वार्षिक प्रतिवेदन

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ICMR - NATIONAL INSTITUTE OF NUTRITION

Indian Council of Medical Research

Hyderabad, Telangana, INDIA

Website: www.nin.res.in

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Annual Report

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RESEARCH HIGHLIGHTS

1. Comprehensive nutrition assessment of tribal population in Attappadi Taluk, Kerala – A rapid exploratory study

In the context of high rates of infant and neonatal mortality, the Indian Council of Medical Research-National Institute of Nutrition (ICMR-NIN) conducted a community-based cross-sectional study in Attappadi, Kerala. The objective was to assess the nutritional status of distinct demographic groups, encompassing young children (0-59 months), children (5-9 years), adolescent girls (10-19 years), currently pregnant women (15-49 years), and lactating mothers (with children aged 0-11 months). The survey encompassed 480 households, covering 523 children, 150 adolescent girls, 40 pregnant women, and 110 lactating mothers across 20 tribal villages.

The study revealed widespread malnutrition and chronic energy deficiency among the tribal population. Young children exhibited high rates of underweight (48.3%), stunting (41%), and wasting (27.4%). Inadequate intake of most food groups was found to be a significant contributor to malnutrition. Anaemia prevailed among young children, adolescent girls, and pregnant women, with 65% of children aged 12-24 months experiencing iron deficiency anaemia. Additionally, deficiencies of vitamin B12, vitamin D, folate, and vitamin A affected 35%, 20%, 16%, and 12% of children, respectively.

To address the malnutrition challenge, the study proposed a comprehensive strategy involving the identification and promotion of locally suitable and culturally acceptable food-based solutions. The implementation of a participatory and community-driven system was emphasized to mainstream health and nutrition information. Such a multifaceted approach is deemed essential to combat malnutrition and safeguard the well-being of the tribal population in Attappadi.

2. Development of maternal nutrition e-modules to strengthen the maternal nutrition secretariat at NIN

Maternal nutrition e-modules is a collaborative project initiated in January 2021 by ICMR-NIN, FOGSI, and UNICEF. These e-modules aim to enhance maternal nutrition assessment and services in antenatal care in India. The project's primary objective is to present the latest knowledge on maternal nutrition interventions, strategies, and innovations in a format accessible to nutritionists, gynecologists, and public health professionals. A total of 12 modules were prepared, focusing on various aspects of maternal nutrition, including maternal mental health, anthropometric measures,

counseling skills, and emergency preparedness. These modules are intended for obstetricians, nutritionists, and other health professionals and will be integrated into routine counselor/maternal health training. The document also highlights key recommendations to accompany the modules, emphasizing the importance of early identification of at-risk women, dissemination of guidelines, nutrition education, and strengthening health and nutrition systems.

3. Identification of the different processed foods and estimation of their AGEs content

Advanced glycation end products (AGEs) are formed within the body as a part of normal metabolism and are also formed as by-products of cooking food. The elevated levels of AGEs in the body are considered pathogenic. The processing and cooking of modern foods contain high levels of AGEs which get incorporated into the body AGEs pool and contribute to diabetic and age-related complications. The objective of the present study is to estimate the cross-linked AGEs (AGE-fluorescence) and the more stable carboxymethyl-lysine (CML) by spectrofluorimetry and ELISA in 58 kinds of foods in India. It was evident from the results that the foods cooked at higher temperatures showed high levels of AGEs. Among the studied foods highest Fluorescence was notice in Osmania biscuits (362 AU) and CML by soya milk (659.3 ng/gm). However, there was less correlation between the AGE-fl and in the CML content of the food samples. The processed foods, packed foods, western foods, and biscuits contain high AGEs contents. With the present database, a better understanding of the foods with AGEs content can be drawn and a healthier diet can be suggested to control the age and diabetic-related complications.

Novelty Impact Statement: The results of the study indicate that a lot of foods contain AGEs which contribute to diabetic and age related complications. There is no existing database of the Indian foods is available as far as dietary AGEs are concerned, hence this study will become a base in choosing the right food for the diabetic patients. Among the studied foods the category of traditional Indian foods contain fewer AGEs compared to the processed foods which make them a healthy option. The present study can be extended to cover other Indian foods.

4. Nutrient composition and antinutrient factors of thirty-three newly developed varieties of chickpea bred for various agronomic traits

Chickpeas or garbanzo beans also called Bengal gram or chana in India, has a record 13.12 million tons production in India during the year 2021-22, which accounts for 73% of the total global chickpea production. It has large variation in its types and nutrient composition, owing to diverse environmental conditions, breeding techniques, and cultivars. Thirty-one improved varieties of chickpea bred for various agronomic traits like high yield, resistance to diseases, tolerance to abiotic stress were analyzed for

their nutrient composition, along with two local varieties. They were found to be rich in proteins (16.09 - 26.22 g/100g) and dietary fiber (10.33 - 26.33g/100g) with moderate amounts of carbohydrates (34.20 - 54.72 g/100g) and to have a significant quantity of minerals like calcium (127.50 - 183.86 mg/100g), iron (4.55 - 8.33 mg/100g) and phosphorous (285.92 - 528.31 mg/100g). They were found to be similar (fat, carbohydrates, dietary fiber) or statistically higher (protein, ash) than the local varieties for all the nutrient parameters that were analyzed. This can address the common misconception among the public about the health and nutritional aspects of crops that are genetically variant or altered for various agronomic traits, in terms of nutrient quantities. A significant difference was also found between the desi and kabuli varieties, where desi variety showed significantly lower fat, available carbohydrates, and high amounts of dietary fiber, making it nutritionally superior in terms of diseases associated with carbohydrate metabolism and gut health. This study signifies that the varietal differences in nutritional composition is significant in chickpea. Varieties like Sasho, ICCV 96030, and Teketay were identified as possessing desirable nutritional qualities associated with moisture, protein, dietary fiber, and minerals like zinc, phosphorous, iron, copper, and calcium. Antinutrients like tannins, saponins, and phenolic acids were also found in smaller quantities. This data will be beneficial for manufacturers in product development and value addition industries for selection of varieties ideal for their need, since the nutrient component also confers several functional and physiochemical properties to the chickpea seed besides providing a nutritionally diverse diet.

5. A study on the macronutrients, micro-elements and anti-nutrient composition of thirteen green gram (*Vigna radiata (L.) Wilczek)* cultivars

Green gram *(Vigna radiata (L.) Wilczek*) is India's fourth most-produced and commonly consumed pulse crop and is regarded as green pearl owing to its high nutrient content (Mekkara nikarthil Sudhakaran and Bukkan, 2021). The objective of the current study is to accurately estimate the macronutrient, mineral and antinutrient composition of 13 different cultivars (ten newly developed and three local varieties) of green gram. From the results, they contain 62.5 to 84.6 g/kg of moisture, 28.3 to 37.4 g/kg of ash, 21.9 to 3.08 g/kg of fat, 484.6 to 535.7 g/kg of carbohydrate, 228.7 to 277.6 g/kg of protein, and 118.3 to 157.9 g/kg of dietary fibre. The most abundant mineral found was phosphorus, ranging from 2716.66 to 4473.49 mg/kg followed by 3183.31 to 3597.61 mg/kg of potassium, 1506.51 to 1713.93 mg/kg of magnesium, 166.38 to 340.62 mg/kg of calcium, 40.16 to 348.79 mg/kg of iron, 27.60 to 34.35 mg/kg of zinc, 5.95 to 12.86 mg/kg of copper and 8.65 to 19.47 mg/kg of manganese. The phytic acid and saponin content varied from 0.453 to1.137% and 1.17 to 1.99% respectively. Tannin content varied from 0.91 to 1.78 mg Catechin equivalents/g. Total phenolic content varied from 17.17 to 25.55 mg/100g. Trypsin inhibitor activity varied from 4.41 to 10.44 TUI/mg sample and chymotrypsin inhibitory activity was not detected. The newly developed varieties of green gram

showed high protein, dietary fibre content and low carbohydrate content, while the local varieties were high in calcium and iron. Hence, both types of varieties are nutritionally significant.

6. Understanding the role of vitamin B12 in diabetic neurodegeneration: Implications in protein quality control processes.

- Dietary B12 deficiency impaired the cognitive behaviour and induced the memory loss in streptozotocin (STZ)-induced diabetic rats.
- Furthermore, B12 deficiency induced the neuronal cell death, astrogliosis, reduced the neurotrophic and synaptic density markers, caused the ER stress, and autophagy in the cerebral cortex (CC) of the STZ-induced diabetic rats.
- However, dietary B12 supplementation improved the cognitive behaviour and ameliorated the neuronal morphology and cell death, reduced the astrogliosis in diabetic rats.
- Dietary B12 supplementation improved the neurotrophic support and synaptic density related proteins and inhibited the ER stress in CC of STZ-induced diabetic rats
- Dietary B12 supplementation ameliorated the activation of autophagy and restored the ubiquitin proteasome system in STZ-induced diabetic rat CC.
- Overall, the findings suggest that B12 acts as neuroprotective agent by inhibiting the neuropathological changes induced by chronic hyperglycemia.

7. A functional food formulation attenuates diabetic nephropathy and retinopathy in rats

- A functional food (FF) formulation attenuates diabetic nephropathy and retinopathy in rats.
- A functional food (FF) mix containing amla, turmeric, black pepper, cinnamon, and ginger was formulated and evaluated for its preventive effect against diabetic nephropathy and retinopathy (DN and DR) in rodent models.
- FF (0.85% and 4.25.% in the diet) attenuated proteinuria and renal abnormalities in diabetic rats.
- FF attenuated loss of retinal function due to diabetes as examined by electroretinogram
- FF prevented DN and DR progression in diabetic rats by inhibiting glycation, neovascularization, polyol pathway activation, oxidative stress, and inflammation.

8. Attenuation of cataracts through a functional food mix in a diabetic rat model

- We tested a functional food (FF) mixture containing six ingredients (amla, turmeric, black pepper, cinnamon, ginger, and fenugreek) for its effect on suppressing the onset and progression of cataracts in a diabetic rat model.
- Two months old SD rats were grouped as control (C), diabetes untreated (D), and diabetic rats treated with FF at two doses (FF1= 1.35 g and FF2=6.25 g/100g of diet). The status of cataracts was monitored weekly by a slit lamp examination for 20 weeks, after which animals were sacrificed to collect eye lenses.
- Results showed that feeding FF1 and FF2 to diabetic rats yielded a significant anti-hyperglycaemic effect and delayed cataract progression. FF2 showed better efficacy than FF1.

9. Dietary zinc inadequacy affects neurotrophic factors and proteostasis in the rat brain

- We investigated whether dietary Zn inadequacy affects neurotrophic factors and proteostasis in the brain.
- Three-week-old Wistar/Kyoto male rats were fed either a Zn-deficient diet (D; < 1 mg Zn/kg diet; *n* = 18) or pair-fed with the control diet (C; 48 mg Zn/kg diet; *n* = 9) for 4 weeks. Subsequently, the rats in the D group were subdivided into two groups (*n* = 9), in which one group continued to receive a Zn-deficient diet, whereas the other received a Zn-supplemented diet (R; 48 mg Zn/kg diet) for 3 more weeks, after which the rats were sacrificed.
- The results showed an altered ubiquitin-proteasome system and autophagy components and increased gliosis, endoplasmic reticulum stress, and apoptosis markers in Zn-deficient rats compared with the control group.
- Zinc repletion for 3 weeks could partially restore these alterations.
- In conclusion, a decline in Zn concentrations below a critical threshold may trigger multiple pathways, leading to brain-cell apoptosis.

10. To assess the prevalence of chronic kidney disease (CKD) in prehypertensive (PHT) urban Indian adult population-A pilot study

- In the present pilot study we have investigated the prevalence of CKD in PHT urban adult subjects in Hyderabad. A total of 337 subjects were screened in the present community-based cross-sectional pilot study and the prevalence of PHT in these subjects was 22.55%.
- Biochemical data from 96 controls (blood pressure<120/80 mmHg) and 53 PHT (blood pressure 120-139/80-89mmHg) subjects indicates that there was an

increase in mean haemoglobin levels, fasting glucose, TG, and TC in PHT subjects compared to controls. However, HDL cholesterol levels were lower among PHT than in controls.

- Serum creatinine, urea and uric acid levels were within the normal range both in control and PHT subjects, however, they were significantly higher among PHT compared to controls. In the present cross sectional pilot study with limited sample size, CKD in PHT subjects was assessed by estimated glomerular filtration rate (eGFR) and urinary albumin to creatinine ratio (ACR).

11. Effect of maternal protein restriction (quantity and quality of protein) on quality control processes in the brain and skeletal muscle of the offspring

The intrauterine environment plays a crucial role during fetal development and affects the health of the adult in later stages. Undernourishment during gestation and lactation affects the skeletal muscle (SM) and brain development.Several studies suggest that the maternal protein content and source can affect the offspring's health.

- Combined prenatal to postnatal protein restriction (PR) (8% calories from protein) lowered body weight, and myofiber cross sectional area in offspring.
- PR caused ER stress, autophagy, and ubiquitin -proteasome system in the skeletal muscle and brain of the offspring.
- Furthermore, chronic PR promoted muscle atrophy by increasing skeletal muscle proteolysis in the offspring.
- Maternal protein restriction (MPR) with postnatal rehabilitation of the offspring from weaning ameliorated skeletal muscle proteolysis.
- The findings indicate that exposure to prenatal PR and PR in the offspring induce muscle atrophy and accelerate skeletal muscle proteolysis in the offpsring via augmenting PQC processes, while MPR shows little or no effect.

12. Effects of Bisphenol-A on lipid accumulation in hepatocytes and adipocytes

Curcumin (2μM) inhibited BPA (10μM) altered expression levels of Fasn and Scd1 HepG2 and 3T3-L1 cell lines involved in fatty acid production. Moreover, curcumin could alter the mRNA expression of Hmgcr, Sqle, Srebf1,Srebf2 Lpl, Apoa1, and Apoa2in BPA treated HepG2 cell lines. Curcumin could inhibit BPA altered mRNA expression of Lpl and apolipoprotein (Apoa1), shows that BPA dysregulate the lipoprotein-apolipoprotein metabolism. BPA could stimulate the expression of PPARγ "the "master transcriptional regulator of adipogenesis", and C/EBPα, resulting in adipogenesis and lipogenesis. BPA increased the aP2 expression, which plays important role in adipocytes differentiation. Co-treatment of Curcumin could inhibit the expression of PPARγ and C/EBPα and can modulate the BPA induced

altered expression of genes and transcription factors responsible for fatty acid synthesis, cholesterol synthesis and lipid metabolism and adipogenesis

13. Oral toxicity study of new salmonella killing bio-control agent NINMB 13076 bacteriophage

In this investigation, a sub-chronic oral toxicity study was conducted on BALB/c mice to evaluate the impact of Salmonella-killing bacteriophages. Following 28 consecutive days of sub-chronic oral administration of the Salmonella phage, no major pathological abnormalities were observed in vital organs such as the lungs, kidneys, heart, liver, and intestines. Furthermore, even after a 24-hour incubation with the Salmonella phage, the growth of native probiotic bacteria remained unaltered. Comparative analyses of urine tests and body weight measurements for both control and test subjects did not reveal any notable changes. An examination of the effect of these phages on probiotic microbiota, specifically Lactobacillus and Bifidobacterium species isolated from the caecum of mice subjected to treatment and those left untreated, showed no significant variations in their populations. Based on the study's findings, oral administration of lytic Salmonella phages exhibited no discernible adverse effects on the animals, did not appear to harm the probiotic gut microbiota, and is likely a safe option for use in food preservation.

14. Evaluation and role of isolated compound from amla fruits on volproic acid induced autism spectrum disorder (ASD) in experimental Balb/C mice

Ethyl acetate fraction of amla extract (EAFA) reduced the autism like symptoms due to its anti-oxidant, anti-inflammatory, and neuroprotective properties. This effect may be due to the presence of compounds namely: quercetin, rutin, pantothenic acid, gallic acid, and ascorbic acid present in EAFA extract. The concentrations of these compounds were quantified using LC-MS/MS.

15. Assessing effectiveness of front-of-pack nutrition labels for processed pre-packaged food products in India-A cross-sectional study on formats, acceptability and potential use

Front of Pack Nutrition Labelling (FOPNL) system is one of the policy tools to help reduce nutrition related non-communicable diseases. Globally various formats of FOPNL have been implemented, however, which FoPNL format will work in Indian context should be based on data on consumer acceptability, and understandability of the different label formats. A cross-sectional study conducted among 3231 participants (Adults – 2616, and adolescents – 615) in the age group 10-60 years in five regions of India namely New Delhi, Jorhat (Assam), Kolkata, Pune and

Hyderabad. The data collection was completed in a single contact with the participants using a validated questionnaire. Apart from socio-demographic details, and packaged food purchasing behaviours, the participants perception on likeability, attractiveness and perceived cognitive workload of the five different formats of the FOPNL i.e., Nutri-Score (NS), Health Star Rating (HSR), Warning label (WL), Multiple traffic lights (MTL), and Nutri-Star (NSR) was assessed. Additionally, 1/5th of the participants was randomized to one of the five FoPNL formats and asked questions about objective understanding, perceived product healthfulness, purchase intention and willingness to change purchase behaviour. The findings showed that WL performed better in influencing purchase intention, product choice, and eating behaviour across the product variants compared to other FOPNL formats. WL and NSR had greater impact in altering the health perception of the food products, as presence of even one octagon or absence of stars prompted more cautious behaviors in choosing the foods. However, among the summary indicators, even presence of 2 stars (in HSR) or Code D (orange shades in NS) prompted higher choice of the same variants of food and lesser willingness to opt for others. In conclusion, to identify healthiest or unhealthiest variants any format of FOPNL can work. However, for promoting healthier food choices among the available variants, summary indicators (NS and HSR) seem to work better, but to deter consumption of even moderately unhealthy foods, Warning Labels (NSR or WL) are a better option.

16. Promoting nutrition and health of corporate employees with workplace intervention-A study using communication for behavioral impact (COMBI) approach" -16ET-07

This study conducted in IT workplaces of various operational levels in India's premium IT hub, Hyderabad reported that at baseline although the food and physical activity environment (FPAE) at workplaces of big operation level provide facilities like gym and walking tracks, utilization remain poor, while at smaller workplaces facilities are limited. However, consumption or distribution of unhealthier food items prevail at all operation levels. The employees reported high prevalence of lifestyle risk factors like average sitting time of ≥8 hours on a working day, 88% employees not meeting recommended intentional physical activity level. Dietary risk factors like frequent eating out, not consumption of 400g of fruits or vegetables per day were also commonly reported. Though the median age of the employees was 30 years (26-35) years, 44.02% were overweight, 16.85% were obese, 48.21% of female employees had waist circumference >80cm, 3.89% were diabetic. In all, 29.87% of the study population were considered to have metabolic syndrome since they had ≥3 metabolic risk scores and reported significantly higher levels of biomarkers like MDA (p=0.003), homocysteine (p=0.001), IL-6 (p=0.017), IL-4 (p=0.000). After baseline situation analysis, a prototype of a complex nutrition-based intervention model of WWP, based on socio-ecological theory was

developed. The intervention components targeted at reducing the risk factors of MetS were delivered using Communication for Behavioral Impact (COMBI) approach at individual, interpersonal and organisation levels over 6 months. These included health screening, one-to-one and telephonic consultations at individual level, bi-monthly group awareness sessions, demonstrations and events at interpersonal level and creating an enabling environment through modification of workplace FPAE at organisation level. The impact of the intervention was evaluated using a pre-post longitudinal method by repeating the baseline survey methods after 6 months. Post-intervention data revealed high employee engagement, positive changes in employee health behaviour and workplace FPAE. A mild but significant reduction occurred in body weight ($z=-3.59$, $p<0.01$), LDL-cholesterol ($z=-4.75$, $p<0.01$), triglyceride ($z=-4.63$, $p<0.01$). Overall, this intensive WWP was found to be partially effective in promoting the health and nutrition status and health behaviour of IT employees working in a medium-sized organization. This comprehensive nutrition-based WWP model showed efficacy in improving employees' nutrition knowledge, health behavior and FPAE and can be adapted to contexts and sectors where sedentary work continues to increase MetS.

I. PUBLIC HEALTH NUTRITION

1. Comprehensive nutrition assessment of tribal population in Attappadi Taluk, Kerala: A rapid exploratory study

The health disparities faced by indigenous populations worldwide stem from factors like geographical isolation, discrimination, and loss of autonomy over their lands and culture. These have led to higher rates of poverty, undernutrition, and limited access to education and social services, resulting in poorer health outcomes, increased disability rates, and shorter life expectancy compared to non-indigenous groups. India has a significant tribal population of over 100 million people, comprising about 8.6% of the total population. Despite special provisions in the Constitution, indigenous communities in India continue to face substantial challenges, making them one of the most underserved and undernourished segments of the population. Recent data highlights alarming rates of undernutrition among tribal children. The study was conducted in Attappadi, a tribal region in Kerala, India. While Kerala has made significant progress in various areas, the indigenous tribes in the state remain marginalized. Tribal children in Kerala experience a higher prevalence of stunted growth compared to non-tribal children, and neonatal/infant mortality rates in Attappadi have been distressingly high. To address the issues of malnutrition and high neonatal/infant mortality in Attappadi, the Indian Council of Medical Research-National Institute of Nutrition (ICMR-NIN) Hyderabad conducted a comprehensive nutrition assessment survey in 2022. The survey aimed to assess the nutritional status of different demographic groups, including young children, children, and adolescent girls.

METHODS

This community-based cross-sectional study employed a quantitative and prevalence approach to investigate malnutrition among children and adolescent girls in Attappadi. The sample size was determined based on the prevalence of underweight (48.5%) among children aged 0–59 months from a previous nutritional assessment. The estimated sample size was initially set at 340, but to account for potential refusal to provide blood samples, it was increased to 400. Data were collected from children aged 0–59 months, children aged 5–9 years, and adolescent girls aged 10–19 years in households with atleast one child aged 0–59 months. The villages for the study were selected randomly from the Integrated Tribal Development Project of Attappadi, with 20 villages chosen, 10 of which had reported infant deaths between January 2019 and April 2022, and the other 10 had not. From each selected village, 20 households were randomly chosen, ensuring each had at least one child aged 0–59 months. Data collection occurred from May to June 2022 by a trained team of seven members. The team collected information on socio-demographics, anthropometric measurements, 24-hour dietary recall, and blood samples for hemoglobin and micronutrient analysis. Haemoglobin status was measured using the Haemocue method, while venous blood samples were collected for micronutrient analysis. The blood samples were transported and

processed at appropriate temperatures at the CHC in Attappadi before being sent to the ICMR-NIN laboratory for micronutrient estimation. A 24-hour dietary recall survey was conducted among 25% of the selected households to gather information on the diet of household members. Ethical approvals were obtained from relevant committees and authorities, and written informed consent was obtained from parents and assent from children aged 5–19 years. Data analysis was performed using Stata-14 software. Descriptive statistics were used to determine the prevalence of malnutrition. Z-scores for height-for-age, weight-for-height, and weight-for-age were generated using WHO-Anthro software for children aged 0–59 months. Z-scores for BMI-for-age and height-for-age were calculated using Anthro-plus software for children aged 5–9 years and adolescent girls aged 10–19 years. WHO child growth standard cut-off values were used to classify nutritional status, and WHO hemoglobin cut-off values were used to determine anemia status. Cut-off values from WHO and the Institute of Medicine were used to classify deficiencies in iron, vitamin A, vitamin D, vitamin B12, and folate.

