village/ward profile, including agriculture productivity, food availability, food security programmes, health, Water, Sanitisation and Health (WASH) and home environment etc.

Methodology

A total of 4166 individuals were covered for socio-economic and demographic particulars. Anthropometry data were collected from 900 HHs in 42 villages and 18 wards in Nalgonda district, Telangana state, India. Food and nutrient intakes were collected from subjects in 337 households.

Information on antenatal care, infant and young child feeding (IYCF) practices as well as coverage for immunization, iron and folic acid tablets, and massive dose of vitamin A supplementation was collected from 163 lactating mothers of <36 months old children. Blood pressure was measured in 1111 subjects and blood samples were collected in a sub-sample of 1300 subjects. Information on personal, environmental hygiene (n=379) and health seeking behaviour (n=362) was obtained from mothers of under 5-year children. A total of 42 *anganwadi* workers (AWWs), 18 auxiliary nurse mid-wife (ANM) and 34 accredited social health activist (ASHA) were also interviewed.

Results

Nutritional Status

Food and nutrient intakes: The diets of the subjects were not adequately diversified. Across the 12 food groups, cereals and millets (384g) formed the bulk of the diet. Except for cereals and fats, the overall intake of remaining food groups was not meeting even 50% of the recommended intake. Though, there was an increase in the intake of milk and milk products and flesh foods, compared to the last survey (NNMB, 2012). The intakes were still below the recommended values. Similarly, except for fat, the intake of all macro and micronutrients was less than the recommended intake levels of ICMR.

Anthropometry: The overall prevalence of undernutrition (<Median -2SD) in terms of stunting, underweight and wasting among under 5 years boys was 38.2%, 32.8% and 17.8%, respectively. While the corresponding figures for girls were 34.3%, 31.1% and 16.2%, respectively. Thus, the prevalence of undernutrition was relatively higher among boys compared to girls. The prevalence of stunting and thinness among school going boys was 24.5% and 34.4%, respectively. While the corresponding figures for girls were 25.4% and 26% respectively.

Among adults, the prevalence of chronic energy deficiency (CED i.e. BMI <18.5) was about 13.1% in men and 18.9% in women. Similarly, the prevalence of overweight (BMI>25) and obesity (BMI≥30) was 28.3% and 5.7%, respectively among adult men. While the corresponding figures for women were 19.9% and 5.6%, respectively.

The prevalence of abdominal obesity (WC≥90 cm for men and ≥80 cm for women) among men and women was 35.8% and 42.7%, respectively. While, the prevalence of truncal obesity (WHR≥0.90 for men and ≥0.80 for women) among men and women was 74.5% and 83.2%, respectively. Similarly, among adolescents, the prevalence of abdominal and truncal obesity were 11.7% and 60.1%, respectively.

Micronutrient deficiencies: The prevalence of anemia among under 5 years children, school going children, adolescents, adult men, adult women were 76.3%, 52.2%, 49.7%, 6.5, and 46.6% respectively. While the prevalence among pregnant and lactating women were 55.4% and 36.6% respectively. The prevalence of sub-clinical deficiency of vitamin A, vitamin D and vitamin B12 among men were 3.3%, 24.3% and 30.1% respectively, while the corresponding values among women were 10%, 35% and 23.2%, respectively. Similarly, the prevalence among adolescent girls was 13.6%, 56.3% and 35.1% respectively.

Diet related chronic non-communicable diseases

The prevalence of diabetes mellitus was 6.4% and 2.5% among men and women, respectively. Whereas, the prevalence of HbA1C ≥ 6.5% was higher among men (7.9%) than in women (2.7%). While all the adolescent girls were having normal blood glucose levels. The prevalence of hypercholesterolaemia, low HDL, high LDL and hypertriglyceridemia among men was 15.5%, 66.5%, 14.8%, and 39% respectively, while the corresponding figures for women was 9.5%, 62.2%,

10.5% and 14.9%, respectively. Thus, dyslipidaemia was higher among men than in women. Similarly, the corresponding values among adolescent girls were 0.8%, 78%,1.9% and 7.6%, respectively. The prevalence of hypertension (SBP≥140 mm Hg and / or DBP ≥90 mm Hg) was about among men and women was 23.1% and 12.3% respectively, while the prevalence among adolescent girls was about 4%.

Infant and young child feeding (IYCF) practices

The proportion of pregnant women for early registration (<12 weeks of gestation) of antenatal check-ups was 51%. About 67% of pregnant women received ≥100 iron and folic acid tablets during their pregnancy. About 6% of mothers reportedly fed pre-lacteals to their babies, while the proportion of mothers-initiated breast feeding within one hour was 71.8 %, and 92 % of mothers fed colostrum to their babies. The proportion of mothers who practiced exclusive breastfeeding for the first 6 months was only 87.3%, while 53% of mothers who initiated complementary feeding to their infants at the age of 6 months.

Conclusions and recommendations

In conclusion, there is a significant decline in the prevalence of under-nutrition although the current prevalence is still a major public health problem in India. To tackle anemia across all age groups, there is a need to promote consumption of diversified diet by including fresh fruits and minimally processed vegetables for increasing the iron bioavailability from Indian diets. However, the determining factors of dietary diversification such as availability, accessibility and affordability needs to be tackled. Regarding the health seeking behaviour of the mother of pre-school children, it is to be noted that there is a need to improve the health workers quality of service, and increase the number and frequency of home visits for health check-ups and create awareness on health programs to the beneficiaries. There is a need to increase the supervision by the heads of ICDS to increase the attendance in refresher program of AWW, which in turn will increase the knowledge of AWW on anemia and ultimately improve the practices of the beneficiaries. The other area that needs to be focussed are (i) improving the IFA tablet distribution in *anganwadi* centres and (ii) encouraging pregnant women to consume the distributed IFA tablets. Considering the higher prevalence of diet related non-communicable diseases, there is a need to create behaviour change among individuals to promote physical activity along with consuming diversified diet among food groups.

2. A COMMUNITY-BASED INTERVENTION ON MATERNAL AND NEW-BORN CARE AMONG THE URBAN POOR LIVING IN NON-NOTIFIED SLUMS THROUGH *JANANI SURAKSHA YOJANA* AND HOME-BASED NEW-BORN SCHEME IN HYDERABAD CITY

Neonatal mortality contributes to the highest percentage of deaths among under five year children and represents a considerable challenge. India accounts for highest proportion of the global burden of neonatal mortality. Efforts to reduce neonatal mortality should focus on the modifiable risk factors and try to minimize the wide variability across regions of the country. The daily risk of

mortality in the first four weeks of a new-born's life is many folds higher than the post-neonatal period, from 1 to 59 months. Yet new-born health did not receive the required attention it needed during the past decade. Since 1995, the Indian government has instituted two major public health programs to improve maternal and child survival outcomes (i.e. *Janani Suraksha Yojana (JSY)* and ''Home-based New Born Scheme''. However, the utilization of JSY in urban areas is mired in problems as the health care system is not structured and organized in urban areas, unlike the rural areas. In addition, the migrant communities are more vulnerable to underutilization of healthcare services because they are alien to the urban system and are away from the traditional and government healthcare systems available in their place of origin. Telangana government has launched a scheme on 2nd June, 2017 which enables the reduction of both NMR and maternal death rate by providing lavished monitory benefits in the name of Arogyalaksmi which is also notably called KCR kit, this scheme offering amount Rs. 12000/- for mothers who deliver boy and Rs. 13000/- for those who deliver a girl child. Keeping in view the need for intervention studies to improve the urban slum dwellers, the study aims to assess various programs implemented in the state of Telangana to improve the health status and create awareness among the migrant urban poor people in Non-notified slums of Hyderabad city which improve the delivery of neonatal health care services.

Methods and Results

This study was conducted in the non-notified slums of the Hyderabad, Telangana from September, 2017 to September, 2019. During the study period, data from various slums in Hyderabad city were collected by administering pretested questionnaires. During the period after launch of KCR kit those who could not avail or had not obtained the KCR kit were also recorded and placed under the category NT-KCR kit. All mothers who delivered 18 months preceding the survey were included in the study. A detailed questionnaire was administered on socio demographic, awareness and utilization of JSY and KCR-kit, pregnancy history, antenatal care, maternal complications during pregnancy and labor, new-born care and complications during birth and post-partum till 28 days. Information on immunization history, morbidity history of the child in the preceding 15 days, anthropometry of mother and child, nutrition history including IYCF practices, child care practices, and consumption expenditure during the last 30 days was collected. In addition, food frequency questionnaire was used to assess food group intakes over the past one year.

The results indicated that the KCR kit group had significantly lower LBW compared to JSY and the group of non-recipients. Forty one percent of children had birth weight less than or equal to 2.5 kg in the JSY group compared to 36.8% in the KCR-kit group. It was further observed that only 50.5% of the deliveries of JSY group happened in government hospital (55.9%) compared to 99% in KCR kit group. The normal deliveries in the beneficiaries of KCR kit were significantly higher in the government hospital (57.8%) compared to JSY group (50.5%). Higher number of mothers in JSY group had 5 or more ANC visits in private facility (30.5%) compared to 12.5%. Late registration of pregnancy was higher in JSY group (48.4%) compared to KCR kit group (42.4%). There was also increased use of government facilities such as diagnosis of pregnancy, consumption of IFA tablets in the KCR kit group compared to the JSY group.

The commonest complication was swelling of legs, which was seen more common in JSY group (26.1%) compared to KCR-kit (16.6%). Child nutrition and health was also better in KCR-kit group. A higher proportion of children in the JSY group had fever and cough than in KCR kit group. Initiation of breast feeding with in the first half an hour after birth was higher in KCR-kit (63.6%) group compared to JSY group (55.6%). Further, a higher proportion in KCR-kit group (97%) fed

colostrum to the new-born compared to JSY group (92.8%). However exclusive breast-feeding rates were higher in JSY group than KCR kit group and so was the initiation of complementary feeding. Bottle feeding was higher in JSY group (20.4%) compared to KCR-kit group (13%). More children in JSY group had fever, diarrhoea, ARI than in KCR-kit group. Hospitalization of the child due to illness was more commonly recorded in JSY group (9.6%) than in KCR-kit group (5%). More mothers in the KCR-kit group consulted government doctor (42.5%) compared to JSY group (20.3%). There was also increased awareness of giving ORS in the KCR-kit group (42.1%) compared to JSY group (37.5%). The mothers from KCR kit recipients group said they received all the items and found all items useful. Consumption expenditure in pregnancy in KCR kit group was lower compared to JSY group. It was observed that KCR-kit increased the awareness of government services available related to maternal and child health, led to increased use of the facilities, which in turn improved maternal and child health indicators. Larger studies across the state including diverse rural and urban communities will be required to assess the overall impact of KCR-kit in improving maternal and child health in the state of Telangana.

3. CORRELATION OF *PRAKRITI (AYURGENOMICS)* WITH DIETARY PATTERNS, GUT MICROBE, HLA-DRB1 GENES AND DISEASE SEVERITY IN RHEUMATOID ARTHRITIS (RA) PATIENTS

Prakriti, refers to genetically determined physical and mental constitution of an individual. Every person has his/her own unique constitution, which determines the biological functions, response to environmental factors, drugs and also susceptibility to diseases making it one of the earliest known concepts of preventive and personalized medicine. HLA- DRB1*04 gene and shared epitope of the same has been shown strong association with RA among almost all population. Developing countries like India are undergoing a rapid nutrition transition. From a healthy, traditional, high-fibre, low-fat, low-calorie diet, a shift is being made toward consumption of refined carbohydrates, high total fat and red meats, along with low intakes of fibre. Earlier studies have showed that differential gut Microbiome has been reported in RA patients when compared with normal individuals.

Primary objective: To assess "*Prakriti*" and "HLA-DRB1" gene association among RA patients.
***Secondary objective*:** Compare dietary pattern and gut microbiome composition among RA patients and healthy controls.

Methodology

RA subjects and age, sex matched healthy control subjects were screened and recruited by strictly following the inclusion and exclusion criteria. Clinical evaluation, *Prakriti* assessment was done using standard questionnaire by an Ayurveda Physician. Dietary pattern was assessed using semi-quantitative FFQ. Rheumatoid Factor (IgM), Anti-Cyclic Citrullinated Peptide antibody (anti-CCP) and high sensitive C-reactive protein (hs-CRP) estimations were done for both patients and

controls by ELISA using commercially available kits. Blood DNA was extracted using commercially available kits and HLA-DRB1 typing was done by PCR-SSOP technique using Histo Type kits.

Results

A total of 1931 RA subjects were screened and among them 300 RA subjects were enrolled. Of 1032 apparently healthy subjects, 310 controls were recruited by following the inclusion and exclusion criteria.

Demographic details of recruited RA and control subjects are discussed (Table 1). In this study, female subjects are more than the males with ratio of 7:1 (F:M) in both groups. Mean age of the recruited RA subjects was 40.68yrs (SD ±7.95) with mean BMI 23.42 (SD±4.71). About 13.7 % of the study subjects had family history of arthritis and only 21% were doing physical activity. Around 20% of the recruited RA subjects were addicted to alcohol, smoking or tobacco chewing.

Table 1. Demographic and auto antibody profile among cases and controls

S. No.	Parameter	RA Subjects	Healthy Controls
1	Total subjects	300	310
2	Sex ratio(F:M)	263:37	274:36
3	Age (Mean ± SD)	40.73 ±7.94	35.00 ± 8.32
4	Age of Females (Mean ±SD)	40.79±7.92	35.12 ± 8.13
5	Age of Males (Mean ± SD)	40.33±8.17	33.27 ± 9.72
6	BMI(Mean ± SD)	23.42±4.71	24.68 ± 3.75
	16- 18.9 (n + %)	49(16.3)	38 (12.25)
	19 – 24.9 (n + %)	149(49.5)	112 (36.12)
	25- 29.9 (n + %)	91 (30.6)	160 (51.61)
7	Family H/O Arthritis (n+ %)	41(13.7)	Nil
8	*Physical activity* No (n+ %) Yes (n+ %)	 235 (78.59) 63 (21.07)	Nil
8	*Addictions* No Alcohol, Smoking, Tobacco Chewing Any two	 80.3 % 15.7 % 1.3 % 1.3 % 1.0 %	Nil
1	*ESR (High) n+%*	158 (52.8)	-
2	*RF (Positive) n+ %*	300 (100)	0
3	*Anti- CCP (Positive) n+%*	230 (76.4)	0
4	*Hs-CRP (Positive) n+%*	102 (67.1)	162 (64.8)

Table.2 Distribution of *Prakriti* among cases and controls

S. No	*Prakriti*	Patients (N=300) N+ (%)	Controls (N=310) N+ (%)	O.R	95%CI	X^2Y	P-value
1	Vata	63 (21)	24 (7.74)	3.16	1.92-5.22	20.845	0.0001
2	Pitta	17 (5.66)	52 (16.77)	0.29	0.16-0.52	17.659	0.0001
3	Kapha	11 (3.66)	6 (1.93)	1.92	0.70-5.28	1.108	0.20
4	VP	85 (28.33)	80 (25.8)	1.14	0.76-1.62	0.374	0.48
5	PK	29 (9.66)	53 (17.09)	0.34	0.28-0.54	6.609	0.0001
6	VK	14 (4.66)	8 (2.58)	1.85	0.76-4.47	1.355	0.18
7	VPK	81 (27)	87 (28.06)	0.95	0.66-1.35	0.207	0.77

A significant increased frequency of Vata prakriti was observed among cases (21% vs 7.74%: OR:3.16; 95%CI 1.9- 5.22, p=0.0001) whereas a significant increased frequency of Pitta and Pitta kapha prakriti (Pitta:5.66 vs. 16.77%; OR 0.29, 95%CI: 0.16-.052, p <0.005: Pitta kapha 9.6% vs 17%, OR 0.34, 95%CI: 0.28-.054, p <0.005) were observed among healthy controls. A trend of increased frequency of Vata kapha prakriti was observed among cases when compared with controls. A significant increased frequency of HLA-DRB1 gene *04(32.8 % vs 16.8%, OR: 2.14, 95%CI: 1.23-3.74 , p< 0.005) was observed in RA patients and while the frequencies of HLA-DRB1 *07 (18.4 % vs 36%, OR: 0.46, 95%CI: 0.27-0.79 , p< 0.005) , and *14(10.4 % vs 21.68%, OR: 0.45, 95%CI: 0.23-0.90 , p< 0.005) were significantly low in cases when compared with controls. Prakriti distribution was compared among shared epitope positive and negative RA subjects, which suggests that vata and vata kapha prakriti frequency was more in cases and pitta and pitta kapha prakriti was more among controls. Comparison of HLA-DRB1 gene frequency among cases and controls are as shown in Table 3.

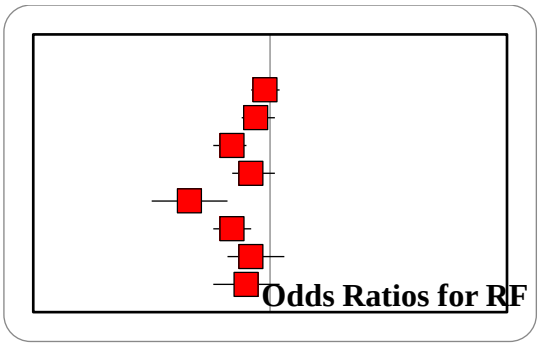
Table 3. Distribution of HLA-DRB1 gene among cases and controls

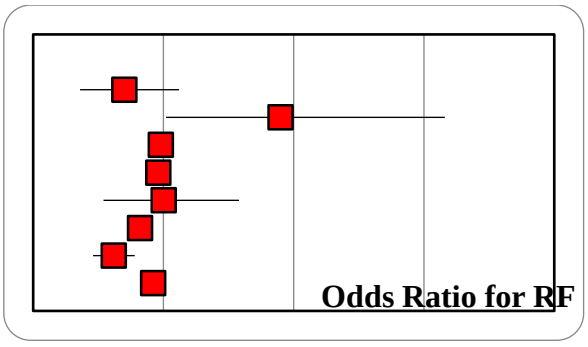
		Cases, n=125	Controls, n=125				
Sl No	DRB1	N+, PF %	N+, PF %	OR	95% CI	X^2Y	P value
1	01	9 (7.2)	5 (4)	1.83	0.60-5.53	0.661	0.28
2	03	16 (12.8)	16 (12.8)	1.00	0.48-2.05	0.000	1.0
3	04	41 (32.8)	21 (16.8)	2.14	1.23-3.74	6.647	0.0076**
4	07	23 (18.4)	45 (36)	0.46	0.27-0.79	7.506	0.0048**
5	08	1 (0.8)	4 (3.2)	0.25	0.028-2.23	0.808	0.21
6	09	2(1.6)	3 (2.4)	0.66	0.11-4.00	0.202	0.65
7	10	38 (30.4)	20 (16)	2.06	1.16-3.65	5.637	0.13
8	11	7 (5.6)	8 (6.4)	0.87	0.31-2.44	0.069	0.79
9	12	8 (6.4)	11 (8.8)	0.72	0.28-1.82	0.219	0.48
10	13	12 (9.6)	10 (8)	1.21	0.51-2.85	0.048	0.66
11	14	13 (10.4)	27 (21.6)	0.45	0.23-0.90	4.592	0.023*
12	15	80 (64)	77 (61.6)	1.06	0.72-1.54	0.037	0.77
13	16	0(0)	3 (2.4)	0.14	0.007-2.75	1.341	0.196

Table 4. Prakriti distribution among Shared Epitope positive and negative genes

Sl.No	Prakriti n+ (%)	SE Pos, n+ (%)	SE Neg n+ (%)
1	**Vata** 25(20)	16(64)	9(36)
2	**Pitta** 9(7.2)	3(33.33)	6(66.67)
3	**Kapha** 4(3.2)	3(75)	1(25)
4	**VP** 36(28.8)	18(50.0)	18(50.0)
5	**PK** 13(10.4)	2(15.38)	11(84.61)
6	**VK** 5(4)	3(60.0)	2(40)
7	**VKP** 33(26.4)	20(60.6)	13(39.4)
	TOTAL	**65**	**60**

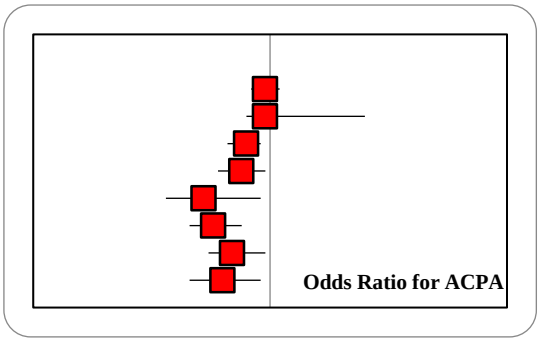
The study also revealed that increased frequency of consumption of red and organ meat in cases compared to controls. The odds ratio value of less than 1 indicates that exposure to that food decreases the value for RF auto-antibodies, which is applicable for cereals, dals, vegetables, fruits, salads, non-veg items like poultry, sea food, milk and milk products as well as snacks, sweets and chutneys. On the contrary, value of more than 1 indicates that exposure to foods like red and organ meats would increase the value of RF. Similar results have been produced by studies previously conducted showing a higher risk of getting RA in people consuming more of red meats as well as alcohol intake.

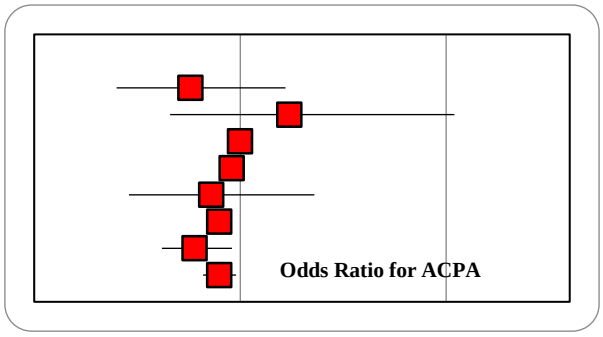




On the contrary, value of more than 1 indicates that exposure to food, like red and organ meats increases the value of Anti-CCP auto antibodies. Alcohol consumption and effect on RA has shown contradicting evidence, where in limited amounts alcohol can have diverse association with RA. Therefore more studies are required to arrive at a conclusion regarding alcohol intake and RA.

Consumption of a healthy balanced diet delays the progression of RA.





4. A STUDY ON ASSESSMENT OF PREVALENCE OF MICROALBUMINURIA AND MACROALBUMINURIA AND THEIR ASSOCIATED FACTORS IN TYPE2 DIABETICS IN URBAN SLUMS OF HYDERABAD, TELANGANA, INDIA

India is the nation with the second largest prevalence of type 2 diabetes mellitus in the world, with an estimated 69.2 million people having the condition as of 2015. In the Southeast Asia region as a whole, 78.3 million people live with type 2 diabetes, which is estimated to be about 9% of the adult population, out of which 52.1% of the cases are undiagnosed. It is these cases that cause the most problems because by the time the diabetes is detected due to secondary complications like retinopathy or neuropathy, it would have already inflicted significant damage. Additionally, it is estimated that 44% of all cases of nephropathy are caused by type 2 diabetes mellitus.

Methods

This is a cross-sectional study, and there are two goals of this study: to measure the prevalence of microalbuminuria and albuminuria in type 2 diabetic patients, and to see if there were any associated factors that elevated diabetic people's risks of ending up with kidney disease. To conduct this study, a lifestyle questionnaire modelled off of the WHO STEPS Questionnaire for NCDs was administered to 100 patients that have had type 2 diabetes for at least 5 years. After the questionnaire was administered, the patient was given a urine collection bottle for urine sample collection. This study was done in the morning, and as much as possible, the first urine of the day was collected. The patient interviews were conducted in Telugu and Hindi. The data from the questionnaires was entered into an Excel spreadsheet, and then copied into Stata 15 in which it was analyzed.

Results

Of the 100 patients that were interviewed, 42 (42 %) were found to be normoalbuminuric (have an albumin to creatinine ratio (ACR) of less than 2.5 mg/mmol in men and 3.5 mg/mmol in women), 46 (46 %) were found to be microalbuminuric (have an ACR of between 2.5 mg/mmol and 25 mg/mmol in men and between 3.5mg/mmol and 35mg/mmol in women), and 12 (12 %) were found to be macroalbuminuric (ACR higher than 25mg/mmol in men and 35 mg/mmol in women). Previous studies suggest that the prevalence of microalbuminuria in a type 2 diabetic population should be between 30 and 40%, but the prevalence from our study was 46%. Additionally, risk factors that were found to be significant were: being older than 44 years, having type 2 diabetes for longer than 6 years, drinking alcohol, smoking, being comparatively more educated. Additionally, eating fruit was considered a protective factor as people who ate fruit more were significantly less likely to have nephropathy. Age and hypertension were not significant factors, even though previous studies suggest that both should be significant. Duration of diabetes was not significant as a continuous variable, but when a categorical variable was made from the continuous it was shown to be significant.

Conclusion

The prevalence of microalbuminuria in type 2 diabetic people is higher than it was in previous studies.

5. PREBIOTIC POTENTIAL AND OTHER BENEFICIAL EFFECTS OF OCIMUM, GINGER AND PIPER NIGRUM ON IMMUNE-INFLAMMATORY DISEASE CONDITIONS

The establishment of a normal intestinal bacterial flora has important implications for health and disease. Gastrointestinal mucosa is the primary interface between the external environment and the immune system. The non-pathogenic probiotic bacteria interact with the gut epithelial cells and the immune cells to start the immune signals. Few reports in the recent past showed involvement of gut bacteria in causing disorders like obesity and diabetes where in the immune and inflammatory markers are the key players. Recent data, both from experimental models and from human studies, support the beneficial effects of particular food products with prebiotic properties that promote growth of beneficial bacteria such as bifido bacteria and lactobacillus.

Objective-1: To document the ethanobotanical profile of *Ocimum sanctum*, *Zingiberofficinale* and*Piper nigrum* following Indian pharmacopoeia and WHO monographs.

Under this objective, the selected herbs (*Ocimum sanctum, Zingiberofficinale* and *Piper nigrum*) authenticated and validated as per the norms of Indian Pharmacopoeia and WHO monographs. Further, the process of 'hydro-alcoholic extract preparation', analysis of active and phytoconstituents of extracts was undertaken and to test the prebiotic potential of *O.sanctum, Z.officinale and P.nigrum* hydro-alcoholic extracts by culturing the *Lactobacillus* and *Bifidobacterium* in presence and absence of extracts along with oral supplementation to healthy SD rats to determine selective gut microbiota and biochemical parameters.

Objective-2&3: To assess impact of supplementation of *Ocimum sanctum, Zingiber officinale* and *Piper nigrum* extracts on selective gut bacteria profile and intestinal permeability in Ovalbumin-(OVA) induced allergic inflammatory disease.

For this objective, the prebiotic potential and effects of *O.sanctum, Z.officinale* and *P.nigrum* extracts on food allergy were evaluated using a mouse model of ovalbumin-(OVA) induced allergic inflammatory disease.

Assess impact of supplementation of *Ocimum sanctum, Zingiberofficinale* and *Piper nigrum* extracts on selective gut bacteria profile and intestinal permeability in Streptozotocin Induced Diabetes Rats

The effect of *O. sanctum, Z. officinale* and *P. nigrum* extract was evaluated on intestinal integrity, biochemical parameters and selective gut microbiota in streptozotocin (STZ) induced diabetes rats.

***Statistical Analysis*:** Descriptive results are presented as Mean ± SD. ANOVA was used to assess the significance of descriptive data. p values < 0.05 were considered significant. Dunnett's test was used to identify the groups that were similar with respect to the mean. The relationships were identified by means of Spearman's rank correlation between Selective gut bacteria and inflammatory index. Computational analysis was performed using SPSS-V22 (SPSS Inc, Chicago, IL).

Results

The ethnobotanical information was collected through extensive literature search and discussion with Ayurveda practitioners/physicians. Information on plant species being used to treat various human diseases and disorders along with their local name, plant parts, method of drug preparation, and mode of application, dosage and duration collected and documented.

Collection of plants/herbs material and authentication and preparation of extracts

The selected plants/herbs were authenticated by Department of Botany, Osmania University, Hyderabad, India based on the 'vochure specimens' submitted (*O. sanctum*-0280, *Z.officinale*-0344and *P.nigrum*-0301).The lyophilized powder obtained from 100g of raw plant material of herbs is 16.4g for *O.sanctum*, 29.6g for *Z.officinale* and 12.1g for *P.nigrum*, indicating an yield of 16.4%, 29.6% and 12.1%, respectively.

Phytochemical screening of extracts

The results of preliminary phytochemical screening of the hydroalcohol leaf extract of *O. sanctum*, dry root extract of *Z.officinale* and dry seed extract of *P.nigrum* showed the presence of tannins, flavonoids, alkaloids, saponins and phenolic compounds. The total polyphenol content of *O.sanctum*, *Z.officinale* and *P.nigrum* hydro-alcoholic extracts were 9.19 ± 0.801g, 11.41± 0.312g and 1.404± 0.121g respectively per 100g gallic acid equivalent.

The HPLC analysis of hydroalcoholic extracts of *O. sanctum, Z. officinale and P.nigrum* revealed presence of eugenol (619.02 ± 12.38), gingerol (800.04 ± 25.54) and piperine (5371.73 ± 122.15) respectively. Presence of active components were confirmed by retention time when compared to standard eugenol, gingerol and piperine. The mean antioxidant activity of O.sanctum, Z.officinale and P.nigrum hydro-alcoholic extracts were 43.81±1.703 µg/ml, 41.29±0.693 µg/ml and 89.41±2.699 µg/ml respectively. Though the antioxidant activity of extracts were not comparable to ascorbic acid (5.89±0.152 µg/mL), these extracts showed a modest antioxidant activity.

Prebiotic potential - *In vitro'*

Prebiotic potential of the herbs were measured by quantifying growth of probiotic bacteria (*Lactobacillus rhamnosus* GG and *Bifidobacteriuminfantis*). The mean colony forming units of LGG was 41.50 ± 2.121 × 10^7 CFU/mL on plain MRS agar, whereas an exponential dose dependent increase of CFU was noted in presence of FOS from 2.5 ppm to 5.0 ppm is 57.50 ± 2.121 × 10^7 CFU/mL to 71.50 ± 3.536 × 10^7 CFU/mL respectively, thereafter the growth of LGG plateaued. When 5.0 ppm of either *O.sanctum*or *Z.officinale* extracts were added to MRS broth a significant increase in LGG colony forming units were noted compared to CFU's on MRS alone. In addition, the growth of LGG on *O. sanctum* and *Z.officinale* agars were higher (p<0.05) when compared to FOS MRS agar. Whereas, comparable levels of CFU's of LGG were noted with higher concentration (25.0 ppm) of *Piper nigrum* extract. The mean *B.infantis* levels on BMS agar was 47.0 ± 1.414 × 10^7 CFU/mL, while FOS supplemented (2.5 to 10.0 ppm) BSM agar have shown exponential increase in *B. infantis* from 48.5 ± 0.707 × 10^7 to 62.0 ± 1.414 × 10^7 CFU/mL, thereafter the growth of *B. infantis* plateaued. Interestingly, a significant ($p<0.05$)increase in *B. infantis* levels was noted upon supplementation of either *O.sanctum*or *Z.officinale* to BMS agar, when compared to control and FOS. In contrast, *P.nigrum* showed an augmented colony count of *B. infantis at* 25.0 ppm (54.5 ± 0.707) and was significantly (p<0.05) more than control, but less when compared to FOS.

Table 1. Polyphenols content in *O. sanctum, Z.officinale, P.nigrum*and combined Extracts

Polyphenols(mg/100gm) (Mean±SD)	*O. sanctum*	*Z. officinale*	*P. nigrum*	Combination*
Gallic acid	4.02±0.12	84.13±1.2	6.49±0.52	12.91±0.47
Protocatechuic acid	23.53±0.1.2	19.26±0.32	15.38±0.25	22.18±0.62
P-hydroxi benzoic acid	12.61±0.32	0.77±0.11	3.35±0.42	8.59±0.43
Catechin	356±2.02	9.70±0.14	28.15±0.62	194±1.66
Caffeic acid	55.68±1.21	1.43±0.01	3.92±0.23	33.32±1.32
Sinapic acid	18.58±0.12	0.95±0.06	2.54±0.31	12.41±0.91
Ferulic acid	7.17±0.23	2.82±0.35	10.45±0.64	8.17±0.33
P-Coumaric acid-4	BDL	3.84±0.11	0.80±0.01	0.82±0.04
Ellagic acid	18.14±0.21	14.64±10.62	2.47±0.26	11.39±0.62
O-Coumaric acid-2	10.19±0.96	7.06±0.14	1.60±0.02	5.22±0.34
Luteolin 7 O Glucoside	430±2.3	BDL	BDL	266±1.8
Myricetin	43.70±1.2	BDL	2.52±0.34	27.52±0.56
Resveratrol	270±2.1	2.72±0.12	BDL	164±2.8
Daidzein	BDL	BDL	2.26±0.41	3.13±0.24
Quercetin	BDL	BDL	BDL	BDL
Luteolin	64.10±1.02	BDL	2.23±0.61	39.09±0.99
Naringenin	6.43±0.31	BDL	24.95±1.51	12.99±0.53
Apigenin	132±3.11	BDL	BDL	79.57±1.65
Kaempferol	57.03±1.21	BDL	BDL	36.78±0.87
Hesperetin	164±2.31	BDL	7.06±0.97	93.66±2.56
Flavone	BDL	21.89±0.91	88.18±1.8	33.36±1.65
*Combination=*O.sanctum+Z.officinale + P.nigrum;* BDL*: Below Detectable Level*				

***In Vivo* Evaluation**

A proportionate increase in food intake and body weight gain was noted during the course of experiment irrespective of treatment regimen. A significant ($p<0.05$) increase in haemoglobin level was noted among *O.Sanctum* and combined treated groups compared to the control. Whereas, all other hematological parameters were comparable between groups and controls. A significant ($p<0.05$) decrease in total cholesterol and triglyceride levels were observed in *O.sanctum, Z.officinale* and *P.nigrum* alone and combined treated rats and FOS (prebiotic control) when compared to control. Interestingly, TG levels of combined treated rats were lower ($p<0.05$) than the FOS treated rats. Similarly, the HDL concentration in extracts administered groups either alone or combined have shown significant ($P<0.05$) increase over control and FOS groups. The effect of extracts on various inflammatory markers (LPS, serum insulin, CRP and IL-6 levels) the serum LPS, a marker of gram negative bacteria translocation from lumen to circulation, was observed to be lower ($p<0.05$) in extracts administered groups i.e. either alone or combined and FOS (prebiotic control) when compared to control rats. Serum insulin, CRP and IL-6 levels were comparable between extract administered groups and FOS along with controls. The histopathological examination of vital organs such as liver, kidney, small intestine, lungs, spleen, heart, stomach, and colon of the FOS, *O.sanctum, Z.officinale* and *P.nigrum* alone and combined treated rats did not reveal any pathological changes and were comparable to control group rats. An increase in the levels of Caecal *Lactobacillus* (1.7-3.4 folds) and *Bifidobacteria* (5.89-28.4 folds), whereas decrease in *Firmicutes* (0.04-0.91 folds) and *Bacteroides* (0.69-0.88 folds) were noted in extracts and FOS administered rats.

Objective 2&3: Assess impact of supplementation of *Ocimum sanctum, Zingiberofficinale and Piper nigrum* extracts on selective gut bacteria profile and intestinal permeability in Ovalbumin- (OVA) induced allergic inflammatory disease.

Treatment of OVA challenged animals with extracts alone or combined showed significant (P<0.05) suppression of both total and differential leukocyte counts in the blood and inflammatory immune cell populations in BALF. Also significant (P<0.05) attenuation of lung alveolar thickening, reduced serum LPS (p<0.05), OVA specific-IgE(21.61%-48.51%) levels was found along with improved intestinal integrity(p<0.05) after treatment with these extracts alone or combined. These extracts also showed reduced (P<0.05) expression levels of IL-4, IL-5, TNF-α, iNOS, NF-kB, TLR4 and up-regulation of occludin, AQP1 and AQP5 levels. Treatment with these extracts improved the growth of *Lactobacilli* and *Bifidobacteria* and inhibited *Bacteroides* and Firmicutes growth in the OVA challenged animals.

Assessing the impact of supplementation of Ocimum sanctum, Zingiber officinale and Piper nigrum extracts on selective gut bacteria profile and intestinal permeability in Streptozotocin Induced Diabetes Rats

The food intake was significantly (p<0.01) increased in the diabetes induced animals initially, when compared to the control group. Treatments with FOS and extracts alone or combined, the food intake was significantly (p<0.01) decreased when compared to the diabetes control group and comparable with control group. The body weight gains of diabetes control and treatments with FOS and extracts alone or combined groups were significantly lower compared to control group during the course of experimental period. The fasting blood glucose concentrations of diabetic group was significantly (p<0.05) higher (>145 g/dL) than the control group at Week 0 (1 week after diabetes induction). After treatment with FOS and extracts alone or combined, the fasting blood glucose levels were decreased insignificantly at Week 1 when compared to diabetic group. Whereas, treatment with FOS and extracts alone or combined the fasting blood glucose levels were decreased significantly (p<0.05) at 2nd, 3rd and 4th weeks of experimental course. Serum insulin levels, a marker of diabetes, were significantly (p<0.05) decreased in the diabetic group. Whereas, the mice exposed to STZ followed by administration of FOS and extracts alone or combination have shown comparable levels of serum insulin as with control group and no significant difference was observed between the diabetic group and treatment groups. Serum LPS levels were significantly (p<0.01) elevated in diabetic mice compared to control mice. Whereas, a significant (p<0.05) decrease in LPS levels of rats administered with STZ subsequently treated with *P.nigrum* extract alone or combined was noted when compared to diabetic group.

A significant (p<0.05) decrease in total cholesterol and TG levels were noted in FOS and extract *Z.officinale, P.nigrum*alone and combined treated rats when compared to control and diabetic control groups. The mean LDL levels were significantly (p<0.05) low in *O.sanctum, Z.officinale* alone and combined treated rats when compared to control and diabetic groups. No considerable change was noted in HDL levels of various treatment groups.

The mRNA expression levels of occlude in were significantly (p<0.05) decreased in the diabetic group when compared to the control. Treatment with extracts combined group significantly (p<0.05) improved occlude in expression when compared to the diabetic group. The mRNA expression levels of TLR4 and NF-kB(p65) increased in the diabetic group when compared to the

control. Treatment FOS and with extracts alone and combined decreased significantly ($p<0.05$) when compared to the diabetic group and comparable with control group.

A decrease in caecal Lactobacillus levels ($p<0.05$) and Bifidobacteria levels was noted in the diabetic group compared to control group rats. Treatments with FOS and extracts alone and combined treated groups improved significantly ($p<0.05$) Lactobacillus levels. Caecal Firmicutes and Bacteroides levels were increased ($p<0.05$) in diabetic group as compared to control rat. Treatments with FOS and extracts alone or combined treatment decreased levels of caecal Firmicutes and Bacteroides and were comparable to control group. An inverse relationship was noted between fasting blood sugar, tight junction marker, LPS with Lactobacillus and Bifidobacterium levels. Whereas, the Bacteroids and Firmicutes were positively correlated with LPS.

Inference & Conclusion

The hydro-alcoholic extracts of *O. sanctum*, *Z. officinale* and *P. nigrum* enhanced the growth of beneficial microbes, Lactobacilli and Bifidobacteria *in vitro* and *in vivo* studies thus establishing the prebiotic potential of these extracts. Administration of extracts to OVA exposed animals prevented airway obstruction by inhibiting cellular infiltration and collagen accumulation in the lung. In another disease model i.e Diabetes induced rats, the extracts regulated fasting blood sugar and serum insulin levels, and reduced inflammatory markers compared to control and/or STZ group.

IV. DIETETICS STUDIES

1. TO STUDY THE EFFECT OF LEGUME PREBIOTIC MILK ON GUT MICROBIOTA, ADIPOSITY AND INFLAMMATION IN OBESE RAT

Recent evidences suggest that gut microbiota is involved in the control of body weight, energy homeostasis and inflammation and thus, play a role in the pathophysiology of obesity. Prebiotics have been shown to alter the composition of gut microbiota and induce to decreased food intake and appetite, body weight composition and metabolic functions through gastrointestinal pathways and modulation of the gut bacterial community. New research reveals that obese animal and human subjects have alterations in the composition of the gut microbiota compared to their leaner counterparts. The bifidobacterial abundance is significantly increased with prebiotics and proved that prebiotic fiber is a potential non-invasive treatment option to reduce overweight and obesity by gut microbiota modulation. Non-digestible oligosaccharides are a new category of low energy sweeteners that share many properties with fermentable dietary fibers and act as prebiotics. The legume raffinose family oligosaccharides such as raffinose, stachyose and verbascose belong to this class of food ingredients. Legumes are important food crops, which are grown and consumed in the tropics and semi arid parts of the world. Carbohydrates constitute the main fraction of grain legumes, accounting for up to 55–65% of the dry matter. Of these, starch and non-starch are the major constituents, with smaller but significant amounts of α-galactosides. Legumes are also complex foods rich in soluble fibers. Replacing energy dense foods with legumes has been shown to have beneficial effects on the prevention and management of obesity and related disorders. In a study, legume derived novel prebiotic ingredient modulated the colonic microbiota in humans since it is shown to significantly increase levels of bifidobacterium spp. and decrease production of pathogenic bacteria. Our study was proposed to assess the effect of legume prebiotic milk on the prevention of overweight and obesity in obese rat.

Objectives

1. To evaluate the effect of legume prebiotic milk on the gut microbiota in obese rat.
2. To determine the effect of legume prebiotic milk on adiposity and inflammation in obese rat.

Methodologies

Preparation of legume prebiotic milk: Legume prebiotic milk was prepared according to the method of Mulimani and Ramalingam. Red gram, green gram, chick pea and soybean seeds were ground to flours. The flours were suspended in distilled water (1:10 w/v) and heated on boiling water bath. Undissolved residue was separated from legume prebiotic milk by centrifugation for 5 min at 12,300 g. The supernatant containing legumes was removed and stored at 4^0C until further use.

Experimental design and animal grouping: Seventy two 4-5 week old WNIN/Ob rats weighing 60-70 g were housed in individual stainless steel cages under standard conditions. The rats were fed with a standard chow diet for 1 week to be acclimatized. Different legume prebiotic milk containing 3% of raffinose family of oligosaccharide was offered in ordinary water drinking bottles with steel sipper

tubes for a period of 18 weeks. After 18 weeks on these experimental diets, 5 ml of blood was drawn and sacrificed by CO_2 inhalation. After sacrificing the rats.

Body composition: DEXA (Dual-energy X-ray absorptiometry)
Ceacum (*Gut bacteria****):*** The ceacum content was be analyzed for gut bacteria (lactobacillus, bifidobacteria, enterobacillus and bacteroides) by PCR based detection using genus specific primers as molecular prob (16s rDNA sequences).
Biochemical Profile: Serum was used for biochemical analyses. The following biochemical parameters were done: Glucose, oral glucose tolerance test (OGTT), lipid profile, leptin, adiponectin, lipoprotein lipase (LPL) mineral content and short chain fatty acids and insulin were measured by Radioimmunoassay (RIA) using commercially available kits.

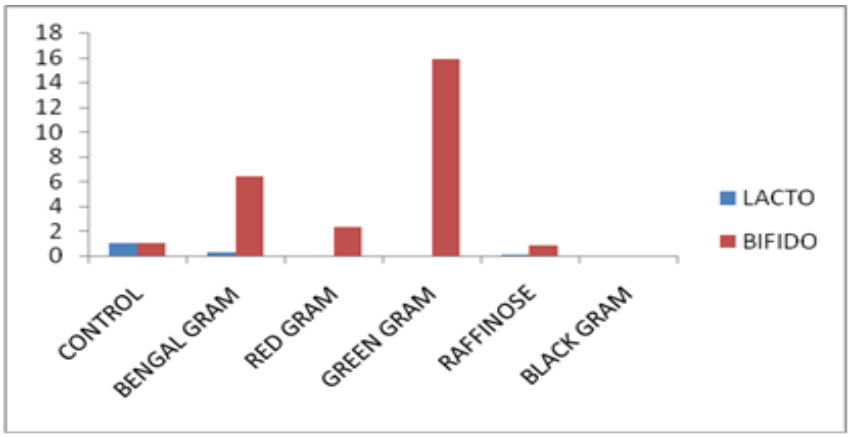
Results

Blood glucose and lipid profile of raffinose, red gram, green gram, black gram and bengal gram oligosaccharide fed obese rat showed promising results when compared to control obese rat. The serum Cholesterol content in all legume oligosaccharide fed group were decreased when compared to control group. The triglycerides, LDL and VLDL cholesterol were decreased and HDL cholesterol level is increased in legume oligosaccharide fed animals when compared to control group. Gain in body weight was significantly lower in legume prebiotic fed group (352.6±90.82) when compared to raffinose fed group (591.9±171.97) and basal diet fed group (635.7±115.32). The body fat percent was much lower in legume fed group (33.96±7.3) when compared to raffinose fed group (53.3±1.92) and basal fed group (57.9±5.4). The ceacum content for gut bacteria by PCR based detection using genus specific primers as molecular probe (16s rDNA sequences) was showed fold increase for lactobacillus is not significant in all the legume prebiotic fed groups when compared to control whereas significant fold increased was showed for bifidobacteria in green gram prebiotic fed group followed by bengal gram prebiotics and red gram prebiotic fed groups.

Table 1. Effect of legume prebiotic milk on the gut microbiota of obese animals

OB/OB group	Fold increase of Lactobacilli	Fold increase of Bifidobacteria
Control	1	1
Raffinose	0.08	0.83
Red gram prebiotics	ND	2.32
Green gram prebiotics	ND	15.91
Bengal gram prebiotics	0.32	6.41
Black gram prebiotics	0.02	0.01

Figure 1. Effect of legume prebiotic milk on the gut microbiota of obese animals



Conclusion

The results of the legume prebiotic milk is promising since there was a decrease in blood glucose level, improved lipid profile, and improved body mass composition. The ceacum content analysis for gut bacteria by PCR based detection using genus specific primers as molecular prob (16s rDNA sequences) showed increased lactobacillus, but was not significant in all the legume prebiotic fed groups when compared to control, whereas significant increase was observed for bifidobacteria in green gram prebiotic fed group followed by bengal gram prebiotics and red gram prebiotic fed groups.

2. EFFECT OF NON-DIGESTIBLE CARBOHYDRATE OF KIDNEY BEANS ON DIET INDUCED METABOLIC SYNDROME IN A RAT MODEL

Metabolic diseases, such as obesity and type 2 diabetes, are world-wide health problems. The prevalence of metabolic diseases is associated with dynamic changes in dietary macro nutrient intake during the past decades. Long-term intake of diets high in fats and meats appear to induce chronic systemic low-grade inflammation, endo toxicity, and metabolic diseases. Recent investigations support the idea of the involvement of intestinal bacteria in host metabolism and preventive and therapeutic potentials of probiotic and prebiotic interventions for metabolic diseases like obesity and type 2 Diabetes mellitus. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve the host health. Kidney bean (Phaseolus vulgaris), a grain legume, is one of the neglected tropical legumes that can be used to fortify cereal-based diets especially in developing countries, because of its high protein content. It is also a rich source of vitamin, minerals and relatively high in crude fiber.

In India, kidney bean is widely consumed in the form of dhal as an economical source of protein. The role of kidney bean prebiotics in health benefits may include reduction of heart and renal diseases risks, cataracts, relieve constipation, improve gastrointestinal integrity, stabilize blood sugar, brain and immune dysfunction. Information on the prebiotic effects of kidney beans with oligosaccharides is scanty. Therefore this study has been proposed with the following objectives. Currently, the data on anti-obesity and anti-diabetic properties of kidney bean (*Phaseolus vulgaris*) are limited in open literature. Hence, the present study was undertaken to determine the prebiotic potential of Kidney Beans (*Phaseolus vulgaris* L.) seeds and also to study its effects on some biochemical and haematological parameters in obesity and diabetes induced rats.

Hypothesis: Consumption of kidney beans prebiotics will have a positive impact in reduction of risk of obesity, and type-2 diabetes.

Objectives

1. To study the role of kidney bean prebiotics in the control of obesity in type 2 diabetes induced rat model
2. To study the role of kidney beans prebiotics in the control of type 2 diabetes in induced rat model

Methodologies

Determination of non-digestible carbohydrate of kidney beans: Determination of oligosaccharides: The database of raffinose family of sugars from different varieties of kidney beans was developed by following the method of Ekvall et al. (1981) & Wang et al. (1998) and estimation was done using HPLC with RI detector.

Animal and diet

*To study the role of kidney bean prebiotics in the control of obesity in high fat induced and high sucrose fed rat model***:** Four-week-old Male Sprague Dawley rats (N=24) were used for the study all the rats were fed with a high-fat diet (59%fat) and high-sucrose diet (60% sucrose) for a period of 4 weeks. After inducing obesity and insulin resistance kidney bean prebiotics were fed for 12 weeks, 5 ml of blood was drawn and sacrificed by CO_2 inhalation. After sacrificing the rats, the ceacal content and other organs were collected for further analysis.

Body composition: DEXA (Dual-energy X-ray absorptiometry)

Ceacum (*Gut bacteria****):*** The ceacum content was analyzed for gut bacteria (lactobacillus, bifidobacteria, enterobacillus and bacteroides) by PCR based detection using genus specific primers as molecular prob (16s rDNA sequences) and short chain fatty acids.

Biochemical Profile: Serum was used for biochemical analyses. The following biochemical parameters will be done: Glucose, oral glucose tolerance test (OGTT), lipid profile, leptin, adiponectin, lipoprotein lipase (LPL) mineral content and short chain fatty acids and insulin was measured by Radioimmunoassay (RIA) using commercially available kits. The inflammatory markers like IL-1 IL- 6, IL-12, SCD-1, TNF-α, C-reactive protein, Interferon–γ, MCP (Monocyte chemoattractant protein) will be estimated by Milliplex- ELISA is based on the luminex xMAP technology.

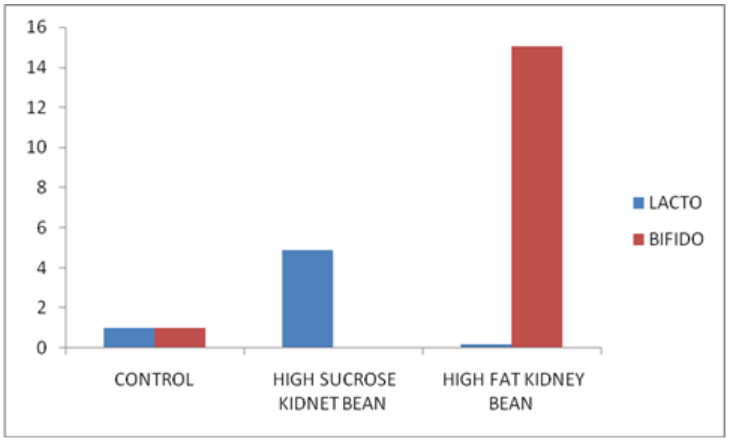
Results

Blood glucose and lipid profile values showed promising results in kidney bean fed prebiotic group rats when compared to high fat and high sucrose fed animals. Wight gain was very much lower in kidney bean fed group (277.3±6.1) when compared to raffinose (324.07±26.76) and high fat group (390.17±9.17). Similar results were observed for high sucrose fed animals i.e kidney bean group showed average weight 274.43±8.09, raffinose group 359.20±11.76 and high sucrose fed groups 434.77±19.88. The fasting blood glucose was significantly lower in kidney bean fed group (84.7±3.1) when compared to raffinose fed group (86±4.6), high fat group (99.7±9.3) and similar results were observed for high sucrose fed group animals. The body fat percent was lower in kidney bean group (12.20±3.05) when compared to raffinose group (13.70±2.62) and high fat fed group (25.03±0.75). The ceacum content for gut bacteria by PCR based detection using genus specific primers as molecular prob (16s rDNA sequences) was showed fold increase for lactobacillus is significant in high sucrose raffinose fed group followed by high sucrose kidney bean and high fat raffinose group when compared to control where as significant fold increased was showed for bifidobacteria in high fat raffinose fallowed by high fat kidney bean fed groups.

Table 1. Effect of non-digestible carbohydrates of kidney bean on the gut microbiota of SD rat animals fed high fat raffinose or high sucrose

OB/OB group	Fold increase of *Lactobacilli*	Fold increase of Bifidobacteria
Control	1	1
High fat raffinose	2.35	113.0
High fat kidney bean	0.21	15.02
High sucrose raffinose	27.14	1.0
High sucrose kidney bean	4.91	0.06

Figure1. Effect of non-digestible carbohydrates of kidney bean on the gut microbiota of SD rat animals



Conclusion

Effect of non-digestible carbohydrate of kidney beans on diet induced metabolic syndrome in rat model was carried out in Male Sprague Dawley rats. The results of the non-digestible carbohydrate of kidney beans decreased blood glucose level, improved lipid profile, and improved body mass composition. The ceacum content analysis for gut bacteria by PCR based detection using genus specific primers as molecular prob (16s rDNA sequences) showed (26.14) fold increase of lactobacilli in high sucrose raffinose fed group followed by high sucrose kidney bean (3.91) and high fat raffinose group when compared to control where as significant fold increase was showed for bifidobacteria in high fat raffinose (112) followed by high fat kidney bean fed groups (14.02).

3. STUDIES ON NITRATE AND NITRITE IN INDIAN FOODS

Nitrate and nitrite ions are ubiquitous in the environment and naturally found in plant foods as a part of the nitrogen cycle. Vegetables are the source of dietary nitrate; however, wide variations in nitrate levels have been observed depending on the type of vegetable, its source, conditions of cultivation, and storage. The amount of available nitrate in soil (depending on the content of artificial fertilizer) appears to be a major factor determining the nitrate content in vegetables. Studies related to the beneficial effect of nitrate, nitrite and nitric oxide consumption are cardiovascular health, glucose and calcium regulation, muscle contractility, mitochondrial bio-synthesis and respiration, and regulation of blood flow. Nitrate is non-toxic even in higher doses, while nitrite can cause serious

harm at lower levels. Nitrites are known to cause methemoglobinemia in infants and cancer and hypotension in adults and therefore its consumption is restricted. Many studies have revealed the effects of nitrates on the human body by its direct consumption in the form of nitrate-rich dietary supplements mainly, or inorganic nitrates. Dietary nitrate intake is determined by the type of vegetable consumed, the levels of nitrate in the vegetables (including the nitrate content of fertilizer), the amount of vegetables consumed, and the level of nitrate in the water supply. As such, the nitrate content of organic vegetables may be less than that of vegetables grown in the presence of nitrogen-containing fertilizers. The primary determinants of nitrite consumption are the levels of nitrites in cured, processed meats and the consumption level of these products. The accumulation of nitrate is subject to factors such as genotype, soil conditions, growth conditions (ie., nitrate uptake, nitrate reductase activity, and growth rate), storage and transport conditions. The World Health Organization (WHO) recommended the upper limit of concentration of daily nitrate and nitrite uptake to be 3.7 mg/kg and 0.06-0.07 mg/kg, respectively. Data on nitrate and nitrite contents of foods are not available in India; hence values from overseas are commonly used. Due to the growing concern of N-nitroso compounds, accurate and robust methods are necessary for long-term monitoring of nitrate and nitrite concentrations in foods for susceptible populations.

Aims and objectives

- Determination of nitrates and nitrites in Indian foods
- Effect of processing/cooking on nitrates and nitrites in Indian foods of plant as well as animal origin foods/products

Materials and methods

The samples were collected from the local markets of the twin cities of Secunderabad and Hyderabad. The samples selected for the study included green leafy vegetables such as spinach, rumex, fenugreek, amaranth, spring onion; vegetables such as French beans, cluster beans, capsicum (red and green), brinjal and cabbage; roots and tubers such as potato, carrot, beetroot and non-vegetarian foods such as chicken, fish (Rohu and Tilipia) which were analyzed for the nitrate and nitrite content in the raw and cooked forms.

Effect of cooking on the nitrate and nitrite content of foods

The green leafy vegetables were cooked in sunflower oil with onion, turmeric powder, chilli powder, salt and seasoned with mustard and cumin seeds and the cooking time was 15-20 min. Vegetables such as French beans, cluster beans, carrot and beetroot were cooked in a similar manner as that of GLV with a cooking time of 30 min while capsicum red and green and potato were cooked for 15-20min and brinjal and cabbage for 25-30min. Chicken was cooked with sunflower oil, onion, ginger garlic paste, mustard, turmeric salt, chilli powder in 25-30min. Chicken fingers were deep fried in sunflower oil for 10min while chicken salami and sausage were sautéed for 10 min in sunflower oil. Rohu and Tilapia were cooked for 25-30min with sunflower oil, onion, tomato, turmeric, chilli powder and salt.

***HPLC method validation for the determination of nitrates and nitrites in plant and animal sources*:** HPLC method for the estimation of nitrates and nitrites were followed.

Results

The results of the nitrite and nitrate in raw and cooked food samples are given in Table 1. The nitrite and nitrate content in green leafy vegetables ranges from 0.36 to 6.63 mg/kg (spring onion and amaranth) and 78.96 to 388 mg/kg (spring onion and sorrel), vegetables 0.04 to 14.43 (cabbage round

head and French bean) and 2.35 to 132.73 mg/kg (capsicum green and cabbage round head), roots 0.00 to 0.91mg/kg (beetroot and potato), 33.94 to 149.46 mg/kg (potato and carrot) non-vegetable foods ranges from 0.00 to 16.82 (chicken salami and chicken sausage) and 12.52 to 36.48 (chicken sausage and chicken fries). The effect of cooking led to decrease in the levels of nitrite and nitrate content of foods tested.

Table 1. Nitrate and nitrite in raw and cooked vegetarian and non-vegetarian foods

Sl. No	Sample name	Scientific name	Nitrite mg/kg	Nitrite mg/kg	Nitrate mg/kg	Nitrate mg/kg
Green leafy vegetables			**Raw**	**Cooked**	**Raw**	**Cooked**
1	Spinach	Spinicia oleracea	2.79	0.010	263.78	148.74
2	Sorrel	Rumex acetosa	4.63	0.126	388.80	141.52
3	Fenugreek	Trigonella foenumgraecum	2.94	0.105	117.75	410.49
4	Amaranth	Amaranthus viridis/spinosus	6.63	0.048	161.89	353.62
5	Spring Onion	Allium cepa	0.36	0.029	78.98	67.40
Vegetables						
6	French beans	Phaseolus vulgaris	14.43	0.009	32.00	31.10
7	Cluster beans	Cyamopsis tetragonoloba	0.03	ND	22.03	71.47
8	Capsicum green	Capsicum annuum	0.02	0.019	2.35	7.23
9	Capsicum red	Capsicum annuum	5.62	1.175	2.77	5.45
10	Brinjal purple round	Solanum melongena	1.63	34.948	13.66	14.77
11	Cabbage round head	Brassica oleracea var. capitata	0.04	0.028	132.73	72.09
Roots and tubers						
12	Potato	Solanum tuberosum	0.91	0.048	33.94	17.84
13	Carrot	Daucus carota	0.64	0.800	149.46	173.06
14	Beetroot	Beta vulgaris	0.00	1.036	130.48	357.45
Non vegetarian foods						
	Chicken					
15	Chicken	Gallus gallus domesticus	0.20	0.804	27.17	20.69
16	Chicken fries	Gallus gallus domesticus	0.90	0.015	36.48	17.54
17	Chicken sausage	Gallus gallus domesticus	16.82	ND	25.63	18.05
18	Chicken salami	Gallus gallus domesticus	ND	ND	12.52	11.19
	Fish					
19	Rohu	Labeo rohita	ND	0.004	2.16	1.88
20	Tilapia	Oreochromis niloticus	ND	0.000	0.95	1.62

Conclusion

The study indicates that green leafy vegetables were high in nitrite and nitrate content as compared to the other food samples analyzed. Among the non-vegetarian foods analyzed chicken was found to have more nitrates as compared to fish. It was observed that in most of the samples in cooked form the nitrite and nitrate content reduced significantly except for a few which could be due to the addition of other ingredients.