RESULTS

Anthropometric malnutrition is a pressing concern in the community of Attappadi, with significant prevalence among children and adolescent girls. The study found that almost half of the children (48.3%) were underweight, while over a third (40.9%) suffered from stunted growth, and more than a quarter (27.4%) experienced wasting. Additionally, 1.4% of the children were overweight or obese. Among children aged 5–9 years, the prevalence of thinness was 35.5%, and 14.6% were stunted. For adolescent girls aged 10–19 years, the rates of thinness were higher among younger girls (25.6%) than older ones (12.5%). The prevalence of overweight/obesity among adolescent girls was 10.5%, while stunting affected 43.3% of them, with higher rates among older girls. Anaemia was a critical public health concern among both children and adolescent girls. The prevalence of anaemia was exceptionally high (91.2%) among children aged 12–59 months, with 5.2% having severe anaemia. Among adolescent girls, the overall prevalence of anaemia was 96.6%, with the majority having mild (53.4%) or moderate (42.1%) anaemia. Iron deficiency was particularly prevalent among tribal children, affecting half of them, and vitamin A, folate, vitamin B12, and vitamin D deficiencies were also observed among the studied children. The food and nutrient intake data indicated that the protein intake of children exceeded the recommended daily allowance (RDA), but energy intake was insufficient. Calcium and iron intake were deficient across all age groups. Intake of other essential vitamins and minerals like vitamin A, thiamine, riboflavin, niacin, vitamin C, and zinc were also below the RDA for children in the community. Folate intake was sufficient for some age groups but inadequate for others. The low intake of essential nutrients corresponded to the prevalence of deficiencies observed in the community, with iron intake and iron deficiency anaemia showing a notable correlation. Overall, children in Attappadi were not meeting the recommended nutrient intake for most essential vitamins and minerals, except for protein intake.

INFERENCE & CONCLUSION

This nutrition survey provides a comprehensive understanding of the prevalence of malnutrition in different age groups among the tribal population of Attappadi. Despite all the efforts, malnutrition rates remain high, with significant numbers of children suffering from underweight, stunting, wasting, and anaemia. Children and women in the community are not meeting recommended nutrient intake levels for essential vitamins and minerals, leading to deficiencies and health challenges. To address the triple burden of malnutrition (undernutrition, overnutrition, and micronutrient deficiencies), the study recommends a multipronged strategy. This includes promoting culturally appropriate food-based solutions, advancing preconception nutrition, and developing culturally sensitive intervention strategies.

2. National Action Plan for Climate Change and Human Health (NAPCCHH) – Nutrition and climate change

The Government of India had constituted the Prime Minister's Council on Climate Change (PMCCC) for a coordinated response towards issues related to climate change at the national level and for providing oversight for formulation of action plans in the area of assessment, adaptation and mitigation of climate change and their monitoring. National Centre for Disease Control (NCDC) is the Nodal Technical Agency for coordination for undertaking activities listed under the National Action Plan for Climate Change and Human Health (NAPCCHH). The NAPCCHH outlines strategies to reduce climate sensitive illnesses through integration with other National Missions for Climate Change of Government of India as well as through health and non-health programmes run by various ministries. NAPCCHH aims to strengthen health system to protect the citizens of India especially the vulnerables like elderly, children, women and marginalized population against climate sensitive illnesses. The ICMR–NIN was identified as one of the Centres of Excellence to work on the area of Nutritional Disorders with reference to Climate change.

METHODS

The ICMR–NIN was entrusted with the deliverables as in Table 1. Various scientists of the institute worked on these documents and content was also developed which were thoroughly reviewed internally before submission. For the development of IEC tools, MS-PowerPoint and online tools like Canva were used. AV tools were developed through outsourcing after content was finalized.

RESULTS

Health Action Plan (HAP): It was prepared with a view to serve as a guiding document for preparation of state specific action plan for climate change and impact on nutrition. The HAP includes various components that will aid for planning the type of actions to be taken, conditions under which the HAP needs to be implemented, and specific personnel that need to be involved while implementing the HAP. The document covers various topics that include overview of the Inter-governmental Panel on Climate Change (IPCC), impact of climate change on the food system, impact of climate change on health and nutrition outcomes, malnutrition in India, consequences and implications of key nutrition indicators, available resources, and evidence on the association of climate change impacts with malnutrition. The document elucidates the plans for Creating awareness, strengthening of health system capacity, performing situational analysis, developing partnerships/collaborations, strengthening monitoring, surveillance and research capacities, etc.

Activity Matrix: From the HAP, SOPs were laid down for implementation of the action plan. These had chalked out short term (upto 2 years), midterm (upto 5 years) and long term (upto 15 years) activities, along with indicators to monitor the progress. These activities and indicators were listed objective-wise, a sample of it is presented in Table2.

Training Modules: Training modules were developed for various functionaries like state nodal officer, medical officers, paramedics and grass root level workers like ASHA, AWW, etc. These training modules covered topics that included–Introduction, Goal, and Objectives of NPCCHH, Components of climate change effecting nutrition, clinical

assessment and Nutritional illnesses/disorder, role of the officer on climate change and nutrition related illnesses or disorders and plan of actions (activity matrix) (Figure 1). A surveillance system was developed to monitor the progress of implementation of the action plan. For IEC (Figure 2) and SBCC, 10 GIFs, 5 audios, 10 posters and 5 videos were developed. All the deliverables were submitted to NCDC and are under review by the expert committee for final approval.

Table 1. Deliverables

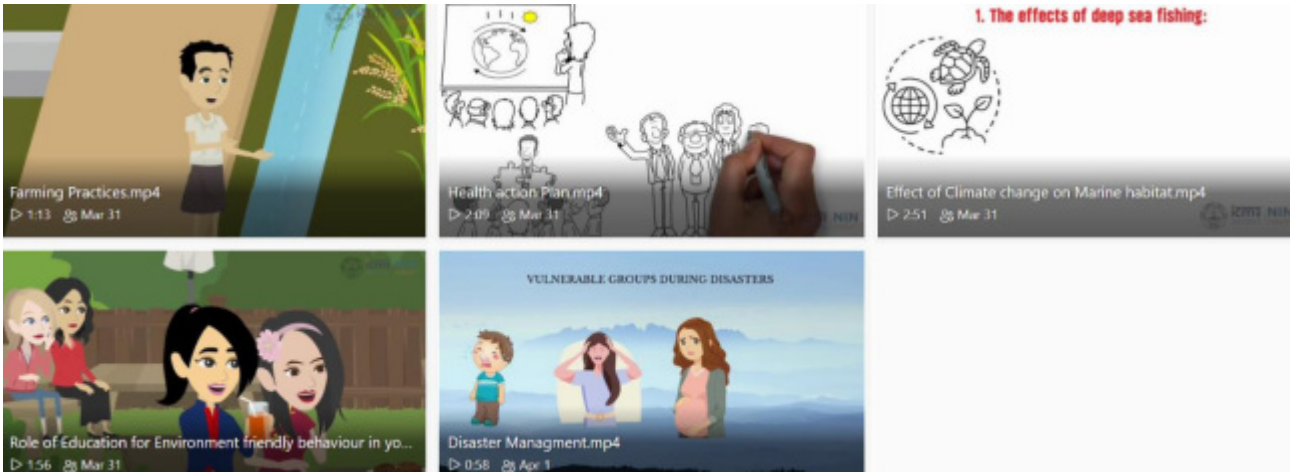
S.No	Deliverables	Minimum deliverable quantity
1	To develop subject specific health action plan (HAP)	01 HAP
2.	To develop Standard Operating Procedures (SOPs) related to implementation of action plan and subject related Guidelines*	03 (one SOP, two guidelines/advisories)
3	To support 36 States/UTs in development of relevant chapter/s in their State Action Plan on Climate Change and Human Health (SAPCCHH)*	7 priority states/UTs (preferably south Indian)
4	To develop training module for a) State Nodal Officer Climate Change, District Nodal Officer Climate Change and Consultant Climate Change (1 day, 1 module including exercises) b) Medical Officers (1 day, 1 module) c) Para medical officers & Health care workers (1 day, 1 module) d) Community level (1 day, 2 module) for vulnerable population group such as women/ children/ elderly/ different type occupations)	Each module in English language (total 5 modules)
5	To conduct training of all • State Nodal Officers Climate Change and Consultants Climate Change (36 States/UTs/) ToT is for 1 day online	4 batches x 30 participants x 1 day *(72 participants (36 SNOs +36 Consultants) /(30 per batch)=2.5 ~ approx. 3 batches + 1 extra batch for the left overs= 4 batches x 1 day each batch)*
6	To design/frame surveillance system for climate sensitive subject or integrate in existing surveillance system. Execute an example.	01
7	To develop monitoring and evaluation system for action plan implementation, including surveillance	01
8	To develop IEC on climate sensitive illnesses/diseases/ subject	01 videos (1 min each)
		01 audios
		01 templates for print IEC poster, banner with graphics
		01 GIF
9	To prepare report and document best practices	To develop half yearly report
		To e-publish quarterly newsletter (3 newsletters) or contribute to newsletter of NAPCCHH

Table 2. Activity Matrix

S.No	Key action	Activity			Indicators
		Short term (upto 2 years)	Medium term (upto 5 years)	Long term (upto 15 years)	
1	**Objective: To create awareness on the impacts of climate change on human health and nutrition among general population (vulnerable community), health-care providers and Policy makers.**				
	i.Development of IEC material on climate change impacts and malnutrition	Perform communication needs assessment Develop communication tools Develop IEC material in English and local languages Evolve strategies for dissemination of IEC	Evolve IEC material for the State action plan	Establish communication and outreach programmes for stakeholders at various levels	-Prepare and display IEC material on climate change impacts on nutrition in various departments of health, teaching institutions, schools, -Conduct public education meetings, seminars focusing on climate change and nutrition -Prepare and distribute brochures in local languages -Assess awareness of the stakeholders
	ii.Conduct awareness campaigns	Conduct awareness campaigns on climate change impacts and risk of malnutrition in various climate sensitive and malnutrition vulnerable regions	Develop communication and outreach plan	Establish communication and outreach programmes for stakeholders at various levels	
2	**Objective:To strengthen capacity of health system (infrastructure, training, development of resource material and HMIS) to respond to and reduce to climate change impacts on malnutrition**				
	i.Developing infrastructure to reduce the risk of malnutrition associated with climate change.	-Setting up interdepartmental/ multisectoral task force for developing health action plan on climate change impacts and malnutrition -Establishing State climate change and nutrition monitoring cell -Identify and Define roles and responsibilities of all stakeholders	-Identify for coordination relevant departments/ agencies involved in programmes or policies concerning food supply chain activities impacting nutrition and nutrition/child care programmes for children and other vulnerable groups. -Establish climate change and nutrition monitoring cell with complete testing and assessment infrastructure in each	Establish coordinated network with identified sectors and stakeholders	Identify stakeholders Define composition, roles and responsibilities of each coordinating relevant sector and State level climate change and nutrition monitoring cell is given in Table 1. Notification to Stakeholders (government and non-government) in various sectors on the State level climate change related nutrition illnesses monitoring cell (Table 2)

Figure 1. Training modules

Figure 2. IEC material developed (screenshots of videos)



INFERENCE & CONCLUSION

The documents and content developed by the ICMR–NIN is expected to aid the stakeholders in mitigating the effects of climate change on human nutrition.

3. Evaluation of food-based nutritional security for the malnourished population of rural households by establishing nutrition-gardens and nutrition education: An intervention research - Kanpur Dehat, Uttar Pradesh

Undernutrition is a major public health problem in India despite the green revolution and implementation of several national nutrition intervention programs over four decades. Micronutrient deficiencies (MNDs), particularly vitamin A deficiency (VAD), iron deficiency anemia (IDA), iodine deficiency disorders (IDD), and zinc deficiencies are major nutritional problems that adversely affect people's health, cognitive function, performance and productivity and income, thereby becoming a major impediment to economic development. MS Swaminathan Research Foundation (MSSRF) initiated a BIRAC funded Nutri-Garden Project and established Nutrition Gardens in about 2000 households (HHs) in four geographical locations in India by including the local Self-Help Groups (SHGs) and Krishi Vigyan Kendra's (KVKs) of the respective states. This approach is expected to provide multiple benefits to the community including bridging the disconnect between agriculture and nutrition, growing nutritionally important crop farms, and awareness about healthy food leading to increased consumption of healthy foods and thus development of healthy society. The project was implemented in four states, one district in each state on a pilot basis in the year 2019 and the four districts are Thiruvallur from Tamil Nadu, Koraput from Orissa, Palaghar from Maharashtra and Kanpur from Uttar Pradesh. ICMR-National Institute of Nutrition (NIN), Hyderabad has taken up a 'Current Situational Analysis Study' in all the sites where MSSRF is in progress and collected information regarding food and nutrient intakes of the beneficiary HHs, nutritional status of the <5 year children and their mothers, food insecurity and the perceptions & practices of the beneficiaries regarding the project under MSSRF.

METHODS

Study design: It was a community-based cross-sectional study by adopting the multi-stage random sampling procedure.

Estimation of sample size: Assuming 30% prevalence of stunting, 95% confidence interval, 5% absolute precision, 1.5 design effect, the sample size required was 486 for all States. With 10% non-response, the total sample required was 540. The sample was equally distributed in 4 states. In each state, 135 HHs were covered. (135 HHs *4 states = 540 HHs). Similarly, 135 HHs *4 states = 540 HHs were covered as control group.

Selection of the villages: Two blocks of Kanpur Dehat district where Nutri-Garden Project is in operation were selected. From the list of villages in which the Nutri-Garden program is in operation, 20 villages from experimental group and 20 villages from control villages were selected randomly for the purpose of the study.

Selection of households: In each district 500 small and marginal farmers were associated with the Nutri-Garden program. From these HHs, 135 HHs with at least one <5year child were randomly selected for the study from 20 villages i.e. 6–7 HHs per village. Information about Socio-economic and demographic particulars were collected.

Information of IYCF practices were carried out among <3 year children. One day 24 hour diet recall was done in 50% of HHs. Anthropometric measurements such as height and weight were carried out.

RESULTS

Total 274 HHs (control: 136; experimental: 138) were covered for anthropometry and dietary survey. A total of 297 children under five years were covered for anthropometric measurements and IYCF practices and 269 mothers of index child for anthropometric measurements. Majority of the HHs surveyed belongs to Hindus (98%) in both control and experimental areas, and 4–45% belongs to Scheduled Caste (SC) in both areas. Majority of the HHs (82% in control & 72.5% in experimental areas) were living in pucca houses. About 7–13 % of fathers and 27–31 % of mothers of index children in both areas were illiterate. The major occupation of head of HHs in control areas was labour (64.7 %) and 46% in experimental areas. The majority of the women in control and experimental villages were housewives. Sanitary latrines facility was available in 93% in control and 91 % in experimental HHs. Majority (82%) of the HHs in experimental areas reported having a kitchen garden, compared to 31% in the control villages. Food and nutrient intakes were below the suggested level in children and women except proteins. Intake of green leafy vegetables were very low in both the areas although iron intake ranged from 70–80% among children and 66–78% among NPNL and lactating women. Intake of energy was about 84-90% among NPNL women and 64–66% among lactating women while it was 70–75% among children. The prevalence of low birth weight (LBW) was more in experimental villages (27%) as compared to control villages (19%). Majority (80%) children received breastfeeding immediately after the delivery in experimental, and 56% in control villages. Pre-lacteal was given to 20 % of children in the experimental group compared to 33% in the control group. Colostrum was given to all the children in experimental groups and 78 % in control areas. Exclusively breast feeding up to 6 months was observed low in the control group (60%) as compared to experimental group (67%). About 92% children of 6–11 months in experimental and 80 % in control villages received complementary feeding (CF) in addition to breast milk, at 6–8 months of age, while only 86% of 12–35 months children in experimental and 95 % in control villages received CF in addition to breast milk at 6–8 months of age. Among 12–59 months children, 47 % children from control and 46 % from experimental villages had received vitamin A supplementation during the previous year, while de-worming was received in 30–36% children of 6–59 months during the year. Prevalence of undernutrition. The overall prevalence of underweight was 36%, and was lower in control (30.9 %) as compared to experimental (41.5 %) villages. The prevalence of undernutrition increases as age increases in the study population. Similarly, stunting and wasting was low in control as compared to experimental areas. The prevalence of chronic energy deficiency (CED) was 56 % among women and was more in experimental (62 %) as compared to control areas (50%). Food insecurity was observed in 7% in control and 13% in experimental villages. All the HHs in experimental areas were beneficiary of Nutri-Garden scheme and received seed.

INFERENCE & CONCLUSION

Both the areas were comparable in socio-demographic variable. IYCF practices were also sub-optimum in both areas although it was better in experimental areas as compared to control areas. The prevalence of low birth weight was high in experimental as compared to control areas. The prevalence of undernutrition among children was also high in experimental as compared to control areas. It was observed that the seeds were not equally distributed among the villages, only few villages received seeds and training while some villages received the seed in the current year only. Also, seeds were not distributed among the beneficiaries' i.e. small and marginal farmers. There is a need to further extend the program with regular monitoring and supervision during distribution of seeds along with regular health and nutrition education to the people in this area.

4. Evaluation of food-based nutritional security for the malnourished population of rural households by establishing nutrition-gardens and nutrition education: An intervention research - Palghar, Maharashtra

Undernutrition is a major public health problem in India despite the green revolution and implementation of several nutrition intervention programs over four decades. Micronutrient deficiencies (MNDs), particularly vitamin A deficiency (VAD), iron deficiency anemia (IDA), iodine deficiency disorders (IDD), and zinc deficiencies are major nutritional problems that adversely affect people's health, cognitive function, performance, and productivity and income, thereby becoming a major impediment to economic development. MS Swaminathan Research Foundation (MSSRF) initiated a BIRAC funded Nutri-Garden Project and established nutrition gardens in about 2000 households (HHs) in four geographical locations in India by including the local Self-Help Groups (SHGs) and Krishi Vigyan Kendra's (KVKs) of the respective states. This approach is expected to provide multiple benefits to the community including bridging the disconnect between agriculture and nutrition, growing nutritionally important crop farms, and awareness about healthy food leading to increased consumption of healthy foods and in turn to the development of healthy society. The project was implemented in four states, one district in each state on a pilot basis in the year 2019 and the four districts are Thiruvallur from Tamil Nadu, Koraput from Orissa, Palaghar from Maharashtra and Kanpur from Uttar Pradesh. ICMR-National Institute of Nutrition (NIN), Hyderabad has taken up a 'Current Situational Analysis Study' in all the sites where MSSRF is in progress and collected information regarding food and nutrient intakes of the beneficiary HHs, nutritional status of the <5 year children and their mothers, food insecurity and the perceptions and practices of the beneficiaries.

METHODS

It was a community based cross-sectional study carried out in 4 states of India by adopting the multi stage random sampling. In each state, one district, two blocks from each district, 20 villages and 135 HHs were covered for socio-demographic and Anthropometric measurements were done. A one-day diet survey by 24-hour recall method and food frequency was done on 50% of the HHs. Information on infant and young child feeding practices were also collected for 0–36 month children. Food insecurity was assessed using FAO food insecurity scale.

RESULTS

A total of 273 HHs (control: 137; experimental: 136) were covered for anthropometry and dietary survey. A total of 319 children under five years were covered for anthropometric measurements (control: 153; experimental: 165) and IYCF practices (control: 133; experimental: 138) and 273 mothers of index child for anthropometric measurements. Majority of the HHs surveyed belongs to Hindus (98%) in both control and experimental areas, and 93% belongs to Scheduled Tribes (ST) in both areas. About half of the HHs (51% in control & 59% in experimental areas) were living in semi-pucca houses, and 45% in control and 40% in experimental areas were living in kutcha houses. About 31% of fathers and 43-45% of mothers of index children in both areas were illiterate. The major occupation of the women in control villages were housewives. Sanitary latrines facility was available in 85% in control and 68% in experimental HHs. The majority of the HHs (90-93%) in both areas was availing of PDS benefits and was receiving wheat/rice monthly. Majority (77%) of the HHs in experimental areas reported having a kitchen

garden, compared to 22% in the control villages. Food and nutrient intakes were below the suggested level in children and women except proteins. Micronutrients such as vitamin A, calcium, iron etc. were grossly deficient in both the areas. The prevalence of low birth weight (LBW) was more in experimental villages (39%) as compared to control villages (27%). Majority (93%) children received breastfeeding immediately after the delivery in control, and 82% in experimental villages. Pre-lacteal was given to 13% of children in the experimental group compared to 3% in the control group. Colostrum was given to all the children in both groups. Exclusive breastfeeding (EBF) was observed in 47-48% children of 6-11months in both the areas. All the children of 6-11 months in control and 95% in experimental villages received complementary feeding (CF) in addition to breast milk, while only 86% of 12-35 months children in control and 73% in experimental villages received CF in addition to breast milk. Among 12-59 months children, it was observed that 72% of children from control villages and 86% from experimental villages had received vitamin A supplementation during the previous year. Prevalence of undernutrition. The overall prevalence of underweight was 55%, and was similar in control (55%) and experimental (56%) villages. The prevalence of undernutrition increases as age increases in the study population. The prevalence of chronic energy deficiency (CED) was 56% among women and was more in experimental areas (62%) as compared to control areas (50%). The prevalence of overweight/obesity was 3-4% in both the areas. Food insecurity was observed in 16% in control and 18% in experimental villages, while severe food insecurity was high (12% each) in both the areas. All the HHs in experimental areas were beneficiary of Nutri-garden scheme and received seed such as bitter gourd, bottle gourds, tomato, cucumber, chilies, ladies finger GLVs and pumpkin and training.

INFERENCE & CONCLUSION

The survey area is tribal dominated with low socioeconomic status, low access to sanitary latrine, safe drinking water and low level of education. IYCF practices were also sub-optimum in these areas. The prevalence of undernutrition among women is very high and thus low birth weight and undernutrition among children. Although the program was initiated in 2019, but because of Covid-19, it could not reach all the beneficiaries. There is a need to further extend the programme with regular monitoring along with health and nutrition education to the people in this area.