V. BASIC STUDIES

1. RELATIVE TELOMERE LENGTH AND MITOCHONDRIAL DNA COPY NUMBER VARIATION WITH AGE: ASSOCIATION WITH PLASMA FOLATE AND VITAMIN B12

Mitochondria are involved in the various cellular processes including energy metabolism, free-radical production and apoptosis. The mitochondrion has 16,569 base pairs with circular double-stranded DNA. Because of lack of efficient DNA repair mechanisms, protective histones & introns; the mitochondrion is more susceptible to oxidative stress which is one of the crucial factors associated with the aging process. Each mitochondrion contains 2-10 mitochondrial DNA genome copies, and each cell may have hundreds or thousands of mitochondria. Mitochondrial DNA copy number (mtCN) is associated with chronological age, and it may be used as a biomarker of aging. Several previous studies have shown that mtDNA substantially declines in individuals with. Further, studies revealed that the telomere shortening is highly associated with decreased mitochondrial biogenesis and its function.

Although TL decreases with age, it can be further affected by a variety of factors, such as lifestyle and genetic background, and is different in diverse tissues. Moreover, TL is epigenetically regulated by DNA methylation; hence, anything that can modulate DNA methylation would, in turn, influence TL as well as mtDNA replication. The B-vitamins: folate and vitamin B12, which are intricately involved in one-carbon transfer pathways, are linked to methylation of DNA. Although, epidemiological reports have shown inconsistent results with regard to the associations of plasma folate and vitamin B12 levels with TL, it is argued that deficiency of these vitamins may affect the stability of the genome and the methylation status of genes. The discrepancies could be due to variations in the age, presence or absence of disease and dietary intake vis-à-vis nutritional status of the study subjects. However, the exact relationship between TL and mtCN in aging and age-associated disorders are not fully understood. Further, there have been no studies about TL and mtCN correlation with micronutrients in the Indian context. Thus, in the present study, we have investigated the association of (i) TL and mtCN variation with age (<60 years and >60 years) and (ii) plasma folate and vitamin B12 with TL and mtCN.

Methodology

A community-based cross-sectional study was conducted on 428 (219 men and 209 women) apparently healthy subjects aged 21-88 years, stratified into two age groups: <60 years (n=242) and ≥60 years (n=186). Sociodemographic and anthropometric data were collected. Blood based biochemical estimations were carried out. Estimation of plasma folate, vitamin B12 and homocysteine was carried by RIA and HPLC. Genomic DNA was extracted from whole blood. The relative telomere length (rTL) was measured by quantitative real-time PCR (qPCR). The mitochondrial DNA copy number (mtCN) was quantified by qPCR method

Results

The gender distribution was approximately the same in both the groups (men and women were 48% and 52% in <60 years age group, 55% and 45% in ≥60 years age group respectively). Median (P_{25}-P_{75}) age of <60 years age group was 39.0 (26.0-50.0) and ≥60 years age group was 65.0 (62.0-70.0). In <60 years age group, 7% of the subjects were underweight, 32% had normal BMI, 37.3% were overweight, and 23.4% were obese, while 6.1% of the subjects were underweight, 26.5% had normal BMI, 45% were overweight, and 22.2% were obese in the ≥60 years age group. BMI was not significantly associated with age (p >0.05). The prevalence of hypertension was significantly higher in the ≥60 years age group (26%) compared to the <60 age group (12%). Around 59% of the <60 years age group and 83% of ≥60 years age group have abdominal obesity as assessed by WC. The prevalence of anemia in the <60 years age group (31.5%) was significantly higher compared to the ≥60 years age group (18%). There is no significant difference between the age groups in the case of plasma folate, but plasma vitamin B12 (p <0.001) and tHcys (p 0.017) levels were significantly higher in the ≥60 years age group. Notably, the rTL (p = 0.043) and mtCN (p <0.001) are significantly lower in the ≥60 years age group compared to the <60 years age group (Table 1).

Table 1: Anthropometric, clinical and biochemical profile of the study subjects

Parameter	<60 years (n=242) Median (P_{25}-P_{75})	≥60 years (n=186) Median (P_{25}-P_{75})	Pooled (n=428) Median (P_{25}-P_{75})	p value
Age, years	39[a] (26-50)	65[b] (62-70)	55 (35-65)	<0.001**
Males, n (%)	117[a] (48.34)	102[a] (54.83)	219 (51.16)	0.657
Females, n (%)	125[a] (51.65)	84[a] (45.16)	209 (48.83)	0.320
BMI, kg/m^2	24.2 [a](21.7-27.3)	24.7[a](22.1-27.2)	24.3 (21.8-27.2)	0.339
FBS, mg/dl	99[a](92-107)	101[a](93-110)	100 (93-109)	0.127
Hb, g/dl	12.9[a](11.7-14.3)	13.6[b](12-15)	13.4 (12-14.5)	0.003**
HbA1c , %	5.5[a] (5-5.9)	6.0[b] (5.8-6.6)	5.9 (5.3-6.2)	<0.001**
rTL	0.89[a] (0.54-1.41)	0.82[b] (0.15-1.68)	0.88 (0.4-1.54)	0.043*
mtCN	0.93[a] (0.5-1.99)	0.47[b] (0.19-0.97)	0.71 (0.29-1.65)	<0.001**
Folate, nmol/l	13.4[a] (9.3-17.6)	14.0[a] (9.3-21.8)	13.6 (9.3-19)	0.176
Vitamin B12, pmol/l	162.4[a] (110- 228)	226.8[b] (140-398)	184.5 (118-303)	<0.001**

P_{25}, 25th percentile; P_{75}, 75th percentile; BMI, body mass index; FBS, fasting blood sugar; Hb, hemoglobin; HbA1c, glycosylated hemoglobin; rTL, relative telomere length, mtCN, mitochondrial DNA copy number.
**Significantly different at p <0.01. *Significantly different at p <0.05. Values represent medians, 25thand 75thpercentiles. Significant differences (p<0.01, p<0.05)of median values between the age groups are indicated by different superscript letters (a, b).

The rTL (r = -0.201, p <0.001) and mtCN (r = -0.150, p = 0.002) showed a significant negative correlation with age (Figure 1). By gender, rTL and mtCN significantly decreased with increasing age in men, but no difference was observed in the case of women (Figure 2). There is no difference in rTL between the genders of respective age groups (Figure 2A). The mtCN of men were significantly higher when compared to women in the <60 years age group, but there was no difference

between the genders in the ≥60 years age group (Figure 2B). A significant positive correlation was observed between rTL and mtCN (r = 0.162, p <0.001) (Figure 3).

Figure 1: Correlation of (A) relative telomere length and (B) mitochondrial DNA copy number with age.

Spearman rank correlation was used to assess the correlation (r-value). While negative r value indicatesinverse relationship between the variables. Values of p <0.05 were considered significant.

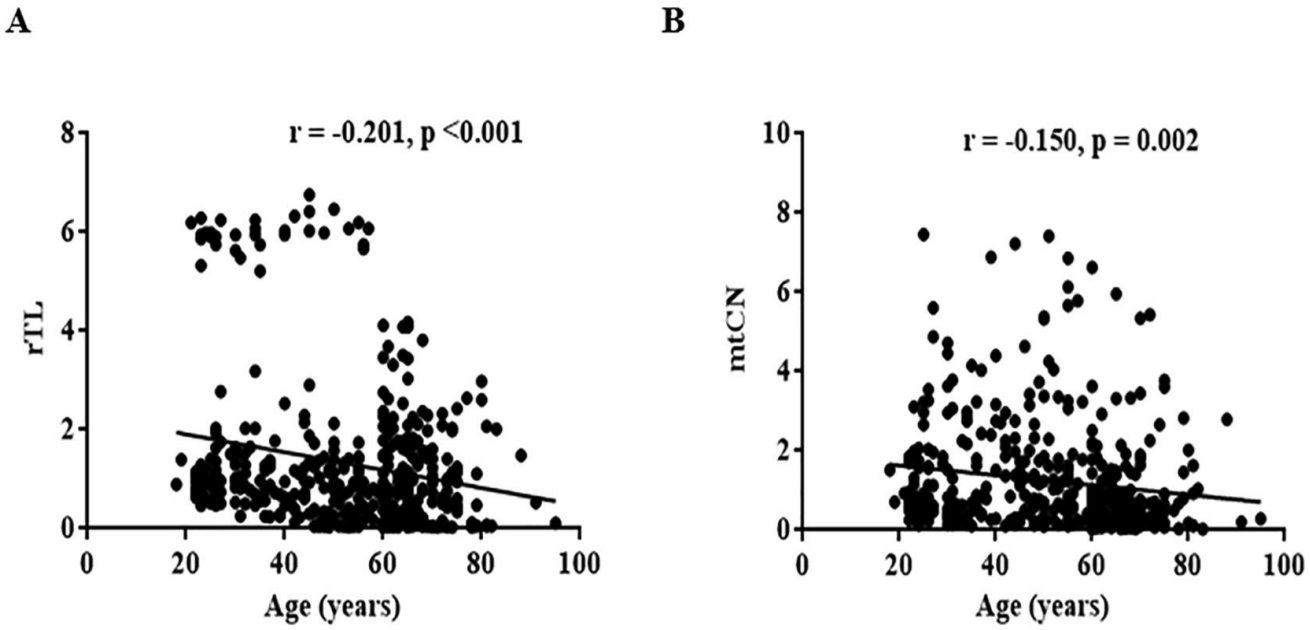


Figure 2: DNA markers (rTL and mtCN) with age and gender

rTL, relative telomere length, mtCN, mitochondrial DNA copy number

Box plot represents the 25-75th percentile; the median is shown as the heavy dark horizontal line. Vertical lines extend to the minimum and maximum values. Significant differences (p<0.05) of median values among the groups are indicated by different letters (a, b, c) above the bars.

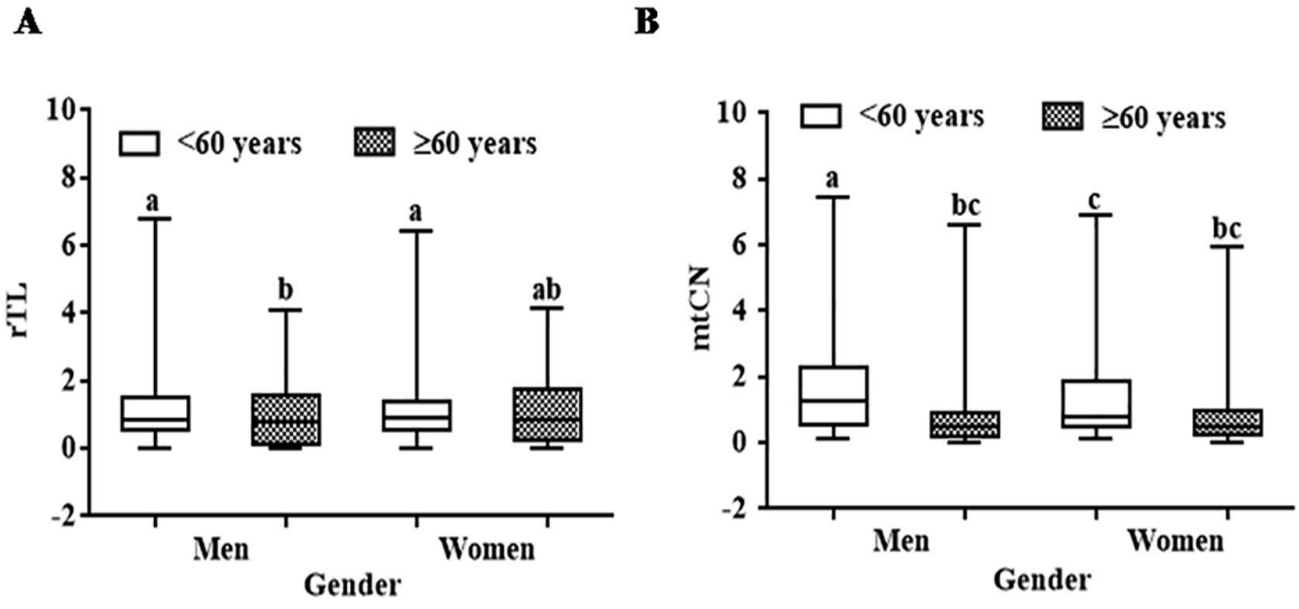
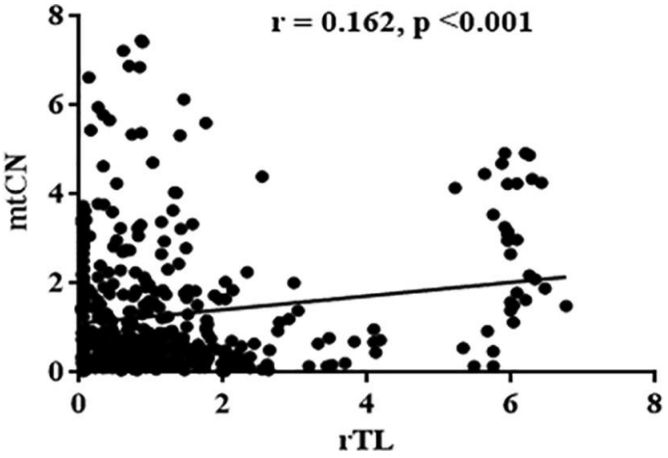


Figure 3: Correlation between relative telomere length and mitochondrial DNA copy number.

Spearman rank correlation was used to assess the correlation (r-value). While positive *r* value indicates a direct correlation between the variables. Values of p <0.05 were considered significant.



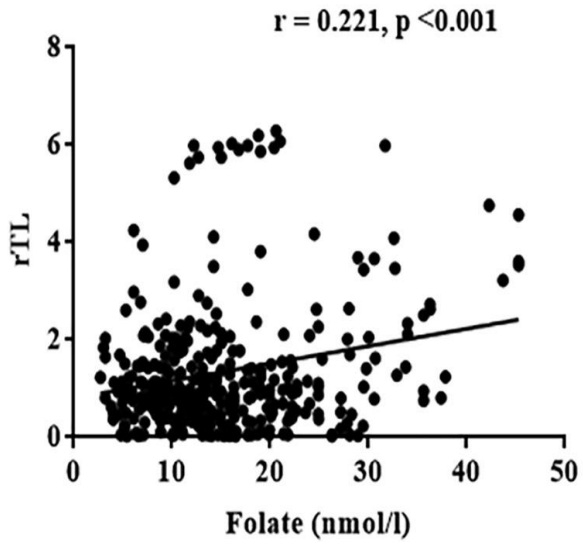
A significant positive correlation of plasma folate (r = 0.221, p <0.001) and vitamin B12 (r = 0.187, p <0.001) levels with rTL was observed (Figure 4). Likewise, a significant positive correlation was observed between the plasma folate (r = 0.202, p <0.001) and vitamin B12 (r = 0.158, p = 0.003) levels with mtCN (Figure 5). A significant positive correlation was observed between plasma folate and vitamin B12 with rTL in the ≥60 years age group, while no difference was observed in the <60 years age group.

In case of mtCN, while there was a significant positive relation with folate in both age groups, a significant positive relation with vitamin B12 in the ≥60 years no difference was observed in the <60 years age group. By gender, a significant positive correlation was observed between rTL and folate in both genders; folate and vitamin B12 with mtCN in women but not in men; vitamin B12 and rTL in men but not in women.

Figure 4: Correlation of (A) folate and (B) vitamin B12 with relative telomere length.

Spearman rank correlation was used to assess the correlation (r-value). While positive *r* value indicates a direct correlation between the variables. Values of p <0.05 were considered significant.

A



B

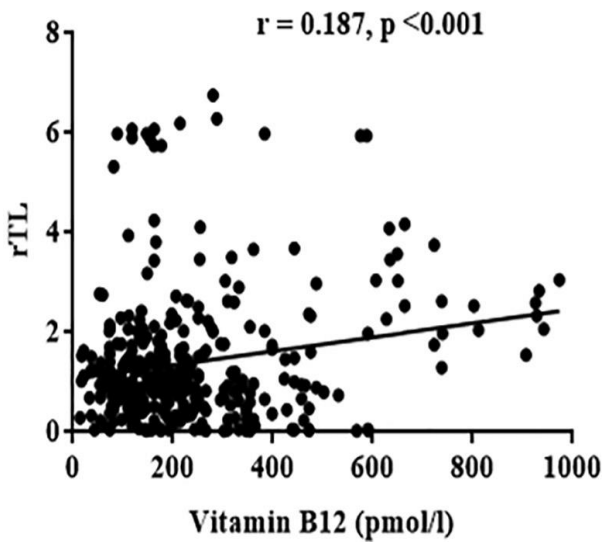
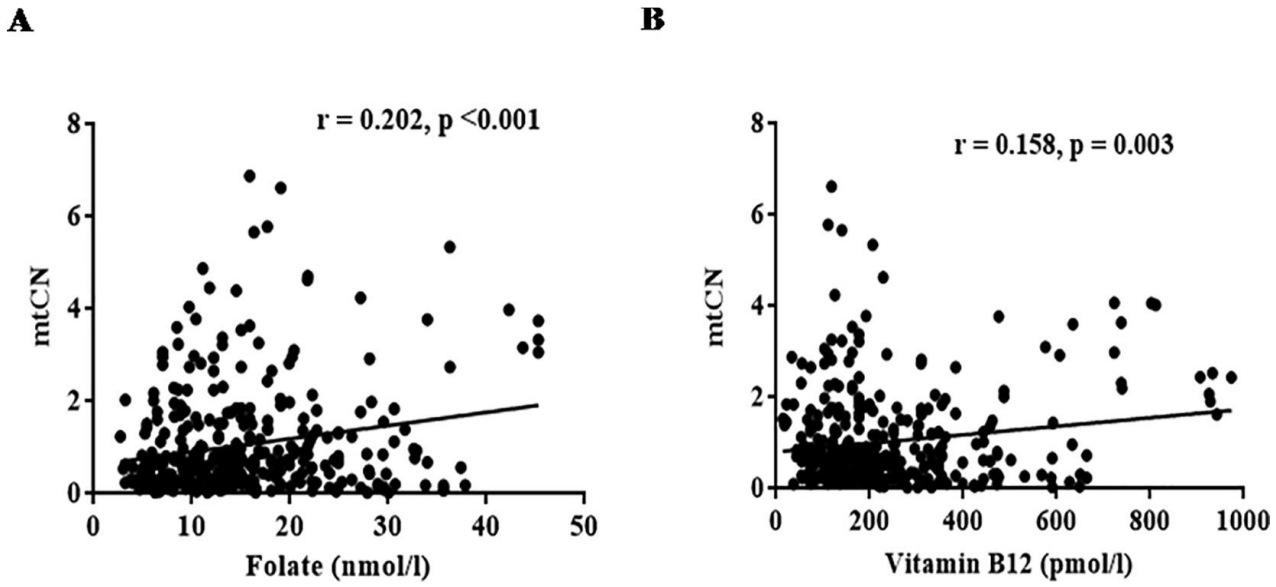


Figure 5: Correlation of (A) folate and (B) vitamin B12 with mitochondrial DNA copy number.
Spearman rank correlation was used to assess the correlation (r-value). While positive *r* value indicates a direct correlation between the variables. Values of p <0.05 were considered significant.



Conclusion

In summary, for the first time, we have shown that the rTL and mtCN decline with age and both are associated with each other in the Indian population. The plasma folate and vitamin B12 levels may influence aging by stabilizing the TL and mtCN. By deciphering the role of these factors in aging may aid in developing effective therapeutic strategies to prevent or delay age and age-associated disorders.

2. SUPPLEMENTATION OF VITAMIN B12 AMELIORATED RETINAL LESIONS IN DIABETIC RATS

Recently, we reported a high prevalence of multiple subclinical micronutrient deficiencies, dietary inadequacies, along with hyperhomocysteineamia in apparently healthy adults (30-70 years) particularly B-vitamins including vitamin-B12. However, to date, only a few studies have evaluated the possible role of nutritional factors in the development of DR. Most importantly, our earlier studies suggest that vitamin B12 deficiency could be an independent risk factor for DR.

Vitamin-B12 plays a very fundamental role in DNA synthesis, optimal haemopoiesis, neuronal and vascular functions. There are several studies signifying vitamin B12 deficiency in diabetes. Meta-analysis showed that treatment with vitamin B12 improved nerve conduction velocity in patients with diabetic peripheral neuropathy. A study reported that vitamin B12 supplementation improved nerve conduction velocity in diabetic rats by preventing impaired neural signalling of protein kinase-C and oxidative stress-induced damage. Another study concluded that exogenous vitamin B12 delayed onset of diabetic peripheral neuropathy via up-regulation of sciatic nerve IGF-1 gene expression. However, the effect of vitamin B12 supplementation in experimental

DR is unknown. Hence, in the current study, we investigated whether vitamin B12 supplementation could influence DR in diabetic rats.

Methodology

Diabetes was induced in rats with a single intraperitoneal injection of streptozotocin (STZ; 38 mg/kg) in citrate buffer (pH 4.5), whereas the control group of rats received vehicle alone. A group of diabetic rats were fed with vitamin-B12 supplemented diet and remaining half were fed normal diet similar to control group of rats. Vitamin B12 supplemented diet consists of 50 µg/kg diet, two-fold of vitamin B12 content to that normal diet (25 µg/kg diet). Body weight and fasting blood glucose levels were measured weekly till the end of 4 months of experimentation period. Animal care protocols were in accordance with and approved by the Institutional Animal Ethics Committee (IAEC). At the end of the experimentation period, overnight fasted rats were sacrificed to collect eyeballs. Four eyeballs per group were fixed in 4% paraformaldehyde for immunohistochemical analysis, and remaining were dissected to collect retina, snap-frozen in liquid nitrogen and stored at -80°C.

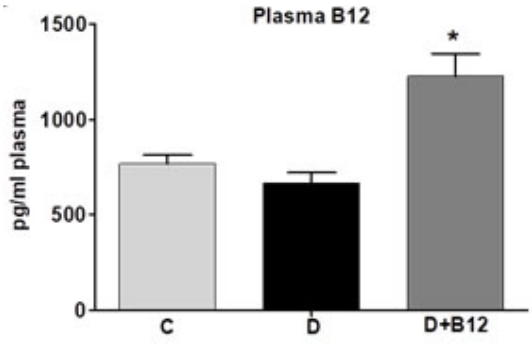
Plasma total vitamin B12 levels were analysed using a solid phase RIA kit. Plasma total homocysteine was determined by reverse phase HPLC method. Immunoblotting was performed to analyze various proteins in the retina. Tubulin served as a loading control. TUNEL assay was performed on ocular sections to investigate apoptosis in the retina.

Results

While there was no significant change in the plasma vitamin B12 levels, supplementation to diabetic rats for four months led to increased plasma-B12 levels over and above the control rats (Figure-1). The overall thickness of the retina in the diabetic rats was decreased significantly when compared with the control rats. Vitamin B12 supplementation in diabetes rats partially prevented retinal thinning (Figure 2). Figure 3 shows reduced rhodopsin (Rho) staining in the retina of diabetic rats, but vitamin B12 treatment considerably prevented loss of Rho in diabetic rats. Glial fibrillary acidic protein (GFAP) is an intermediate filament protein present in retinal glial cells. Retinal glial cells respond to the retinal injury and have been shown to be activated in diabetes. Immunofluorescence staining for GFAP on rat retinal sections showed minimal staining (red) in control rats, especially in the ganglionic cell layer and nerve fiber layer (Figure-4). In the diabetic rats, the GFAP staining spanned all the retinal layers indicating activation of Müller cells/gliosis. Nevertheless, vitamin B12 treatment in diabetic rats for four months completely prevented diabetic gliosis. Hypoxia-inducible factor 1-alpha (HIF1α) is a master regulator of cellular response to hypoxia. Vascular endothelial growth factor (VEGF) is a prominent angiogenic factor that induces vascular permeability. Immunoblotting for HIF1α and VEGF displayed higher protein levels in diabetic rats indicative of hypoxia and elevated vascular permeability in diabetic rat retina (Figure 5). Vitamin B12 intervention to diabetes rats prevented overexpression of HIF1α and VEGF.

Figure 1: Plasma vitamin-B12 levels in rats.

Data are mean±SEM. C=control, D=diabetes, D+B12=diabetic rats treated with vitamin-B12.



As ER stress was shown to cause DR pathology, hence we examined ER stress markers such as GRP78, ATF6, XBP1, CHOP and caspase-12 by immunoblotting (Figure 6), and XBP1 and CHOP by immunofluorescence. Diabetes rats showed higher levels of GRP78, ATF6 and XBP1 proteins indicative of ER stress. Immunofluorescence for XBP1 and CHOP showed higher staining (green and red respectively) in outer nuclear and inner nuclear layers of the retina in diabetic rats. Further, CHOP and caspase-12 overexpression is indicative of maladaptive ER stress, as they trigger apoptosis. Interestingly vitamin B12 feeding to diabetes rats prevented over-expression of ER stress markers in the retina. After observing maladaptive ER stress, we next examined for apoptosis in the retina. Immunoblotting for BAX protein showed higher levels in diabetic rats (Figure 6). Further, TUNEL assay was performed to confirm apoptosis, and the results showed increased TUNEL positive cells as shown in Figure 7. In case of vitamin B12 feeding to diabetes rats, considerably prevented apoptosis in the retina.

Figure-2: Retinal morphology (H&E staining) and retinal thickness of rat

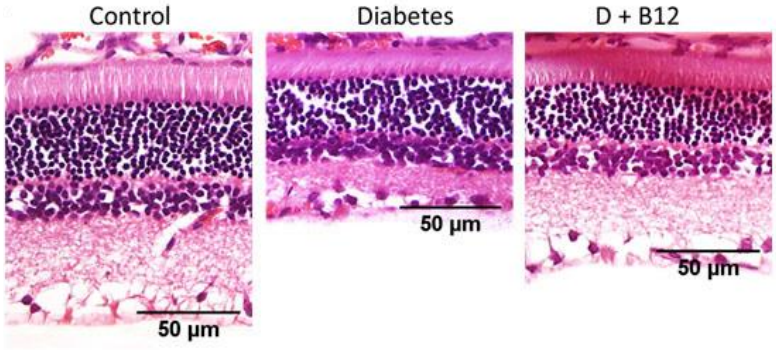


Figure-3: Immunofluorescence staining for rhodopsin in rat retina. C=control, D=diabetes, D+B12=diabetic rats treated with vitamin B12.

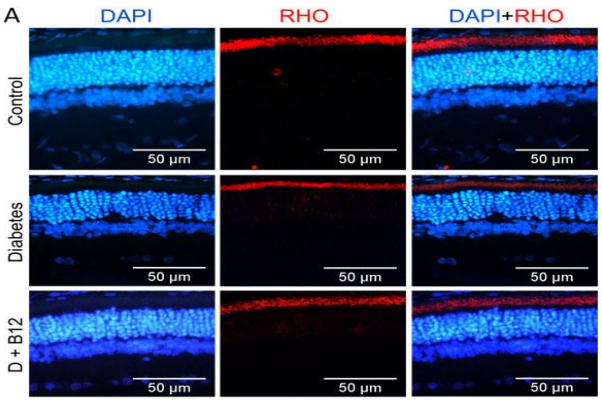


Figure 4: Immunofluorescence staining for GFAP in rat retina. C=control, D=diabetes, D+B12=diabetic rats treated with vitamin B12.

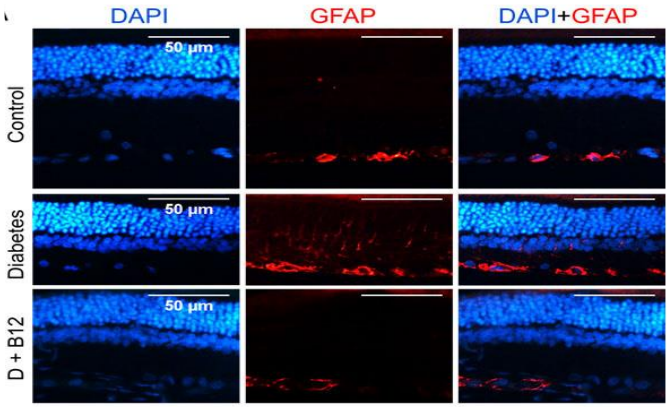


Figure 5: Immunoblotting for HIF1α and VEGF in rat retina. (A) Immunoblots for HIF1α and VEGF (B) Quantification of data. The protein expression was normalised to the tubulin and was represented as %control. Data are means±SEM. C=control, D=diabetes, D+B12=diabetic rats treated with vitamin B12.

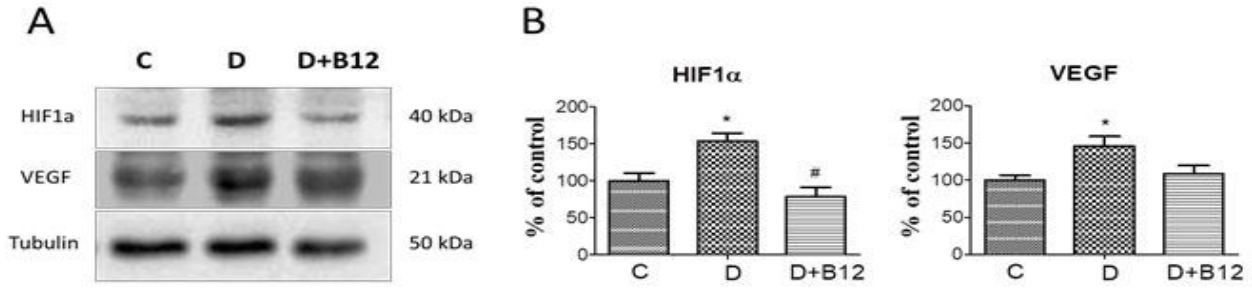


Figure 6: Immunoblotting for ER stress and apoptotic markers in rat retina. C=control, D=diabetes, D+B12=diabetic rats treated with vitamin-B12.