II. MATERNAL AND CHILD HEALTH

1. Development of maternal nutrition E-modules to strengthen the maternal nutrition secretariat at NIN

The maternal nutrition technical e-Dialogue series has been started with the aim to strengthen the maternal nutrition assessment and services in antenatal care in India was initiated in January 2021 as a collaborative project of ICMR-NIN, FOGSI and UNICEF with partners Alive and Thrive, IFPRI, SAS, IEG, Sight and life, and Nutrition International. To extend the current work on the technical e-dialogues on maternal nutrition to training modules on maternal nutrition we have taken up this project which was initiated in October 2021.

METHODS

The primary aim is to make the latest knowledge on maternal nutrition interventions, strategies, and innovations accessible to nutritionists, gynecologists, and public health professionals in an easily digestible format. Preparation of online training modules on maternal nutrition for practicing obstetricians, nutritionists, and other health professionals working for pre-pregnant, pregnant, and lactating women. These training modules would be later integrated into routine counselor/maternal health training The existing materials including draft agenda, training power point presentations and cards for algorithm, gestational-month-wise cards, nutrition risk specific cards etc. were reviewed initially followed by their enhancement as required in order to develop into a one day training session for integration in government systems either online or self-taught online. Procedure: Review and technical vetting of all the modules, Establishment of expert committee, Revision of content based on the suggestions made by the expert committee.

RESULTS

We have completed the development of 11 maternal nutrition e-modules based on the existing and evidence based maternal nutrition interventions, strategies etc. presented and discussed during the maternal e-technical dialogues. Following which an expert committee has been constituted by Director, ICMR-NIN and all these modules were thoroughly reviewed by the experts and submitted for further video development.

INFERENCE & CONCLUSION

Development of 11 maternal nutrition e-modules. Features of the e-modules: Comprehensive including Mental health elaborate module on counseling skills, Dietary recommendations based on EAR and also for different health conditions. Caters to different professionals involved in maternal care.

MN E-LEARNING MODULES

S. No.	Module Name
SB Mod 1	Maternal Nutrition and Mental Health in India
SB Mod 2	Maternal Nutrition ANC package

SB Mod 3a	Anthropometric Measures
SB Mod 3b	Maternal mental health & screening tool
SB Mod 4	Nutritional Inadequacies during Pregnancy
SB Mod 5a	Maternal Nutrition Counselling skills
SB Mod 5b	Group and Individual Counselling
SB Mod 6	Recipes and Thali Models
SB Mod 7	Routine antenatal screening tests
SB Mod 8	Recording, Reporting & Reviewing of Maternal Nutrition services
SB Mod 9	Planning for Maternal Nutrition in PIP–Rapid assessment
SB Mod 10	Supplies-how to plan and prevent stock outs
SB Mod 11	Planning T3 Campaigns on maternal nutrition and life style-considerations

III. DIETITICS STUDIES

1. Identification of the different processed foods and estimation of their age's content

Advanced glycation end products (AGEs) are formed by the Maillard process, a non-enzymatic reaction between ketone group of the glucose molecule or aldehydes and the amino groups of proteins that contributes to the aging of proteins. In hyperglycemia elicited by diabetes, the process begins with conversion of reversible Schiff base adducts to more stable irreversibly bound moieties known as AGEs. The formation and accumulation of AGEs have been known to progress at an accelerated rate under diabetes, thereby being involved in diabetic vascular complications, atherosclerosis, Alzheimer's disease and normal aging.

Modern diets are largely heat-processed; as a result contain high levels of AGEs. Dietary advanced glycation end products (dAGEs) are known to contribute to increased oxidant stress and inflammation, which are linked to the recent epidemics of diabetes and cardiovascular disease. Due to food preparation methods, modern diets contain large amounts of AGEs and their daily consumption ranges from 25 to 75 mg of mainly pyrraline and carboxymethyllysine (CML). It has been suggested that about 10–30% of the ingested load of dietary AGEs (dAGEs) gets absorbed into the body and becomes incorporated in the body AGE pool. Among the better-studied AGEs are the stable and relatively inert N^{ε}-carboxymethyllysine (CML) and the highly reactive derivatives of methylglyoxal (MG). The formation of new dAGEs during cooking was prevented by cooking with moist heat, using shorter cooking times, cooking at lower temperatures and by the use of acidic ingredients such as lemon juice or vinegar.

Hence, in the present study the dietary AGEs content of the different day to day foods including fats, protein, carbohydrates, packaged and canned food which are heat processed were estimated in order to suggest a better diet for diabetics.

RESULTS

A total of 58 food samples were evaluated for the AGE-Fluorescenc and CML content. All the food samples were divided in to five categories including beverages and sauces, milk and milk products, Indian foods, snacks and biscuits and Western foods. The fluorescence of the food samples were measured as arbitrary units (AU). Among the beverages and sauces, tomato sauce showed highest AU of 141.3. Among the milk and milk products, cheese showed highest AU of 134.2. Among the Indian foods, *pesarattu* (moong dal dosa) showed highest fluorescence of 194.7. Among the snacks and biscuits, osmania biscuits showed highest fluorescence of 353.4 AU. Among the Western foods, chicken nuggets showed highest fluorescence of 292.2 AU (Figure 1-5).

Apart from AGE-Fluorescence the CML content of the 58 food samples were tested using double sandwich ELISA technique. The results were represented in nanograms per gram weight. Among the beverages and sauces highest CML was noted in tomato sauce (408.4 ng/ml) which correlates to its high AGE-fluorescence. Among the

milk and milk products, the highest amounts of CML was noted in soymilk (659.3ng/gm) which is also the highest CML content among the 58 studied food samples. Among the Indian foods, highest CML content was noted in rice kichadi (309.ng/gm). Among the snacks and biscuits, the highest CML content was noted in Haldiram's moong dal (465 ng/gm). Among the Western foods category, all the tested foods showed CML values among which highest CML content was noted in Chicken nuggets (362.3 ng/gm) (Figure 6-10).

Figure 1. AGEs in beverages and sauces

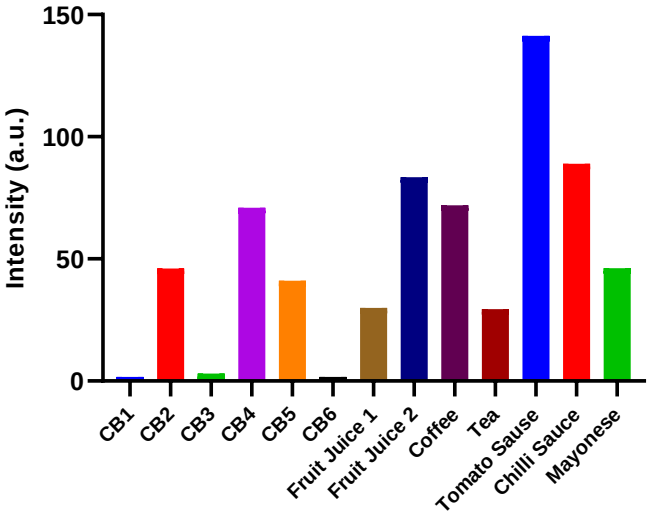


Figure 2. AGEs in milk and milk products

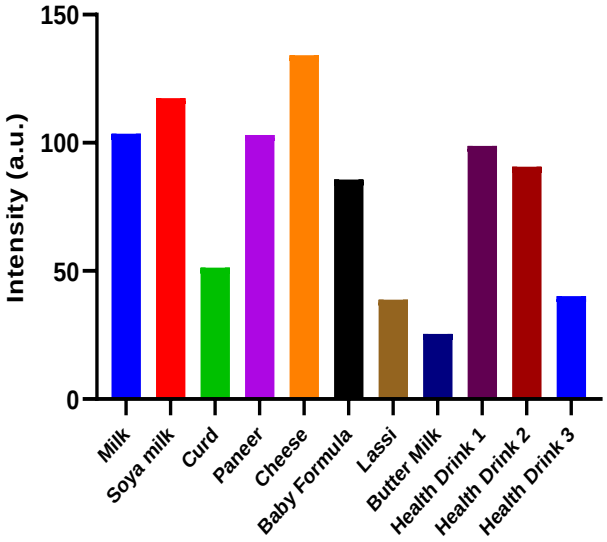


Figure 3. AGEs in breakfast and lunch foods

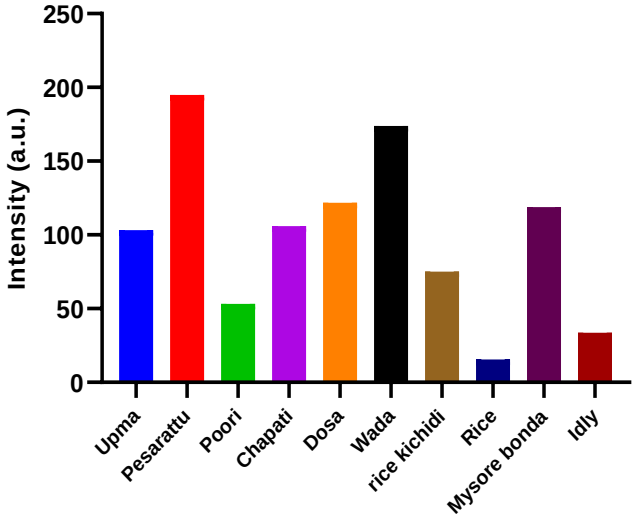


Figure 4. AGEs in snacks and biscuits

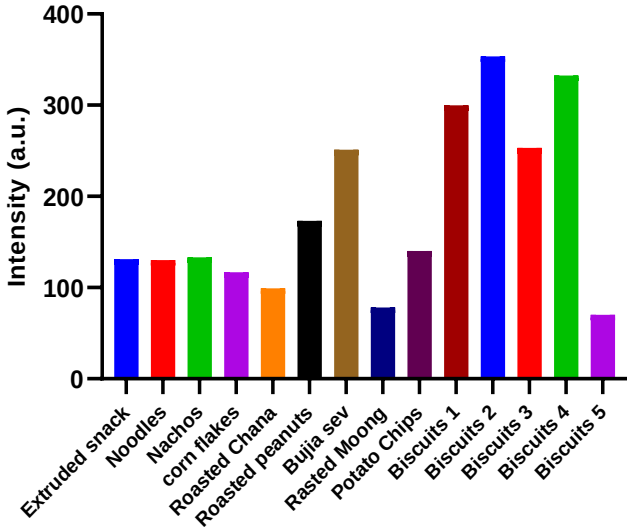


Figure 5. AGEs in Western foods

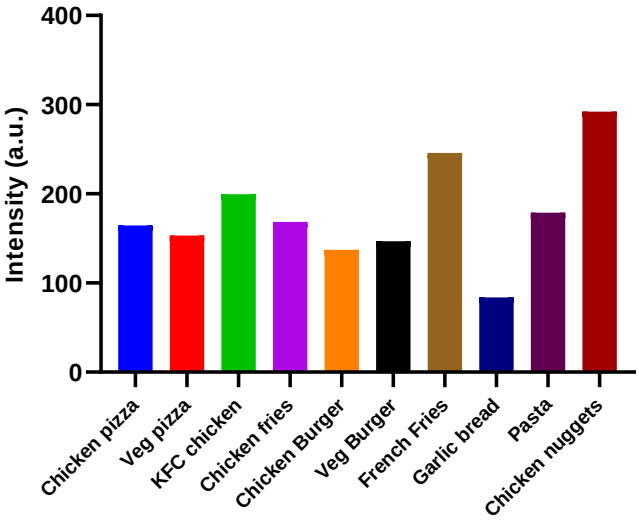


Figure 6. CML in beverages and sauces

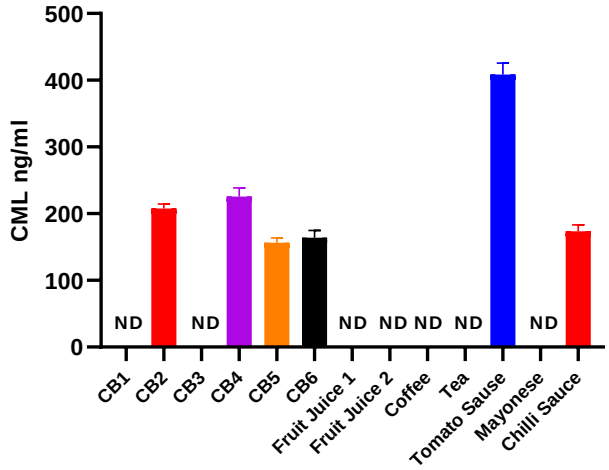


Figure 7. CML in milk and milk products

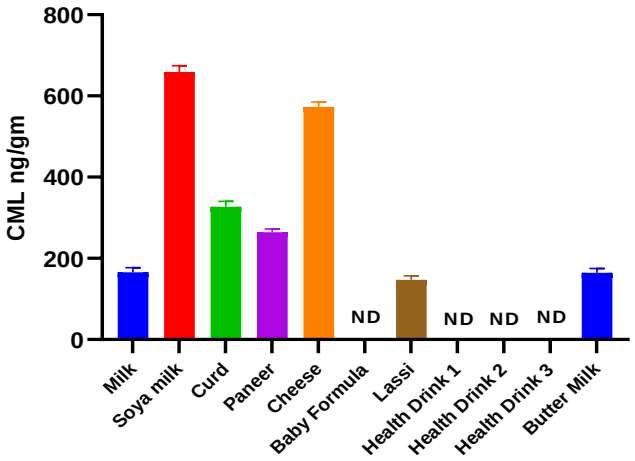


Figure 8. CML in Breakfast and Lunch foods

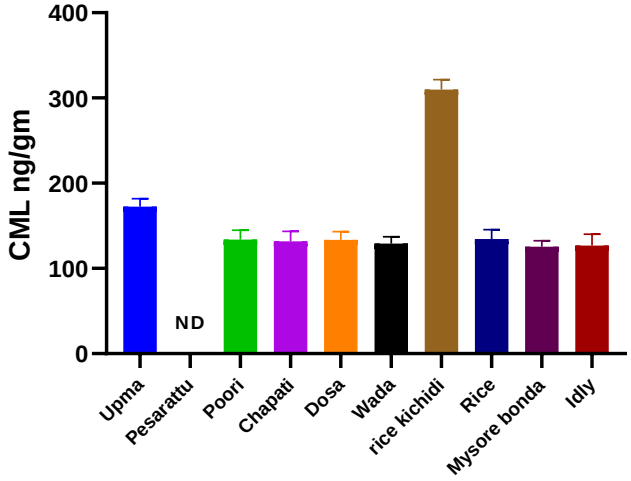


Figure 9. CML in snacks and biscuits

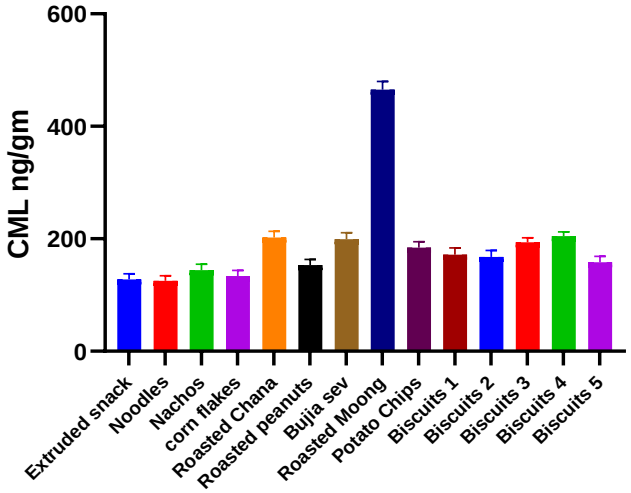
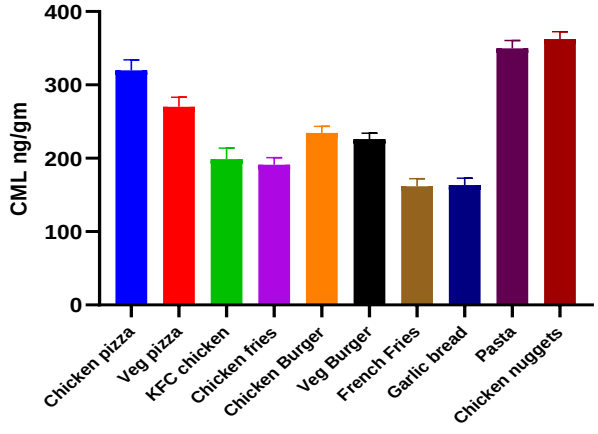


Figure 10. CML in western foods



CONCLUSION

The present study is taken up to estimate the dietary AGEs content in commonly consumed foods in India. A total of 58 foods are tested. The results of the study indicate that a lot of foods contain AGEs which contribute to diabetic and age related complications. There is no existing database of the Indian foods is available as for as dietary AGEs are concerned, hence this study will become a base in choosing the right food for the diabetic patients. Among the studied foods the category of traditional Indian foods contains fewer AGEs compared to the processed foods which make them a healthy option. The present study can be extended in elaborating the database which includes most of the Indian foods.

2. Nutrient composition and anti-nutrient factors of thirty-three newly developed varieties of chickpea bred for various agronomic traits

Chickpeas or garbanzo beans also called Bengal gram or *chana* in India, have a second place next to dry beans for food legume production in the world. The total consumption (Production quantity + Import – Export) of pulses in India in 2021 was approximately 27.83 million tons, out of which 12.05 million tons chickpea, approximating about 43% of the nation's combined pulse consumption (FAO 2021b). *Kabuli*, which has larger off-white seeds, and *desi*, which has leaner brown seeds, are the two major market classes of chickpea. Additionally, chickpea has a large variation in its types and nutrient composition, owing to diverse environmental conditions, breeding techniques, and cultivars. Yegrem (2021) reported that since there is a large variation in the nutrients of chickpea among different cultivars, it should be determined whether phenomenal differences are consistent within and across different environments. This data would be useful in identifying chickpea's nutritional potential. This research study is therefore aimed at providing a methodologically current and accurate database of the nutritional and anti-nutritional composition of newly developed varieties of chickpea bred through various breeding techniques for different agronomic traits like high yield, heat and cold tolerance, drought tolerance, pest resistance, wide adaptation, early maturity, and bigger seed size.

RESULTS

Thirty-three varieties of chickpea were analyzed (in duplicate) for their proximate composition, macro-elemental and micro-elemental constituents, and anti-nutrients (oligosaccharides, tannins, and total phenolic compounds), and the results are given in Table 1, 2(a), 2(b), 3, and 4 respectively. The *Dilaji* variety showed the highest protein content (22.98 ± 0.02 g/100g). The fat content ranged from 4.67 ± 0.05 (NBeG 49) to 7.60 ± 0.46g/100g (Yezin 11), and the available carbohydrate varied from 33.56 ± 1.15 (JAKI 9218) to 46.73 ± 0.19 g/100g (Chania Desi 1). Dietary fibre was highest in Himachal chana 2 (30.76 ± 0.58 g/100g).

Calcium (184.66 ± 0.07 mg/100g), copper (1.45 ± 0.005 mg/100g), and iron (8.33 ± 0.002 mg/100g) was found to be highest in *Teketay* variety. Sodium (31.52 ± 0.02 mg/100g), potassium (1264.58 ± 0.007 mg/100g), magnesium (179.12 ± 0.008 mg/100g), phosphorus (528.31 ± 6.31mg/100g), manganese (2.83 ± 0.02mg/100g), and zinc (5.29 ± 0.13mg/100g), were found to be highest in *Mastewal, Ahero 1, Chania Desi 1, ICCV 96030, NBeG 47,* and *Sasho* respectively.

On oligosaccharide estimation, verbascose (0.22 ± 0.02 g/100g) and stachyose (0.32 ± 0.02 g/100g) was found to be highest in *Local 1,* raffinose (0.47 ± 0.16 g/100g) was highest in *Yezin 12*, and sucrose (0.72 ± 0.22 g/100g) in *Mastewal.* Tannin content ranged from 0.46 ± 0.32 (JG 16) to 1.06 ± 0.06 mgCE/100g (Sasho) and TPC ranged from 4.15 ± 0.25 (Saina K1) to 9.75 ± 0.40 mgGAE/100g (Himachal chana 2).

Figure 1: Chickpea samples and their types

Table 1. Proximate composition of chickpea varieties (g/100g of wet weight)

Sample name	Moisture	Protein	Fat	Ash	Available CHO	Dietary fibre
Dilaji	8.05±0.003	22.98±0.02	4.78±0.13	2.46±0.01	37.22±0.28	24.40±0.33
JG 16	8.27±0.02	21.28±1.37	6.20±0.15	2.38±0.05	40.93±1.47	20.88±0.63
Himachal chana 2	7.49±0.28	21.88±0.10	5.18±0.13	2.84±0.02	30.92±1.31	30.76±0.58
JAKI 9218	7.25±0.03	19.59±0.04	6.57±0.27	2.70±0.01	33.56±1.15	30.45±0.24
Mastewal	8.11±0.01	19.17±0.03	5.99±0.32	2.73±0.02	34.28±0.08	29.82±0.09
LDT 068	8.15±0.09	19.30±0.06	6.23±0.09	2.66±0.00	34.98±1.49	27.66±0.01
Minjar	7.35±0.14	21.44±0.43	5.66±0.18	2.77±0.02	45.07±0.33	17.89±1.06
Chania Desi 1	8.06±1.45	20.43±0.39	5.48±0.12	2.68±0.08	46.73±0.19	17.62±1.74
Natoli	7.26±0.29	21.19±0.04	5.34±0.02	2.88±0.02	43.88±0.52	20.31±0.45
RVG 203	7.21±0.04	20.44±0.03	5.93±0.21	2.61±0.15	44.73±0.22	19.89±0.04
Chania Desi 3	7.54±0.25	22.78±0.08	6.24±0.01	2.51±0.09	40.31±0.10	20.56±0.62
NBeG 47	7.47±0.03	16.09±1.06	6.19±0.14	2.52±0.05	44.00±0.18	23.62±0.55
Dimtu	7.60±0.03	21.51±0.08	5.46±0.10	2.43±0.09	38.07±0.22	24.87±0.11
NBeG 49	8.05±0.12	20.39±0.00	4.67±0.05	2.44±0.04	43.76±0.25	20.54±0.83
Yezin 12	6.42±0.28	21.04±0.10	6.39±0.00	2.49±0.02	43.88±0.52	20.63±0.07
JG 14	6.45±0.18	21.91±0.02	5.08±0.01	2.41±0.00	40.82±0.15	22.95±1.21
Phule Vikram	7.93±0.12	18.63±0.01	5.74±0.09	2.32±0.03	44.73±0.22	20.57±0.69
ICCV 96030	7.69±0.13	22.78±0.09	6.04±0.27	2.56±0.04	41.20±0.01	20.11±0.35
KAK 2	7.06±0.07	22.67±0.03	6.82±0.06	2.44±0.04	39.12±0.01	21.81±0.03
Sasho	6.28±0.12	26.22±0.03	6.07±0.21	2.58±0.14	38.39±0.07	19.98±1.08
Virat	7.67±0.06	22.46±0.25	6.31±0.02	2.31±0.04	38.25±0.04	22.44±0.86
Vihar	7.64±0.09	21.62±0.25	6.86±0.02	2.42±0.09	43.39±0.06	18.02±0.91
JGK 2	7.99±0.23	19.65±0.02	6.33±0.06	2.66±0.02	39.60±0.48	22.79±0.77
LDT 065	7.08±0.13	21.63±0.01	6.71±0.20	2.85±0.01	43.96±0.18	17.76±0.58
Yezin 8	8.07±0.05	20.39±0.16	7.25±0.22	2.69±0.00	37.14±0.35	23.38±0.21
Saina K1	7.68±0.17	21.29±0.09	6.88±0.22	2.49±0.02	38.79±0.07	22.73±0.82
Yezin 11	7.92±0.14	20.33±0.06	7.60±0.46	2.13±0.00	36.10±0.47	24.39±0.19
NBeG 119	7.11±0.05	20.20±0.07	7.14±0.36	2.37±0.04	44.72±1.16	18.45±1.99
Teketay	7.09±0.26	20.64±0.21	6.65±0.13	2.72±0.04	42.06±0.04	21.27±0.93
Ahero 1	8.07±0.06	21.37±0.05	6.25±0.25	2.27±0.03	39.33±0.50	22.48±0.66
Haraka	8.38±0.20	19.78±0.10	5.92±0.05	2.25±0.01	46.32±0.14	17.50±0.87
Local 1	6.49±0.01	17.38±0.12	6.19±0.04	1.76±0.12	43.99±0.44	23.09±0.58
Local 2	6.30±0.07	17.45±0.00	7.26±0.01	1.79±0.12	41.90±0.24	24.99±0.77

Results are expressed as mean ± standard deviation of duplicate determinations.