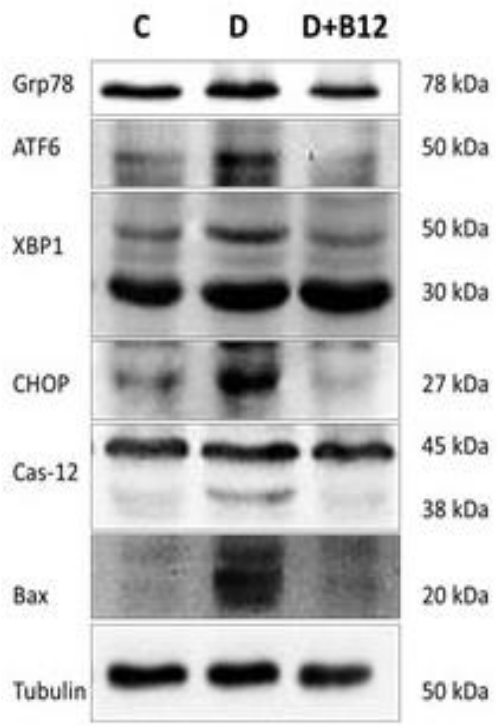
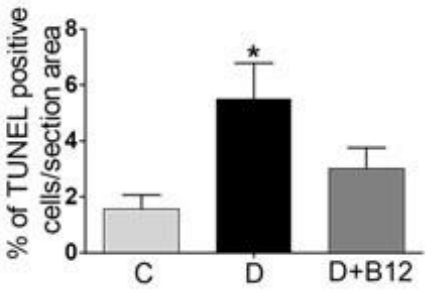


Figure 7: Number of TUNEL positive cells on rat retinal sections. Data are means ± SEM. C=control, D=diabetes, D+B12=diabetic rats treated with vitamin-B12.



Conclusion

Vitamin B12 supplementation to diabetic rats showed to be beneficial by preventing retinal hypoxia, VEGF overexpression, and ER stress-mediated cell death in the retina. Considering the general prevalence of micronutrient deficiency and its contribution to many metabolic and age-related disorders, such as diabetes, and cardiovascular diseases in India, ameliorative effects of vitamin-B12 on DR merits attention.

3. COMBINATION OF THERAPEUTIC POTENTIAL OF DIETARY COMPONENT STIGMASTEROL AND MSCS IN MANAGEMENT OF OSTEOARTHRITIS – AN *IN VIVO* AND *IN VITRO* APPROACH

Osteoarthritis (OA) is the most common joint disorder affecting about half of the aged (≥65 years) population, representing a leading musculoskeletal health and socioeconomic burden worldwide. Multiple risk factors including obesity, ageing, elevated levels of certain lipids, local and systemic inflammation predispose to OA development in humans. Till now there is no single therapeutic agent that has been deemed safe and effective for treating OA.

Combination therapy employing agents with complementary mechanisms of action has been shown to benefit effective disease management in osteoarthritis. Mesenchymal Stem Cells (MSCs) present a newer paradigm for the treatment of OA. In addition to their regenerative capacity, MSCs have also been shown to exhibit potent anti-inflammatory and immunomodulatory effects. Besides, stigmasterol has been reported to counteract the expression of the key mediators and matrix metalloproteinases involved in cartilage degradation. The current study aimed at exploring the therapeutic potential of combination of stigmasterol and MSCs in the management of osteoarthritis employing WNIN/GR-Ob mutant rats as an experimental model. The WNIN/GR-Ob rats depict biochemical and metabolic traits such as obesity, hypercholesterolemia, and impaired glucose

tolerance which form the confounding factors and contribute mechanistically to the development of OA in humans.

Methods

Exploring the Feasible Application(s) WNIN/Gr-Ob mutant rats Model for osteoarthritic Research

a) The study was approved by the Institutional Animal Ethical Committee (IAEC) (P29F/III-IAEC/NIN/12/2016/SSJ/WNIN(CG)-6F/WNIN-Gr-Ob-42F).

For standardization of baseline data, WNIN/Gr-Ob rats of varying ages (3, 6 and 9 months) were obtained from the Animal Facility, National Institute of Nutrition, Hyderabad. Age matched WNIN Wistar rats served as controls. The rats were grouped according to their age with six rats in each group.

b) Body weight, abdominal circumference and BMI were measured in the WNIN and WNIN/Gr-Ob rats. The body composition analysis to estimate fat mass and lean mass in the rats was carried out by magnetic resonance imaging using an EchoMRI (Echo Medical Systems, Houston, TX, USA) at CCMB, Hyderabad. Levels of serum TNF-α were measured in WNIN and WNIN/Gr-Ob rats at 3, 6 and 9 months using commercially available ELISA kit (PeptroTech, USA).

c) Micro-CT assessment of knee joints: was carried out in formalin fixed knee joints of the ratsusing SkyScan 1176 Micro-CT system and softwares (SkyScan, Kontich, Belgium). After scanning, knee joints were three-dimensionally reconstructed by SkyScanReCon software. For analysis of subchondral plate, the load- bearing region with an area of 1.04 x 1.04 mm^2 was selected as Region of Interest (ROI). Post micro-CT examination, the formalin fixed knee joints of the rats were decalcified at 4°C for 4-5 weeks with 0.5 M ethylenediaminetetraacetic acid (EDTA), dehydrated and embedded in paraffin by the standard method. Serial sections of tibia were prepared in the sagittal plane at 5-7µm and stained with Hematoxylin and Eosin (H&E). Images were captured using Nikon Eclipse TE2000-S Microscope (Japan).

d) For ultra-structure analysis, the tibial tissues were fixed overnight in Karnovsky's fixative, washed in PBS, dehydrated in graded series of ethanol, vacuum dried, sputter-coated. The cartilage surfaces were scanned using Hitachi S-3400N scanning electron microscope (Gomes et al, 2016).

e) For immunofluorescence studies, the chondrocytes (P1) grown on cover slips were fixed in 4% paraformaldehyde, washed with PBS, permeabilized with 50% chilled methanol, and was probed with COL2A1(1:25) as per our published protocol The mounted samples were scanned using a Leica SP5 confocal microscope.

Therapeutic potential of Human placental mesenchymal stem cells in combination with stigmestrol in averting osteoarthritic lesions.

Transplantation studies

a) Maintenance and Characterization of primary cultures of Human placental mesenchymal stem cells (hPMSCs), the hPMSCs were obtained from Manipal Institute of Regenerative Medicine as a part of the collaborative work. Mesenchymal stem cells were isolated from the placenta using the method of Semenov et al (2008) with minor modifications.

b) ***In vivo biofluorescent imaging : Tracking the homing of the transplanted hPMSCs - Pilot study***

Data were analyzed using the Living Image software (PerkinElmer, USA) to evaluate the average signal intensity of regions of interest (ROI). 4 weeks post the injections, the knee joints were collected and formalin fixed and processed for histopathology using the procedures as mentioned above. The MSCs *per se*as well as in combination with stigmasterol showed a smooth cartilage

surface. Since we could not appreciate benefits of the combinatorial approach with WNIN/Gr-Ob rats, we adopted a chemically induced rat OA model system to better appreciate the efficacy of the treatments in tissue regeneration in a shorter period of time.

c) ***Induction of osteoarthritis (OA)***

Monosodium iodoacetate (MIA; Sigma Aldrich, USA) was dissolved in sterile saline solution. The rats were anesthetized with 5% isoflurane and OA was induced with 2 mg of MIA in a total volume of 50 µl. MIA was injected intra-articularly through the patellar ligament of the right knee, using a 26-gauge needle while the rats were under anesthesia. Monosodium iodoacetate stigmasterol and indomethacin were procured from Sigma Aldrich, USA.

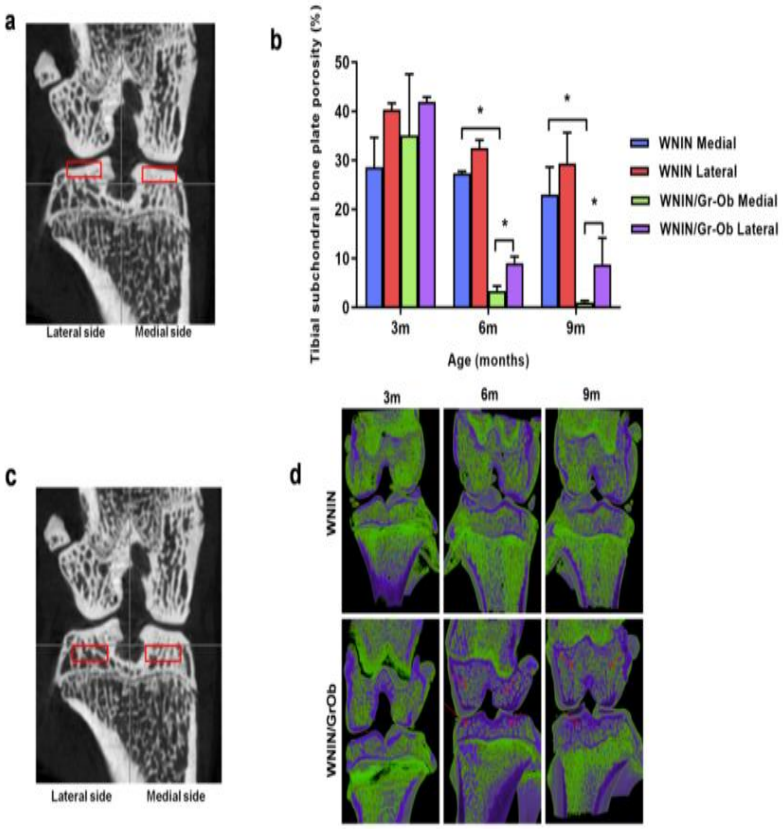
d) ***Experimental design***

The control rats did not receive MIA injection and Experimental rats demonstrated OA like lesions as evident by swelling of the knee joints and difficulty in walking. One week after the MIA injection, the OA rats were randomly divided into 5 groups and were subjected to the following treatments as follows: Untreated OA rats, OA rats treated hPMSCs (2 x10^6 cells in PBS) [OA + hPMSCs], OA rats treated with stigmasterol (20µg/ml in PBS) [OA + Stigma] , OA rats treated with a combination of hPMSCs (2 x10^6 cells) and stigmasterol (20µg/ml) [OA + hPMSCs + Stigma], OA rats treated with indomethacin (2mg/kg body weight in PBS) – a reference non-steroidal anti-inflammatory drug (NSAID) [OA + NSAID].

All injections were administered intra-articularly in a volume of 100µl using a 26-G needle. The hPMSCs (2 x10^6 cells) *per se* or in combination with stigmasterol (20µg/ml) were administered as a single dose. Stigmasterol and indomethacin were administered weekly once for 4 weeks. After the treatment regimen, the animals were euthanized, the right hind limbs were formalin fixed and evaluated by Micro-CT and histopathology as described above. The rats were also imaged using IVIS Spectrum as mentioned above to ensure homing of the cells.

Results

a) ***Micro-CT analysis of subchondral trabecular bone: Higher BV/TV in WNIN/Gr-Ob rats (Fig. 1- beside)***

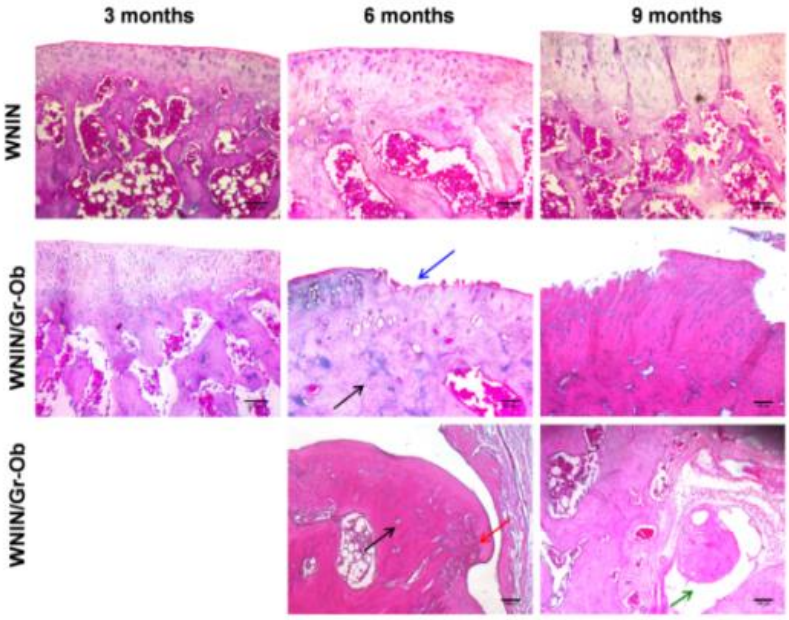


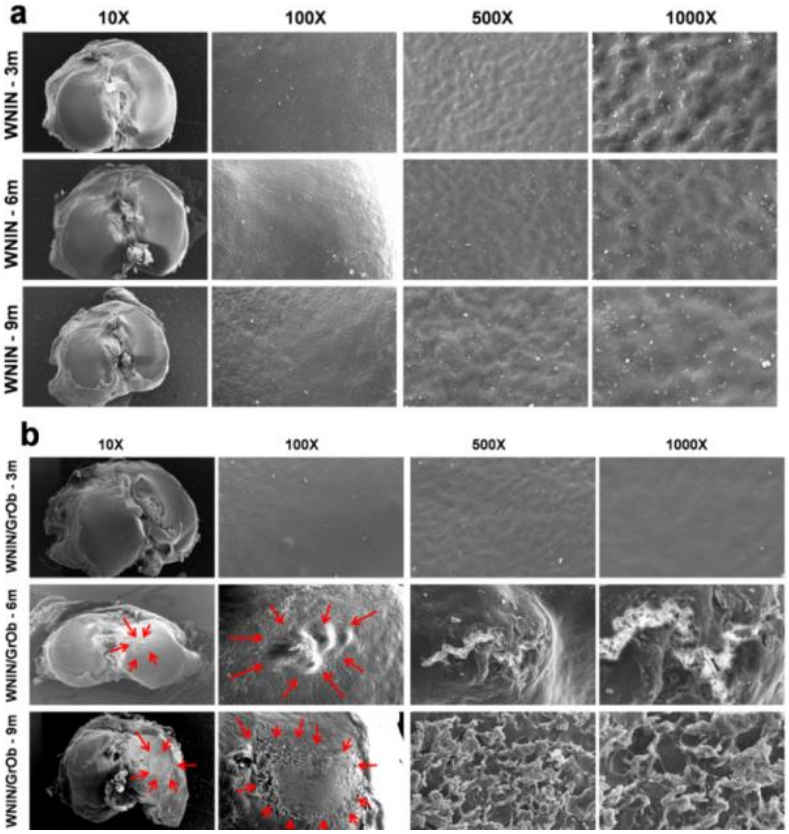
Micro-CT analysis of tibial subchondral plate and subchondral bone in WNIN and WNIN/Gr-Ob rats (a) Demographic image of coronal view of ROI selection of medial and lateral tibial subchondral bone plate; (b) Quantitative analysis of subchondral plate porosity showed significant difference between WNIN/Gr-Ob and WNIN rats on medial sides at age of 6 and 9 months, while within WNIN/Gr-Ob group, significant difference was defined between medial and lateral sides at age of 6 and 9 months; (c) Demographic image of coronal view

of ROI selection of medial and lateral tibial subchondral bone plate; (d) Reconstructed cross-sections through the intact knee joint (femur and tibia) showed significant reduction in bone porosity (green areas) and increased bone formation (purple areas) marked by red arrows in subchondral trabecular bone of WNIN/Gr-Ob rats compared to WNIN rats with at 6 and 9 months of age.

b) ***Histological findings in tibia: Cartilage erosion and subchondral sclerosis in WNIN/Gr-Ob rats (Fig. 2)***

Tibial histology with H&E staining of WNIN and WNIN/Gr-Ob rats. OA-like degenerative changes like cartilage erosion (blue arrow), subchondral sclerosis (black arrow), osteophyte formation (red arrow) (6 months) and bone cyst (green arrow) (9 months) were observed in WNIN/Gr-Ob rats with no detectable abnormalities in WNIN rats at all three age groups.



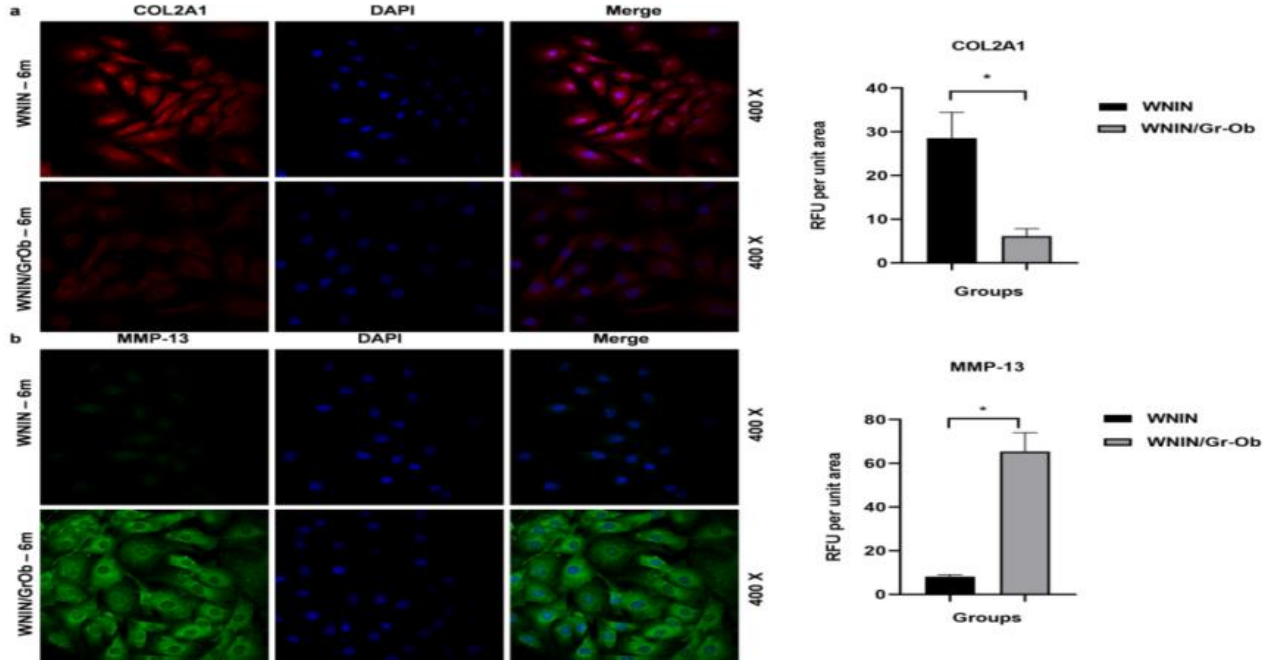


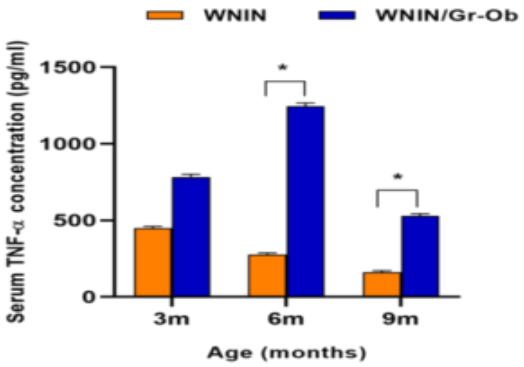
c) **Scanning electron microscopy assessment of tibial cartilage in WNIN and WNIN/Gr-Ob rats. (Fig 3)**

(a) Micrographs of WNIN tibial surface (3, 6 and 9 months) exhibited a smooth cartilage surface with no erosions/abrasions at all three age groups at different magnifications; (b). Micrographs of WNIN/Gr-Ob rats showed a smooth cartilage surface at 3 months of age while at 6 and 9 months of age exhibited a rough cartilage surface with degeneration of cartilage tissue (red arrows) resembling OA in humans.

d) **Immunofluorescence studies in primary articular chondrocytes of WNIN and WNIN/Gr-Ob rats at 6 months (Fig. 4**)

(a). Confocal imaging showed a significantly lower COL2A1 expression in WNIN/Gr-Ob articular chondrocytes at 6 months compared to WNIN group; (b) Confocal imaging showed a significantly higher expression of MMP-13 in WNIN/Gr-Ob articular chondrocytes compared to WNIN group at 6 months.

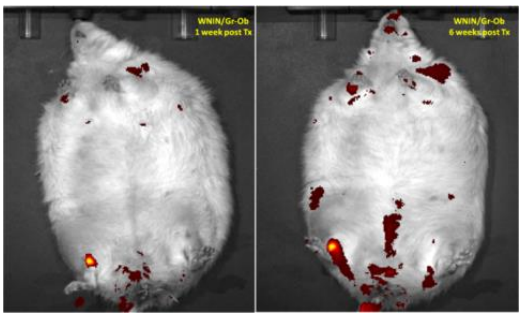




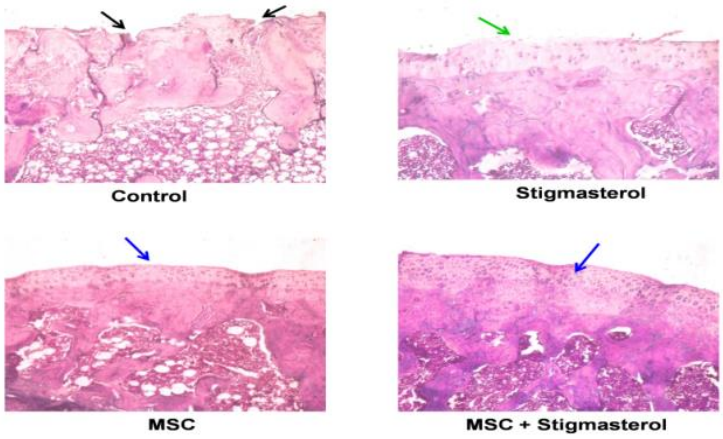
e) **Increased circulating levels of pro-inflammatory cytokine TNF-α in WNIN/Gr-Ob rats (Fig. 5)** Quantitative analysis of serum TNF-α showed a significant increase in WNIN/GrOb rats compared to WNIN rats at 6 and 9 months of age.

f) **Biofluorescent imaging in WNIN/Gr-Ob rats (Fig. 6)**

The WNIN/Gr-Ob mutant rats which were injected (intra-articularly) with hPMSCs were monitored for a period of six weeks post transplantation. The injected cells homed and were localized into the intra-articular space ensuring site specific transplantation.

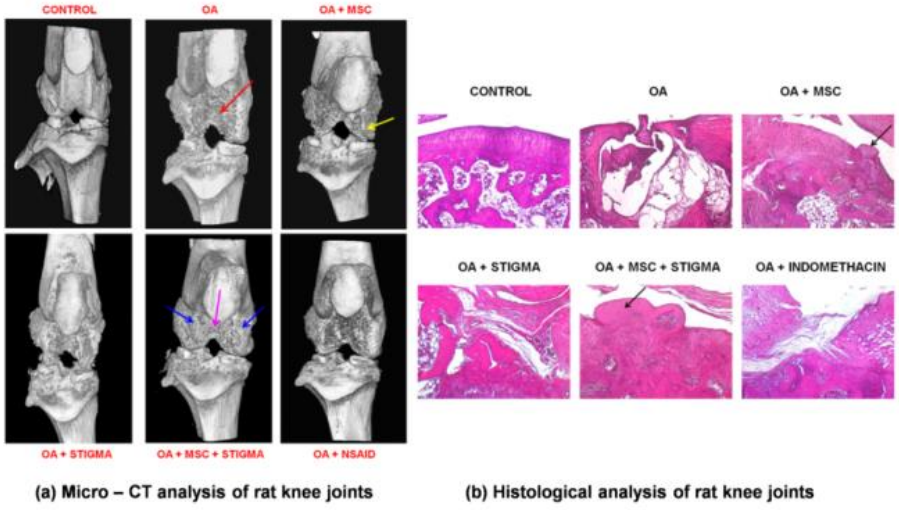


g) **Histopathological exami-nation of WNIN/Gr-Ob rat knee joints 6 weeks post MSC transplantation (Fig 7)**



The control WNIN/Gr-Ob rats exhibited severe cartilage erosion (black arrow) indicating onset of OA. The Stigmasterol treated WNIN/ Gr-Ob rats exhibited disconti-nuity in the cartilage surface with erosions (green arrow). The WNIN/Gr-Ob rats treated with hPMSCs per se and in combination with stigmasterol exhibited a smooth cartilage surface (blue arrow).

h) **Chemically Induced – Monosodium iodoacetate (MIA) rat OA model**



In control rats, the surface of the joint tissues was smooth without erosions as observed by micro-CT. In MIA induced OA rats, severe erosion of the femoral condyle (red arrows) and tracheal groove cartilage exposing the subchondral cancellous bone was observed by micro-CT. In OA rats treated with mesenchymal stem cells (MSCs) alone, minimal tissue repair/regeneration was observed (yellow arrows). OA rats treated with a combination of MSCs and Stigmasterol showed a very significant regenerative effect in the medial and lateral femoral condyles (blue arrow) as well as the trochlear groove (pink arrow) which was comparatively smoother with the subchondral cancellous bone being undetectable. These findings are in line with the histological findings where cartilage regeneration was observed (black arrows).
OA rats treated with stigmasterol per se or the non-steroidal anti-inflammatory drug (NSAID) Indomethacin did not show any significant ameliorative effects. The combination of Stigmasterol (anti-inflammatory/ chondroprotective effects) and MSCs (immunomodulatory/ anti-inflammatory/ multipotent functions) resulted in better disease modification via tissue regeneration in experimental OA in rats and offers promise to be evaluated in a clinical setting.

Inference and conclusion

- In the chemically induced rat OA model, the combination of Stigmasterol (possessing anti-inflammatory/ chondroprotective effects) and MSCs (having immunomodulatory/anti-inflammatory/ multipotent functions) resulted in better disease modification via tissue regeneration compared to the individual treatments in experimental OA in rats and offers promise to be evaluated in a clinical setting.

4. TARGETED STUDIES TO ASSESS THE ANTI-METASTATIC AND ANTI-ANGIOGENIC (LYMPH ANGIOGENESIS) POTENTIAL OF 6-GINGEROL AND 6-SHOGAOL USING BREAST CANCER CELL LINES - A 3-D CO-CULTURE APPROACH

Breast cancer (BC) is one of the major public health concerns in recent times in both developed and developing countries. There is an urgent need for a new comprehensive treatment strategy combining anti-angiogenic and anti- lymphoangiogenic agents with conventional cyto reductive treatments in the control of breast cancer.

Of great interest in this context are the beneficial effects of nutrients and functional foods with inherent antioxidant and anti-inflammatory functions, which play a pivotal role in suppressing the tumorigenic effects, isolated from Ginger (*Zingiber officinale Rosc*). Amongst the naturally occurring food ingredients, 6-Gingerol and 6-Shogaol are renowned food components known for their contribution to human health and nutrition, more so to target the mechanistic interplay between Ginger/ its derivatives (6-Gingerol and 6-Shogaol) to stop the angiogenesis/ lymphoaniogenesis and metastatic progression, which might help patients with therapeutic intervention. Till date, there are no reports in literature documenting beneficial effects of Ginger/ its derivatives (6-Gingerol and 6-Shogaol) against the lympho angiogenesis resulting in metastasis arrest.

Hypothesis

The potent anti-inflammatory and antioxidant property inherent with 6-Gingerol and 6-Shogaol would be beneficial to ameliorate the robust proliferation and angiogenesis (lymphoangiogensis) of the metastatic BCs, advocating its role in the regulation of EMT.

Objectives

- Development of 3D cultures to create *in vivo* environment by using breast cancer cell lines (MCF-7, MDA-MB-231) with endothelial cell line (HUVEC).
- To examine the potential effects of 6-Gingerol and 6-Shogaol (before and after treatment) on markers of angiogenic and lympho angiogenic (ANGPTL3, STAB1, COL4A3, NRP1, NRP2, VEGFC, VEGFD, LYVE1, PROX1, PDGF and *SERPINF1*) genes by qRT-PCR analysis using breast cancer cell line 3D Co-cultures (fibroblast, endothelial cells, lympho endothelial cells, MCF-7, MDA-MB-231) in comparison with non-tumorigenic cell line (MCF10A).
- Detection of above-mentioned proteins by Flow cytometry using 3D Co-cultured cell lines

Methodology

a) ***Breast cancer cell lines and maintenance*:** Breast cancer cell lines were obtained from the National Center for Cell Science (NCCS), Pune, India. MCF-7 and MDA-MB-231 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotics in tissue culture flasks under a humidifying atmosphere containing 5% CO2 and 95% air at 37°C. The cells were seeded at a density of 5×10^4 cells/mL, then washing the monolayers with phosphate-buffered saline (PBS) followed by a brief incubation with trypsin/EDTA.

b) ***Establishment of 3D cultures / Co- cultures of different celllines*:** 3D Co-cultures were established.

c) ***Cell Viability Assay:*** Briefly, the dose-dependent effects of 6-Gingerol and 6-Shogaol on the viability of MCF-10A, MCF-7 and MDA-MB-231 cell lines in combinations lymphoendothelial cell lines were done by trypan blue dye exclusion assay. The effect of 6-Gingerol and 6-Shogal on growth inhibition were assessed as % of cell viability, where control untreated cells will be taken as 100 % viable. All experiments were repeated three times for confirmation of the results.

d) ***Cytotoxic test in 6-Gingerol and 6-Shogaol treated breast cancer cell lines:*** Cytotoxic effects at various concentrations of 6-Gingerol and 6-Shogaol were tested on cell lines using MTT assay. The IC50 expressed as the extract concentration in µg/ml that caused a 50% inhibition of growth of cell lines were deduced.

e) ***Cell migration or cell invasion assay:*** Boyden chamber cell migration/invasion test can be used to determine the migratory response of breast cancer 3 D Co-culture cells before and after treatment of 6-Gingerol and 6-Shogaol to know the formation of angiogenesis and metastasis. The breast cancer cell lines were placed on the upper layer of a cell permeable filter and permitted to migrate in response to a test factor placed in the medium below the filter (5-8µm).

f) ***RNA isolation (TRIzol method*):** RNA was isolated by std methods. The purity was estimated with the help of Nano drop 1000 (Thermo scientific), and RT PCR was followed, Fold change of expression calculated by $2^{-\Delta\Delta Ct}$ method.

g) ***Estimation of protein by flow cytometry*:** The procedures were well standardized. Cells were washed 2 times with 1X PBS at 4°C and aliquoted as number 1-2x10^6 into each tube, 1 mL blocking buffer, vortexed briefly and incubated on ice for 30 min followed by centrifugation. Subsequently aspirate supernatant, cell pellet resuspended with 125 µL FACS buffer containing diluted primary antibody as per manufacturer's recommendations.