Table 2(a). Macro elements composition of chickpea varieties (mg/100g of wet weight)

Sample name	Macroelements				
	Sodium	Potassium	Calcium	Magnesium	Phosphorous
Dilaji	23.74±0.02	953.68±0.004	164.40±0.03	154.64±0.13	449.12±6.09
JG 16	22.96±0.004	1034.47±0.002	155.93±0.01	167.36±0.05	457.54±3.14
Himachal chana 2	25.89±0.08	968.34±0.01	148.53±0.06	144.16±0.004	505.42±4.90
JAKI 9218	26.80±0.99	991.33±0.01	183.74±0.01	149.54±0.001	421.07±2.54
Mastewal	31.52±0.02	886.17±0.001	172.98±0.01	159.60±0.09	418.23±2.43
LDT 068	30.55±0.10	942.75±0.02	127.50±0.09	156.40±0.01	437.13±0.45
Minjar	29.53±0.08	983.15±0.002	138.60±0.11	166.88±0.05	463.12±0.60
Chania Desi 1	27.66±0.001	945.89±0.003	154.78±0.12	179.12±0.01	425.13±0.69
Natoli	25.10±0.01	979.19±0.004	146.71±0.18	150.72±0.03	435.77±2.16
RVG 203	23.87±0.12	1174.88±0.06	171.72±0.15	161.45±0.12	374.39±5.15
Chania Desi 3	28.54±0.10	954.18±0.01	146.61±0.12	146.70±0.09	451.34±1.82
NBeG 47	26.16±0.03	1064.28±0.01	137.86±0.05	166.64±0.001	397.77±5.84
Dimtu	22.74±0.03	1217.73±0.01	129.57±0.003	164.83±0.003	376.26±2.19
NBeG 49	28.57±0.001	1084.58±0.01	173.57±0.02	160.86±0.01	397.34±6.62
Yezin 12	26.92±0.02	1005.78±0.01	164.12±0.06	159.16±0.001	398.85±3.23
JG 14	30.42±0.001	992.15±0.0004	138.39±0.13	173.23±0.0005	405.53±7.40
Phule Vikram	27.75±0.11	935.05±0.001	144.81±0.13	159.01±0.0005	381.64±5.85
ICCV 96030	25.75±0.07	883.5120±0.07	159.11±0.10	153.87±0.001	528.31±6.31
KAK 2	24.03±0.03	928.12±0.0003	156.68±0.02	178.27±0.03	480.29±0.70
Sasho	26.82±0.15	1055.74±0.01	141.09±0.03	149.53±0.001	508.49±5.01
Virat	30.00±0.004	957.73±0.005	162.70±0.07	153.08±0.0001	415.05±3.16
Vihar	31.42±0.08	1148.03±0.0004	183.52±0.10	156.98±0.01	512.02±3.11
JGK 2	27.50±0.03	1159.26±0.001	172.62±0.15	165.62±0.001	421.77±5.47
LDT 065	27.86±0.04	1058.47±0.002	168.11±0.01	163.55±0.02	489.38±1.22
Yezin 8	26.04±0.03	1079.17±0.02	177.00±0.002	154.89±0.001	445.14±0.68
Saina K1	29.66±0.11	975.23±0.05	152.57±0.09	153.94±0.001	420.51±0.61
Yezin 11	22.90±0.02	946.67±0.01	157.15±0.02	159.05±0.001	376.58±0.76
NBeG 119	25.80±0.17	950.66±0.05	178.57±0.06	166.33±0.03	385.36±1.11
Teketay	30.31±0.02	1127.07±0.02	184.66±0.07	158.65±0.003	430.30±1.37
Ahero 1	28.36±0.10	1264.58±0.01	130.40±0.07	170.18±0.07	391.38±4.27
Haraka	22.70±0.05	1147.43±0.001	146.66±0.08	164.09±0.04	407.69±5.68
Local 1	24.89±0.02	962.54±0.003	134.13±0.10	145.03±0.16	285.92±4.97
Local 2	27.63±0.11	996.16±0.01	168.77±0.08	167.94±0.02	352.97±2.70

Results are expressed as mean ± standard deviation of duplicate determinations.

Table 2(b). Micro elements composition of chickpea varieties (mg/100g of wet weight)

Sample name	Microelements			
	Manganese	Zinc	Copper	Iron
Dilaji	2.47±0.07	4.87±0.08	1.15±0.03	5.69±0.55
JG 16	2.12±0.001	4.66±0.08	1.24±0.02	5.24±0.11
Himachal chana 2	2.26±0.002	4.67±0.03	1.03±0.03	5.90±0.37
JAKI 9218	2.33±0.04	4.04±0.02	0.84±0.02	4.55±0.20
Mastewal	2.13±0.05	3.78±0.05	0.97±0.01	5.67±0.14
LDT 068	2.20±0.05	3.79±0.08	0.92±0.001	7.45±0.07
Minjar	2.35±0.03	4.81±0.02	1.01±0.01	5.75±0.10
Chania Desi 1	2.26±0.04	4.11±0.01	0.96±0.01	6.32±0.49
Natoli	2.31±0.03	4.33±0.01	1.16±0.01	6.14±0.14
RVG 203	2.41±0.15	3.67±0.16	0.99±0.01	6.04±0.10
Chania Desi 3	2.37±0.05	3.92±0.06	0.95±0.01	7.37±0.0005
NBeG 47	2.83±0.02	4.90±0.04	1.37±0.03	5.96±0.09
Dimtu	2.45±0.03	3.62±0.08	1.12±0.03	5.53±0.11
NBeG 49	2.09±0.03	3.65±0.02	1.06±0.02	6.11±0.05
Yezin 12	2.70±0.03	3.92±0.02	1.12±0.01	5.93±0.11
JG 14	2.42±0.005	3.72±0.20	1.153±0.01	5.94±0.27
Phule Vikram	1.85±0.01	3.49±0.07	1.11±0.01	5.93±0.17
ICCV 96030	2.71±0.05	4.44±0.13	1.14±0.01	5.84±0.50
KAK 2	1.87±0.02	4.16±0.03	1.20±0.002	5.68±0.07
Sasho	1.89±0.03	5.29±0.13	1.39±0.03	7.15±0.62
Virat	1.74±0.02	4.95±0.01	1.29±0.01	5.93±0.30
Vihar	1.64±0.02	5.23±0.01	1.39±0.01	5.85±0.23
JGK 2	1.66±0.01	4.31±0.01	1.19±0.01	5.45±0.04
LDT 065	2.10±0.03	4.74±0.01	1.32±0.002	6.11±0.03
Yezin 8	1.59±0.02	4.64±0.08	1.37±0.01	5.60±0.06
Saina K1	1.66±0.01	4.51±0.06	1.38±0.01	5.99±0.04
Yezin 11	1.66±0.002	3.59±0.04	1.26±0.01	5.26±0.01
NBeG 119	1.65±0.01	3.67±0.03	1.25±0.01	5.22±0.06
Teketay	2.04±0.01	4.53±0.03	1.45±0.005	8.33±0.003
Ahero 1	2.02±0.04	3.94±0.01	1.35±0.01	5.28±0.12
Haraka	1.56±0.02	3.42±0.03	1.30±0.01	5.12±0.01
Local 1	1.69±0.04	2.94±0.01	1.23±0.03	4.96±0.06
Local 2	1.37±0.01	4.02±0.003	1.39±0.004	5.62±0.01

Results are expressed as mean ± standard deviation of duplicate determinations.

Table 3. Oligosaccharide composition of chickpea varieties (g/100g of wet weight)

Sample	Verbascose	Stachyose	Raffinose	Sucrose
Dilaji	0.07±0.01	0.21±0.03	0.28±0.04	0.61±0.05
JG 16	0.09±0.05	0.17±0.05	0.28±0.01	0.53±0.12
Himachal chana 2	0.08±0.02	0.13±0.02	0.25±0.02	0.52±0.06
JAKI 9218	0.17±0.07	0.25±0.07	0.29±0.04	0.61±0.14
Mastewal	0.11±0.02	0.26±0.00	0.29±0.01	0.72±0.22
LDT 068	0.11±0.00	0.25±0.05	0.33±0.01	0.67±0.09
Minjar	0.14±0.08	0.24±0.10	0.27±0.06	0.48±0.01
Chania Desi 1	0.10±0.08	0.21±0.02	0.26±0.03	0.54±0.09
Natoli	0.12±0.02	0.18±0.06	0.31±0.04	0.50±0.06
RVG 203	0.17±0.01	0.12±0.04	0.19±0.01	0.44±0.00
Chania Desi 3	0.10±0.05	0.16±0.01	0.26±0.05	0.63±0.17
NBeG 47	0.14±0.02	0.20±0.00	0.25±0.01	0.63±0.21
Dimtu	0.20±0.11	0.22±0.00	0.26±0.02	0.42±0.11
NBeG 49	0.18±0.06	0.25±0.02	0.33±0.00	0.63±0.03
Yezin 12	0.12±0.05	0.26±0.02	0.47±0.16	0.62±0.07
JG 14	0.13±0.00	0.28±0.04	0.36±0.00	0.63±0.10
Phule Vikram	0.11±0.01	0.16±0.09	0.32±0.02	0.63±0.06
ICCV 96037	0.11±0.02	0.25±0.01	0.25±0.01	0.50±0.01
KAK 2	0.07±0.00	0.14±0.01	0.25±0.01	0.60±0.08
Sasho	0.15±0.03	0.27±0.12	0.20±0.01	0.44±0.13
Virat	0.09±0.00	0.15±0.02	0.25±0.04	0.66±0.17
Vihar	0.10±0.00	0.17±0.01	0.34±0.08	0.72±0.01
JGK 2	0.10±0.01	0.26±0.00	0.40±0.06	0.57±0.01
LDT 065	0.16±0.04	0.28±0.03	0.41±0.01	0.49±0.04
Yezin 8	0.14±0.01	0.31±0.11	0.42±0.01	0.56±0.02
Saina K1	0.13±0.00	0.29±0.01	0.36±0.01	0.50±0.00
Yezin 11	0.13±0.02	0.26±0.03	0.47±0.00	0.28±0.04
NBeG 119	0.12±0.00	0.24±0.04	0.37±0.04	0.29±0.07
Teketay	0.09±0.00	0.25±0.02	0.34±0.03	0.47±0.06
Ahero 1	0.09±0.01	0.15±0.02	0.34±0.04	0.38±0.08
Haraka	0.21±0.02	0.28±0.14	0.41±0.04	0.53±0.14
Local 1	0.22±0.02	0.32±0.02	0.40±0.03	0.57±0.00
Local 2	0.13±0.01	0.22±0.02	0.42±0.03	0.36±0.15

Results are expressed as mean ± standard deviation of duplicate determinations.

Table 4. Anti-nutrient components of chickpea varieties

Sl.No.	Sample	Tannins (mg CE/g)	TPC (mg GAE/g)
1	Dilaji	0.80±0.27	5.93±0.55
2	JG 16	0.46±0.32	7.38±0.29
3	Himachal chana 2	0.72±0.09	9.75±0.40
4	JAKI 9218	0.78±0.18	7.83±0.47
5	Mastewal	0.79±0.12	6.42±0.64
6	LDT 068	0.84±0.02	6.69±0.01
7	Minjar	0.95±0.21	7.76±0.26
8	Chania Desi 1	0.81±0.01	7.73±0.46
9	Natoli	0.96±0.04	7.10±0.16
10	RVG 203	0.96±0.04	6.94±0.37
11	Chania Desi 3	0.73±0.14	6.95±0.43
12	NBeG 47	0.55±0.04	6.73±0.45
13	Dimtu	0.88±0.09	7.19±0.55
14	NBeG 49	0.71±0.02	6.94±0.31
15	Yezin 12	0.94±0.01	7.20±0.33
16	JG 14	0.81±0.00	9.37±0.18
17	Phule Vikram	0.78±0.02	7.71±0.37
18	ICCV 96037	0.83±0.00	7.45±0.13
19	KAK 2	0.79±0.04	5.17±0.67
20	Sasho	1.06±0.06	5.14±0.38
21	Virat	0.82±0.48	4.84±0.02
22	Vihar	0.75±0.18	5.73±0.45
23	JGK 2	0.67±0.35	5.09±0.10
24	LDT 065	0.79±0.07	5.67±0.40
25	Yezin 8	0.79±0.47	4.39±0.34
26	Saina K1	0.82±1.16	4.15±0.25
27	Yezin 11	0.84±0.04	5.92±0.24
28	NBeG 119	0.72±0.50	5.06±0.27
29	Teketay	0.79±0.14	6.69±0.37
30	Ahero 1	0.81±0.44	7.17±0.73
31	Haraka	0.73±0.48	7.23±0.07
32	Local 1	0.72±0.04	7.28±1.06
33	Local 2	0.84±0.47	5.53±0.16

Results are expressed as mean ± standard deviation of duplicate determinations.

CONCLUSION

The data from the study infers that chickpea is a good source of protein, comparatively low in available carbohydrates, and an excellent source of dietary fibre. It is a repository of minerals like phosphorus, potassium, and magnesium and is much higher in minerals like calcium, sodium, and iron in comparison with other legumes. Phosphorus was determined to be in high amounts compared to previously reported studies. Antinutrients are also present in smaller quantities. Further research needs to be conducted to study the effect of processing methods on anti-nutritional factors and the direct health benefits of chickpea consumption.

3. A study on the macronutrients, micro-elements and anti-nutrient composition of thirteen green gram (*vigna radiata (l.) wilczek*) cultivars

Green gram, locally known as mung bean, has been present in the Indian region since the neolithic period. It is known to be emanated from India, and is widely grown in the central region of Africa, east and south regions of Asia, South and north regions of America and a few regions of China, mainly due to its high protein content. According to the Green gram Outlook Report-January to December 2021 released by Acharya N.G. Ranga Agricultural University, green gram is the third most important pulse crop in India, contributing 16% to total pulse area and 12% to total pulse production in the year 2020–21. India has acquired a green gram productivity level of 729.1 kg/ha with a total production of 30.9 lakh tones from 42.38 lakh hectares area in the year 2020-21. Green gram is a healthy, nutrient-dense and economical food choice comprising high-quality and easily digestible proteins, carbohydrates, dietary fibre, macro and micro minerals, and low amounts of fat. Like other legumes, green gram is also rich in protein and essential amino acids except for cysteine and methionine. Also, it contains an adequate amount of soluble carbohydrates, crude fibre, and macro minerals like potassium and phosphorus. Therefore, the current study has undertaken an analysis to generate data on the proximate mineral and anti-nutrient composition of newly cultivated varieties and local varieties of green gram by using well-established and direct methods of analysis.

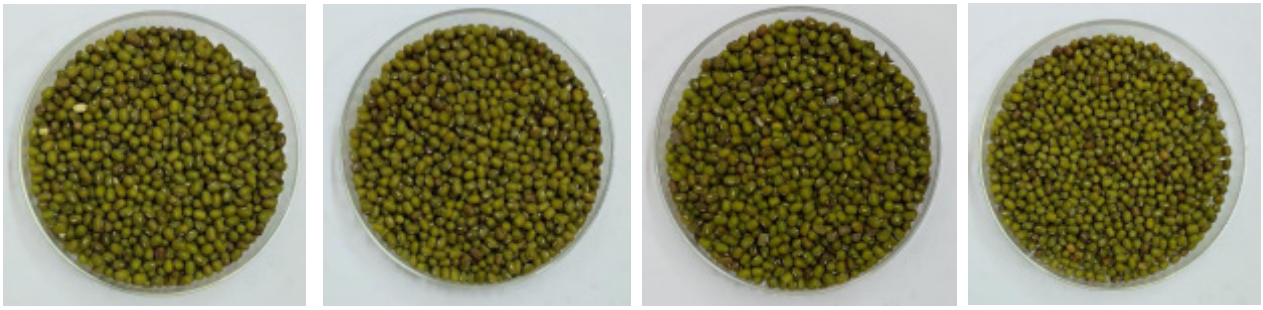
RESULTS

The moisture content of green gram varieties varied from 6.25 to 8.46%. Ash content of the green gram varieties varied from 2.83 to 3.74%. Fat content ranged from 2.19 to 3.08%. Available carbohydrate content of green gram varieties varied from 48.46 to 53.57%. The protein content varied from 22.87 to 27.76%. The highest protein content was found in the newly cultivated variety, IPM 302-2 (Kanika) and the lowest was found in the locally available variety Local-3 (Black Green gram). The dietary fibre content varied from 11.83 to 15.79% (Table 1).

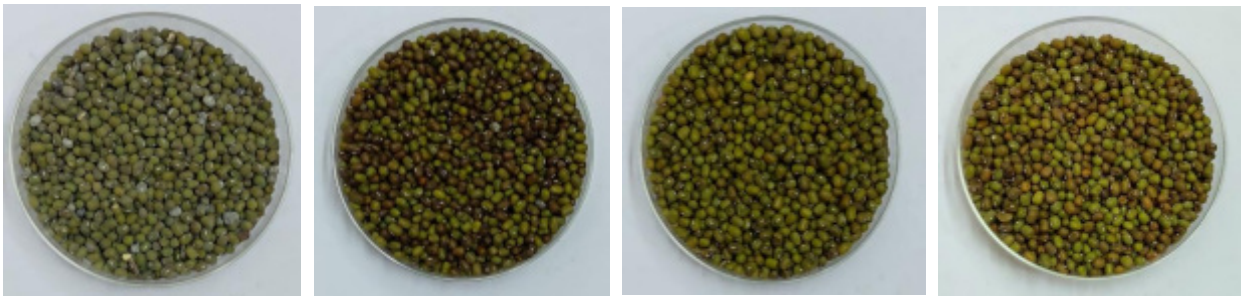
The potassium content varied from 318.33 to 359.76 mg/100g and the calcium content varied from 18.14 to 34.06 mg/100g. Magnesium content in green gram varieties varied from 150.65 to 171.39 mg/100g and the phosphorus content ranges from 282.49 to 447.35 mg/100g. Iron content varied from 2.76 to 34.88 mg/100g and Copper content varied from 0.59 to1.29 mg/100g for local varieties (Table 2).

The phytic acid and saponin content varied from 0.453 to 1.137% and 1.17 to 1.99% respectively. Tannin content varied from 0.91 to 1.78 mg Catechin equivalents/g. Total phenolic content varied from 17.17 to 25.55 mg/100g. Trypsin inhibitor activity varied from 4.41 to 10.44 TUI/mg sample and chymotrypsin inhibitory activity was not detected (Table 3).