Results

a) **Cell viability**: MTT assay

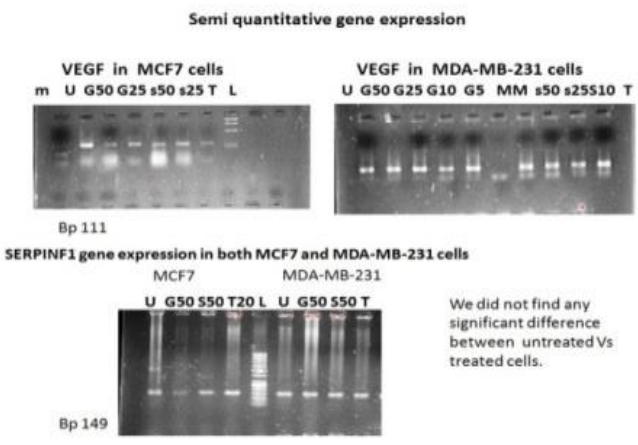
***Observation: On MCF 7*:** MTT results had shown that 31.25 µM concentration of 6-Gingerol can inhibit the 47% of cell inhibition and 28.45µM concentration of 6-shogaol can inhibit the 50% of cell inhibition.

Observation: MDA-MB-231 cells: MTT results had shown that 31.25 µM of 6-Gingerol and 25µM concentration of 6-Shogaol can inhibit 50% of the cell reduction.

Cell cycle analysis: To elucidate the mechanism of 6-Gingerol and 6-Shogaol on cell cycle arrest mechanism, we treated MCF7 and MDA-MB-231 cells with Ginger compounds (6-Gingerol and 6-Shogaol) with IC50 concentrations and further analyzed by flow cytometry.

Results: Cell cycle analysis revealed that 6-Gingerol (25µM) and 6-Shogaol (10 µM) on MCF7 cells arrested in G1 phase of the cell cycle. However, the data with MDA-MB-231 cells showed no difference.

***Apoptosis assay*:** Apoptosis assay was performed with Annex in V assay kit. This assay revealed that around 35% and 26.7% of the cells showed apoptosis with 6-



gingerol and 6-Shogaol samples as compared to controls (0.2%). This indicated that around 30% of the cancer cells can be inhibited by ginger compounds.

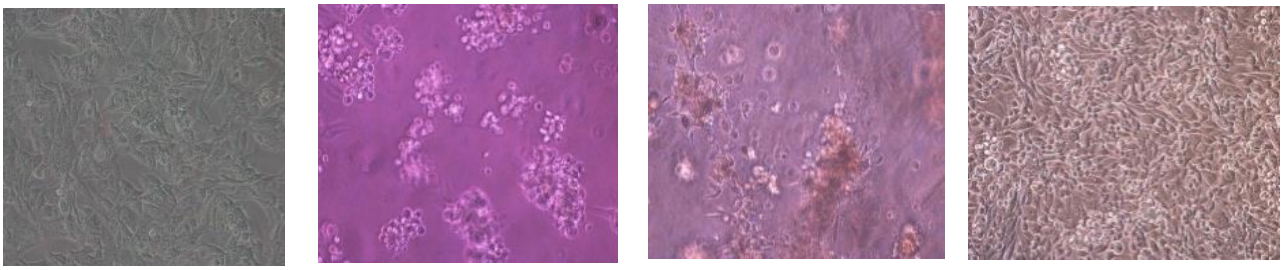
VEGFC expression in MDA-Mb-231 cells: VEGFC (vascular endothelial growth factor) is important marker for vascular genesis (Angiogenesis), cell proliferation and migration. Hence, we selected to check VEGFC expression through quantitative method (Flow cytometry) in treated with 6-Gingerol and 6-shogaol. VEGFC expression was decreased in 6-Gingerol and 6-Shogaol treated MDA-MB-231 cells samples as compared to untreated MDA-MB-231 cells. These results indicate that 6-Gingerol and 6-Shogaol inhibit the angiogenesis mechanism through VEGF signaling.

VEGFc expression in MDA-MB-231 cells

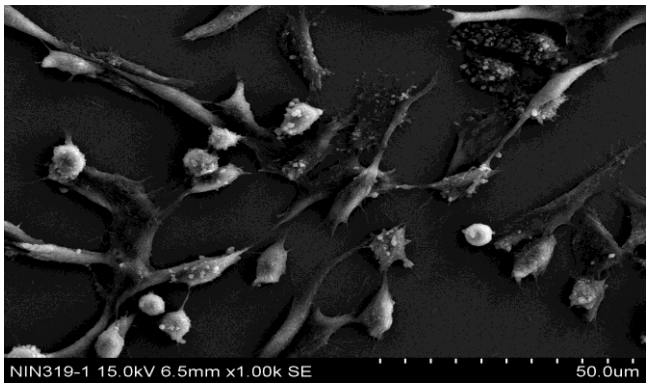
Untreated : 97%	**Tamoxifen: 89.5%**
Gingerol low : 84.2%	Shogaol low 84.9%
Gingerol IC50: 66.5%	Shogaol IC50: 91.6%
Gingerol high: 67.6%	Shogaol high conce: 89.9%

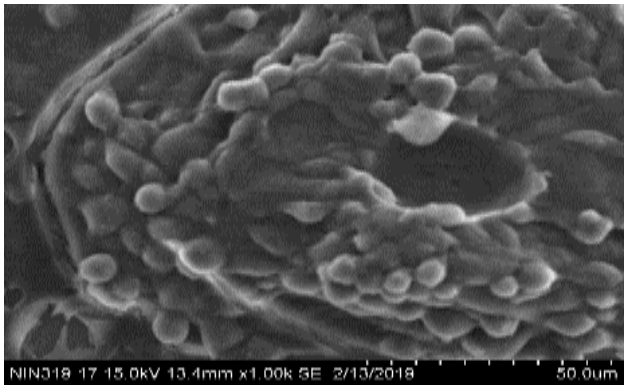
The difference between the monolayer cultures and 3D cultures of MCF7 cells and *MDA-MB-* ***231 cells :*** MCF7 and MDA-MB-231 cells are grown in the ECM gel from ***Engelbreth-Holm-Swarm murine sarcoma*** consist of laminin as a major component, collagen type IV, heparin sulfate proteoglycan, entactin, and other minor components to develop 3 D cultures. MCF7 and MDA-MB-231 cells were observed 6 days with changing media after than these tumor spheres are treated with 6-Gingerol and 6-Shoggaol and fixed the cells for marker expression through flow cytometry and isolated RNA for expression analysis.

MCF7 cellMDA-MB-231



SEM Analysis MDA-MB-231 and MCF, 3D cultures and formation of tumor sphere





Molecular Docking Studies: The selected proteins and ginger compounds (ligand) interactions were observed by docking studies (Autodock vina 2.0). In *silico* analysis suggested that the role of ginger compounds (6-Gingerol and 6-Shogaol) as growth inhibitors and modulators of lympho angiogenesis, angiogenesis (VEGF-A, VEGF-C, Nrp2, Agiopoietin-2, PDGF-B, *SERPINF1*, KDR..Etc) which are involved in metastatic progression of breast cancer. The q PCR primers are strandized for selected genes involved in angiogenesis and metastasis. The *in silico* approach might

be useful for therapeutic intervention of novel drugs and control of angiogenesis (lympho angiogenesis) after clinical trials on cancer patients.

Inference and conclusions

The present study demonstrated the beneficial effects of Ginger compounds (6-Gingerol and 6-Shogaol) in suppressing the angiogenesis and metastasis in breast cancer by modulating the genes involved in this mechanisms.

5. BENEFICIALS EFFECTS OF METFORMIN IN COMBINATION WITH VITAMIN B12 IN ALLEVIATING HIGH FAT MILEU

Metformin is being prescribed worldwide for diabetic care and the treatment. Over a long period, the prevalence of metformin-induced vitamin B12 deficiency may have also significantly increased. More importantly recent study shows that it activates an enzyme called AMPK (AMP-activated protein kinase) that plays an important role in insulin signaling, systematic energy balance, and the metabolism of glucose and fats. Activation of AMPK via inhibition of STAT 3 is one mechanism that may explain why diabetics prescribed metformin have sharply lower cancer rates. We therefore set out i) to study the effect of metformin on human placental derived mesenchymal stem cells in vitro under palmitate induced High fat diet to mimic the obese milieu ii) and further to estimate the per se role or combined effects of Metformin and Vit B12 to under adipogenesis and angiogenesis

Methodology and Results

a) Human placental mesenchymal stem cells (hPMSCs), characterization

The hPMSCs were obtained from Manipal Institute of Regenerative Medicine as a part of the collaborative work. Mesenchymal stem cells were isolated from the placenta using the method suggested by Semenov et al (2008) with minor modifications. The hPMSCs were maintained in Dulbecco's Modified Essential Medium/ Ham's F12 (1:1) (Invitrogen, CA, USA), supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher, Scientific, CA, USA), penicillin (100 units/ml) and streptomycin (100 µg/ml) at 37°C, 5% CO_2 and 95% humidity in a CO_2 incubator. The isolated cells were characterized for MSCs specific markers such as CD90 & CD105 using a BD FACSAria II flow cytometer and the data analyzed using FACS Diva software. The hPMSCs were also evaluated for their multi-lineage differentiation potential (adipo, chondro and osteogenic lineages) using commercial kits (Life Technologies, Gibco).

b) The experimental design

Group	Description
Control	hPMSCs
B12	hPMSC + Vitamin B12 (50 mM)
Met	hPMSC + Metformin (50 mM)
Met + B12	hPMSC + Metformin (50 mM)+ Vitamin B12 (50 mM)
Pal	hPMSC + Palmitate (500 µM)
Pal + B12	hPMSC + Palmitate (500 µM) + Vitamin B12 (50 mM)
Pal + Met	hPMSC + Palmitate (500 µM) + Metformin (50 mM)
Pal + Met + B12	hPMSC + Palmitate (500 µM) + Metformin (50 mM)+ Vitamin B12 (50 mM)

c) Approach and Results

The hPMSCs were grown in 6 well plates in DMEM/F12 medium containing 10 % fetal bovine serum, L-glutamine, penicillin/streptomycin (1X) at 37°C in a 5% CO_2 humdified incubator until the cells reached 100% confluency. Following differentiation (adipocytes) the medium was replaced with fresh Adipogenesis Induction/ Maintenance Medium [DMEM/F-12 medium containing a final concentration of 10% heat-inactivated FBS, Insulin (10 µg/mL), P/S (1X)] every 2-3 days for 21 days. The cells were fixed at various time intervals (Day 0, Day 7, Day 14 and Day 21) for Oil Red O staining and RNA extraction for further analysis.

h) Oil Red O staining and quantification

At the end of each experimental period, the cell cultures were washed with PBS 0.1M pH 7.4, fixed for 20 min with 4% formalin in PBS 0.05M. Cells were washed with sterile double distilled water and subsequently with 60% isopropanol for 2 min and stained with a filtered 0.35% Oil Red O solution in 60% isopropanol for 10 min at room temperature. Then, cells were washed again with sterile double distilled water.

Triglyceride content with treatment: There was a significant increase in triglyceride content in palmitate treated group and upon treatment with metformin and vitamin B12, there was a significant reduction in levels of triglyceride released.

e) Leptin levels with treatment: The levels of leptin released into the (i) cell culture medium (expressed as pg/ml) and (ii) extracted from cell lysates (expressed as pg/mg protein) were estimated using ELISA from samples collected at various time points. The levels were not significant when compared among various groups.

f) Estmation of Glucokinase and Pyruvate Kinase activity in cell lysates

Assessment of Glucokinase (GK) and pyruvate kinase (PK) activity were as per the standard method (Teslaa T et al., 2014). The specific activity of glucokinase is expressed as nM of NAPD formed per mg protein/min.

Results

There was a significant decrease in GK and PK activity observed during differentiation from day-0 to day-21, indicating the metabolic shift compared to control.

Inference and conclusion

- Treatment with metformin was protective to the obesogenic milieu and negated high levels of Triglyceride content, although leptin levels showed a decrease was not statistically significant. Supplementation with Vit B12 was not very beneficial.
- However, combination of Vit B12 with Metformin was able to increase the energy status of the cells evidenced by glucokinase and pyruvate kinase pathways.

6. FEASIBILITY OF MESENCHYMAL STEM CELLS (MSCS) OF HUMAN PERINATAL ORIGIN TO AMELIORATE TYPE 2 DIABETES WITH INSULIN RESISTANCE IN WNIN/GR-OB MUTANT RAT MODEL SYSTEM

Despite multiple drugs being used in the management of obesity/preclinical diabetes, there are still several side effects and need to be taken into account. Hence, alternate approach such as targeting Mesenchymal therapy to improve insulin resistance and inflammation underlining Obesity/preclinical diabetes become promising.

Hypothesis: Multiple intramuscular injections (IM) of MSCs derived from hPDMSCs to WNIN/GR-Ob (Mutants) rat model system with IR, would negate the inflammatory response due to its inherent paracrine and autocrine effects.

Aims & Objectives

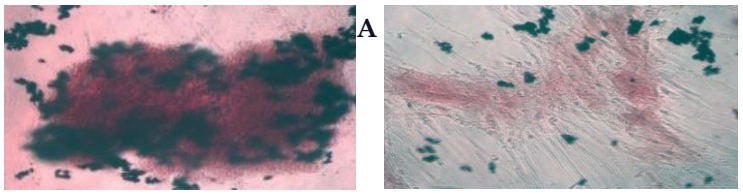
- To explore the possible therapeutic effect of MSCs of perinatal origin hPDMSCs, in mutant rats/ WNIN/GR-Ob, and Parental Control (Controls) by evaluating their effect on glycemic status and other parameters related to IR.
- These will be addressed in mutants of 6 months age due to their inflammatory Milieu.
- *Invivo* imaging of the injected MSCs to assess the homing sites.

Methods and Results

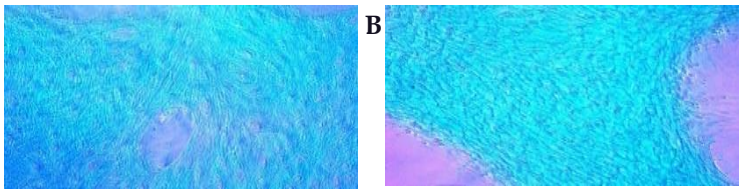
a) Characterization Placental–derived MSCs.

Multipotent functions: Cells demonstrated multipotent functions**,** using the specific cocktail as indicated below.

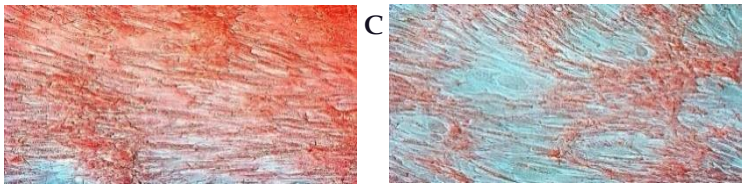
Induction of adipogenic, chondrogenic, osteogenic differentiation



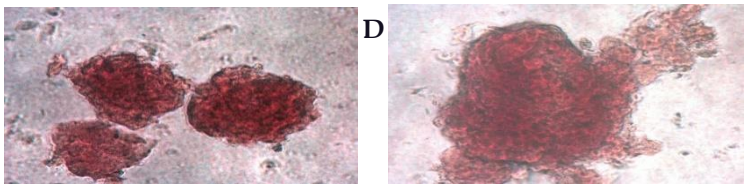
Adipocyte lineage/ Oil Red O



Chondrocytes/Alcin Blue



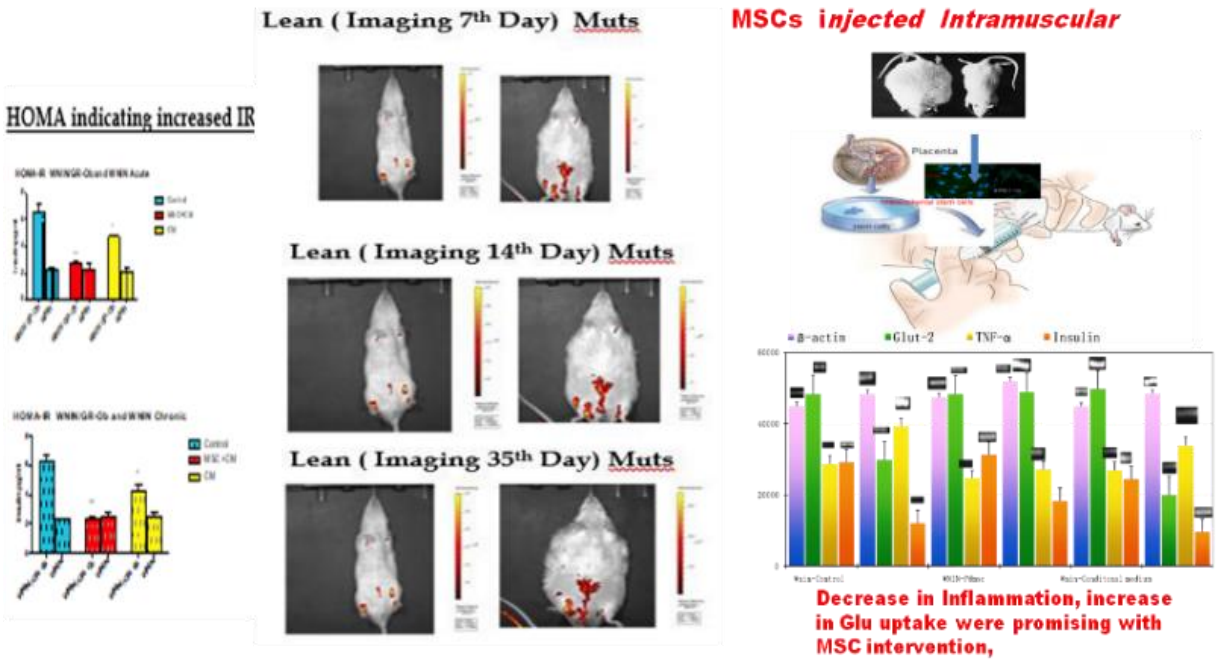
Osteogenic/Alzarin Red



Islet Staining /DTZ staining

b) ***In vivo tracking of placental derived mesenchymal stem cell (Pd-MSC):***

Cells after 4th passage were harvested, counted and stained with one of membrane fluorescent dyes: DID. Placental derived mesenchymal stem cells stained with DID suspended in 100 µl of phosphate-buffered saline (PBS) were injected into the muscular region of rats.



Interestingly, data show that the cells specifically homed near the visceral site in the Mut rats as compared to Controls, which were localized at the site of injection.

Glucose (2-NBDG) uptake study by flow cytometer

Adipocytes and myotubes have been isolated according to the pervious protocols. Where adipose tissue was subjected collagen V digestion for 30-45 min at room temperature and passed through 100µ filtration sieve and spun at 1200 rpm for 5 min and collected the SVF. SVF is then treated with 2-NBDG at 10µm/ml concentration for 10 min and washed with PBS and analysed further using flow cytometer. Similarly skeletal muscle tissue was subjected for tissue digestion using collagen II and myotubes were collected after the enzymatic digestion and treated with 2-NBDG for flow cytometer analysis.

Results: From the above results It is clear that PMSCs increased uptake of glucose in myotubes and adipocytes from Mut rats as compared to Controls.

Glycolysis enzyme activity assay: *Hexokinase and Pyruvate Kinase:*

Results: The decrease in the activity of hexokinase ,pyruvate kinase, fructose-1, 6-bisphosphatase has been linked to insulin resistance and obesity in experimental animal models of diabetes. In the present study, the reduced activities of both HK and PK have improved after the admiration of PdMSCs from Mut rats as compared to Controls.

Flow cytometric analysis of CD11b: Adipose tissue was subjected to collagen V digestion for 30-45 min at room temperature and passed through 100µ filtration sieve and spun at 1200 rpm for 5 min and collected the SVF. SVF is then treated with CD11b at 10µl / 10000 cells for 10 min and washed with PBS and analysed further using flow cytometer

Results: CD11b/macrophage recruitment was greatly decreased in the Mut rats which are treated PdMSC as compared to Controls.

Inference and Conclusions

Salient finding:

- MSCs localized predominantly in visceral adipose tissue as compared to subcutaneous.
- MSC improved insulin sensitivity as well as negated the adipocyte inflammation.as compared to Controls.

7. EFFECT OF ALOE VERA EXTRACT ON DIABETES AND OBESITY IN WNIN/GR-OB RATS

In the present study, we evaluated the anti-diabetic and anti-obesity potential of *Aloe vera* through the restoration of beta-cell mass and its function, effect on blood lipid profile and changes in body composition monitored through DXA scan in WNIN/GR-Ob rats with STZ induced diabetes which is an ideal model to study Frank diabetes as well as obesity, as evident from its altered physiological and biochemical parameters, and attempt to elucidate the mechanism by which *Aloe vera* imparts its therapeutic benefits.

Objectives

1. To evaluate the ability of *Aloe vera* extract to restore the function and mass of pancreatic b-cells in WNIN/GR-Ob rats.
2. To evaluate the anti-adipogenic activity of the *Aloe vera* extract.

Methods: The animals were divided into five groups as follows:

Groups	Animals
GR-Ob rats	6
GR-Ob + STZ	6
GR-Ob + STZ+ Aloe vera extract	6
GR-Ob + STZ + Sitagliptin	6
GR-Ob + Aloe vera	6
Total	**30**

Recommended Dose: Streptozotocin (STZ): 35mg/kgbw; Aloe vera Extract: 300 mg/kg bw; Sitagliptin: 10 mg/kg bw. After 4 weeks the following parameters were estimated:

Effect of four weeks' daily dose administration of A.vera and Sitagliptin on fasting blood glucose and serum insulin levels of WNIN/GR-Ob streptozotocin-induced diabetic rats.

STZ treatment successfully induced hyperglycemia in rats. The fasting blood glucose was elevated throughout the trial (* $p<0.05$) and insulin levels (* $p<0.05$) were reduced when compared to the control. Both A.vera extract (300mg/kg bw) and Sitagliptin (10mg/kg bw) showed a significant (<0.05) reduction in the blood glucose levels and increased insulin levels after 28 days of treatment, but not enough to restore it to the normal levels .

Effect of four weeks daily dose administration of A.vera and Sitagliptin on DPP-IV activity and active GLP-1 levels

In the present study, DPP-IV activity has significantly (** p< 0.001) increased in diabetic control as compared to GR-Ob control. Sitagliptin group (positive control) significantly (* p< 0.05) reduced the DPP-IV activity when compared to diabetic control. While, administration of *A.ve*ra extract for four weeks has reduced DPP-IV activity, but not enough to restore it to normal levels. While there was an increase in the active GLP-1 upon administration of Sitagliptin and A.vera extract, the differences were not statistically significant.

Effect of four weeks daily dose administration of A.vera and Sitagliptin on HOMA-IR and HOMA-β

The increase in insulin resistance was observed in both control and diabetic control, with a significant decrease in β- cell function in the diabetic control group based on HOMA. Oral administration of *A.vera* and Sitagliptin has decreased HOMA-IR but the difference was not statistically significant, with a significant increase in HOMA-β. GR-Ob control group receiving A.vera treatment showed a decrease in HOMA-IR when compared to GR-Ob control, while β cell function (HOMA- β) did not show a significant difference.

Primary Islet Cell Cultures

Islet cell clusters after digestion with collagenase (Type-V) and observed using ACT-2U/1.7 version software attached to Nikon inverted microscope (TE-2000s). Islet cell integrity was assessed using DTZ stained (crimson red) islet cell clusters Fig 6B and was observed using ACT-2U/1.7 version software attached to Nikon inverted microscope (TE-2000s). The average area of islets was measured using Image J software and significant reduction was observed in the size of islets in STZ induced diabetic rats. However, no significant difference was observed in the treated group.

SEM Analysis

The cell surface showed increased structural irregularities upon streptozotocin induction. However, the cell surface was relatively smooth upon treatment with *A.vera* and Sitagliptin as observed in the control group. The diameter of beta cells calculated through SEM.

Glucose stimulated insulin Secretion Assay

Freshly isolated size-matched islets from GR-Ob control, Diabetic control (DC) and DC-Sitagliptin and DC-A.vera animals (4 weeks treatment) were tested *in vitro* in static glucose-stimulated insulin secretion (GSIS) assay.

Islets from GR-Ob control animals displayed steep increase in insulin secretion upon high glucose stimulation while islets from the DC animals, in sharp contrast, displayed a weak insulin secretory response (95% decrease when compared to GR-Ob control *p<0.05) indicating a significant loss of β-cell sensitivity to glucose. However, islets from the DC-Sitagliptin displayed 60% and DC-A.vera displayed a 57% increase in insulin secretory response at basal challenge and 68% and 58% increase at stimulated glucose challenge respectively. When compared to that observed in islets of diabetic control, indicating there might be an improvement in β-cell sensitivity to glucose upon treatment with A.vera.

Effect of four weeks daily dose administration of A.vera and Sitagliptin on lipid profile

There was a significant increase in the hyperlipidemic parameters such as serum triglycerides, VLDL levels in diabetic control, compared with levels in corresponding control groups. Upon treatment

with *A.vera* (* p< 0.05) and Sitagliptin (** p< 0.001), there was a significant decrease in triglyceride and VLDL levels. TG/ HDL ratio, which is an indicator for insulin resistance was significantly higher in diabetic control and on treatment with A.vera and Sitagliptin it has reduced significantly.

Body Composition

DXA scan showed that there was a significant difference in the lean body mass, fat mass and fat% between the groups. GR-Ob control group had significantly higher fat mass, fat %, and low lean body mass. In diabetic control, LBM was reduced further as compared to GR-Ob control groups and there was a decrease in fat mass as well as fat %. However, there was a significant increase in lean body mass upon the administration of A.vera and Sitagliptin. Interestingly, fat % reduced in the treated groups as compared to control and diabetic control, but fat mass increased as an age-dependent effect, even though it was lower than the GR-Ob control group.

To assess the degree of visceral obesity, these parameters were also calculated in the abdominal cavity, especially the retroperitoneal region. The lean body mass increased significantly in the groups treated with A.vera and Sitagliptin, and fat mass increased as an age-dependent effect. However, there was no difference observed in fat % among diabetic control and treated groups, even though it was less than the GR-Ob control group.

Inference and Conclusion

The present study has demonstrated that *Aloe vera* extract could reduce FBG levels with a concomitant increase in the insulin levels, ameliorate the blood lipid profile, improve insulin sensitivity in GR-Ob rats and reduce DPP-IV activity.

8. CHARACTERIZATION OF REPRODUCTIVE FUNCTION AND EPIGENETIC CHANGES DURING OMEGA-3 (N-3) FATTY ACID DEFICIENCY IN PREGNANT MICE

Intakes of maternal n-3 PUFAs during pregnancy and lactation are much lower in Indian as compared with the developed nations. Levels of these fatty acids are further reduced by higher intake of n-6 PUFA rich oils that possibly leads to the n-3 deficiency. Excess intake of n-6 PUFAs negatively regulates the endogenous ability to synthesize and incorporates n-3 PUFAs in tissues, particularly when their requirement is high during development. The effects of increasing LA (n-6) and decreasing ALA (n-3) over generations on development and epigenetic functions of the placenta have not been studied earlier. Creating a deficiency of n-3 fatty acids is ethically not acceptable in humans, therefore, a model of dietary n-3 fatty acid deprivation in mice was used.

This work aimed to investigate the impacts of long-term deficiency of n-3 fatty acids on placental function with reference to its vascular development, and epigenetic functionalities using dietary deprived model of n-3 fatty acid deficiency in mice.

Methods

All the procedures involved in the animal experiments were conducted as per the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA),

Government of India. Institutional animal ethical committee approved the research protocol (No. NCLAS/IAEC/02/2017). The diets were formulated to ensure that each mouse receives the same calories and nutrients but variable amounts of n-3 and n-6 fatty acids in the total mixture of PUFA. The weanling female mice were randomly divided into two groups and provided diet as n-3 deficient (0.13% energy from ALA; n-6: n-3 PUFA= 50:1) and n-3 sufficient (2.26% energy from ALA; n-6: n-3 PUFA= 2:1). After confirming n-3 PUFA deficiency, age matched male mice (chow fed) were introduced (male to female ratio 1:2) for mating. Few pregnant mice were euthanized to analyze uterine artery remodelling (gD 8.5-12.5) and analysis of placenta (gD14.5-17.5) and liver. Remaining pregnant mice were continued on similar diet and proceed to next generation. F1 female pups were continued to follow the similar diets and mating cycle till the collection of F1 placenta (gD 14.5-17.5) as shown in Fig.1. Diet induced n-3 PUFA deficiency was confirmed at F0 and F1 generation. Fatty acid composition was analyzed in plasma, milk and placental tissue. Placental early development was assessed by uterine spiral artery remodeling (8.5-12.5 gD). The labyrinth of placenta region was analyzed by arterial thickness, lumen areas and vessel diameters to assess vascular remodeling. Placental angiogenesis was further characterized by measuring the expression of several vasculogenesis, and invasive growth factors in the labyrinth region of the placenta by real-time PCR, and immunoblots. Epigenetic modification of the placental tissue was measured by 5-methylcytosine levels in F0 and F1 placenta and liver.