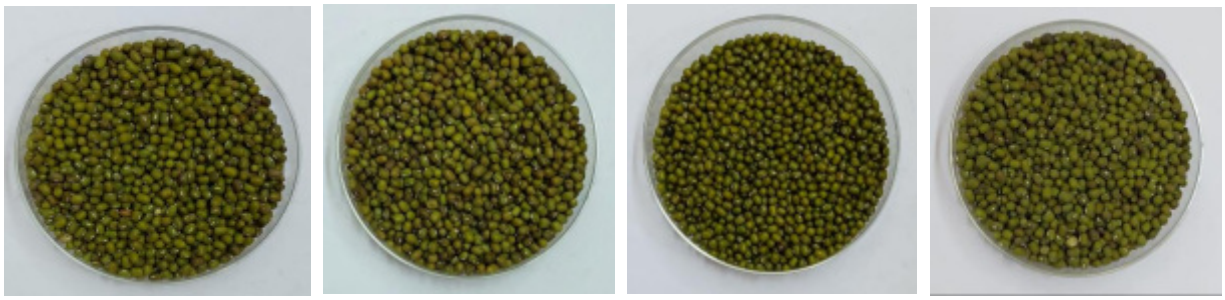
Figure 1. Green gram varieties selected for the study



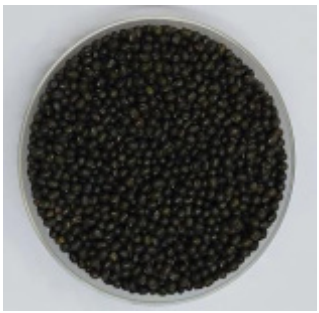
IPM 02-3 | **IPM 2-14** | **IPM 2K 14-9 (VARSHA)** | **IPM 99-125 (MEHA)**



IPM 205-7 (VIRAT) | **IPM 302-2 (KANIKA)** | **IPM 312-20 (VASUDHA)** | **IPM 409-4**



IPM 410-3 (SHIKHA) | **IPM 512-2 (SOORYA)** | **LOCAL-1 (SHINY)** | **LOCAL-2 (ROUGH)**



LOCAL-3 BLACK GREEN GRAM

Table 1. Proximate composition of different varieties of green gram (g/100g)

Green gram samples	Moisture	Ash	Fat	Available carbohydrate	Protein	Dietary fibre
IPM 2K14-9 (Varsha)	8.0 ± 0.23	3.07 ± 0.04	2.81 ± 0.01	49.66 ± 0.18	26.58 ± 0.68	15.61 ± 0.23
IPM 410-3 (Shikha)	7.43 ± 0.13	2.86 ± 0.00	2.19 ± 0.29	52.76 ± 0.27	26.45 ± 0.02	15.79 ± 0.08
IPM 302-2 (Kanika)	7.97 ± 0.00	2.91 ± 0.11	2.40 ± 0.19	48.46 ±0.03	27.76 ± 0.05	13.55 ± 1.24
IPM 512-2 (Soorya)	8.06 ± 0.04	2.87 ± 0.03	2.75 ± 0.27	50.86 ± 0.43	25.49 ± 0.02	14.22 ± 0.07
IPM 205-7 (Virat)	8.12 ± 0.07	3.01 ± 0.04	3.07 ± 0.11	50.88 ± 0.62	24.59 ± 0.10	15.22 ± 1.04
IPM 99-125 (Meha)	7.63 ± 0.15	2.86 ±0.00	2.32 ± 0.05	49.76 ± 0.12	24.14 ± 0.03	14.887 ± 1.22
IPM 312-20 (Vasudha)	8.21 ± 0.12	2.92 ± 0.02	2.33 ± 0.07	50.46 ± 0.01	24.41 ±0.11	13.99 ± 0.50
IPM 02-3	8.46 ± 0.27	2.83 ± 0.26	2.98 ± 0.01	51.53 ± 0.14	24.31 ± 0.33	14.21 ± 0.60
IPM 2-14	8.22 ± 0.29	3.15 ± 0.04	2.74 ± 0.05	50.92 ± 0.62	25.23 ± 0.67	13.82 ± 0.32
IPM 409-4	8.38 ± 0.26	3.26 ± 0.05	2.93 ± 0.28	49.86 ± 0.07	26.21 ± 0.41	14.25 ± 0.49
Local-1 (Shiny)	6.72 ± 0.17	3.33 ± 0.03	2.98 ± 0.11	52.05 ± 0.10	23.47 ± 0.16	12.02 ± 1.27
Local-2 (Rough)	7.63 ± 0.11	3.74 ± 0.14	3.08 ± 0.26	52.21 ± 0.58	22.93 ± 0.26	11.83 ± 0.33
Local-3 (Black green gram)	6.25 ± 0.11	3.27 ± 0.22	2.28 ± 0.06	53.57 ± 0.76	22.87 ±0.06	13.78 ± 0.32

Each value is the average of duplicate determinations expressed as Mean ± SD.

Table 2. Mineral composition of different varieties of green gram (mg/100g)

Sl. No.	Green gram samples	Macro minerals				Micro minerals			
		K	Ca	Mg	P	Fe	Zn	Cu	Mn
1	IPM 2K14-9 (Varsha)	359.76 ± 8.91	23.92 ±2.74	158.57 ± 0.84	438.21 ±3.80	4.08±0.56	3.43 ±0.02	0.73 ±0.01	1.11 ± 0.01
2	IPM 410-3 (Shikha)	318.33±9.66	20.38 ±0.30	150.65 ± 0.05	438.78 ±2.16	4.44±0.57	2.76 ±0.04	0.69 ±0.03	1.08 ± 0.01
3	IPM 302-2 (Kanika)	344.28±6.61	18.14 ±0.67	159.30 ± 2.15	415.68 ±4.09	5.51±1.65	3.14 ±0.29	0.75 ±0.29	1.02 ± 0.01
4	IPM 512-2 (Soorya)	340.94±0.92	28.84 ±1.82	164.05 ±4.41	441.55 ±4.94	5.88±0.47	3.02 ±0.03	0.96 ±0.03	1.11 ± 0.02
5	IPM 205-7 (Virat)	358.05±5.47	24.76 ±0.61	156.06 ± 0.13	444.73 ±3.02	4.02±0.33	3.40 ±0.02	0.76 ±0.02	0.98 ±0.01
6	IPM 99-125 (Meha)	356.90±5.76	16.64±0.78	170.54 ± 0.06	407.97 ±1.09	4.59±0.14	2.86 ±0.02	1.06 ±0.02	0.86 ±0.00
7	IPM 312-20 (Vasudha)	346.03±087	25.21±1.13	157.24 ± 0.44	403.87 ±6.70	4.64±0.50	2.84 ±0.06	0.99 ±0.06	1.08 ±0.01
8	IPM 02-3	332.92±6.62	22.56±2.80	150.68 ± 1.06	411.96 ±0.73	4.80±0.03	2.94 ±0.03	0.59 ±0.02	1.04 ±0.02
9	IPM 2-14	348.96±3.80	18.45±0.32	170.59 ± 2.30	425.24 ±10.39	5.18±0.41	2.90 ±0.06	1.08 ±0.06	0.91 ±0.01
10	IPM 409-4	351.32±1.45	26.69±3.21	152.99 ± 0.99	447.35 ±5.69	4.72±0.02	3.03 ±0.09	1.18 ±0.09	1.20 ±0.01
11	Local-1 (Shiny)	353.57±1.63	20.21±1.33	171.39 ± 15.37	428.63 ±8.87	5.30±0.11	3.11 ±0.06	1.16 ±0.05	0.86 ±0.00
12	Local-2 (Rough)	340.06±3.51	23.13±1.20	167.66 ± 0.28	382.33 ±4.28	34.88±0.89	3.21 ±0.03	1.23 ±0.03	1.95 ±0.03
13	Local-3 (Black Green Gram)	326.93±0.35	34.06±12.95	151.71 ±0.84	282.49 ±7.57	23.67±0.03	3.41 ±0.13	1.29 ±0.13	1.36 ±0.01

Each value is the average of duplicate determinations expressed as Mean ± SD.

Table 3. Antinutrient composition of different varieties of green gram.

Green gram samples	Phytic acid (g/100g)	Saponins (g/100g)	Tannins (mg Catechin equivalents/g)	Total phenolic content (mg/100g)	Trypsin inhibitor activity (TUI/mg sample)	Chymotrypsin inhibitor acvity (CIU/mg sample)
IPM 2K14-9 (Varsha)	1.137± 0.044	1.30±0.02	1.63±0.65	20.27±0.70	7.11±0.39	ND
IPM 410-3 (Shikha)	1.098±0.019	1.38±0.06	1.74±0.25	22.09 ±0.13	7.04±0.06	ND
IPM 302-2 (Kanika)	0.832±0.043	1.48 ±0.04	1.06±0.21	18.28±0.26	5.52±0.20	ND
IPM 512-2 (Soorya)	0.999±0.048	1.99±0.10	1.55±0.86	20.16±0.47	5.79±0.53	ND
IPM 205-7 (Virat)	1.098 ±0.014	1.29±0.04	0.91±0.42	21.50±0.86	5.82±0.36	ND
IPM 99-125 (Meha)	0.882±0.020	1.98±0.10	1.10±0.55	22.42±0.86	6.03±0.25	ND
IPM 312-20 (Vasudha)	1.059 ±0.094	1.17±0.03	1.27±0.04	17.17±0.37	6.58±0.35	ND
IPM 02-3	0.856±0.059	1.81±0.08	1.63±0.43	19.13 ±0.64	5.04±0.40	ND
IPM 2-14	0.831 ±0.064	1.55±0.05	1.74±0.82	20.65±0.37	5.02±0.30	ND
IPM 409-4	0.873±0.045	1.63±0.12	1.78±0.33	18.77±0.35	4.41±0.47	ND
Local-1 (Shiny)	1.059±0.023	1.18±0.16	1.12±0.24	20.30±0.69	6.45±0.01	ND
Local-2 (Rough)	0.790±0.076	1.39 ±0.10	1.72±0.64	20.63 ±0.04	10.44±0.22	ND
Local-3 (Black green gram)	0.453±0.028	1.73±0.04	1.19±0.73	25.55±0.15	6.23±0.62	ND

Each value is the average of duplicate determinations expressed as Mean ± SD. ND- Not Detected

CONCLUSION

This study was conducted to quantify the nutrient and antinutrient content of different varieties of green gram using well established methodologies. Thirteen varieties of green gram were analysed to quantify their proximate mineral and antinutrient content. It was found that, compared to the locally available varieties; the newly cultivated varieties of green gram contained high protein, dietary fibre and low carbohydrate content. In contrast, local varieties of green gram contained a high amount of calcium and iron content compared to the newly cultivated varieties. Based on the above results, the green gram varieties analysed are nutritionally significant and rich source of nutrients.

IV. BASIC STUDIES

1. Understanding the role of vitamin B12 in diabetic neurodegeneration-Implications in protein quality control processes

The chronic effects of diabetes on the brain are displayed at neurological, patophysiological and structural level, and several pathogenic factors seem to be implicated in the aetiology of the neuropathological processes in diabetes, such as advanced glycation end products, astrogliosis, impaired synaptic plasticity, cognitive impairment, and neuronal apoptosis.

Mammalain cells are equipped with several protein quality-control (PQC) processes such as unfolded protein response (UPR) and autophagy. Altered chaperones, UPR and impairment in autophagy are implicated in development of diabetes, neurodegenerative diseases, and obesity. Recent animal experimental studies suggest involvement of ER stress and impaired autophagy in the aetiology of diabetes complications, such as nephropathy, retinopathy, peripheral neuropathy. Vitamin B12 is a water soluble vitamin important for maintenance of neuronal and cognitive health. Several case reports and cross-sectional studies have documented the increased frequency of vitamin B12 deficiency in diabetic patients. Besides, several studies proposed that metformin therapy in diabetic patients associated with a higher prevalence of B12 deficiency.

Deficiency of vitamin B12 has been shown to induce UPR and, impair autophagy in cell lines and rodent models. Previously, we have reported that vitamin B12 deficiency was associated with hyperhomocysteinemia and retinopathy in diabetic patients. Further, vitamin B12 supplementation alone or in combination with other micronutrients to diabetic patients showed beneficial effects. However, underlying molecular and biochemical basis for neuroprotective effect of B12 supplementation in diabetes remains poorly understood. Therefore, we aimed to understand the impact of B12 in diabetic neuropathological processes using diabetic rodent model.

MATERIALS AND METHODS

Animal Care and Induction of Diabetes

Two-month-old male Sprague-Dawley rats (NIN animal facility) maintained at a temperature of 22±2^{0}C, 55% humidity, and 12-hour light/dark cycle controlled conditions with food (AIN-93; Research Diets Inc., New Brunswick, NJ) and water *ad libitum*. The control rats (CN; n=8) were injected with vehicle (0.1 M sodium citrate buffer, pH 4.5) alone. Diabetes was induced by a single intraperitoneal injection of STZ (38 mg/kg) in the same buffer (n=16). Fasting blood glucose levels were measured 72 h after STZ injection and animals with blood glucose levels >150 mg/dL were considered for the experiment. In STZ-induced diabetic (D) group, half of the animals (n = 8) were fed a a normal diet similar to that of the control group of rats and the remaining half were fed a vitamin B12-supplemented diet (DBS; n=8). The vitamin B12-supplemented diet consists of 50 µg/kg diet, whereas normal

diet consists of 25 µg/kg diet. At the end of 4 months, rats were sacrificed by CO_2 asphyxiation. Animals were dissected, cerebral cortex (CC) of brain was harvested and frozen in liquid nitrogen and maintained at -80°C. All experimental protocols involving animals were approved by the Institutional Animal Ethical Committee (IAEC) of the National Institute of Nutrition.

Histopathology

The collected tissue was immediately placed in 4% paraformaldehyde in phosphate buffer (pH-7.2), fixed overnight, embedded in paraffin blocks, and cut into 4 µm sections and used for Haemotoxylin and Eosin (H&E) staining, Nissl body staining and TUNEL assay.

Immunoblotting

The frozen cerebral cortex was homogenized in liquid nitrogen in tris-lysis buffer (pH 7.5) containing protease inhibitors. After protein estimation with BCA method, protein samples were subjected to 12% SDS-PAGE and transferred to nitrocellulose membranes by western blot transfer system. The blots were blocked with 5% skim milk powder in PBST and incubated overnight at 4°C primary antibodies diluted in PBST. Blots were washed with PBST and incubated with peroxidase conjugated secondary antibodies. Protein bands were visualized using enhanced chemiluminescence detection kit by Image analyzer and protein bands were quantitated using image J software.

Immunohistochemistry

The harvested tissue was immediately placed in 4% paraformaldehyde in phosphate buffer (pH-7.2), fixed overnight, embedded in paraffin blocks, and cut into 4 µm sections. Sections were deparafinized by incubating in xylene for 5 min followed by dehydration in decreasing grades of ethanol. Deparafinized sections were boiled in 0.01 M Na-citrate pH 6.0 for 10 minutes at 60°C and blocked with blocking solution in PBS. After blocking, slides were incubated with primary antibodies in PBS and allowed to incubate overnight at 4°C. Binding of primary antibodies was visualized by Alexafluor-488 conjugated anti-rabbit (1:1000) IgG antibody for 1 hour. Sections were mounted with VECTASHIELD mounting medium containing DAPI. Slides were visualized using a Leica fluorescence microscope

Statistical analysis

The data are expressed as the mean ± SE. Statistical significance between groups were determined by the one way ANOVA followed by Tukey's post-hoc for multiple comparisons. Values of $p< 0.05$ were considered significant.

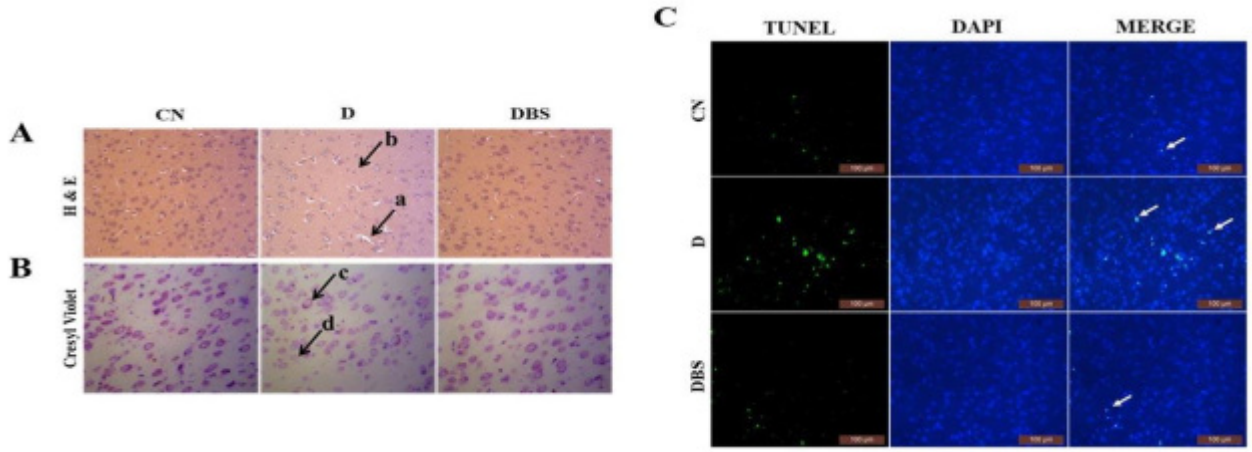
RESULTS

Effect of B12 supplementation on plasma vitamin B12 and Hcy levels:

Plasma vitamin B12 levels were significantly higher in vitamin B12 supplemented group compared to control and diabetic rats. Vitamin B12 levels in diabetic rats were tended to be lower even though there was no statistical significance compared to control. Plasma Hcy levels were significantly lower in diabetic rats irrespective of B12 supplementation compared to control. Though statistically not significant, Hcy levels were tended to higher in B12 supplemented group when compared to untreated diabetic rats.

Vitamin B12 supplementation ameliorates the neuronal morphology and cell death in diabetic CC

We determined the effect of B12 supplementation on neuronal morphology and cell death using H&E, Nissl body staining and TUNEL assay. The H&E, Nissl body staining of the CC showed the cellular degeneration in diabetic group compared to control (**Figure 1A-B**). Nevertheless, B12 supplementation reduced the cellular degeneration. Furthermore, TUNEL staining revealed increased TUNEL immunostaining in diabetic rats compared to control rats whereas B12 supplementation reduced the TUNEL immunostaining (**Figure 1C**).

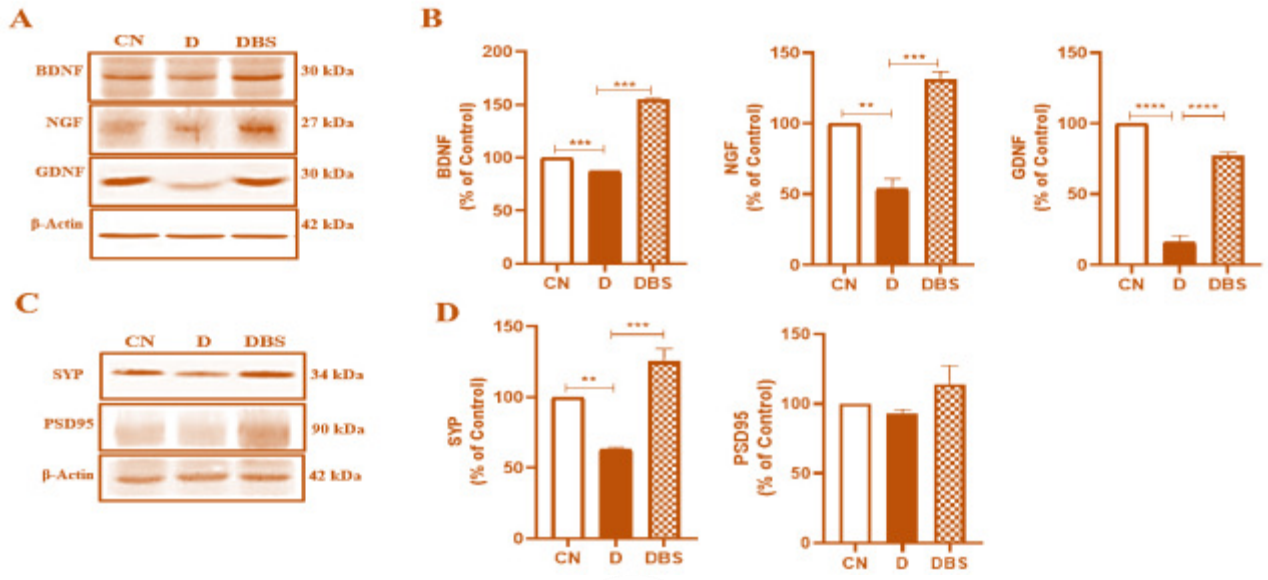


Vitamin B12 supplementation attenuates astrogliosis in diabetic CC

Astrogliosis or reactive gliosis is a hallmark feature of diabetic brain, thus we determined the GFAP levels which is a marker of activated astrocytes using immunostaining and immunoblot. Immunostaining of GFAP and its protein levels were increased in untreated diabetic rats compared to control which was reduced upon B12 supplementation.

Vitamin B12 supplementation ameliorates neurotrophic support and synaptic density in diabetic CC

We investigated the neurotrophic factors such as NGF, GDNF, and BDNF in CC of the rats by immunoblotting. Therefore NGF, GDNF and BDNF levels were significantly decreased in untreated diabetic group compared to control (**Figure 2A-B**). B12 supplementation significantly restored the neurotrophic factors in diabetic rats. Diabetes significantly decreased the synaptic density marker SYP and showed downward trend for PSD95 compared to control (**Figure 2C-D**). However, B12 supplementation significantly restored the SYP levels.

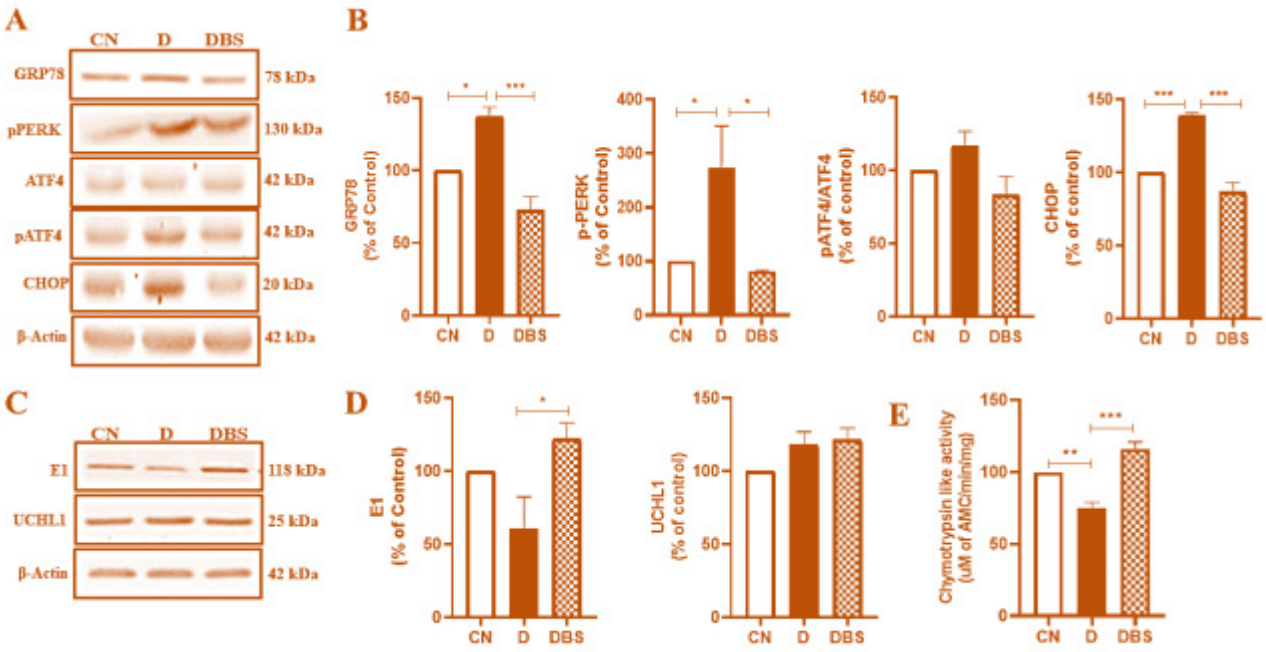


Vitamin B12 supplementation ameliorates neuronal markers, α-synuclein, and tyrosine hydroxylase in diabetic CC

Diabetes significantly decreased the MAP2, myelin basic protein (MBP) and Tyrosine hydroxylase (TH) levels while α-synuclein levels were tended to be higher compared to control, while B12 supplementation restored the MAP2, MBP, α-synuclein and TH levels compared to diabetes.

Vitamin B12 supplementation inhibits the UPR and improves ubiquitin-proteasome system (UPS)-related markers in diabetic CC

UPR markers such as Grp78, pPERK, and CHOP levels were significantly increased while pATF4 and UCHL1 levels were tended to be higher in untreated diabetic group compared to control (**Figure 3A-B**). However, B12 supplementation significantly restored the levels Grp78, pPERK, and CHOP levels to that of control. The UPS components including E1 ligase was significantly increased; UCHL1 levels were tended to higher while chymotrypsin like activity of 20S proteasome was significantly decreased in untreated diabetic group compared to control; while B12 supplementation significantly restored the CLA activity to that of control (**Figure 3C-E**).



Vitamin B12 supplementation improves the autophagy in diabetic CC

Diabetes impairs the autophagy by altering the important players in brain. However, B12 supplementation significantly improved the autophay markers diabetes.

INFERENCE AND CONCLUSION

In this study, we demonstrated that vitamin B12 acts as a neuroprotective agent in diabetic brain using experimental diabetic rat model. The key findings of the study are that vitamin B12 supplementation (i) ameliorated the neuronal morphology and cell death, (ii) reduced astrogliosis (iii) improved the neurotrophic support and synaptic density related proteins (iv) inhibited the UPR and restored the UPS and (iv) ameliorated the activation of autophagy in STZ-induced diabetic rat CC.

In conclusion, our data indicate that supplementation B12 to diabetic rats alleviates the chronic hyperglycemia-induced neuronal apoptosis and neuropathological processes in CC and suggest its neuroprotective role.

2. A functional food formulation to attenuate diabetic nephropathy and retinopathy in rats

Diabetes is one of the leading causes of mortality and morbidity by the several complications of it. Globally, more than 537 million people are affected by diabetes mellitus. Diabetic Nephropathy (DN) and Retinopathy (DR) are the most common and chronic microvascular complications of diabetes mellitus (DM). The development of diabetes complications is multifactorial and related to increased glycosylated hemoglobin, increased age, and duration of diabetes. Hyperglycemia leads to the production of advanced glycation end products (AGEs), increased oxidative stress, and activation of the polyol pathway, each of which has been implicated in the development of DN and DR. Earlier, we identified some promising natural compounds in the course of screening dietary functional foods (FF) for aldose reductase (AR) inhibitors and antiglycation agents. For these studies, we reported the beneficiary effects of various FF, including turmeric, cinnamon, ginger, pepper, and amla, against diabetic complications under in vitro conditions and rodent models. Although each of these FF individually delayed the onset and halted the progression of diabetic complications, individual FF was partially effective in preventing the complications at the tested levels. Hence, the present study was conducted to test a FF mixture containing the above-mentioned FF ingredients to evaluate its preventive effect against DN and DR using rodent models.

METHODS

Animal experimentation: Two months old male Sprague Dawley rats were collected from the National Institute of Nutrition animal facility and allowed to acclimatize for two weeks. To induce diabetes, a group of animals was injected intraperitoneally with 39 mg/Kg body weight of streptozotocin dissolved in sodium citrate buffer (pH:4.5) and fed on a chow diet. The development of diabetes was confirmed by a fasting blood glucose level of >160 mg/dL. The control animals were injected with saline and fed a chow diet. A group of diabetic animals was provided with a functional food mix at two levels. The animals were maintained for 20 weeks, and body weight, food consumption, fasting glucose, and cataract progression were monitored regularly. At the end of the experiment, animals (n=7/group) were kept for overnight fasting and sacrificed by CO2 asphyxiation to collect blood and ocular tissue. The study was approved by Institutional Animal Ethical Committee (IAEC Ref no: ICMR-NIN/IAEC/02/004/2020).

Functional food mix: Amla, Black pepper, Cinnamon, Ginger, and Turmeric were obtained from local markets of Hyderabad, INDIA. Dried turmeric, black pepper, and cinnamon bark were powdered. The fresh pericarp of amla and ginger were freeze-dried before making the powder. Powder of amla (1g), black pepper (0.5g), cinnamon (1g), ginger (1.5g), and turmeric (0.25g) were mixed in 100g of AIN-93 diet and given to rats as Functional Formulation 2. The amounts used in formulation (FF2) were based on our early individual experiments. We have also used a five-fold lower amount (FF1) expecting the possibility of synergism as well as understanding dose dependency. The amount of lower dose Functional Formulation 1 (FF1) was 0.85g/100g of diet.

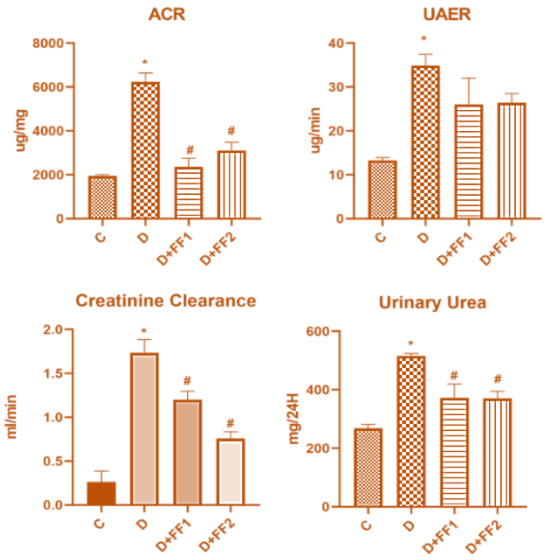
Electroretinogram: Electroretinogram was recorded to assess the retinal function. The JET electrode was placed 1mm away from the temporal limbus on the cornea sclera of each eye, while the ground electrode was attached to the tail. Responses received were amplified at 10,000 gain at 0.3-500 Hzs and digitized at a 10-kHz rate and eliminated 60Hz noise. Scotopic responses were averaged depending on the intensity with a stimulus interval of 0-180 sec. Animals were light-adapted for 10 minutes before recording photopic responses. Photopic responses from light-adapted animals were averaged with a stimulus interval of 1 sec.

Metabolite collection and Kidney processing: At the end of the experiment, 24h urine was collected by placing animals in the metabolic cages and blood was collected from the retroorbital plexus. Animals were sacrificed by CO2 asphyxiation and the kidney and eyeball were excised for histological sectioning, and the few were snap frozen and stored at -80C for RNA and protein analysis.

Biochemical Estimation: Fasting glucose was measured twice a month in the blood drawn from the tail vein using an Accu check glucometer. Albumin, Creatinine, Urea, Cholesterol, Triglycerides, and LDL were measured using commercially available kits.

RESULTS

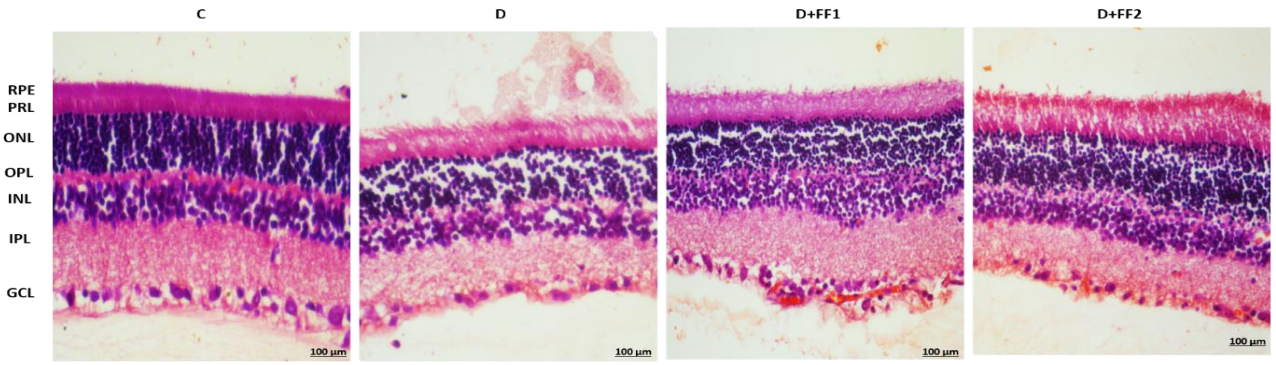
FF attenuated proteinuria and renal pathological changes: We estimated urinary albumin, creatinine, and urea levels to assess renal functioning in the rats. Elevated levels of albumin, creatinine, and urea were observed in the diabetic group compared to the non-diabetic controls. These levels were significantly reduced upon feeding FF to the rats. FF2 showed more effect than FF1. Treatment with FF diminished proteinuria which is also reflected in albumin and creatinine ratio (ACR) (Figure 1).



Effect of FF on retinal function: Retinal function was assessed by electroretinography and observed that the photoreceptor activity (a-wave), bipolar and Muller cell activity (b wave) in scotopic was significantly reduced compared to control and photopic ERG also showed similar activity. Dietary supplementation of functional formulation improved both a-wave and b-wave activity. We also noticed a significant decrease in the oscillatory potentials (OP) in the diabetic rats compared to the control and feeding functional food improved the OP.

Retinal histology and thickness: Morphological examination in H&E stained retina reveals a significant decrease in the thickness of plexiform and nuclear layers in diabetes compared to the control. Feeding functional formulation to diabetic rats prevented these changes.

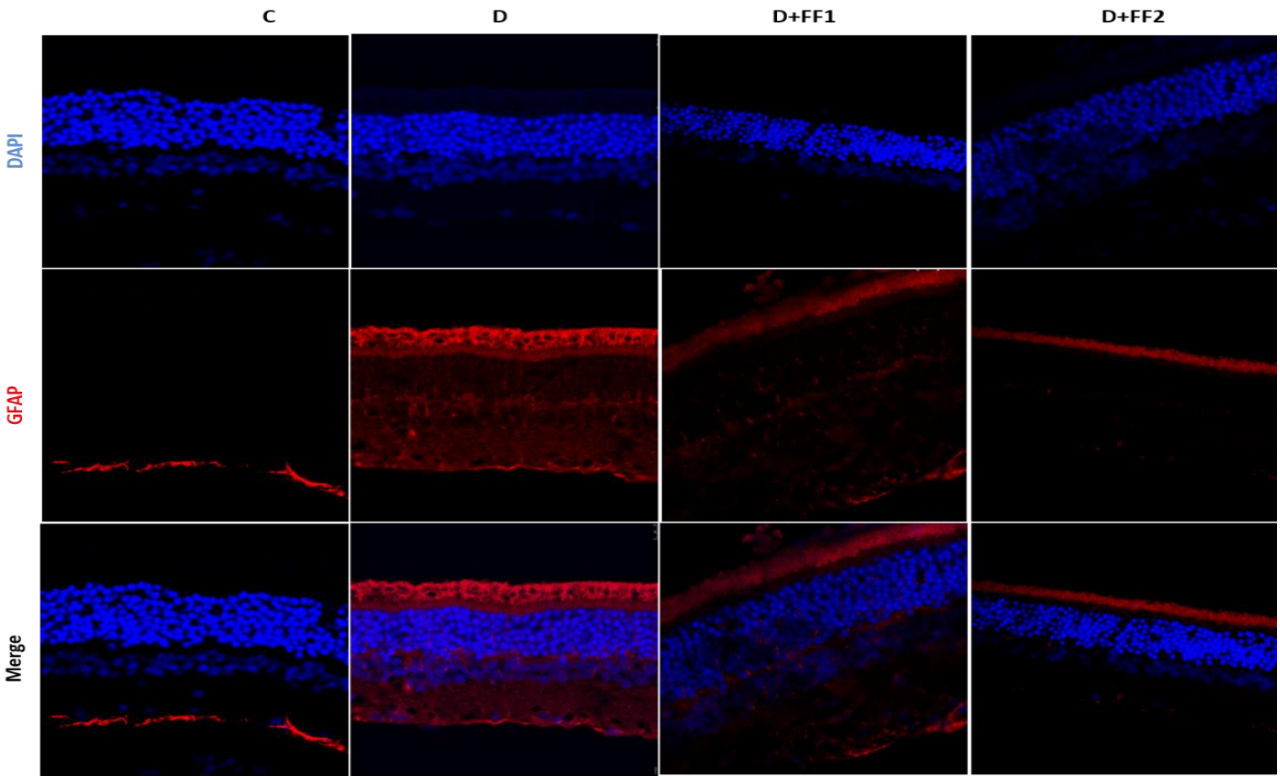
Figure 2: Histology of retina. Representative retinal sections of Control (C), Diabetes (D), Diabetes+FF1 (D+FF1) and Diabtes+FF2 (D+FF2) stained with H&E. RPE-retinal pigment epithelium layer, PRL-photoreceptor layer, ONL-outer nuclear layer, OPL-outer plexiform layer, INL-inner nuclear layer, IPL-inner plexiform layer, GCL- ganglion cell layer. 40X magnification and scale bars, 100µm.



FF prevented the depletion of podocyte slit diaphragm proteins: We observed reduced mRNA expression of both in the diabetic rats compared with the controls. Feeding FF improved nephrin and podocin expression compared with diabetes. Immunohistochemistry results are in synchrony with the RT-PCR analysis. Feeding FF to hyperglycaemic rats ameliorated loss of nephrin and podocin glomerular expression.

Expression of VEGF, HIF1-α, and GFAP: Hyperglycemia-mediated neovascularization induced by vascular endothelial growth factor is one of the implications in DR pathogenesis. VEGF is an angiogenic factor that induces vascular permeability and was controlled by HIF1-α. We observed a significant increase in expressions of both in diabetes compared to control and feeding FF ameliorated this effect. Glial fibrillary acidic protein (GFAP) is a filament protein that manages the cytoskeletal structure of glial cells. We observed GFAP staining on the ganglionic cell layer of control retinal sections, whereas GFAP expression was all over the retinal layers in diabetes, indicating gliosis. In FF-treated diabetic rats, GFAP expression was ameliorated compared to diabetes.

Figure 3: GFAP expression. Representative retinal sections of Control (C), Diabetes (D), Diabetes+FF1 (D+FF1) and Diabtes+FF2 (D+FF2). 40X magnification and scale bars, 100µm.



INFERENCE & CONCLUSION

In conclusion, the FF with the mixture of amla, turmeric, cinnamon, black pepper, and ginger effectively prevented DN and DR progression in diabetic rats by inhibiting glycation, neovascularization, polyol pathway activation, oxidative stress, and inflammation. This data suggests synergism among the individual components of FF supports the continued development of natural products as therapeutic leads in the treatment/prevention of diabetic complications.

3. Attenuation of cataracts through a functional food mix in a diabetic rat model

Globally, more than 537 million people are affected by diabetes mellitus. This number is expected to increase by 46% to 783 million by 2045, according to the International Diabetes Federation. Diabetic patients are 3- to 4 times more likely to develop a cataract, already recognized as a leading cause of blindness, affecting approximately 18 million people. Hence, there is a need for a safe intervention strategy to prevent cataracts including diabetic cataracts. The development of cataract in diabetes is multifactorial and is related to increased glycosylated hemoglobin, increased age, and duration of diabetes. Hyperglycemia leads to the production of advanced glycation end products (AGEs), increased oxidative stress, and activation of the polyol pathway, each of which has been implicated in the development of cataracts. Earlier, we identified some promising natural compounds in the course of screening dietary functional foods (FF) for aldose reductase (AR) inhibitors and antiglycation agents. For these studies, we reported the beneficiary effects of various FF, including turmeric, cinnamon, ginger, pepper and amla, against diabetic complications under in vitro conditions and rodent models. Although each of these FF individually delayed the onset and halted the progression of diabetic complications, including cataract, individual FF was partially effective in preventing the complications at the tested levels. Hence, the present study was conducted to test a FF mixture containing the above-mentioned functional food ingredients to evaluate its preventive effect against diabetic cataract. Moreover, fenugreek (methi), another popular spice shown in earlier studies as anti-hyperglycemic, is included in this formulation.

METHODS

Animal experimentation: Two months old male Sprague Dawley rats were collected from the National Institute of Nutrition animal facility and allowed to acclimatize for two weeks. To induce diabetes, a group of animals was injected intraperitoneally with 39 mg/Kg body weight of streptozotocin dissolved in sodium citrate buffer (pH:4.5) and fed on a chow diet. The development of diabetes was confirmed by a fasting blood glucose level of >160 mg/dL. The control animals were injected with saline and fed a chow diet. A group of diabetic animals was provided with a functional food mix at two levels. FF1 food mix contained 1.35% FF, whereas FF2 food mix contained 6.25% FF. The animals were maintained for 20 weeks, and body weight, food consumption, fasting glucose, and cataract progression were monitored regularly. At the end of the experiment, animals (n=7/group) were kept for overnight fasting and sacrificed by CO2 asphyxiation to collect blood and ocular tissue. The study was approved by Institutional Animal Ethical Committee (IAEC Ref no: ICMR-NIN/IAEC/02/004/2020).

Functional food mix: Amla, turmeric, black pepper, cinnamon, ginger, and fenugreek seeds were obtained from local markets in Hyderabad. Dried turmeric, cinnamon, and pepper were ground to fine powder separately. Fresh ginger and pericarp of amla were freeze-dried and powdered separately. The powders of 1 g of amla, 0.25 g of turmeric, 0.5 g of pepper, 1 g of cinnamon, 1.5 g of ginger, and 2 g of fenugreek powder; a total of 6.25 g were mixed in 100 g of AIN-93 rodent diet. This level (FF2) of function food mix is arrived at based on the effect shown by individual components of the mix in our earlier experiments. Further, with the possibility of synergism between the components of the mix and action on multiple pathways, we also used a (5 times) lower-level functional food mix (FF1; 1.35 g / 100 g of diet).

Slit-lamp examination & cataract scoring: After dilating the pupils with atropine sulphate eye drops, each animal eye lens was examined for the presence and extent of lens opacity using a slit-lamp biomicroscope (Kowa Portable: Kowa, Ltd., Japan). The cataract development and its stage were noted by scoring on weekly follow-ups until the end of the experiment. Initiation, progression, and maturation of lenticular opacity were graded into five stages. Each eye of each rat in the study was assigned a lens opacity score weekly.

Biochemical Estimations: Fasting blood glucose was measured in the blood drawn from the tail vein by an Accu-check glucometer (Roche diabetes care, India). All the parameters were done in soluble fractions of the lens except for the malondialdehyde (MDA), estimated in the total homogenate. In addition, total lens protein, superoxide dismutase (SOD), glutathione-s-transferase (GST) protein carbonyls and MDA, aldose reductase (AR), sorbitol, non-tryptophan fluorescence of advanced glycation end products (AGE), were determined as previously reported

RESULTS

Animal characteristics: As expected, diabetic rats showed increased food intake and decreased body weight compared with the control animals. Intervention with FF2 prevented high food intake in diabetes. Feeding of FF1 and FF2 to diabetic rats resulted in 13.6 and 17.2 % weight loss, respectively, when compared with the control group and thus showed a significant effect in preventing the loss of 20.9 and 17.3 % body weight respectively when compared with the diabetic animals (34.6 % weight loss). Furthermore, fasting blood glucose levels were elevated in diabetic rats and persisted for 20 weeks of the study compared with control animals. At the end of the animal experiment, the fasting blood glucose of groups FF1 and FF2 was significantly lower than the diabetic group. These data demonstrate that feeding of FF1 and FF2 to diabetic rats for 20 weeks produced a significant anti-hyperglycaemic effect.

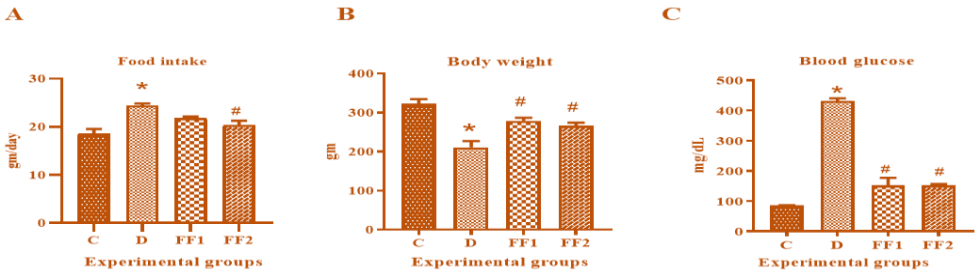


Figure 1: Effect of FF treatment on animal characteristics in STZ-induced diabetic rats. (A) Food intake, (B) Body weight, and (C) Fasting blood glucose of experimental rats. C-control, D-diabetes, FF1-diabetic rats treated with functional food dose-1, FF2-diabetic rats treated with functional food dose-2. Data are mean ± SEM. Statistical significance was determined by one-way ANOVA with multiple comparisons. *, significantly different from the group C; ($P < 0.05$). #, significantly different from group D ($P < 0.05$).

Cataract Progression: All the lenses in the control group appeared normal and free of opacities during the experimental period. The onset of cataract due to hyperglycemia was observed in diabetic animals around eight weeks of STZ injection and progressed to mature cataract by 20 weeks (Figure 2). Feeding of FF1 and FF2 did not prevent the onset of cataracts but delayed their progression to more severe forms in diabetic rats. At the end of 20 weeks, the severity of cataract was significantly lower in both FF1 and FF2 groups than in untreated diabetic rats. The progression of lens abnormalities and maturation of cataract due to STZ-induced hyperglycemia was reduced considerably with the feeding of FF, with FF2 showing a more pronounced slowing down of cataract progression than FF1.

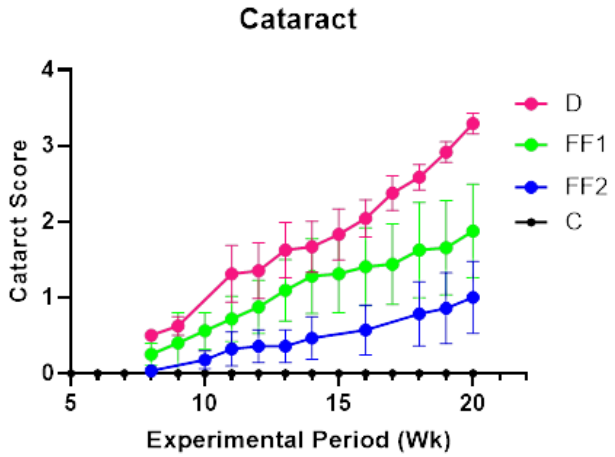
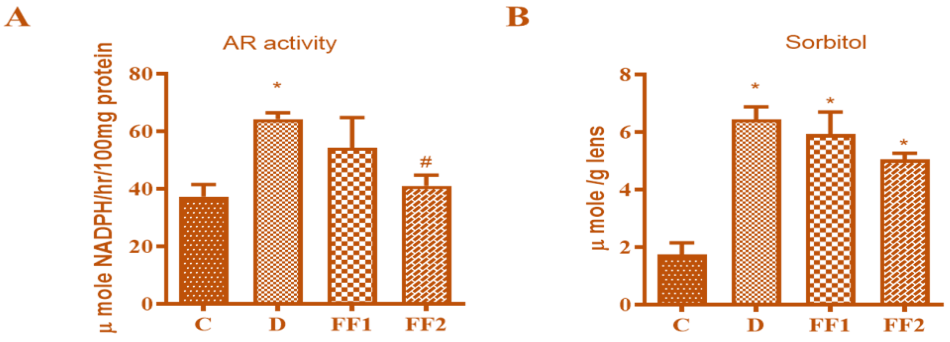


Figure 2: FF delayed the progression of cataract in diabetic rats. Quantitative representation of cataract progression in the experimental groups with time. Data is mean ± SEM. C-control, D-diabetes, FF1-diabetic rats treated with functional food dose-1, FF2-diabetic rats treated with functional food dose-2.