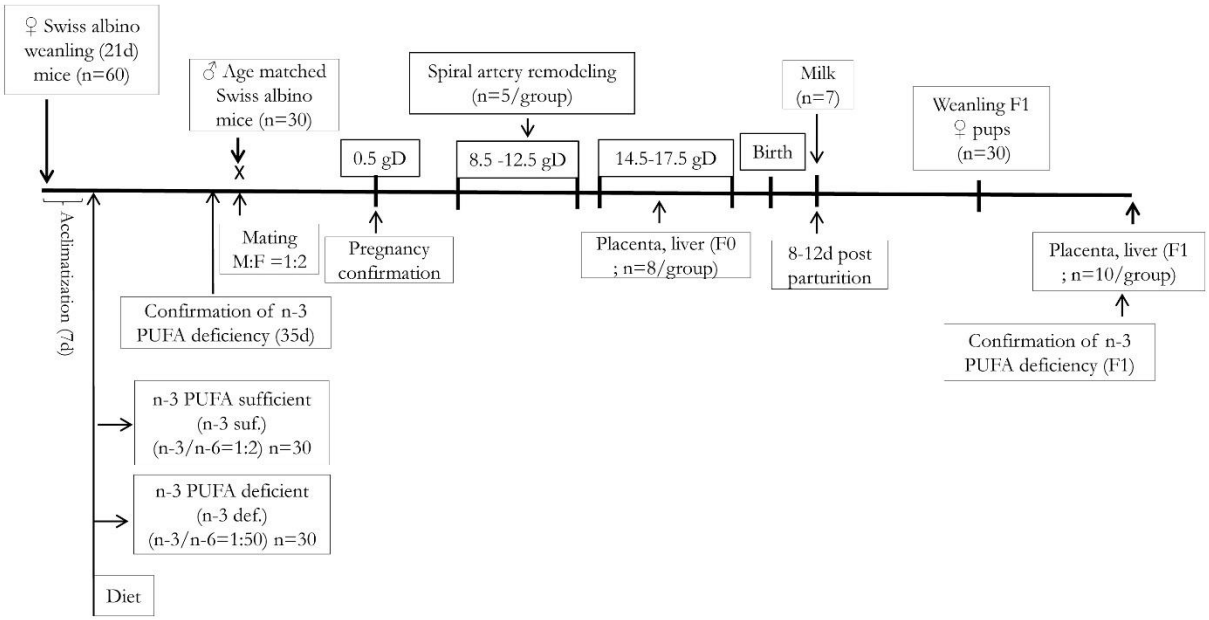


Fig 1. Data collection and timelines of mice fed with n-3 PUFA sufficient (n-3 suf.) and n-3 PUFA deficient (n-3 def.) diets over two generations. F1 female pups were continued with respective diets till the end. Deficiency of n-3 PUFA was induced in mice by depriving dietary (n-3) fatty acids in the diet.

Results

ALA deficient diet-induced n-3 deficiency in mice was confirmed by fatty acid composition in plasma and placental tissue. Pregnant mice with deficient intake of ALA significantly lowered the levels of DHA and DPA in breast milk. In order to assess the arterial remodeling during n-3 deficiency state, measurement of vessel and lumen areas as well as vessel diameters were performed in the labyrinth and junctional zone of the placenta of 8.5-12.5 gD mice. The luminal area in relation to vessel area were significantly decreased (n-3 sufficient vs. deficient: 18.11 ± 1.02 % vs. 12.0 ± 1.31 %, n=15) in n-3 deficient mice (Fig.2). Mice fed with n-3 deficient diets (ALA 0.13% en) until early gestation showed a significant alteration in the arterial remodeling parameters as compared to group fed with high ALA diet (2.26% en). This data suggest that n-3 deficiency state influences the

uteroplacental morphology and vasculature by altering arterial luminal and vessel area in mice models of n-3 deficiency.

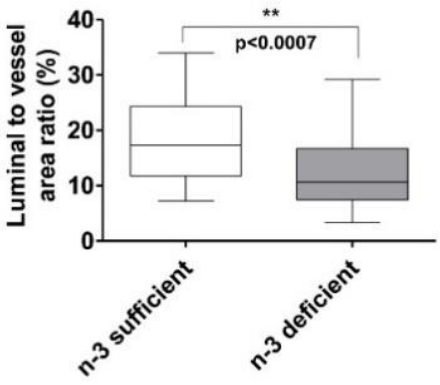


Fig 2. The effects of n-3 fatty acid deficiency on uterine artery remodeling at gD 8.5-12.5 of Swiss albino mice. Percentage of luminal to vessel ratio. Data are analyzed by paired test between n-3 deficient and n-3 sufficient group and expressed as mean ± SEM (n=15); ** p< 0.005

The DNA methylation was estimated in placenta and liver obtained from F0 and F1 mice. Percentage of 5-mC was significantly increased in the placenta tissue of n-3 deficient F0 and F1 mice (Fig 3) as compared to mice fed with n-3 sufficient diet (n-3 supp. vs n-3 def. : F0 = 11.84 ± 1.234 vs 17.61 ± 2.034, p =0.032, and F1 = 4.35 ± 0.635 vs. 9.42 ± 1.146, p= 0.0017). Although, 5-mC percentage was decreased in F1 as compared to F0 mice, however, chronic n-3 PUFA deficiency has a greater impact on the proportion of 5-mC increase in F1-placenta (F0 vs F1 : p=0.032 vs. p=0.0017, Fig 3). However, this trend remained insignificant in F0 and F1 liver tissues of both these groups. Thus, increased 5-mC DNA methylation was noted specifically in the placenta (F0 and F1) derived from n-3 PUFA deficient mice as compared to mice from n-3 PUFA sufficient diet.

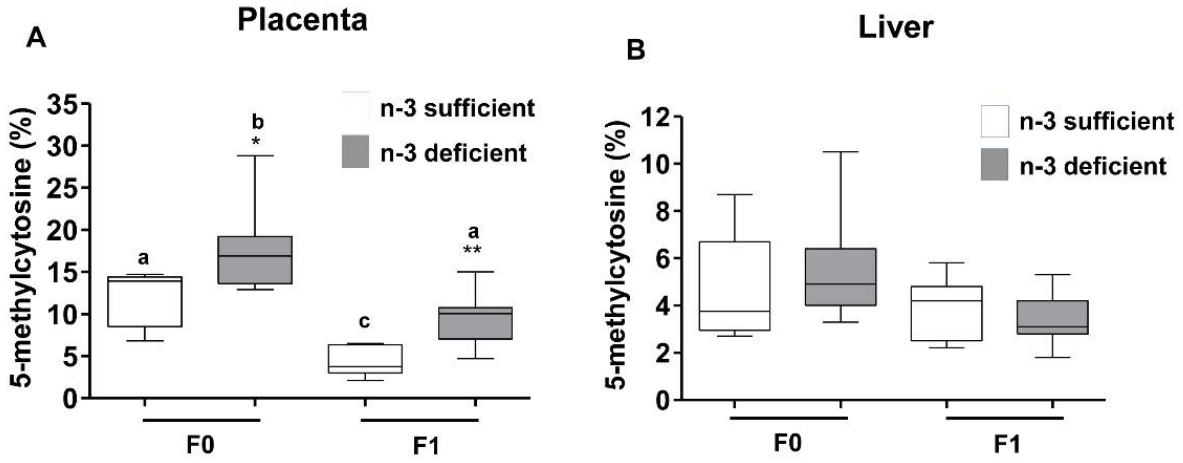


Fig 3. Effects of dietary n-3 fatty acid deficiency on changes of DNA methylation by measuring total percentage of 5-methylcytosine (5-mC) in placenta (A) and liver (B) tissues obtained from F0 and F1 mice (n=8). Data are reported as percentage of mean ± SEM , * p<0.05, ** p<0.005 vs n-3 sufficient diet (Student's t-test). Mean values with different superscript letters are significantly different at p<0.05 level by one-way ANOVA with Tukey's multiple comparison tests.

Maternal intake of ALA deficient diet over generations altered the tissue composition of LCPUFAs significantly in the placenta of F1 mice (Table 1). Increasing LA: ALA (50:1) in the n-3 deficient diet was associated with a significant decrease in the placental LCn-3PUFA such as DHA (C22:6 n-3) and DPA (C22:5 n-3) by ~4.3 (p<0.0001) and ~14 folds (p<0.0001) respectively as compared to n-3 sufficient diet. This was accompanied by a progressive increase in the proportion of LCn-6 PUFAs levels such as ARA (C20:4 n-6) and ADA (C22:4 n-6) by ~1.4 (p=0.0001) and ~1.9 (p=0.0005) folds respectively. Intake of n-3 deficient diet led to significant decrease in total LCn-3 PUFAs (C20:5 n-3, C22:5 n-3 and C22:6 n-3) by 5.5 folds (p=0.002) with simultaneous increase in total LCn-6 PUFAs levels (C20:4 n-6, C22:4 n-6 and C22:5 n-6) in the placenta by 1.8 folds (p=0.067) (Table 1). Due to chronic feeding of n-3 deficient diet, total ratio of LC n-6: n-3 PUFAs was raised by ~10 folds in the placental tissue of the n-3 deficient F1 mice.

Table 1. Placental fatty acid composition in mice fed with n-3 deficient and n-3 sufficient diet where 0.13 % and 2.26% of total energy was derived from alpha-linolenic acid respectively till gestation day gD 14.5-17.5 in F1 (n=5)

Fatty acids (nmol %)	n-3 sufficient (n=5)		n-3 deficient (n=5)		p value	Fold change[$]
	Mean	SEM	Mean	SEM		
C14:0	0.87	0.12	0.88	0.23	0.958	-
C16:0	24.54	0.36	23.2	0.83	0.178	-
C16:1	1.13	0.05	1.12	0.04	0.928	-
C18:0	23.48	0.52	22.92	0.16	0.340	-
C18:1	14.63	0.60	14.69	0.14	0.926	-
C18:2 n-6	11.24	0.37	10.45	0.23	0.114	-
C20:4 n-6	11.11	0.47	16.38	0.62	**0.0001*** **	1.4 (+)
C22:4 n-6	2.05	0.31	4.02	0.13	**0.0005** **	1.9 (+)
C22:5 n-6	ND	ND	4.3	0.27	-	-
C18:3 n-3	0.45	0.02	ND	ND	-	-
C20:5 n-3	0.99	0.05	ND	ND	-	-
C22:5 n-3	1.67	0.11	0.12	0.03	**<0.0001*** **	14.0 (-)
C22:6 n-3	7.50	0.34	1.73	0.07	**<0.0001*** **	4.3 (-)
[1]ΣLC n-6 PUFA	13.16	0.78	24.7	1.02	0.067	1.8 (+)
[2]ΣLC n-3 PUFA	10.16	0.5	1.85	0.1	**0.002** **	5.5 (-)
ΣLC(n-6) : (n-3)	1.3 :1	-	13.3: 1	-	-	10.2 (+)

ND = non-detectable

[$] Fold change in fatty acid concentration are depicted by increase (+) or decrease (-) over n-3 sufficient group.

[1] ΣLC n-6 PUFA is the sum of C20:4 n-6, C22:4 n-6 and C22:5 n-6

[2]ΣLC n-3 PUFA is the sum of C20:5 n-3, C22:5 n-3 and C22:6 n-3

Paired test between n-3 deficient and n-3 sufficient group; Mean ± SEM, ** p< 0.005; *** p< 0.0005;

Inference & Conclusion

We observed that n-3 deficiency state negatively influences the uteroplacental morphology and vasculature by lowering arterial luminal to vessel area in mice with concomitant decrease in the expression of proteins involved in placental vasculature (data not presented). The maternal uterine vasculature remodeled during early gestation to facilitate smooth delivery of nutrient and blood from maternal circulation to the fetus. The uteroplacental vasculature undergoes tremendous adaptations during pregnancy, including vasculogenesis and remodeling of arteries. The successful remodeling has a larger implication for fetal growth.

Maternal intake of n-3 PUFA during pregnancy is required for the placental vascular development and it maintains the epigenetic stability during development of the fetus. Placenta is the susceptible target organ for epigenetics changes in particular during the deficiency n-3 fatty acids.

VI. PATHOLOGY AND MICROBIOLOGY

1. ROLE OF ALDOSE REDUCTASE (AR) IN DIABETES AND CANCER – EFFECT OF DIETARY 'AR' INHIBITORS (ARIS)

Recent work conducted at NIN showed AR levels in RBCs of cancer patients was 1.5 to 2 fold increase as compared to 'controls', and the same was observed in 'tumor' tissues as compared to 'non-tumor' areas and more so in post-chemotherapy surgical samples of organs like breast, colon, rectum etc. Based on the results and observations from earlier studies wherein AR levels in RBCs of blood samples from cancer patients were increased along with a similar increase in tumor tissues, we attempted to reconfirm the same in the present study. This may probably reinforce the earlier observation of possibly using RBC AR levels as a biomarker to indicate possible tumorogenesis. The role of ARIs on the same background needs to be explored further so as to be useful as probable adjuvants in chemotherapy for cancer patients.

Aims and Objectives

1) To look into the role of AR and sorbitol on a background of metabolic syndrome (obesity, IR, DM) and development/progression of cancer.
2) To study AR expression and activity in cancer patients with or without DM.
3) To examine the relation between AR expression and histological staging of tumor as well as type of tumor.

Methodology

- A total of 65 breast cancer subjects were recruited from MNJ Cancer Hospital and South Central Railway Hospital of which 20 patients had diabetes and 45 did not.
- Additionally, 45 healthy controls(C) and 45 type 2 diabetic patients without cancer, who volunteered to participate in the study were enrolled by conducting health camps in different locations of Hyderabad and their blood samples were collected.
- All the collected samples were analyzed for the below mentioned parameters.
- Analysis of clinical parameters: Fasting blood glucose (FBG) was estimated using a glucometer (Accu-Chek® Active Roche Diagnostics GmbH, Mannheim, Germany). Glycosylated haemoglobin (HbA1c) was estimated in whole blood by Afinion AS100 Analyser (Axis-Shield, Norway) based on the principle of fully automated boronate affinity assay and haemoglobin (Hb) by the cyanmethaemoglobin method using a spectrophotometer (Shimadzu UV 2600). Lipid profile [total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL)] was analyzed in plasma using commercially available kits from BioSystems (Barcelona, Spain). Low-density lipoprotein cholesterol (LDL) concentrations were calculated using the Friedewald formula. Fresh post-chemotherapy tissues (including tumor and non-tumor areas) were collected and transported to the lab immediately on ice which are used for histopathological examination (typing and staining) and immunohistochemistry.

Histopathological examination: For the identification of tumor area and type/ stage/ grade, a smaller representative portion was taken from the freshly collected samples after surgery. For histopathological study, Hemotoxylin and eosin staining was done for both tumor and non-tumor tissues. The remaining tissue was used for immunoblotting and enzyme activity studies.

Aldose reductase (AR) assay: AR assay was carried out in the blood and tissues (tumor and non-tumor). Tumor and non-tumor tissues were homogenized using a hand homogenizer (Omni) by subsequent adding of AR buffer. The homogenized mixture was then subjected to centrifugation. The supernatant thus obtained contained the protein lysate. The protein concentration of the lysates was estimated using Lowry's method.

Aldose reductase activity was then carried out in these samples using a spectrophotometer. The aldose reductase activity was expressed as units of NADPH oxidized per min at 37 °C for 1 mg of protein.

Inference

- The expression of AR was relatively low in the non-tumor samples with and without diabetes. Although a statistically significant, the difference was not observed in diabetic and non-diabetic samples, only a trend was observed.
- Immunohistochemistry results also indicate the overexpression of aldose reductase in higher grades of tumors compared to their corresponding non-tumor samples.
- The AR levels in the RBCs of control subjects were lower compared to the diabetic subjects. In the cancer cases, subjects with and without diabetes had similar levels of aldose reductase activity.

2. ISOLATION OF *SALMONELLA* INFECTING BACTERIO-PHAGES TO CONTROL *SALMONELLA* CONTAMINATION IN FOOD

Salmonella belongs to the genus of gram-negative bacteria and it causes Salmonellosis which is one of the most common and widely distributed foodborne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Nontyphoidal *Salmonella* enterica is one of the leading causes of illnesses among the top known foodborne pathogens. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths. In India, the infectious disease burden is among the highest in the world and a recent report showed the inappropriate and irrational use of antimicrobial agents against these diseases, which led to increasing in the development of antimicrobial resistance. India has emerged as the world's largest consumer of antibiotics, with a 62% increase in use over the past decade. This has re-energized the search for new treatments to control the bacteria. Recently the use of bacteriophages has emerged as a promising tool for food preservation and safety. The technique of usage of lytic bacteriophages can represent a viable alternative to antibiotics for the treatment of bacterial infection. Therefore, this study is proposed to determine the effectiveness of bacteriophages to control foodborne pathogens like *Salmonella* etc.

Objectives

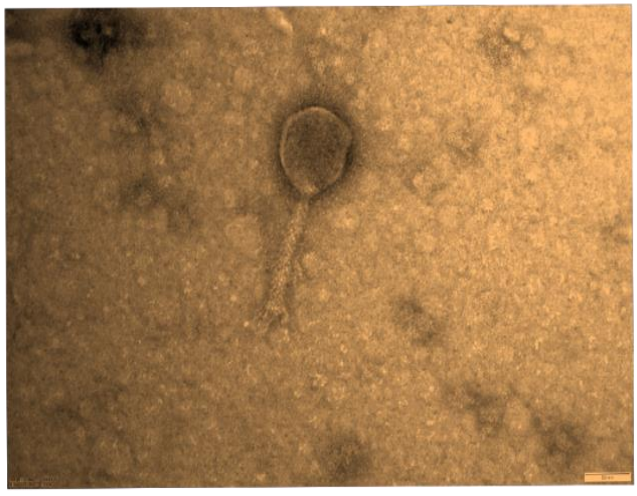
1. Isolation of bacteriophages infecting *Salmonella* spp.
2. To determine the effectiveness of a bacteriophage on several food matrices experimentally contaminated with *Salmonella*.

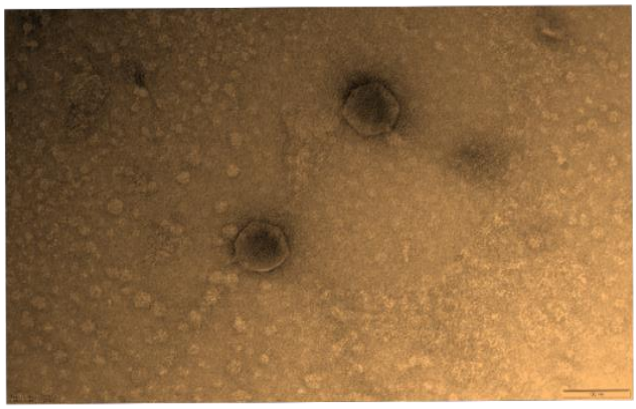
Results

1. Two new phages were isolated and designated as NINP13076 and NINP1162 based on their ability to propagate host strains ATCC13076 and MTCC1162 respectively. A lawn of *Salmonella* containing plaques formed by lytic phages (region of cell destruction) (Fig 1).
2. Both the phages formed clear plaques (0.5–1 mm) on the lawns of their respective hosts in the spot test assay.
3. The TEM (Hitachi) images of phage NIN13076 shown in Salmonella specific bacteriophages exhibit icosahedral heads and flexible tails indicating typical members of the *Siphoviridae* family (The bars represent 50 nm) (Fig. 2) in Transmission electron micrographs.

Fig. 1. A lawn of *Salmonella* containing plaques formed by lytic phages (region of cell destruction)

Fig. 2. Transmission electron micrographs (TEM) of *Salmonella* specific bacteriophages (100000- 260000 magnification)





4. The isolated phage could cause lysis. Inhibition zones were seen on phage spotted regions of the plate after overnight incubation at 37°C. Clear zones on bacterial strains indicated the bactericidal ability of isolated phages.
5. The host range of NINMB13076 and NINMB1162 suggested that the phage NINMB13076 could form plaques on two Salmonella cultures MTCC1162 and ATCC13076, but it did not form plaque on MTCC733. Phage NINMB 1162 showed a lytic effect by forming plaques on ATCC13076 but not on MTCC1162 and MTCC733).
6. The effect of phage mediated lysis of *salmonella* by seeing optical density (600 nm wavelength) at 0, 3, and 24 h at 37 °C indicated that a significant reduction in optical density/turbidity observed after three and 24h of incubation
7. A similar experiment at the gap of every 2 h to see the effect of phages on Salmonella indicated a significant reduction in the optical density of *salmonella*. The mean values across groups were significant at $p < 0.001$.

8. The two isolated Salmonella killing phages are stored in separate buffers of pH 5, 7, and 9. The two phages were not affected by storage at different pH stored up to one week.
9. A similar kind of experiment was done to see the effect of temperature on isolated phages. There was no significant change in the number of phages when stored at four ℃, 26 ℃, and 37 ℃.
10. Approximately 2 log reductions in both NINMB13076 and NINMB1162 were observed at 55℃ after 1d storage. Stability in both phages was seen at 37 ℃ up to two weeks of storage time.
11. Raw chicken samples (In-vivo) contaminated with *Salmonella* spp, treated with bacteriophages showed significant reduction (6.7–5.4 log CFU/g) of Salmonella in raw chicken after 3 hr of incubation at room temperature when compared to untreated control (Fig. 5). The sample sprayed with phage suspension at 10^8 pfu/ml for 3 hr resulted in a 1.3 log CFU/cm^2 reduction (Fig-3).
12. The results of the experiment on the lytic effect of phage on *Salmonella enteritidis* (log CFU/ml) in experimentally contaminated carrot indicated that a significant reduction in *Salmonella* population (1 log) observed after 4h of incubation (Fig-4).

Fig. 3. Lytic effect of Bacteriophage on *Salmonella* in experimentally contaminated raw chicken

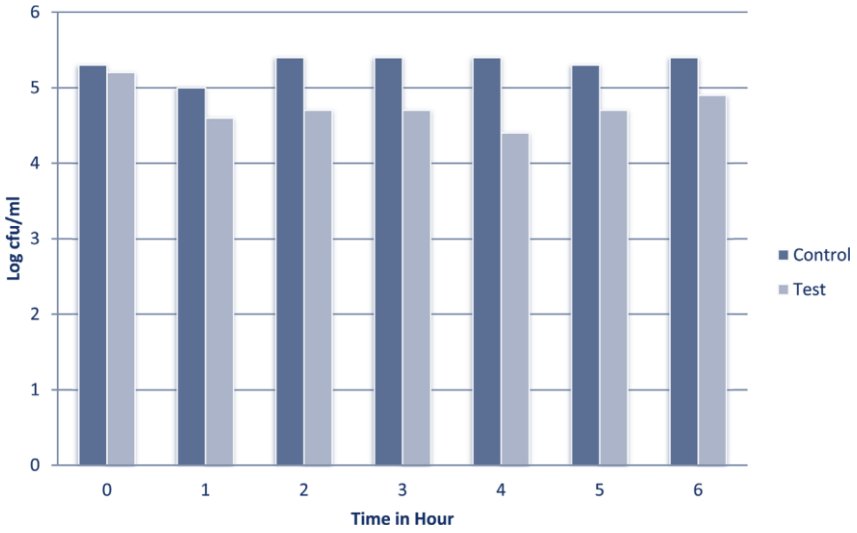
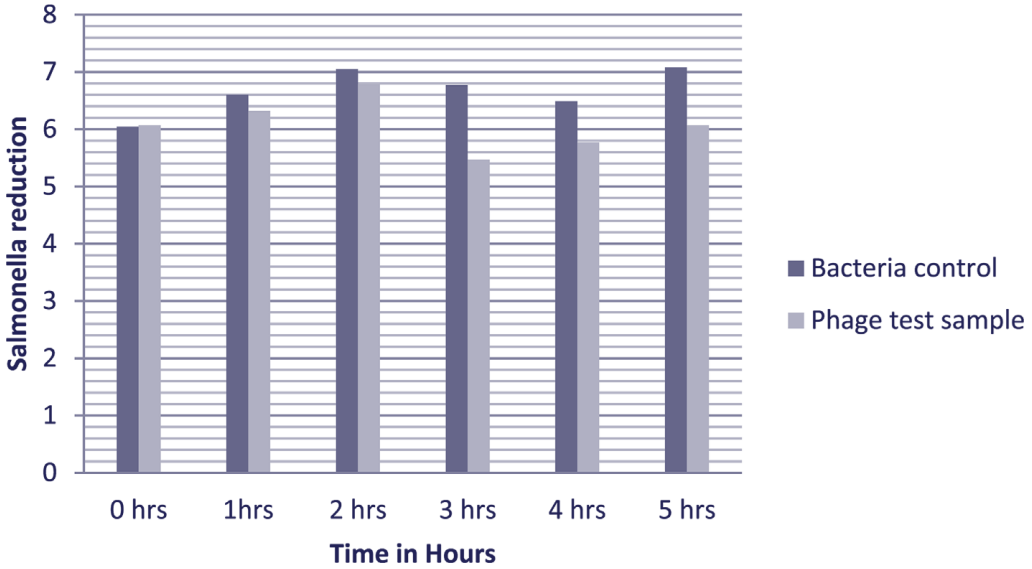


Fig. 4. Lytic effect of phages on *Salmonella enteritidis* (log CFU/ml) in experimentally contaminated carrot



13. The phage-free control group showed an increase in the *Salmonella* population with an increase in time. The experiment was conducted three times, and the values in the graph represent the mean values at 0, 1, 2, 3, 4, 5, and 6 h.
14. The stability of bacteriophage (NINMB13076) concentration in raw chicken samples was monitored for about 48h. No significant loss in the phage titer was observed at 4℃ and room temperature (25℃).

Inference and Conclusion

The present study demonstrated that phage treatment has the potential to be developed as an alternative strategy to prevent Salmonella infection in food safety. The study proved that lytic bacteriophages have great potential as a natural bio-control agent of a foodborne pathogen such as *Salmonella enteritidis*.

VII. EXTENSION & TRAINING

1. DEVELOPMENT OF E-LEARNING MODULES ON NUTRITION AND HEALTH UNDER POSHAN *ABHIYAAN* INITIATIVE OF GOVT. OF INDIA (IN HINDI LANGUAGE)

Nutrition and health education plays a vital role for overall development of the health and nutritional status among the population. The ICMR- National Institute of Nutrition has been entrusted with the responsibility of developing e-learning modules on various nutritional themes.

Objectives

- To develop scientific content/ background on selected nutrition themes with appropriate nutrition messages.
- To provide creative content for the 15-20 minute e-learning module by using appealing and effective multimedia inputs like computer graphics, videos, modeling techniques with inbuilt assessment and evaluation procedures to gauge increment in nutrition knowledge.
- To upload the modules on appropriate platforms/ portals for wider use after pretesting and usage of security procedures.
- To develop a teacher training module to aid in the training of trainers working with the target groups at the grass root levels.

Methods

Production of videos including animation and graphics by incorporating messages on health & nutrition and to make it available to public.

Results

The videos on the following themes were completed and uploaded on the *SWAYAM* portal and NIN website as well.

- **Basic Nutrition**
- **Infant & Young Child Feeding Practices**
- **Mother's Health and Nutrition**
- **Iron deficiency Anaemia**
- **Diarrhoea**
- **Nutrition & Health for adolescents**
- **Food fortification**
- **Immunization**
- **WaSH**
- **Non Communicable diseases (NCDs)**
- **Physical Activity (Yoga)**

Inference & Conclusions

- The e-Learning modules on various nutritional themes have been developed to impart nutrition knowledge among the general public and girls & boys in adolescent age groups on the significance of nutrition and physical exercise quite early in life, especially during their rapidly growing stage of life.
- Nutrition education, thus imparted, has the potential of reaching out to vast segments of population all over the country.
- The Master trainers (paramedics, *Anganwadi* workers, and others) working alongside the adolescents at the grass root levels would facilitate meaningful learning among these much ignored population groups.

VIII. FOOD TOXICOLOGY

1. AMELIORATIVE POTENTIAL OF TAMARIND FRUIT EXTRACT ON THE NAF - INDUCED ALTERATIONS IN THE BONE RELATED PARAMETERS IN SAOS-2 CELL LINE

Exposure to high levels of fluoride (F) over a long period causes damage to osseous tissue, which results as dental and skeletal fluorosis. The toxic effects of F interfere with the mineralization process, and the defects that result are generally irreversible. Significantly increased levels of bone turnover markers like serum alkaline phosphatase and tartrate resistant acid phosphatase were found in the fluorotic patients. The absence of safe and effective ameliorative agents to reduce the F toxicity at earlier stages in human and other domestic animals puts the vulnerable population at risk in the fluorotic area. However, drugs are not available for the treatment of fluorosis making medical intervention impossible. It is reported that chemical compounds such as ascorbic acid, boron and aluminum sulphate have been tried in treating the experimental F toxicity; however, the success rate was very less. These compounds are generally not recommended for prolonged use due to their toxic side effects, which makes it necessary to evaluate a nontoxic-safe agent that can reduce F toxicity in the body. Few *in vitro* studies showed tamarind (*Tamarindus indica* L.) has a great potential to ameliorate fluoride toxicity. Previous *in vivo* studies in dogs, humans and rats also have reported that tamarind ingestion could reduce the accumulation of F in bone and facilitated its excretion through urine.

The osteoblast cell line Saos-2 is used to study the various NaF-induced cytotoxicity in the cells. The ameliorative potential of tamarind fruit extract on the bone related parameters in the osteoblast cell line Saos-2 in NaF-induced cytotoxicity were not studied. Hence this study assesses the ameliorative potential of tamarind fruit extract on the NaF –induced alterations in the bone related parameters such as alkaline phosphatase, tartrate resistant acid phosphatase, carbonic anhydrase in the osteoblast cell line Saos-2.