Polyol pathway: In addition to hyperglycemia, dicarbonyls such as glyoxal and methylglyoxal are aldose reductase (AR) substrates known to activate the polyol pathway under hyperglycemic conditions. Hence, we evaluated the polyol pathway of the lens upon FF feeding. The enzymatic activity of AR was significantly increased in group-D animals compared to group-C (Fig. 3). Feeding FF2 but not FF1 to diabetic rats resulted in the normalization of AR activity. Further, in tune with the increased AR activity, group-D showed increased sorbitol levels in the lens compared with group-C. On the other hand, feeding with FF2 showed a decreased trend in lens sorbitol levels but was not statistically significant.



INFERENCE & CONCLUSION

As the diabetic cataract is multifactorial and caused by glycation of crystallin, polyol pathway activation, oxidative stress, and inflammation, the FF, with its multiple activities, showed promise in preventing cataract progression. The FF2 group displayed 68.7% of lower cataract scores when compared with untreated diabetes at half of the dosage of components tested separately. Our earlier studies on individual ingredients (amla, turmeric, ginger, and cinnamon) showed a 37–70% reduction in cataract scores. Further, the FF1 showed a 42% lower cataract score with a one-tenth concentration of constituents tested separately. This data suggests synergism among the individual components of FF.

4. Dietary zinc inadequacy affects neurotrophic factors and proteostasis in the rat brain

Zinc is the second most essential metal abundantly present in the human body performing a critical role in maintaining human health, especially in terms of antioxidant, anti-inflammation, immunity, proliferation, proteostasis, and apoptosis. Zinc deficiency has many adverse effects including growth retardation, loss of appetite, retarded sexual development, poor immunity, and risk of diabetes incidence. Other problems like short-term memory, impaired cognitive and thinking ability, brain structural malformation, and behavioral issues are also due to inadequate zinc intake.

Proteostasis is vital for any cell type's survival and functioning, in particular postmitotic neuronal cells. Most neurodegenerative disorders are characterized by a snag in proteostasis. Protein aggregation has been associated with Alzheimer's, Parkinson's disease, amyotrophic lateral sclerosis, and frontotemporal lobar dementia. Some enzymes involved in processing amyloid precursor protein and amyloid-β are zinc metalloproteases, with an essential requirement for zinc in their catalytic activity. Zinc was shown to influence neurotransmission and sensory processing and activate both pro-survival and pro-death neuronal signaling pathways in the brain. However, the effect of dietary zinc deficiency on brain proteostasis mechanisms is still unknown. Hence, we investigated mechanistic insight into the fate of brain proteolytic pathways (ubiquitin-proteasome system and autophagy), unfolded-protein response in the endoplasmic reticulum (ER), and apoptosis in dietary zinc deficiency in the growing rats

METHODS

Animal experimentation: All animal experimentation procedures were approved by the Institutional Animal Ethics Committee of ICMR- National Institute of Nutrition, Hyderabad, India (IAEC-P31F/III-IAEC/NIN/12/2016/PR/WKY). Wistar/Kyoto male weanling (3 weeks old, n=27) rats were obtained from and maintained at the institute's animal facility. After one week of acclimation, the rats were fed on either a zinc deficient diet (D; <1 mg/kg diet of Zn; n=18) or pair-fed with the control diet (C; 48 mg/kg diet of Zn; n=9) for a period of 4 weeks. Subsequently, the rats in the D group were subdivided into two groups (n=9). One group continued to receive a zinc deficient diet while the other received a zinc-supplemented diet (R; 48 mg of Zn/kg diet) for three weeks. At the end of the experiment, animals were euthanized by CO2 inhalation, and brain tissue was collected for further analyses.

Zinc analysis in the brain tissue: Rat brain tissues were washed thoroughly with PBS buffer and placed in microwave acid digestion tubes. To this, 5 ml of 0.1 N HCl, 2 ml HNO3 and 1 ml of H2O2 were added and subjected to microwave digestion by a MARS digester. The digested samples were filtered through Whatman filter paper; from this, 2 ml of sample was taken and diluted with 2 ml of 0.1 N HCl to determine zinc levels by atomic absorption spectrophotometry (MERK-multi-element standard # 111355).

Proteasome activity assay: The chymotrypsin-like proteasome activity in the rat brain was assayed by the fluorometric method using the 'Proteasome 20S activity assay kit' from Sigma-Aldrich (#MAK172) according to instructions by the manufacturer.

Statistical analyses: The data are presented as the mean ±SEM of at least four rats in each group for immunoblotting. The data were subjected to statistical analyses using GraphPad Prism 8 software. To identify significant differences between groups, results were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. P values less than 0.05 were considered statistically significant and indicated with an asterisk.

RESULTS

Effect of dietary zinc restriction on brain zinc status and neurotrophic proteins: Zinc levels in the brain of zinc-deficient rats were significantly lower when compared with control rats (Fig. 1). However, Zinc repletion for three weeks in zinc-deficient rats resulted in marginally improved zinc levels and showed no significance with both control and deficient groups. Synaptophysin and postsynaptic density protein 95 (PSD95) proteins were significantly decreased in the zinc-deficient group compared to the control group, while zinc replenishment completely restored synaptophysin and partially restored PSD95. Brain-Derived Neurotrophic Factor (BDNF) showed a decreased trend in the zinc-deficient group and restored in the zinc repletion group. Glial fibrillary acidic protein (GFAP), a marker of gliosis, was increased in the zinc-deficient group but prevented in the zinc repletion group.

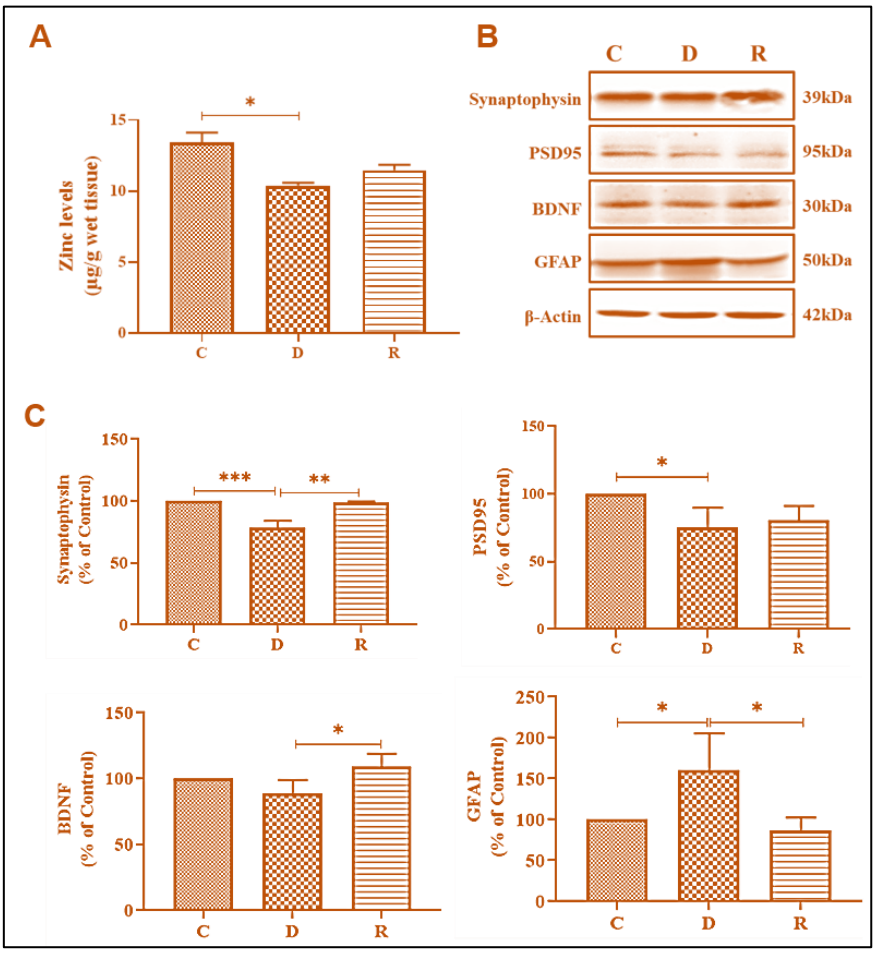


Fig. 1. Dietary zinc deficiency diminishes brain zinc levels and alters neurotrophic factors. A: Dietary zinc deficit for seven weeks resulted in depletion of brain zinc levels but three-week repletion could partially restore zinc levels (n=9). B: Representative immunoblot images of neurotrophic-related proteins in the rat cerebral cortex. C: Quantification data of immunoblots after normalized to β-Actin and is represented as the percentage of control. Data are mean ± SEM. *P < 0:05. C= pair-fed control (zinc sufficient): D= zinc deficient: R=zinc repleted.

Effect of zinc deficiency on ER stress markers: ER is a vital organelle involved in protein quality control. It functions as a leading site for the biosynthesis of proteins, post-translational modification, folding, processing, and trafficking of newly synthesized proteins. We observed increased protein expression of XBP1s, IRE1α, CHOP, and Caspase12 and unaltered protein expression of GRP78 and ATF6 in the zinc deficiency group compared with the control group indicating the existence of ER stress in these rats. However, elevated CHOP and Caspase12 proteins suggestive of maladaptive ER stress were restored in the zinc repletion group. These results indicated the activation of ER stress response in zinc-deficient rats but not in the zinc-replenished group.

Effect of zinc deficiency on the ubiquitin-proteasome system (UPS): UPS is a requisite intracellular molecular machinery essential for protein homeostasis, thereby regulating several vital cellular functions in eukaryotic cells. Therefore, any alteration of the UPS components of neurons may affect brain function. We performed immunoblotting of key UPS components like ubiquitin-activating enzyme E1, deubiquitinating enzymes (ubiquitin C-terminal hydrolase-1 (UCHL1), and UCHL5), and ubiquitinated proteins. The expression of E1 and ubiquitinated proteins in the zinc deficiency group was increased when compared with the control and zinc repletion groups (Fig. 2). The UCHL1 protein expression was unaltered, and UCHL5 was increased in the zinc deficiency group when compared with the control. The proteasome activity declined in the zinc-deficient group compared with the control group but the zinc repletion group showed no significance with both other groups. These results indicate that zinc depletion could alter the protein expression of brain UPS components.

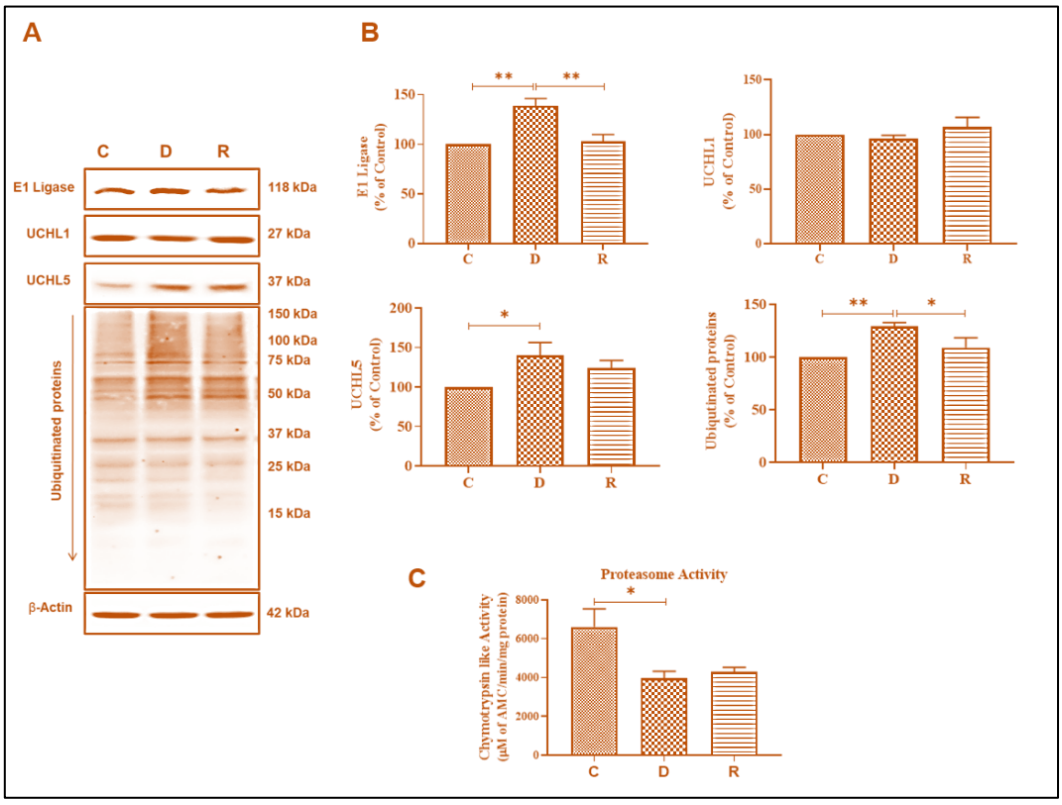
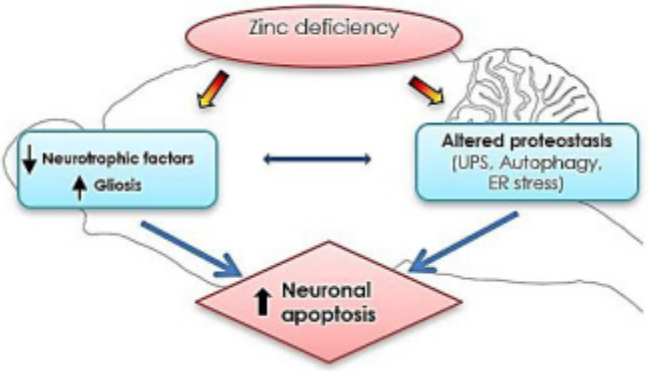


Figure 2. Zinc deficiency alters brain UPS. A: Representative immunoblot images of UPS-associated proteins in the rat cerebral cortex. B: Quantification of immunoblots after normalized to β-Actin and is represented as the percentage of control. C: Chymotrypsin-like proteasome activity in the brain of rats. Data are mean ±SEM (n=4 for immunoblotting and n=9 for proteasome activity) analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. *P < 0:05. C= pair-fed control (zinc sufficient): D= zinc deficient: R=zinc repleted.

Effect of zinc deficiency on Autophagy markers: Autophagy is a conserved catabolic and intracellular process that plays a crucial role in neuronal homeostasis. Therefore, immunoblotting analysis was done to detect the levels of autophagy proteins ATG5, LC3II, Beclin1, and p62 in the cerebral cortex of rats. ATG5, LC3II, and Beclin1 protein expression were enhanced, and p62 declined in the zinc-deficient group but restored with zinc repletion.

Effect of zinc deficiency on apoptosis: We next investigated the status of apoptotic protein levels in the cerebral cortex of rats by immunoblotting. Cleaved caspase 3, the marker of both early and late stages of apoptosis, was found to be increased in zinc deficiency compared with the control group. In contrast, zinc repletion could partially restore caspase expression. Pro-apoptotic protein Bax was raised. Anti-apoptotic protein Bcl2 decreased in the zinc deficiency group compared to the control, and zinc repletion could partially restore these alterations. The Bax/Bcl2 ratio was higher in the zinc-deficient while restored in the zinc repletion group. Further, ER stress-specific apoptotic mediators CHOP and caspase12 were increased in the zinc-deficient group and restored in the zinc repletion group. These results indicate increased apoptosis in the cerebral cortex of zinc-deficient rats.



INFERENCE & CONCLUSION

In conclusion, insufficient dietary zinc intake among developing rats has a negative impact on neurotrophic factors and brain proteostasis mechanisms, ultimately leading to brain-cell death.

5. To assess the prevalence of chronic kidney disease (CKD) in prehypertensive urban Indian adult population: A pilot study

Chronic kidney disease (CKD) is a slow and progressive loss of kidney function over several years. CKD is an increasing global public health problem with an estimated overall prevalence of 8%-16%. The prevalence of CKD in six regions of the world was 14.3% in general and 36.1% in high-risk populations from low and middle-income countries like India. According to previous reports, the prevalence of CKD in Indian adults varies from 6.3% to 20.93%. Apart from all the risk factors studied; diabetes mellitus and hypertension (HT) are the two leading contributory factors to the development of CKD in India. In addition, earlier cohort studies from Western & Asian countries have demonstrated the association of prehypertension (PHT) with a significant increased risk of CKD in the general population. These studies indicate that the onset of CKD may initiate at the PHT stage. Although the prevalence of PHT is high in the urban (40.8% to 55%), and rural Indian adults (34.0% to 52.6%), there are no

studies on the association between CKD and PHT. Hence the present study is proposed to investigate the prevalence of CKD in PHT urban adult subjects in Hyderabad.

Hypothesis: The onset of CKD may occur in the PHT stage among adult subjects.

OBJECTIVES

- To assess the prevalence of PHT among the urban adult subjects.
- To assess the prevalence of CKD in PHT urban adult subjects.
- To study the association between PHT and CKD.

METHODS

A community-based cross-sectional pilot study was conducted using purposive sampling. Institutional Human Ethics Committee approval was obtained from the ICMR-NIN, Hyderabad, for conducting the study.

Inclusion criteria: Subjects aged between 19 to 59 years were included in the study.

Exclusion criteria: Subjects aged below 19 years and above 59 years, not willing / those who do not give consent to participate in the study.

Data collection: A pre-tested questionnaire was used to collect information on demographic particulars, lifestyle habits, physical activity, and history of non-communicable diseases of the study participants. Height and weight were measured using standard equipment and adapting standard procedures. The body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters square (m2). Hip circumference (HC) and waist circumference (WC) were also measured using standard methods. Blood pressure was measured three times at 5-minute intervals on the left arm of the subjects using the OMRON digital BP apparatus. The mean of the three measurements was taken to calculate final systolic and diastolic blood pressures. Subjects with systolic blood pressure (SBP) of 140mmHg and diastolic blood pressure (DBP) of 90mmHg and those on medication for high BP were considered as hypertensive. Subjects with SBP of 120-139mmHg and DBP of 80-89mmHg were considered as PHT. A total of 337 subjects were screened and collected fasting blood samples (6.0 ml) from 96 controls and 53 PHT subjects in blood collection tubes, centrifuged, and serum samples were collected. Haemoglobin was estimated in whole blood immediately after the collection of blood samples. Fasting glucose, creatinine, urea, and uric acid levels were measured in serum samples. Spot urine samples were also collected from these study subjects. Urinary albumin, creatinine, and albumin to creatinine ratio (ACR) were measured in urine samples using the Afinion auto analyzer. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study prediction equation. Lipid profile (Triglycerides, Cholesterol, and HDL) was also estimated by kit (Biosystems) method. LDL was calculated using the Friedewald formula. Statistical analysis: All variables are expressed as Mean ±SD.t-test was used to compare all the parameters between two groups and significance was considered when the p value is <0.05.

RESULTS

A total of 337 adults (230 males and 107 females) were recruited and majority (68%) of the study subjects were males. The prevalence of PHT in these subjects was 22.55% (n=76), while the prevalence of HT was 14.84% (n=50), of which 29 were newly diagnosed. The essential characteristics and biochemical variables of the control and PHT study subjects are presented in Table-1. The mean age, BMI and other anthropometric measurements were

significantly high among PHT subjects compared to controls. There was an increase in mean haemoglobin levels, fasting glucose, TG, and TC in PHT subjects compared to controls. However, HDL cholesterol levels were lower among PHT than in controls (Table-1). Serum creatinine and uric acid levels were significantly high among PHT subjects than controls. However, serum creatinine, urea and uric acid levels were within the normal range both in control and PHT subjects. Although, there was no difference in mean eGFR values between control and PHT subjects, 6 control subjects and 2 PHT subjects had eGFR< 90 mL/min/1.73m2. However, urinary ACR of these subjects was < 30 mg/g. On the other way, two control and one PHT subjects have their ACR values are >30mg/g, but their eGFR values are >90 mL/min/1.73m2.

INFERENCE & CONCLUSION

Although the previous cohort studies demonstrated the association of PHT with increased risk of CKD, in the present cross sectional pilot study with limited sample size we did not observe CKD in PHT subjects.

6. Effect of maternal protein restriction (quantity and quality of protein) on body composition and protein quality control processes in the brain and muscle of the offspring

The intrauterine environment plays a crucial role during fetal development and affects the health of the adult in later stages. Undernourishment during gestation and lactation affects the skeletal muscle (SM) development. Maintaining functional protein homeostasis (proteostasis) or protein quality control (PQC) is a lifelong challenge for cellular health. Molecular chaperones, unfolded protein response (UPR), autophagy, and ubiquitin-proteasome system (UPS) constitute the PQC machinery. The balance between muscle protein synthesis and degradation pathways can determine SM mass and performance. The imbalance in proteostasis pathways leads to muscle mass loss and muscle weakness, called muscle atrophy. Maternal low protein (MLP) during pregnancy and lactation followed by a normal protein (NP) diet-induced changes in SM morphology. Further, effect of PR in non-pregnant rats for a shorter period on proteolysis was reported. Additionally, several experimental studies solely focused on PR during gestation and/or lactation and do not mimic human condition in much of undeveloped, developing countries where malnutrition is not only confined to fetal life but rather a lifelong condition. Thus, understanding the impact of chronic PR along with maternal protein restriction (MPR) on SM is warranted. However, the effect of chronic PR (combined prenatal to postnatal PR) and PR during premating, pregnancy, and lactation, followed by the NP diets by the offspring, after weaning on SM proteolysis in the offspring has not been investigated. In this background, we explored the effect of chronic PR and MPR on SM proteolysis and PQC machinery in adult offspring.

METHODS

Animal care, study design, and diets

Female Wistar-NIN (WNIN) rats of approximately 90 days of age were obtained from the animal facility of ICMR-NIN, Hyderabad, India. Animals were housed in individual cages and maintained at ambient temperature (22 ± 2 oC, 50% humidity, and 12-h light/dark cycle). After one week of acclimatization, animals were randomly

assigned to be fed an AIN93-based isocaloric diet containing either normal protein, (NP; 20% casein, n=4) or restricted or low protein, LP; 8% casein, n=4) for 7 weeks. After 7 weeks, female rats were mated with normal male rats At birth, litter size, sex, and pup weight were recorded, and the day of delivery was designated as day 0 of postnatal life. At weaning (postnatal day 21), both male and female offspring weight was recorded. After weaning, offspring is divided into the following groups: offspring born to mothers fed with an isocaloric diet containing either NP diet (NP; 20% casein, n=12), or LP diet (LP; 8% casein, n=12; chronic PR), or a group of offspring from LP group rehabilitated with NP diet (LPR; 20%, n=12; MPR) and maintained on their respective diets for 16 weeks.