Aims and Objectives

1. To study the ameliorating effect of tamarind fruit extract on the accumulation of fluoride in the Saos-2 cell line.
2. To study the effect of tamarind fruit extract on the amelioration of NaF-induced alterations on the bone parameters like alkaline phosphatase, parathyroid hormone, tartrate resistant acid phosphatase, carbonic anhydrase, vitamin D receptor in the Saos-2 cell line.
3. To study the effect of NaF on the cell morphological changes in the presence and absence of tamarind.

Preparation of tamarind fruit extract (TFE): The fruit pulp extracts were prepared as previously described by Razali et al, 2008. Briefly, the fruit pulp was separated from the seeds, air-dried and then powdered. The powdered T. indica fruit pulp (2.5 g) was then placed in a conical flask and soaked in 50 ml methanol at room temperature for 24 h. The resulting extracts were then filtered, roto-evaporated and redissolved in 10% DMSO. The samples were kept at -20^{o}C until further analysis.

LCMS-MS of TFE: The LCMS-MS analysis of TFE was performed at IICT, Hyderabad.

Culturing the Saos-2 cells: Saos-2 cell line procured from American Type Culture Collection (Chromachemie Laboratory Pvt. Ltd., Bengaluru, India) were grown in McCoy's 5A complete media supplemented with heat inactivated 15% Fetal Bovine Serum (FBS).

MTT assay: The MTT assay was performed to determine the optimum doses of NaF and TFE. Cells were exposed to different concentrations of NaF (1.6 mM, 0.8 mM, 0.4 mM, 0.2 mM, 0.1mM) and TFE (100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml, 20 µg/ml) alone and in combination for 24, 48 and 72 h.

***Treatment of cells for further analysis*:** The differentiated cells were seeded into 6 well plate and incubated overnight to obtain cell morphology in CO_2 incubator. Then the cells were treated with 1.6 mM NaF and 60 µg TFE along with negative controls for 72 h. Later, the cells and the supernatant were taken for studying various parameters.

***F estimation*:** The F estimation in the cell lysate and culture supernatant was done by F ion selective electrode method (Thermo Scientific Orion Star A214).

***Calcium (Ca), Total protein and ALP*:** The cell lysate and culture supernatant were analyzed for Ca, total protein and ALP using an autoanalyser (Roche Cobas C 311).

PTH enzyme immunoassay: The culture supernatant PTH was determined by using ELISA kit (Sigma-Aldrich PTH/Parathyroid Hormone EIA Kit (RAB0412).

***Cell morphology using scanning electron microscope (SEM*)**

The cells were seeded in 6 well flat bottom plate over an autoclaved glass coverslip. A coverslip was placed in each well and cells were seeded with the density of 2 lakh cells/well with complete medium. Once cells were adhered the coverslips were transferred to new 6 well plate and treatments (1.6 mM NaF, 1.6 mM NaF + 60 µg TFE, 0 and -ve controls) were added in each well containing coverslip with cells. The change in morphology after 72 h was photographed by using SEM (S3400N *Hitachi Japan*) at 15KV, pictures were taken in different magnifications x250 to 2k.

Results

LCMS-MS analysis of TFE

The different components present in the TFE are tabulated in Table 1. The analysis shows that Cyanocobalamin (Vit B12) is the most abundant component in the methanolic TFE with 42.25±0.88 ng/g concentration, followed by Naringenin with 24.09±1.43 ng/g and Pyridoxine with 20.63±0.19 ng/g concentration (Table. 1).

MTT assay

The percentage proliferation of cells with different concentrations of F and TFE for different time points (24, 48 and 72 hours) by MTT assay was shown in Fig. 1 A and 1B. The 1.6 mM concentration of NaF at 72 h exhibit around 60% cell viability. Hence, this concentration and treatment time point was selected for further assays and analysis 9Fig. 1A). The 60 µg/ml TFE treatment and 72 h time period showed highest cell viability (more than 80%) and was selected for further assays (Fig. 1B). Hence, the 1.6 mM NaF concentrations and 60 µg/ml TFE concentration for 72 h treatment was selected for further assays.

Fluoride estimation

The accumulation of F in the Saos2 cells (cell lysate) with the NaF and TFE treatments is shown in Fig. 2A. The accumulation of F in 1.6 mM NaF treated cells was significantly increased

compared to 1.6 mM NaF + 60 µg/ml TFE treated cells. The intracellular accumulation of F in TFE treated cells were comparable to control. The extracellular accumulation (culture supernatant) of F in all the groups in shown in Fig. 2B. There was a significant increase in the F levels in the culture supernatant of NaF +TFE treated cells compared to NaF treated cells.

Table. 1 Analytes present in TFE by LCMS-MS analysis along with their concentrations (ng/g) and LOD (ng/mL)

S.No	Analytes	Concentration± SD (ng/g)	LOD (ng/mL)	Calibration range (ng/mL)
1.	Pyridoxine	20.63±0.19	8.04	25-1000
2.	Niacin	16.58±0.42	10.01	50-1000
3.	Pantothenic Acid	18.17±0.08	9.74	50-1000
4.	Biotin	ND	2.28	25-1000
5.	Quercetin	ND	4.08	25-1000
6.	Hespartin	ND	3.32	25-1000
7.	Riboflavin	8.79±0.36	5.91	25-1000
8.	Folic Acid	ND	6.99	25-1000
9.	Cyanocobalamin	42.25±0.88	22.61	100-1000
10.	Naringenin	24.09±1.43	6.71	25-1000
11.	Naringin	ND	14.02	50-1000
12.	Calciferol	ND	18.08	50-1000
13.	Tocoferol	ND	10.33	50-1000
14.	Curcumin	ND	1.29	25-1000

As per International Conference on Harmonisation (ICH),
The limit of detection (LOD) may be expressed as: LOD = 3 x σ/ S
Where, σ = the standard deviation of the lowest concentration response
S = the slope of the calibration curve

Fig 1A. Saos2 cell viability (% proliferation) after treatment with different concentrations of NaF for 24, 48 and 72 hours

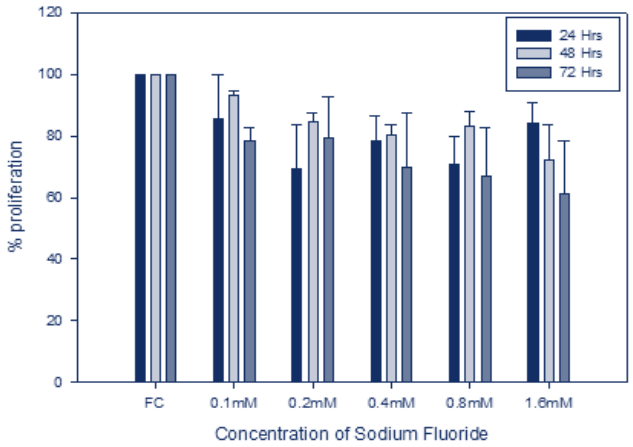
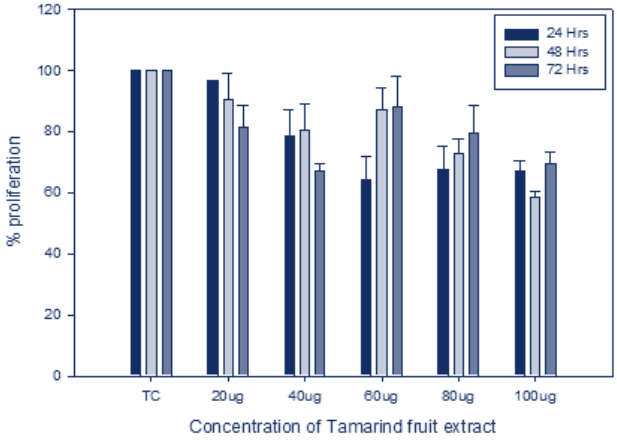


Fig 1B. Saos2 cell viability (% proliferation) after treatment with different concentrations of TFE for 24, 48 and 72 hours



***Alkaline Phosphatase*:** The cell lysate treated with NaF showed increased levels of ALP compared to the cell lysate treated with NaF+TFE. The ALP levels in NaF+TFE treated cells were comparable with the control.

***Ca and total protein*:** The Ca levels in the cell supernatant of NaF treated cells was slightly lesser than the NaF+TFE treated cells, but statistically not significant. Amount of total protein present in the cell lysate and culture supernatant does not show any significant difference in the control and treatment groups.

Fig 2A Accumulation of fluoride in the cell lysate in different treatment groups.
a=compared to control, b=compared to control TFE, c=compared to 1.6 mM F groups. Statistically significant $p<0.05$.

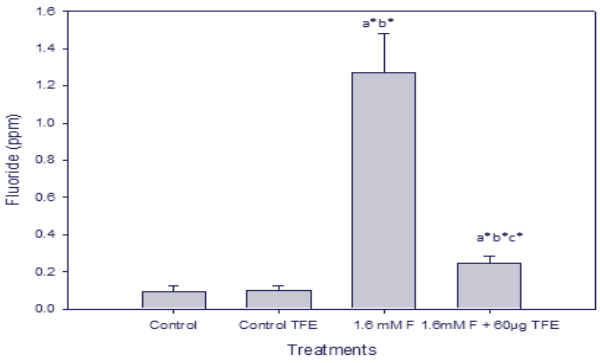
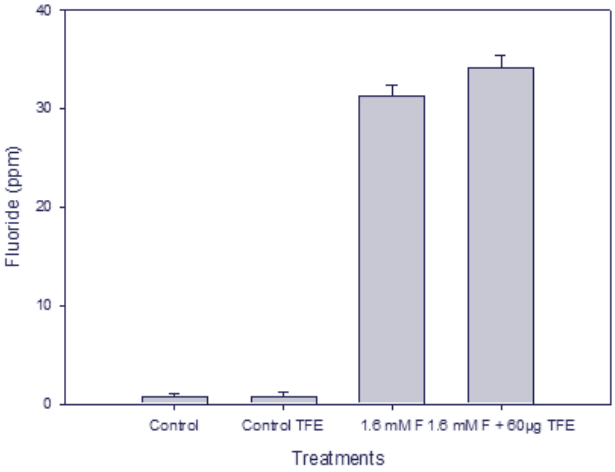


Fig 2B Accumulation of fluoride in the culture supernatant in different treatment groups



PTH: The PTH levels in the NaF treated cells were higher than the NaF+TFE treated cells. The PTH levels in the NaF+TFE treated cells were comparable with the control.

Morphology of cells: The Saos-2 cells in control group showed regular cellular margins and uniform cellular surface, whereas the cells treated with NaF showed larger, irregular and distorted morphology of cells compared with the control group cells. The cells treated NaF+TFE showed smooth cellular margins and cell size was observed to be comparable with that of control group.

Conclusions

The study concludes that TFE treatment has ameliorative potential to control the NaF induced alterations in bone related parameters in Saos-2 cell line. Tamarind can be used as an intervention as it can mitigate the fluoride toxicity.

2. PREVALENCE OF FLUOROSIS IN THE COMMUNITY OF SELECTED DISTRICTS OF INDIA AND DEVELOPMENT OF AN APPROPRIATE INTERVENTION MODEL FOR PREVENTION AND CONTROL OF FLUOROSIS

Fluorosis is a public Health Problem in India, it is slow, progressive and crippling malady affecting most of the organs in the body where fluoride (F) in drinking water is > 1.0ppm. The available data suggest about 62 million people in India suffer from dental, skeletal and non-skeletal fluorosis, out of these 6 million children were below the age of 14 years. To assess the prevalence

and severity of the fluorosis at different levels of fluoride in their drinking water and to develop appropriate intervention model for prevention and control of fluorosis, the present study has been undertaken.

Aims and Objectives:

- ✓ To assess the prevalence of dental, skeletal and non-skeletal fluorosis in the community of selected districts in the country.
- ✓ To find out the severity of dental fluorosis among areas with different fluoride levels in potable water.
- ✓ To assess fluoride level in potable water and urine samples
- ✓ To develop an appropriate intervention model for prevention and control of fluorosis together with its feasibility of adoption with local stakeholders.

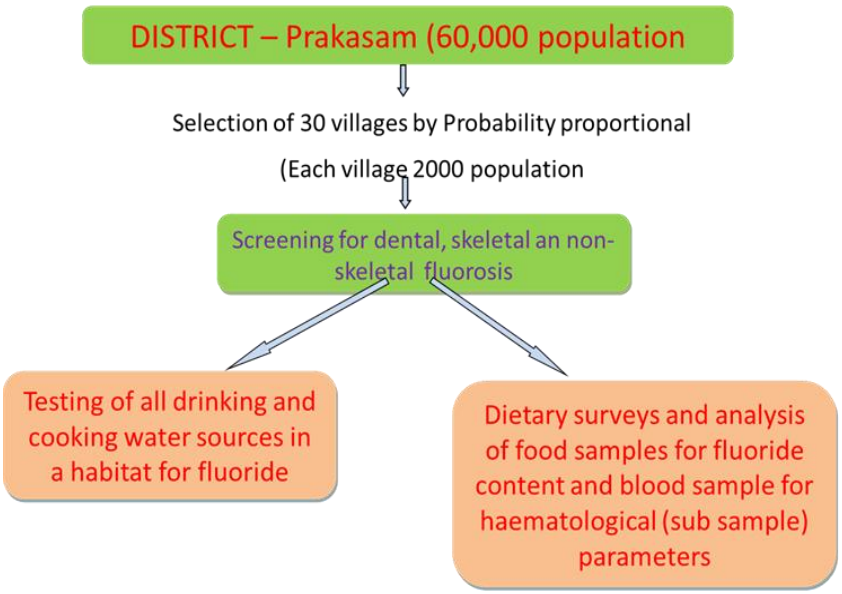
Ethical Committee *Clearance*: Institutional Ethical committee (IEC) clearance has been obtained.

Collection of water samples from Ongole district: A total of 457 groundwater samples used for drinking and cooking have been collected from Ongole district (13 mandals, 59 villages) and sent to Jabalpur for F analysis. On receiving the results of F content in all 457 water samples the villages have been categorized in to three categories ie ,1.5, 1.5-3 and > 3 ppm F (Table 1).

Collection of blood, urine and food samples: According to ICMR Criteria, The sampling design is as shown in Fig. 1.
One time morning urine (15 ml) was collected to analyze urinary fluoride levels. 5 ml venous blood is being collected to analyze the blood parameters.

Diet survey and anthropometry: The diet survey was done by administering a questionnaire and the height and weight was measured in the population.

Fig. 1 The sampling design



Results

Thirty villages from different categories (Fluoride <1.5ppm; 1.5-3.0ppm; <3.0ppm), with 60,000 population have been covered for survey including anthropometry, clinical examination. In 600 households, 24 h dietary recall survey was conducted and 600 blood and urine samples were collected. A total of 630 food samples comprising rice, wheat, red gram, green gram, black gram, cucumber, brinjal, potato, beans etc., were analyzed for fluoride.

Water fluoride and urinary fluoride: The water fluoride and urinary fluoride values are given in Table 2.

Blood parameters: The blood parameters like Ca, P, SGOT, SGPT, T3, T4, TSH, haemoglobin, ferritin, vitamin B_{12} were analyzed among all the different categories in the study population (Table 3).

Table 1. List of 30 villages (10/category) in three categories of fluoride levels

Below 1.5 ppm Fluoride					
S no	**Mandals**	**Villages**	**Village code**	**Avg ppm**	**Population**
1	Maddipadu	Annangi	701	0.945	2243
2	S.N.padu	Chilakapadu	702	0.96	2202
3	Chimakurthy	Ilapavulur	703	1.028	3048
4	N.G.padu	Maddiralapadu	704	0.69	2488
5	Maddipadu	Nelaturu	705	0.553	2807
6	Chimakurthy	Nipatlapadu	706	1.36	2798
7	N.G.padu	Pothavaram	707	0.99	2669
8	Korisapadu	Rachapudi	708	1.3	2897
9	Kothapatnam	Rajupalem	709	0.077	3427
10	Kondepi	Vennur	710	0.89	3312

1.5-3.0 ppm fluoride					
S no	**Mandals**	**Villages**	**Village Code**	**Avg ppm**	**Population**
1	S.N.padu	Batla machavaram	711	1.87	3861
2	Chimakurthy	Budawada	712	2.63	3442
3	Korisapadu	Dyavalaravuru	713	1.51	3205
4	S.N.padu	Enikapadu	714	1.6	2119
5	Maddipadu	Gundlapalli	715	2	4627
6	N.G.padu	Kallagunta	716	1.65	3851
7	N.G.padu	Machavaram	717	1.968	2183
8	Kondepi	Muppavaram	718	1.7	3406
9	N.G.padu	Raparla	719	1.52	3475
10	Podili	Tallamalla	720	1.6	2144

> 3.0 ppm Fluoride					
1	Kanigiri	Boggulagundi colony	721	5.78	300
2	Podili	Challavaripalem	722	3.3	
3	Kondepi	Kuppalapadu	723	3.2	2968
4	Kanigiri	Kammavari palli	724	3.77	
5	Korisapadu	Korisapadu	725	3.012	4009
6	Kandukooru	Kovuru	726	3.5	2246
7	S.N.padu	Mangamuru	727	3.45	3851
8	Singaraya konda	Old singaraya konda	728	4.25	4924
9	Zarugumalli	Pachava	729	3.2	2721
10	H.M.padu	Papireddy palli	730	4.65	1932
11	Podili	Rajupalem	731	5.41	662

Table 2. Water fluoride and urinary fluoride in different categories of the study population

Water Fluoride (ppm)	N	Mean urinary fluoride (mg/L)
<1.5	120	1.23±0.867
1.5-3.0	100	1.28±1.223
>3.0	80	1.61±0.994*ab

Table 3. Blood parameters in different categories in the study population

	Category I	Category II	Category III
Urinary fluoride	(N=204) 1.15±0.89	(N=199) 1.62±1.23a*	(N=203) 2.07±1.40a*b*
Ferritin	(N=194) 22.72±18.34	(N=187) 25.99±19.68	(N=180) 29.88±19.70
Hb	(N=201) 121.39±23.55	(N=194) 120.73±22.81	(N=201) 124.12±22.41
T3	(N=205) 113.78±27.04	(N=200) 115.17±27.72	(N=203) 123.85±29.54a* b*
T4	(N=205) 8.68±1.97	(N=201) 8.48±1.79	(N=204) 8.56±1.90
TSH	(N=204) 2.70±2.05	(N=200) 2.46±1.53a*	(N=203) 2.19±1.35a*
Urinary Iodine	(N=186) 230±144.60	(N=181) 197.94±124.32	(N=194) 200.21±119.96
Vit.B_{12}	(N=205) 308.34±157.18	(N=200) 306.96±179.47	(N=204) 324.40±191.28

3. TOXICOKINETICS OF COMMON ORGANOPHOSPHATE COMPOUNDS IN ACUTE POISONING CASES

Organophosphorus pesticides (OPs) interfere with the activity of acetylcholinesterase (AChE), a vital enzyme that regulates the functioning of the nervous system, resulting in acetylcholine (Ach) accumulation at the synapses and myoneural junctions. It remains unknown whether the commonly used OPs in South India also interfere with the AChE activity and their toxicokinetics in humans remains poorly understood. We collected peripheral blood samples from OP-associated suicide cases (hospitalised) and analysed the pesticide concentration and AChE

activity, and the toxicokinetics of six commonly used pesticides. LC-MS/MS was used for the estimation of pesticide concentration.

Based on a comparison of six pesticide kinetic profiles and toxicokinetic parameters, we concluded that chlorpyrifos ingestion resulted in the highest concentration of chlopyrifos among the identified pesticides, followed by acephate, triazophos, propanil, while dimethoate exhibited the lowest concentration. Based on a timecourse analysis, we observed a faster elimination phase for monocrotophos and dimethoate. We observed that there was a significant decrease in the mean concentration of monocrotophos (64 ng/mL) (P ¼ 0.015), while the mean value of AChE (1.08 unit/mL) increased over time. While monocrotophos and dimethoate elimination phases were remarkable in human subjects, the other pesticides did not demonstrate similar elimination phases owing to their low rate of metabolism and high stability.

Ops are a heterogeneous group of compounds, composed of a phosphoric acid derivative with two organic side chains and an additional side chain, that can be either acyanide, phenoxy, and thiophenoxy or carboxylate group. Several organs/Tissues like skin, respiratory, gastrointestinal tract, and conjunctiva, rapidly absorb most of the Op pesticides. Toxicity of Op compounds varies greatly and is mainly because of their interference with activity of Acetylcholinesterase, an enzyme essential for the proper functioning of the human nervous system. Organophosphate compounds causes accumulation of ACh in the synaptic cleft.

This overflow of Ach stimulates the nicotinic, muscarinic and central nervous system receptors leading to clinical complications in patients. Initial effects observed are usually respiratory and symptoms include coughing, chest discomfort, and difficulty in breathing or short breath, bloody or running nose and wheezing due to constriction or excess fluid in the bronchial tubes. Other systemic effects may be observed within few minutes or may be delayed up to 12 hours. In severe cases there may also be involuntary defecation or urination, psychosis, irregular heartbeats, unconsciousness, convulsions and coma. Death may be caused by respiratory failure or cardiac arrest.

Objectives

- Characterization and confirmation of OPs and their metabolites in biological samples;
- Quantification of unknown organophosphates and their metabolites in biological samples
- Impairment in enzyme activities in blood samples due to organophosphate compounds
- Correlation of biomarkers status with the clinical picture of poisoned cases
- Correlation of concentration of Ops on different time interval with the clinical picture of poisoned patient.

Method

Subjects: The target population of this study was the organophosphate poisoning cases referred to the Osmania general hospital in Hyderabad, Telangana, India. These cases were referred by various general and private hospitals of Hyderabad. Serial blood samples from acute OP poisoning cases were collected, extracted and stored at –80o C for further identification.

Selection of pesticides:

Organophosphate pesticides, simazine, aldicarb, alachlor, malathion, chlorpyrifos, chlorfenvinphos, phosalone, diazinon, acephate, fenitrothion, monocrotophos, imidacloprid, triazophos, ethion, atrazine, propanil, quinalphos, and metribuzin, commonly used in and around Hyderabad were used in this study.

Sample collections: Blood samples and clinical data of poisoning patient were collected from Osmania Hospital, Hyderabad, India for analysis as early as possible.

Extraction: Blood samples were collected from Osmania hospital and transferred into a vacutainer by keeping in icebox and stored at –80o C till the date of analysis. The extraction process has been diagrammatically has shown as below: 100 □l of blood is taken into a 10ml test tube, Add 900 □l of acetonitrile (ACN) to the blood sample, Vortex for 1 min, Pass the sample through a 0.2 □m syringe filter column, Collect the adequate into a clean 1ml vial tubes, Analyse with LC-MS, Correlate with clinical and enzyme activity with pesticides concentration.
LC-MS/MS analysis:

Instrumentation: The common eighteen pesticides were analyzed using a 4000-QTRAP triple quadrupole hybrid mass spectrometer (Applied Biosystems Sciex, USA) equipped with an ultra-fast liquid chromatography apparatus (UFLC, Shimadzu, LC 20 AD, binary pump) and operated in the positive turbo ion spray (ESI) mode. The LC chromatography was fitted with a reverse phase column.

Results

The results of the pesticide exposure and AChE concentrations in patients who had ingested pesticides are reported. The clinical findings, including tachycardia, hypertension, respiratory distress, and increased blood urea levels, varied among patients who ingested the pesticides.

OP exposure trends over time and enzyme (AChE) mean activity data

Data on the pesticide and enzyme concentrations over time are presented in Table 1. The monocrotophos concentration was observed to be 64 ng/mL at 12 h, and the concentration significantly reduced to 0.06 ng/mL at 48 h, whereas the enzyme concentration was 1.08 unit/mL at 12 h and increased to 2.79 unit/mL at 48 h. The dimethoate concentration was 45.43 ng/mL at 12 h, which reduced to 2.66 ng/mL at 36 h, while the enzyme concentration was 5.59 unit/mL at 12 h, and the concentration increased to 7.5 unit/mL at 36 h. Chlorpyrifos concentration at 12 h was 3367 ng/mL, which decreased to 986 ng/mL at 36 h, whereas enzyme concentration increased to 5.47 unit/mL at 36 h from 4.14 unit/mL at 12 h. The triazophos concentration was 5886.9 ng/mL at 12 h, and the same reduced to 1139.7 ng/mL at 72 h with empirical evidence indicating that the decrease in the pesticide concentration was not significant; the enzyme concentration was 4.14 unit/mL at 12 h and increased to 7.6 unit/mL at 72 h.

The acephate concentration was 1181 ng/mL at 12 h, and the concentration reduced to 646.2 ng/mL at 48 h, whereas the enzyme concentration was 4.7 unit/mL at 12 h, which increased to 6.2 unit/mL at 48 h. The propanil concentration was 1036.85 ng/mL at 12 h, and the concentration reduced to 532.08 ng/ mL at 36 h, whereas the enzyme concentration was 5.8 unit/mL at 12 h, which increased to 8.67 unit/mL at 36 h. There was no statistical significance observed due to the high variation in both the concentrations and the limited number of subjects (a markedly small sample size).

Table 1: Analysis of relation between pesticide exposure and enzyme activity in human blood with respect to time (hrs)

				Pesticide Concentration (ng/mL)			Enzyme Concentration (unit/mL)		
S.No	Toxin Consumed	Time (Hrs)	n	Mean (SD)	Median (P25-P75)	P-value	Mean(SD)	Median (P25-P75)	P-Value
1	Monocrotophos	12	4	64.32 (55.20)	48.28 (25.62-103.02)	0.015	1.08 (0.72)	0.96 (0.58-1.59)	0.245
		24	4	1.64 (1.98)	1.04 (0.21-3.07)		1.67 (0.87)	1.69 (1.24-2.22)	
		36	4	0.15 (0.11)	0.11 (0.07-0.22)		2.12 (1.10)	2.31 (1.24-3.00)	
		48	4	0.06 (0.03)	0.05 (0.03-0.08)		2.79 (1.67)	2.86 (1.50-4.09)	
2	Dimethoate	12	4	45.41 (48.44)	29.49 (6.94-99.80)	0.196	5.59 (5.70)	3.96 (0.89-11.93)	0.883
		24	4	5.48 (5.37)	5.26 (0.23-10.96)		6.96 (4.43)	4.92 (3.92-12.05)	
		36	4	2.66 (2.35)	3.01 (0.16-4.82)		7.53 (4.27)	5.48 (4.67-12.43)	
3	Chlorpyrifos	12	3	3367.69 (2429.12)	2429.12 (1538.81-5196.57)	0.252	4.14 (3.95)	3.87 (0.80-7.48)	0.881
		24	3	1834.07 (1226.14)	1608.87 (1004.46-2663.68)		4.54 (3.58)	4.74 (1.54-7.54)	
		36	3	986.11 (736.58)	987.00 (364.77-1607.44)		5.47 (3.86)	5.95 (2.36-8.58)	
4	Triazophos	12	4	5886.92 (6624.28)	5726.31 (161.23-11612.62	0.826	4.14 (0.90)	4.25 (3.43-4.85)	0.073
		24	4	2943.80 (5804.88)	61.44 (5.18-5882.42)		5.18 (1.42)	5.38 (4.04-6.33)	
		36	4	2699.96 (5237.45)	54.20 (4.20-5395.71)		6.09 (1.82)	5.71 (4.64-7.55)	
		48	4	2489.63 (4949.81)	21.88 (2.87-4976.40)		6.91 (1.79)	6.95 (5.47-8.35)	
		60	4	1882.54 (3761.98)	2.14 (0.81-3764.27)		6.95(2.18)	7.08 (5.27-8.64)	
		72	4	1139.74 (2278.07)	0.92 (0.56-2278.93)		7.63 (1.52)	7.38 (6.40-8.87)	
5	Acephate	12	4	1181.01 (744.56)	1379.30 (714.90-1647.12)	0.769	4.71 (3.36)	3.89 (2.29-7.13)	0.941
		24	4	835.77 (749.45)	747.07 (225.78-1445.75)		5.14 (3.42)	4.58 (2.50-7.77)	
		36	4	783.28 (717.47)	672.89 (213.46-1353.10)		5.56 (3.40)	5.11 (2.80-8.32)	
		48	4	646.18	501.73				

				(737.52)	(41.60-1250.76)		6.16 (3.56)	5.74 (3.19-9.13)	
6	**Propanil**	12	3	1036.85 (725.10)	1035.43 (312.46-1762.65)	0.517	5.83 (3.11)	4.37 (3.71-9.40)	0.520
		24	3	676.56 (355.05)	750.62 (290.32-988.73)		7.72 (2.72)	7.17 (5.31-10.67)	
		36	3	532.08 (416.31)	651.82 (69.03-875.40)		8.67 (2.96)	7.94 (6.15-11.93)	

The monocrotophos concentration increased at a rate of approximately 19.43 ng/mL h1, whereas enzyme concentration increased at a rate of 0.6 unit/mL h1. Dimethoate concentration decreased at a rate of approximately 21.4 ng/mL h1, but the enzyme concentration increased at a rate of 0.97 unit/mL h1. Similarly, chlorpyrifos' concentration decreased at a rate of 1190.8 ng/mL h1, and enzyme concentration increased at a rate of 0.66 unit/mL h1. The triazophos concentration decreased at a rate of nearly 775 ng/mL h1, whereas enzyme concentration increased at a rate of 0.67 unit/mL h1. Similarly, acephate reduced at a rate of nearly 165.7 ng/mL h1, whereas enzyme concentration increased at a rate of 0.48 unit/mL h1.

Correlation between pesticides and enzyme concentration with time

Monocrotophos concentration was negatively correlated with AchE concentration, with the relationship showing statistically significant results, while for chlorpyrifos and Other OPs, dimethoate, triazophos, acephate, and propanil, showed a negative correlation with the AChE concentration, which was not statistically significant.

Toxicokinetic analysis of the pesticides

Based on the available time vs. concentration data obtained from the OP-poisoned cases, toxicokinetic parameters such as peak concentration (Cmax), peak time (Tmax), and area under curve (AUC) values were calculated. Whenever a remarkable elimination phase with sufficient time points was observed, elimination phase-based parameters such as half-life (t1/2) and AUCInf were calculated. Toxicokinetics data are shown in Table 3. Owing to dose information unavailability, dose-based parameters such as distribution volume and clearance were not calculated. The time vs. concentration data for monocrotophos were available at six time points over a period of 12e72 h. Based on this data, a Cmax of 313.24 ng/mL was observed at Tmax of 12 h; overall exposure, i.e., AUClast (AUC 12e72 h), was 3999.776 ng*h/mL. The t1/2 of 10.92 h indicates that monocrotophos is not moderately eliminated and can be eliminated completely from the body within three days.

Discussion

In this study, we estimated pesticide residue concentration present in the blood of victims of poisoning and correlated it with AChE activity. Based on the study design, we performed a toxicokinetic analysis of each pesticide. Since the data were generated by analysis of suicide poisoning cases, it was nearly impossible to identify the initial dose of pesticide ingested. It could be presumed that individual physiology of the victims could significantly impact the absorption, metabolism, and elimination processes. Our study presents exciting observations on the inter-relationship between pesticides and AChE enzyme concentrations. Our findings may assist researchers in understanding the relationship between OPs and AChE levels in acute poisoning cases.

We observed a significantly higher concentration of monocrotophos in the patients' peripheral blood and a corresponding significant decrease in AChE concentrations. Since, monocrotophos

concentration was high, even treatment with 2-PAM and atropine was not sufficient to markedly increase the AChE concentration. We observed a similar pattern of pesticide and AChE concentration with chlorpyrifos. The remaining pesticides analysed in this study, namely dimethoate, triazophos, acephate, and propanil, showed an increase in AChE concentration with the injection of 2-PAM and atropine. Thus, our study emphasises minimisation of the handling of monocrotophos and chlorpyrifos by humans. We also measured the Cmax and Tmax of the pesticides using the peripheral blood samples obtained from the acute OP poisoning cases. Cmax and Tmax depend on the pesticide's absorption rate and their deposition profile. The rate of pesticide absorption plays a vital role in the study of pharmacokinetics.

A fraction of an oral dose may be absorbed at the early stage of administration in the gastrointestinal tract or the liver, while few victims may present with malabsorption resulting in incomplete bioavailability of the pesticide in the blood that directly indicates the ineffectiveness of the treatment provided. We observed that chlorpyrifos had the highest Cmax of 9029.36 ng/mL at a Tmax of 12 h, and dimethoate showed the lowest Cmax of 4.05 ng/mL at a Tmax of 12 h in the acute poisoning cases analysed in this study. Notably, all pesticide concentrations reached a maximum value in the blood at 12 h. AUC was also calculated to the determine the concentration of each pesticide accumulated in the blood in a given period. AUC is a way of representing the total pesticide exposure over time. In the present study, we observed that chlorpyrifos followed by triazophos showed the highest AUC values. Dimethoate, in contrast, with a Tmax similar to that of monocrotophos (12 h), did not exhibit a higher AUC because of its low Cmax value. In fact, dimethoate showed the least AUC value among all the pesticide-associated poisoning cases analysed in the current study. The AUC of chlorpyrifos was 232,234.52 ng*h/mL with a half-life of 32.83 h. The chlorpyrifos was either eliminated from the body or was converted into other metabolites within 32.83 h, which was an interesting finding compared with the other

Conclusion

Based on these observations, we suggest that in monocrotophos and dimethoate poisoning cases, the maximum point-of-treatment care should be provided before 12 h post-exposure, while with the other pesticides, it should be provided before 36e72 h, according to the peak circulating pesticide levels. Further, AChE enzyme levels correlate with the pesticide concentration, where a higher pesticide concentration is more detrimental to the enzyme level, and chances for enzyme restoration post-standard treatment using 2-PAM and atropine injections are low. This indicates that toxicokinetics or pesticide concentration level and enzyme level determination help analyse toxic exposure, and accordingly, treatment can be administered to save human lives. We further emphasise the importance of enforcing restrictions or strict safe handling precautionary measures when handling monocrotophos and chlorpyrifos. Further, there is a need to verify the biotransformation and correlate metabolite levels and pesticide levels to enzyme levels and to determine the toxicological effects of various pesticides after human exposure through accidental and/or unfortunate events to collect relevant data; this can provide inputs in guiding decision making processes for treatment.

LIST OF PhD SCHOLARS 2019-2020

S No	Name of the Scholar	Year of Registration/ completion	University	Thesis title	Name of the Guide
1	**Mr. K. Naga Surya Prasad**	Registered (2017)	Osmania University	Beneficial effects of Mesenchymal Stem cells in ameliorating effects of type-2 diabetes	Dr. V. Vijayalakshmi
2	**Ms. Pooja Desai**	Registered (2017)	Osmania University	Therapeutic role of BDNF and PUFAs in type 2 diabetes	Dr. V. Vijayalakshmi
3	**Ms. B. Sindhoora**	2019	Osmania University	Association of candidate gene SNPs with the risk & pathogenesis of PCOS- a hospital based study.	Dr. B. Dinesh Kumar
4	**Dr.Vandana Singh**	2016	NTR Health University, Vijayawada	Translation of traditional formulation Cynodondactylon (Swarasa of Durva) in Menopausal Syndrome Complication (Rajonivrittijanyavikritavvastha) - A Reverse pharmacology approach	Dr. B. Dinesh Kumar
5	**Ms.Kiranmai**	2015	Osmania University	Effect of vitamin D deficiency on Statin induced myalgia: Genetic Polymorphism	Dr. B. Dinesh Kumar
6	**Ms. Anitha Singh**	2015	Osmania University	Evaluation of traditional plant based formulation (Cocculushirsutus, Cuscutareflexa, Tinosporacordifolia and Achyranthusaspera) for immunomodulatory and anti-inflammatory potential	Dr. B. Dinesh Kumar
7	**Mr.M.Sivaprasad**	Awarded (2019)	Osmania University	Status of micronutrients and its influence on molecular mechanisms in diabetic nephropathy	Dr. G. Bhanuprakash Reddy
8	**Ms.T.Shalini**	Awarded (2019)	Osmania University	Assessment of nutritional status of geriatric population	Dr. G. Bhanuprakash Reddy
9	**Mr. K.Rajesh Kumar**	Awarded (2020)	Osmania University	Role of aurora kinase B in chronic tissue remodeling	Dr. G. Bhanuprakash Reddy
10	**Mr. Ch.Uday Kumar**	Registered (2017)	Osmania University	Obesity-induced cardiomyopathy: molecular mechanisms and effect of the dietary intervention	Dr. G. Bhanuprakash Reddy
11	**Ms.P.Swathi Chitra**	Registered (2017)	Osmania University	Studies on biochemical pathways and micronutrient status in age-related cataracts	Dr. G. Bhanuprakash Reddy
12	**Mr.H.E. Harsha-vardhana**	Registered (2018)	Osmania University	Profibrotic mechanisms in diabetic complications: Role of dietary agents	Dr. G. Bhanuprakash Reddy
13	**Ms.Paromita Banerjee**	Registered (2018)	Osmania University	Promoting nutrition and health of corporate employees with workplace intervention-A study using communication for behavioral impact (COMBI) approach	Dr. G. Bhanuprakash Reddy
14	**Mr.S.Udaykanth**	Registered (2018)	Osmania University	Role of vitamin B12 in diabetic neurodegeneration	Dr. G. Bhanuprakash Reddy
15	**Ms.A Santhoshi Vani**	To be Registered	Osmania University		Dr. G. Bhanuprakash Reddy
16	**Mr.K. Krishna Kalyan**	To be Registered	Osmania University		Dr. G. Bhanuprakash Reddy
17	**Ms.Yvette Wilda Jywra**	January-2017	Osmania University	Studies on the bioavailability of iron on fortified foods	Dr.P.Raghu
18	**Ms.Hunuma Naik**	July-2019	Osmania University	Mechanism of iron and zinc interaction in cells	Dr.P.Raghu
19	**Ms.Puneetha Singh**	March-2019	Osmania University	Modulation of iron storage and recycling by zinc in caco2 cells	Dr.P.Raghu
20	**Mr.Palsa Kondaiah**	Completion March 2020	Osmania University	Effect of zinc on intestinal iron absorption: *In vitro* and *in vivo* studies	Dr. P. Raghu

	Name of the Scholar	Year of Registration/ completion	University	Thesis title	Name of the Guide
21	**Ms.Srujana Medithi**	2019 (Completion)	Osmania University, Hyderabad	Assessment and evaluation of micronutrient status and biochemical parameters in relation to pesticide exposure among farm women and their children	Dr. J. Padmaja
22	**Ms Summaiya Alam Lari**	2016 (Registration)	Acharya Nagarjuna University, Guntur	Assessment of pesticide residues penetration into the skin of farmers and farmwomen through protective gear in field conditions	Dr. J. Padmaja
23	**Mr.Arun Pandiyan P.**	2019 (Registration)	Osmania University, Hyderabad	Association between pesticide residue concentrations in tissues and with the lymphoma, leukemia and breast cancers	Dr. J. Padmaja
24	**Mr.Venna Naresh Kumar**	2017	Osmania University	Synergistic effects of cowpea isoflavones, beta carotene and vitamin D on the osteoblasts differentiation	Dr.C.Suresh
25	**Ms Lakshmi Jaya Madhuri B**	2019	Osmania University	Alterations in the mitochondrial function and energy metabolism in human brain cells by the exposure of environmental pollutant, Lead and Amyloid peptide combo: Possible synergistic invitro deleterious intracellular effects.	Dr.C.Suresh
26	**Mr.Srinivas Vilasagaram**	2015	Osmania University	First trimester placental development: Investigating the role of fatty acids and glucose on cellular and molecular mechanisms of placenta related disorders	Dr.Sanjay Basak
27	**Mr.Varma Saikanth**	Yet to register	Osmania University	Maternal exposure of endocrine disrupters during development: impact on reproductive and metabolic homeostasis in the offspring	Dr.Sanjay Basak

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>. 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.nin.res.in).

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	3
Reports	12
Thesis / Dissertations	13
CDROMS	-
General CD's	-

2. Other activities

Journals Bound	318
Visitors using the Library	2,911
Circulation of Books/Journals etc	419
No. of E-mails sent outside	225
No. of E-mails received	4,225
Photocopying (No. of pages)	1,49,043
No. of INTERNET Searches provided	110
No. of Reprints sent	45

3. Total library collections

Books	18,278
E – Books	36
Journals subscribed for 2019	153
E – Journals subscribed for 2019	23
Journals (Bound Volumes)	41,467
Journals received (Gratis/Exchange) for 2019	108
Microforms (Microfiche)	1,080
Slides	280
Reports	14,084
Theses & Dissertations	466
MEDLINE CDROMS Discs	383
Current Contents on Diskettes with abstracts	664
Proquest (Full Text E-Journals) on CD ROMS	495
General CD's	331

SCIENTIFIC PUBLICATIONS - 2019

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Aleksandrowicz L, Green R, Joy EJM, Harris F, Hillier J, Vetter SH, Smith P, Bharati Kulkarni, Dangour AD, Haines A: Environmental impacts of dietary shifts in India: A modelling study using nationally-representative data. Environ int. 126: 207-215, 2019. (IF 7.943)
2. Archana K, Nair KM, Balakrishna N, SubbaRao GM: A sequential, exploratory, mixed-methods approach for development and validation of a context-specific knowledge, attitude and practice questionnaire on micronutrients for literate mothers of school-age children. Public Health Nutr. 22: 2120-2131, 2019. (IF 2.483)
3. Aruna T, Alekhya G, Deepika T, Devindra S: Food processing methods and their effects on oligosaccharide content of commonly consumed legumes. Int J Curr Agric Sci. 9: 359-364, 2019. (IF 0.654)
4. Ashwini VI, Aniket PS, Pramod SP, Khandare AL, Sinha SN, Kshipra SK, Yogesh WM, Dattatraya NP: Simple chromium catalyzed oxidative synthesis of quinazolinones and benzoxazinones from 2- aminobenzamide and anthranilic acid with arylaldehydes. Eur. Chem. Bull. 8: 180-187, 2019.
5. Babu Geddam JJ, Naveen Kumar B, Raja Sriswan M, Mahesh Kumar M, Sreenu P: Associated factors of stunting and thinness among rural adolescent girls in Telangana, South India. Ind J Nut Diet. 56: 381-396, 2019.
6. Bharati Kulkarni, Raja Sriswan M: Nutrition rehabilitation of children with severe acute malnutrition: Revisiting studies undertaken by the National Institute of Nutrition. Indian J Med Res. 150: 139-152, 2019. (IF 2.061)
7. Bidyalakshmi L, Ananthan R, Daniella AL Chyne, Longvah T: 12th IFDC 2017 Special Issue - Nutrient and phytonutrient profiles of some indigenous vegetables of Manipur, Northeast India. J Food Comp Anal. 79: 12-22, 2019. (IF 1.985)
8. Blessy Prabhu Priyanka S, Padma Vijayasree A, Devraj JP, Santosh Kumar B, Mahesh Kumar M, Naveen Kumar B: A study of serum lipid profile in normal pregnancy and pregnancy induced hypertensive disorders: a case-control study. Int J Reprod Contracept Obstet Gynecol. 8: 2071-2075, 2019.
9. Brahmaiah U, Amruth Rao P, Hemalatha R, Laxmaiah A: Pellagra: A forgotten entity. Clin Dermatol Rev. 3: 126-129, 2019.
10. Curto A, Ranzani O, Milà C, Sanchez M, Marshall JD, Bharati Kulkarni, Bhogadi S, Kinra S, Wellenius GA, Tonne C: Lack of association between particulate air pollution and blood glucose levels and diabetic status in peri-urban India. Environ int. 131, 2019 (105033). (IF 7.943)
11. Curto A, Wellenius GA, Milà C, Sanchez M, Ranzani O, Marshall JD, Bharati Kulkarni, Bhogadi S, Kinra S, Tonne C: Ambient particulate air pollution and blood pressure in peri-urban India. Epidemiology. 30: 492-500, 2019. (IF 5.071)
12. Daniella AL Chyne, Ananthan R, Longvah T: Food compositional analysis of indigenous foods consumed by the Khasi of Meghalaya, North-East India. J Food Comp Anal. 77: 91-100, 2019. (IF 1.985)
13. Dripta Roy Choudhury, Nair KM, Balakrishna N, Radhakrishna KV, Sudip Ghosh, Sylvia Fernandez Rao: A food synergy approach in a national program to improve the micronutrient status of preschoolers: a randomized control trial protocol. Ann N Y Acad Sci. 1438: 40-49, 2019. (IF 4.728)
14. Harikumar R, Khandare AL, Laxmaiah A, Meshram II, Arlappa N, Vakdevi V, Venkaiah K, Amrutha Rao P, Sunu PV, Bhaskar V, Toteja GS: Prolonged consumption of grass pea (64

g/Cu/day) along with millets and other cereals causes no neurolathyrism. Nutritional Neuroscience. 2019. (https://doi.org/10.1080/1028415X.2019.1642641). (IF 3.765)

15. Himadri Singh, Samuel JP, Vijayalakshmi Venkatesan: Emerging therapeutic targets for metabolic syndrome: lessons from animal models. Endocrine Metab Immune Disord - Drug Targets. 19: 481-489, 2019. (IF 1.973)
16. Ishwari AK, Nilema SG, Aniket PS, Bhagwan KS, Khandare AL, Sinha SN, Kshipra SK, Dattatraya NP: Peel extract associated oxidative green dakin synthesis of some phenols using aqueous banana extract catalyst. Eur Chem Bull. 8: 160-163, 2019.
17. Joe W, Rajpal S, Kim R, Laxmaiah A, Harikumar R, Arlappa N, Meshram II, Balakrishna N, Radhi ka MS, Swaminathan S, Subramanian SV: Association between anthropometric-based and food-based nutritional failure among children in India, 2015. Matern Child Nutr. 2019. (https://doi.org/10.1111/ mcn.12830) (IF 2.789)
18. Kondaiah P, Aslam MF, Purnachandra M, Sharp PA, Raghu P: Zinc induces iron uptake and DMT1 expression in Caco-2 cells via a PI3K/IRP2 dependent mechanism. Biochem J. 476: 1573-1583, 2019. (IF 4.331)
19. Li Y, Mallinson PAC, Bhan N, Turner C, Bhogadi S, Sharma C, Aggarwal A, Bharati Kulkarni, Kinra S: Neighborhood physical food environment and cardiovascular risk factors in India: Cross-sectional evidence from APCAPS. Environ int. 132, 2019. (105108) (IF 7.943)
20. Mariachiara Di Cesare, Maroje Sorić, Pascal Bovet, J Jaime Miranda, Zulfiqar Bhutta, Gretchen A Stevens, Laxmaiah A, Andre-Pascal Kengne, James Bentham: The epidemiological burden of obesity in childhood: a worldwide epidemic requiring urgent action. BMC Medicine. 17, 2019. (https://doi.org/10.1186/s12916-019-1449-8) (IF 6.782)
21. Meghamala M, Koushik Das, Dilip Kumar N, Balaram Das, Sreenivas Rao J: Analysis of fatty acids from oil of green tea (camellia sinensis l) by gas chromatography coupled with flame ionization detector and its anticancer and antibacterial activity in vitro. Int J Pharmac Pharmaceut Sci. 11: 104-111, 2019. (IF 2.029)
22. Meshram II, Mallikharjun Rao K , Balakrishna N, Harikumar R, Arlappa N, Sreeramakrishna K, Laxmaiah A: Infant and young child feeding practices, sociodemographic factors and their association with nutritional status of children aged is <3 years in India: findings of National Nutrition Monitoring Bureau (NNMB) survey, 2011-2012. Public Health Nutrition. 22: 104-114, 2019. (IF 2.483)
23. NCD Risk Factor Collaboration (Laxmaiah A et al.): Rising rural body-mass index is the main driver of the global obesity epidemic in adults. Nature. 569: 260-264, 2019. (IF 42.778)
24. Neha Reddy R, Sneha Manju M, Souris K, Santosh Kumar B: Nutraceuticals and Nutrivigilance-present scenario in India. Int J Food Biosci. 2: 35-40, 2019.
25. Paromita Banerjee, Vishnuvardhan Rao M, Damayanthi K, SubbaRao GM: Calorie counting smart phone apps: effectiveness in nutritional awareness, lifestyle modification and weight management among young Indian adults. Health Informatics J. 2019. (https://doi.org/10.1177/1460458219852531) (IF 1.833)
26. Pomeroy E, Mushrif-Tripathy V, Bharati Kulkarni, Kinra S, Stock JT, Cole TJ, Shirley MK, Wells JCK: Estimating body mass and composition from proximal femur dimensions using dual energy x-ray absorptiometry. Archaeol Anthropol Sci. 11: 2167-2179, 2019. (IF 2.063)
27. Raghu P, Bharati Kulkarni, Hemalatha R: Ironing out the iron requirements of children and adolescents. Indian Pediatrics 56: 547-548, 2019. (IF 1.186)
28. Rahul Mallick, Sanjay Basak, Duttaroy AK: Docosahexaenoic acid, 22:6n-3: Its roles in the structure and function of the brain. Int J Dev Neurosci. 79: 21-31, 2019. (IF 2.025)
29. Rajesh KK, Bhanuprakash Reddy G, Anil GJ, Satish KM: Dysregulation of mesenchymal cell survival pathways in severe fibrotic lung disease: The effect of nintedanib therapy. Front Pharmacol. 10, 2019. (https://doi.org/ 10.3389/fphar.2019.00532) (IF 4.225)
30. Rajeswara Reddy M, Indrapal Singh A, Utkarsh Reddy A, Bhanuprakash Reddy G, Kundane Ingle, Mukhopadhyay S: Synthesis of 4-C-β-D-Glucosylated isoliquiritigenin and analogues for aldose reductase inhibition studies. Eur J Org Chem. 2019: 3937-3948, 2019. (IF 2.889)
31. Ravindar Naik R, Harishankar N, Suresh P, Sailaja P, Satyavani M, Quadri SSYH, Srinivas M, Brahmanaidu P: Obesity-alleviating capabilities of acalypha indica, pergulari ademia and tinospora

cardifolia leaves methanolic extracts in WNIN/GR-Ob rats. J Nutr Intermediary Metab. 16, 2019. (100090) (doi.org/10.1016/j.jnim.2019.02.001) (IF 0.630)

32. Sanjay Basak, Srinivas V, Kishore Naidu, Duttaroy AK: Insulin-dependent, glucose transporter 1 mediated glucose uptake and tube formation in the human placental first trimester trophoblast cells. Mol Cell Biochem. 451:91-106, 2019. (IF 2.057)
33. Santosh Kumar B, Balakrishna N, Rajanna A: Combined anticonvulsant effect of nifedipine and pentazocine in experimentally induced seizures in rats. Int J Basic Clin Pharmacol. 8: 1875-1879, 2019.
34. Santosh Kumar B, Rajanna A, Balakrishna N: Combined anticonvulsant effect of nifedipine and pentazocine in experimentally induced maximal electro shock method in mice. J Drug Delivery and Therapeutics. 9: 288-291, 2019. (IF 2.2558)
35. Sathibabu Uddandrao VV, Rameshreddy P, Brahmanaidu P, Ponnusamy P, Balakrishnan S, Ravindar Naik R, Swapna, Suresh P, Harishankar N, Balaji M, Vadivukkarasi S, Nivedha PR, Saravanan Ganapathy: Antiobesity efficacy of asiatic acid: down-regulation of adipogenic and inflammatory processes in high fat diet induced obese rats. Arch Physiol Biochem. 2019. (doi: 10.1080/13813455.2018.1555668) (IF 1.730)
36. Saurabh Sharma, Arlappa N, Balakrishna N, Aroonima Misra, Laxmaiah A: Validation of APLS, Argall and Luscombe formulae for estimating weight among Indian children. Indian J Pediatr. 86: 648–650, 2019. (IF 1.508)
37. Sharvari Deshpande S, Harishankar N, Suresh P, Kushaan K, Anita Kumar, Prathap Reddy K, Nafisa H. Balasinor: Genetically inherited obesity and high- fat diet-induced obesity differentially alter spermatogenesis in adult male rats. Endocrinology. 160: 220-234, 2019. (IF 3.961)
38. Sharvari Deshpande S, Harishankar N, Suresh P, Nafisa H. Balasinor: Altered endocrine, cytokine signaling and oxidative stress: A plausible reason for differential changes in testicular cells in diet-induced and genetically-inherited – obesity in adult rats. Reprod Biol. 19: 303-308, 2019. (IF 1.862)
39. Shritesh D J, Aniket P S, Khandare AL,Ishudeep K N, Kshipra S K, Dattatraya N P, Rohini N S: Thionyl chloride induced convenient synthesis of benzamides from 3-bromo-5-nitrobenzoic acid and amines under solvent free conditions. Eur Chem Bull. 8: 123-127, 2019.
40. Shujauddin Mohammed, Hemalatha R, Quadri SSYH, Annapurna VV: Semi-synthetic diet versus diet using natural ingredients—.comparative study in female golden syrian hamsters. J Anim Physiol Anim Nutr. 103: 687-694, 2019. (IF 1.597)
41. Sivakesava Rao K, Sai Santhosh V, Srinivas M, Uday Kumar P, Raghava Rao T, Suryanarayana P: Cinnamon attenuated long-term IGT-induced retinal abnormalities via regulation of glucose homeostasis in neonatal streptozotocin induced rat model. Indian J Clin Biochem. 2019. (DOI 10.1007/s12291-019-00842-z)
42. Sivaprasad M, Shalini T, Yadagiri Reddy P, Seshacharyulu M, Madhavi G, Naveen Kumar B, Bhanuprakash Reddy G: Prevalence of vitamin deficiencies in an apparently healthy urban adult population: assessed by subclinical status and dietary intakes. Nutrition. 63-64: 106-113, 2019. (IF 3.42)
43. Sreenivasa Rao J, Durga Sai Sri T, Shivudu G, Veenita kumari: Micro greens: A new initiative and harmonizing approach for promoting livelihood and nutritional security-India. Int J Food Biosci. 2: 1-5, 2019.
44. Sreenivasa Rao J, Shivudu G, Hrusikesh Panda, Kalyan Reddy P: Livelihood strategies resource and nutritional status of forest dependent primitive tribes chenchu in Andhra Pradesh and Telangana States. Nutr Food Sci Int J. 8: 1-9, 2019. (DOI: 10.19080/NFSIJ.2019.08.555735) (IF 0.849)
45. Sreenivasa Reddy S, Shruthi K, Dorit Joy, Bhanuprakash Reddy G: 4-PBA prevents diabetic muscle atrophy in rats by modulating ER stress response and ubiquitin-proteasome system. Chem Biol Interact. 306: 70-77, 2019. (IF 3.723)
46. Srinivasarao DA, Sreenivasa Reddy S, Bhanuprakash Reddy G, Katti DS: Spatio-temporal control on the delivery of triamcinolone acetonide using polymeric nanoparticles reduces steroid induced cataract. Int J Pharmac. 568, 2019: (doi: 10.1016/j.ijpharm.2019.118474) (IF 4.845)
47. SubbaRao GM: Nutrition communication – Rhetoric and reality. Indian J Med Res. 149: 333-344, 2019. (IF 1.503)

48. SubbaRao GM, Hemalatha R: Thought for food: Mahatma's views on nutrition, controlled and balanced diets. Indian J Med Res. 149(Suppl.): 119-127, 2019. (Invited article for special issue on Gandhi and Health @150). (IF 1.503)
49. Sudha Narayanan, Lentz E, Fontana M, Anuradha De, Bharati Kulkarni: Developing the women's empowerment in nutrition index in two states of India. Food Policy. 89, 2019. (https://doi.org/10.1016/j.foodpol.2019.101780) (IF 3.788)
50. Sudhakar Reddy V, Trinath J, Bhanuprakash Reddy G: Implication of homocysteine in protein quality control processes. Biocheimie. 165: 19-31, 2019. (IF 3.413)
51. Surekha MV, Sapna Singh, Sarada K, Sailaja G, Balakrishna N, Srinivas M, Uday Kumar P: Study on the effect of severity of maternal iron deficiency anemia on regulators of angiogenesis in placenta. Fetal Pediatr Pathol. 2019. (doi: 10.1080/15513815.2019.1587120) (IF 0.596)
52. Swapna K, Sathibabu Uddandrao VV, Brahmanaidu P, Ravindar Naik R, Suresh P, Ponnusamy P, Balakrishnan S, Vadivukkarasi S, Harishankar N, Prathap Reddy K, Nivedha PR, Saravanan Ganapathy: Effects of asiatic acid, an active constituent in *Centella asiatica* (L.): Restorative perspectives of streptozotocin-nicotinamide induced changes on lipid profile and lipid metabolic enzymes in diabetic rats. Comp Clin Pathol. 28: 1321–1329, 2019.
53. Teena D, Selvaraj K, Raghu P, Bharati Kulkarni: Animal source foods for the alleviation of double burden of malnutrition in countries undergoing nutrition transition. Animal Frontiers. 9: 32-38, 2019. (IF 1.382)
54. Udgata A, Dolasia K, Sudip Ghosh, Mukhopadhyay S: Dribbling through the host defence: Targeting the TLRs by pathogens. Crit Rev Microbiol. 2019. (https://doi.org/10.1080/1040841X.2019. 1608904) (IF 5.697)
55. Vakdevi V, Gopalan V, Khandare AL: Comparison of fluoride levels (total and extracted) in young, old tea leaves and market tea samples along with impact of tea infusion on dental fluorosis in fluoride endemic villages of Nalgonda district, India. Adv Dent Oral Health. 10, 2019. (DOI: 10.19080/ADOH.2019.10.555793) (IF 1.092)
56. Varsha V, Kiranmayee A, Ashok Kumar KS, Balakrishna N, Dinesh Kumar B: Sex-specific role of *CYP24A1* rs2762939 in the risk of essential hypertension based on the serum vitamin D and total renin concentrations. Genomics, 2019. (https://doi.org/10.1016/j.ygeno.2019.05.013) (IF 6.205)
57. Venkanna B, Vijayalakshmi Venkatesan: Conventional polymerase chain reaction and amplification refractory mutation system-multi-gene/ multi-primer PCR in the diagnosis of female genital tuberculosis. Arch Microbiol. 201: 267–281, 2019. (IF 1.884)

B. PAPERS PUBLISHED IN PROCEEDINGS/ BOOKS/ CONFERENCES/ ABSTRACTS

1. Himaja N, Devraj JP, Hemalatha R, Manoj Kumar: Food borne illnesses and environmental factors. In “Recent trends and advances in environmental health” edited by Vinod Verma, New York, Nova Biomedical. 2019.
2. Sinha SN: Air pollution from solid fuels. In “Encyclopedia Environ health” 2nd ed. By Nriagu J, Vol 1, Elsevier. 2019, 49-60pp.

Scientific Advisory Committee

Chairperson – Dr. Nikhil Tandon
Professor & Head,
Dept. of Endocrinology and Metabolism
AIIMS, New Delhi

Dr.Kamala Krishnaswamy
Former Director, NIN
Hyderabad

Dr. Umesh Kapil
Professor, Dept. of Gastroenterology & Human Nutrition, AIIMS, New Delhi

Dr. Bikash Medhi
Addl. Medical Superintendent
Dept. of Pharmacology, PGIMER,
Chandigarh

Dr. R.M.Pandey
Prof. & Head Dept. of Biostatistics
AIIMS, New Delhi

Prof. B.P.Sanjay
Manipal Institute of Communication
Manipal, Karnataka

Dr.Dheeraj Shah
Professor, Dept. of Paediatrics
GTB Hospital, New Delhi

Dr.Geeta Trilok Kumar
Director
Institute of Home Economics
New Delhi

Dr.Anand K. Kondapi
Dept. of Biotechnology
University of Hyderabad

Dr.M.V.Murali
Prof. of Paediatrics (Retd.)
Habsiguda, Hyderabad

Prof. Shobha Udipi
Director, Division of Integrative Nutrition and Nutraceuticals, Kasturba-Health Society's Medical Research Centre

Dr. Gangadhar Taduri
Professor & Head
Department of Nephrology (Unit-1)
Nizams Institute of Medical Sciences
Hyderabad