All procedures involving animal experiments were approved by the Institutional Animal Ethical Committee of the ICMR-National Institute of Nutrition (ICMR-NIN). Histology of the SM The harvested tissue was immediately placed in 4% paraformaldehyde in phosphate buffer (pH 7.2), fixed overnight, embedded in paraffin blocks, cut into 4 μm sections, and used for Haemotoxylin and Eosin (H&E) staining for determining the myofibre cross-sectional area. Measurement of muscle protein degradation Total protein degradation (TPD) was measured in the GM as the rate of tyrosine released into the media as muscle neither synthesizes nor degrades tyrosine, and tyrosine does not accumulate in the intracellular pool of the muscle. Further, 3-methyl histidine (3-MH) in urine was estimated by HPLC as described earlier. 2.6. RNA extraction and quantitative real-time PCR (qRT-PCR) Total RNA was extracted from GM muscle tissue using Tri-reagent according to the manufacturer's instructions using RT-PCR. Immunoblotting Gastrocnemius muscle tissue (100 mg) was homogenized in TNE buffer (pH 7.5) and protease inhibitors. The homogenate was centrifuged at 12,000×g at 4°C for 20 min.

The protein concentrations were estimated using the bicinchoninic acid method. Equal amounts of protein were resolved by 12% SDS-PAGE and transferred to nitrocellulose membranes by the western blot transfer system. Nonspecific binding was blocked with 5% nonfat dry milk powder in PBST and incubated overnight at 4°C with primary antibodies diluted in PBST. After washing with PBST, membranes were incubated with anti-rabbit or anti-mouse IgG secondary antibodies conjugated to HRP. The immunoblots were developed and band intensity was quantitated using ImageJ software. 2.10. Statistical analysis The data are expressed as the mean ± SE. The significance of differences between the various groups was measured by one-way analysis of variance (ANOVA) using the Graph Pad Prism, version 7.0. The multiple comparisons were assessed using the Tukey post hoc test. Values of $p < 0.05$ were considered significant.

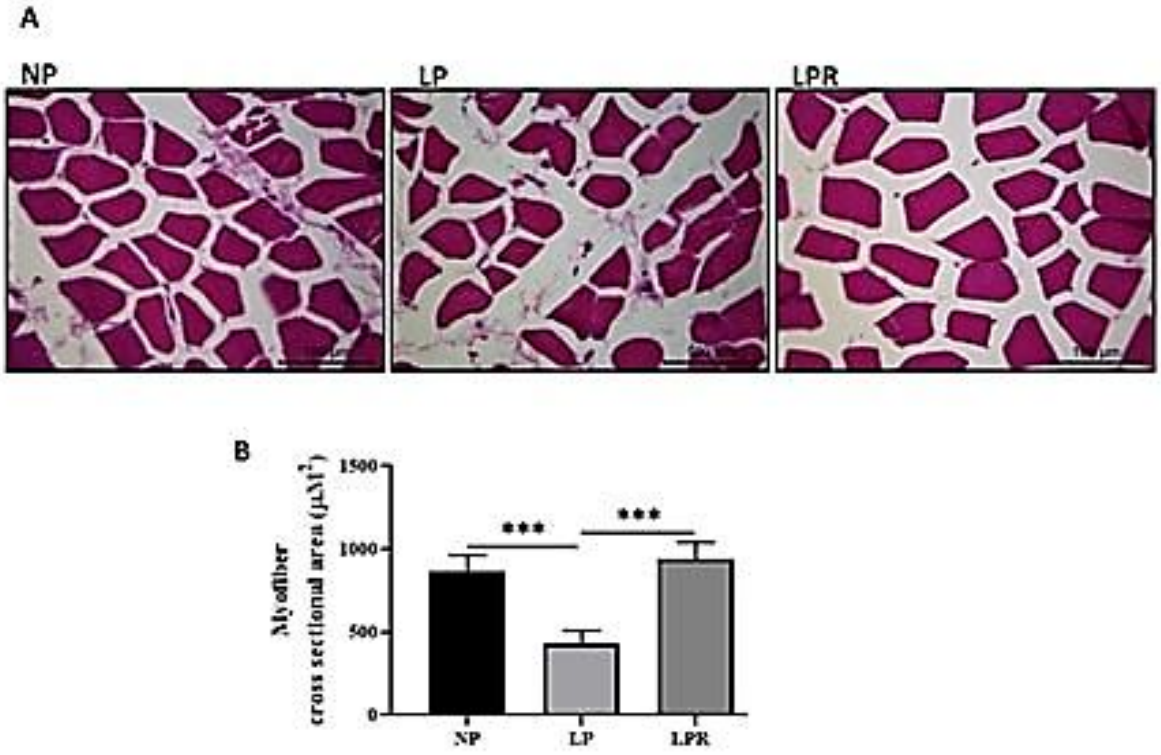
RESULTS

Effect of chronic PR and MPR on food intake and body weight

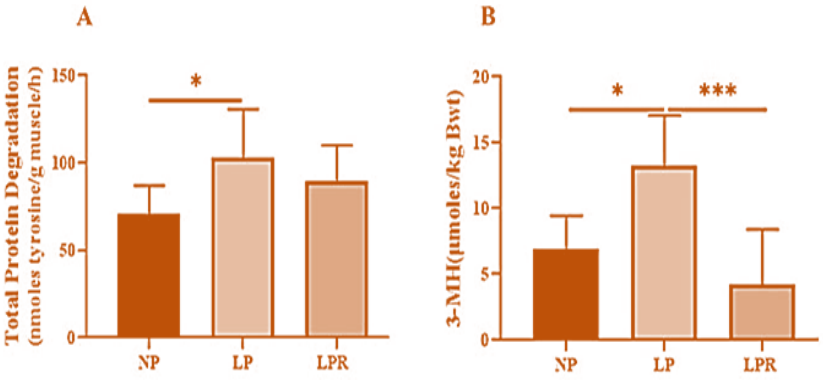
The BW was significantly lower in LP (52%) and LPR (23%) than NP offspring at the end of the experiment. Adding to this, lean body mass (LBM) was significantly lower in LP (62.5%) and tended to be lower in LPR (22.5%) than the NP.

Effect of chronic PR and MPR on myofiber cross-sectional area (CSA) in the SM of the offspring

Interestingly, myofiber CSA was decreased in LP offspring compared to NP (Fig 1A-B). However, myofiber CSA in LPR offspring was higher compared to LP offspring and comparable to NP. H& E staining also revealed the increased gap between myofibers and distorted myofibers in LP and LPR offspring compared to NP.



Effect of chronic PR and MPR on SM protein degradation in the offspring



The TPD was significantly higher in the LP offspring compared to NP (Figure 2A) and tended to be higher in LPR than NP offspring. Furthermore, TPD tended to be lower in LPR offspring than in the LP. It has been reported that 3-MH is a reliable index for SM protein degradation. In the same line, 3-MH was also significantly higher in the LP compared to NP offspring (Figure 2B) while tended to be lower in LPR compared to NP offspring Moreover, excretion of 3-MH was significantly lower in LPR offspring compared to LP offspring (Figure 2B).

Effect of chronic PR and MPR on UPR in the SM of offspring

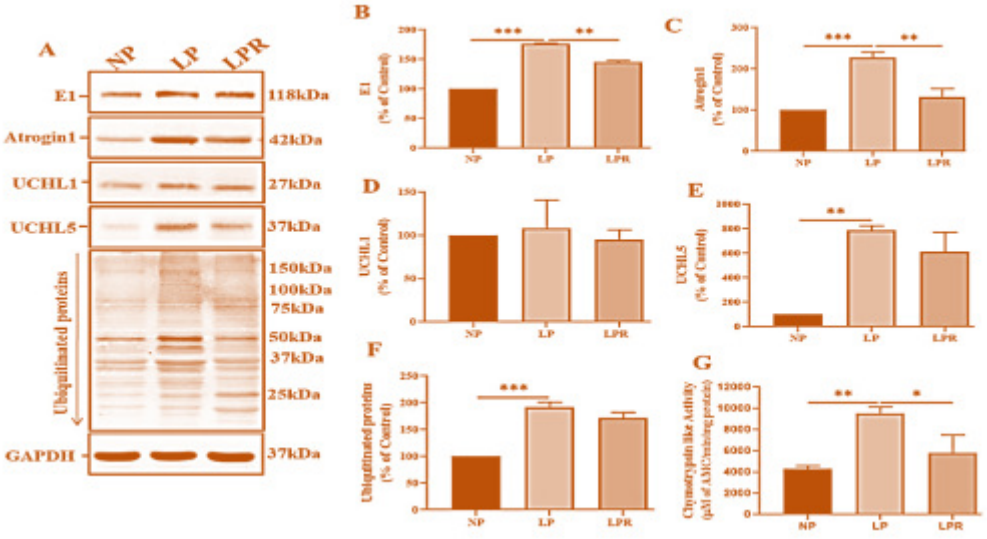
Chronic PR significantly increased the Grp78, IRE1α, XBP1S, ATF4, CHOP, ATF6, and caspase-12 levels in the GM muscle of LP offspring compared to NP. MPR significantly increased the Grp78 levels in LPR while IRE1α, XBP1S, ATF4, CHOP, ATF6 and caspase-12 levels were comparable to NP offspring.

Effect of chronic PR and MPR on UPS in the SM of offspring

We examined the key components of UPS, including E1, UCHL1, UCHL5, ubiquitin-conjugates, and proteasome activity and found that prolonged PR significantly increased the E1, atrogin1, UCHL5, ubiquitin-conjugates while decreased chymotrypsin-like activity (CLA) in LP than the NP offspring (Fig 3A-G). MPR significantly increased the E1, UCHL5 and ubiquitinated proteins in LPR, while atrogin 1, and CLA of proteasome was comparable to NP offspring. But, E1 levels were significantly decreased while UCHL5, ubiquitinated proteins tended to decrease in LPR compared to LP offspring.

Effect of chronic PR and MPR on the expression of muscle-specific E3 ubiquitin ligases, myostatin (MSTN), and myogenin (MYOG) in the SM of offspring

Here, we investigated the mRNA expression of E3 ubiquitin ligases (atrogin1/MAFbx, MuRF1, and TRIM72), a negative regulator of SM growth (MSTN), and myogenesis (MYOG) using qRT-PCR. Chronic PR caused a significant increase in the expression of the atrogin1, MuRF1, TRIM72, and MSTN while a significant decrease in MYOG in the muscle of the LP offspring compared to NP. Moreover, the expression of MuRF1 was higher in LPR than NP, while atrogin1, TRIM72, MYOG and MSTN levels were comparable to NP. Nevertheless, TRIM72 and MSTN were significantly decreased in LPR.



INFERENCE & CONCLUSION

This study reported the impact of combined prenatal to postnatal PR and MPR on SM proteolysis by modulating protein quality control processes in adult offspring. The important findings of the study are: chronic PR diets (i). decreased the myofibre size and increased the SM protein degradation, (ii). altered the molecular chaperone levels and activated UPR (iii). activated the major proteolytic systems; UPS, caspase-3, and autophagy (iv). increased the expression of genes associated with muscle-specific E3 ubiquitin ligases, myostatin and decreased myogenin (v). increased the apoptosis in the SM of the offspring, while MPR showed little effect on the SM proteolysis.

7. Quercetin inhibits hephaestin expression and iron transport in intestinal cells: Possible role of PI3k pathway (part of the work in project titled 'Effect of zinc on intestinal iron absorption: *in vitro* and *in vivo* studies')

Iron is a crucial micronutrient essential for growth and overall health, and its systemic balance is meticulously regulated through the process of intestinal iron absorption. In the realm of dietary iron, two primary forms exist: heme and non-heme iron, with the latter being the predominant source in typical vegetarian diets. Divalent metal ion transporter 1 (DMT1), located at the apical membrane of enterocytes, plays a pivotal role in the

absorption of ferrous iron. Within the enterocytes, iron undergoes one of two fates—it is either stored in ferritin or transported to the serosal side. This transport is mediated by hephaestin (HEPH) and ferroportin (FPN1), essential proteins responsible for iron export. At the apical surface, duodenal cytochrome-B (DcytB) reduces ferric iron, making it suitable for absorption, while HEPH carries out the oxidation of ferrous iron at the basolateral side of enterocytes. The iron status of intestinal cells is regulated by iron regulatory proteins 1 and 2 (IRP1 and IRP2), which modulate the translation of proteins involved in iron transport and storage. Hepcidin, a liver derived iron hormone, regulates the systemic iron homeostasis. It functions to inhibit iron absorption from enterocytes and restrict its release from the liver and macrophages by promoting the internalization and degradation of FPN1.

Moreover, beyond the internal dynamics of the host's iron status, various dietary factors have been identified as modulators of intestinal iron absorption. Notably, ascorbic acid (vitamin C) has been found to stimulate non-heme iron absorption, while phytic acid and polyphenols, such as quercetin, inhibit this process. Interestingly, clinical studies have reported a reduction in iron absorption when iron-rich foods are consumed alongside polyphenol-rich sources, with the chelation of dietary iron by anionic polyphenols being proposed as a potential mechanism. Intriguingly, studies conducted on intestinal cells have revealed that grape seed polyphenols exhibit a specific inhibition of the transport of both non-heme and heme iron. Similarly, quercetin has been shown to reduce iron transport by down-regulating the expression of FPN1 through a miRNA-dependent mechanism. Additionally, the intraperitoneal administration of quercetin has been observed to stimulate the expression of hepcidin mRNA in the liver of rats.

Our previous research has shed light on the involvement of the phosphoinositide 3-kinase (PI3K) pathway in regulating the uptake and basolateral transport of iron within intestinal cells. Activation of the PI3K pathway by zinc has been found to enhance iron uptake and transport, with DMT1 expression being influenced by IRP2 and HEPH expression being influenced by CDX2. Interestingly, polyphenols, including quercetin, have been identified as inhibitors of the PI3K pathway. Consequently, it has led us to hypothesize that quercetin-induced inhibition of iron transport in intestinal cells may involve the modulation of PI3K-dependent HEPH expression.

OBJECTIVES

The primary objective of the current study was to investigate the impact of quercetin on intestinal iron transport, with a particular focus on understanding the potential intermediary role of HEPH, if any, in differentiated Caco-2 cells.

METHODS

Treatments and cell lysis: A day before the treatments, the cells were washed with 10 mmol/L phosphate buffer saline pH 7.2 (PBS), and the spent media was replaced with serum-free MEM. Next day cells were treated with quercetin (100 µmol/L) and/or Zn (100 µmol/L) for indicated times (0 to 24 h). Wherever present quercetin was added 2 h prior to the zinc treatment. After the treatments, the cells were rinsed thrice with ice-cold PBS and lysed in Cell Lytic M containing protease (1X) and phosphatase (1X) inhibitor cocktail. The micro-BCA kit was used to estimate the protein concentration in cell lysates. A set of cell lysates are used for the immune blotting and other set was used for Real time PCR.

^{55}Fe uptake and transport: Briefly, after the treatment with quercetin (24 h), the cells grown on transwell plates were washed with HEPES buffer and incubated in the same buffer for 10 min, while the basal chamber contained 2.5 mL of MEM with 0.5% FBS. To assess the uptake and transport, the apical chamber buffer was supplemented

with 10 µmol/L FeCl3 (traced with 1.1 µcurie/mL 55FeCl3) and 1 mmol/L ascorbic acid (prepared fresh in 0.1N HCl). At the end of 1 h, 55Fe radioactivity in basolateral media and in cells was estimated by β-scintillation.

Statistics: Sigma Plot was used for Unpaired t-tests or One-way ANOVA followed by Tukey's post hoc test was used to compare the differences between groups, and $p < 0.05$ was considered significant.

RESULTS

To investigate the impact of quercetin on iron absorption within intestinal cells, we conducted a series of experiments using differentiated Caco-2 cells cultivated in transwell plates. The effects of quercetin on ^{55}Fe uptake and transport were assessed. Remarkably, quercetin treatment led to a significant increase in iron uptake, as evidenced by the elevated cell-associated ^{55}Fe activity (Figure 1, top panel), while concurrently reducing basolateral efflux of ^{55}Fe into the media (Figure 1, bottom panel) compared to control cells. Moreover, the ratio of cell-associated ^{55}Fe to that found in the basolateral media was fourfold higher in quercetin-treated cells compared to untreated controls.

To gain deeper insights into the underlying mechanisms, we analyzed the expression of various iron transporters at both the protein and mRNA levels, including DMT1, HEPH, FPN1, IRP2, and CDX2. Quercetin treatment resulted in a significant reduction in HEPH and FPN1 protein expression over a 12 to 24-hour period (Figure 2A). Consistently, it also markedly inhibited the mRNA expression of HEPH and FPN1, without affecting DMT1 mRNA expression (Figure 2B, C). Furthermore, quercetin exhibited a notable inhibitory effect on phospho-CDX2 (pCDX2) expression, particularly the slower-migrating upper band, while the levels of unphosphorylated CDX2 (bottom band) remained similar to untreated controls (Figure 2A).

In previous studies, we had demonstrated that zinc-induced iron transport and HEPH expression in enterocytes are regulated by the PI3K-dependent phosphorylation of CDX2. Consequently, we explored the influence of quercetin on zinc-induced CDX2 phosphorylation and HEPH expression. Zinc treatment led to a significant induction of pCDX2 (upper band) and increased HEPH expression compared to untreated control cells. However, all of these effects were significantly reversed in the presence of quercetin (Figure 3). Additionally, quercetin inhibited the phosphorylation of Akt at Ser 473 induced by zinc. Both pCDX2 and HEPH expression in quercetin-treated cells were significantly lower compared to untreated control cells (Figure 3).

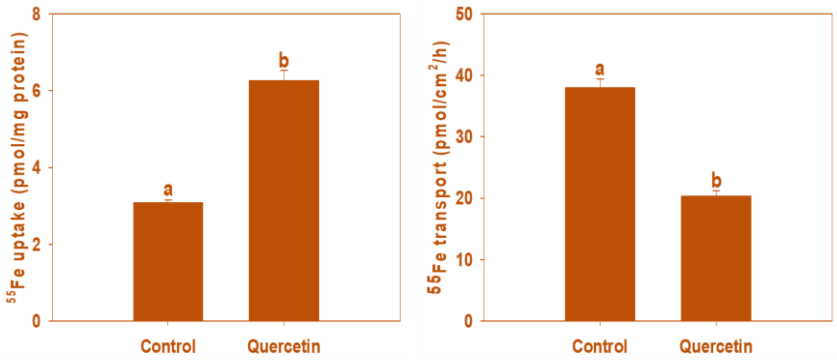


Figure-1. Effect of quercetin on ^{55}Fe iron uptake and transport in Caco-2 cells: Differentiated Caco-2 cells grown in transwell plates were incubated with quercetin (100 µmol/L) for 24 h, after which iron uptake (**Left panel**) and transport (**Right panel**) were measured as described in methods. The experiments were performed in triplicate and repeated twice to generated 6 independent observations ($n = 6$). The bars (mean ± SEM) without common superscripts differ significantly ($p < 0.05$), unpaired *t*-test.

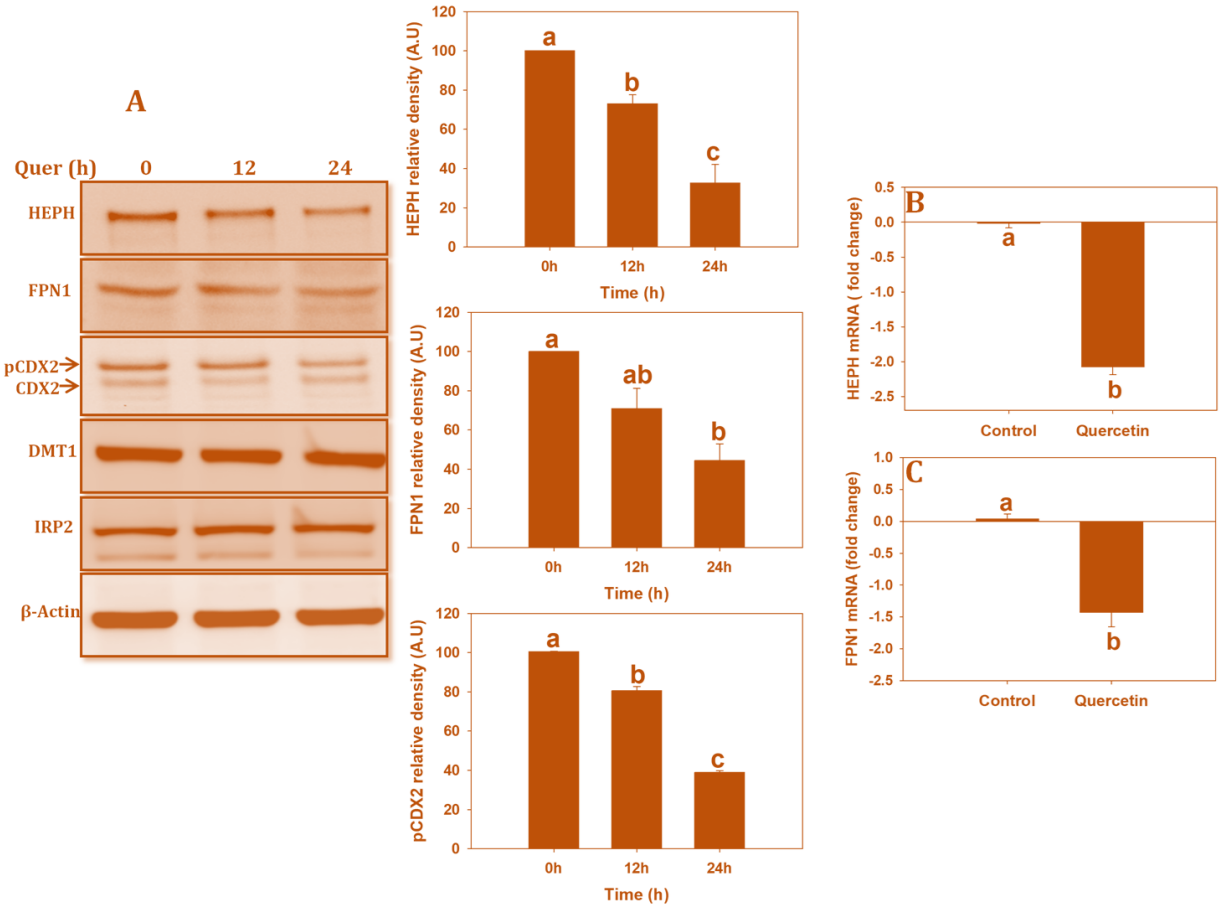
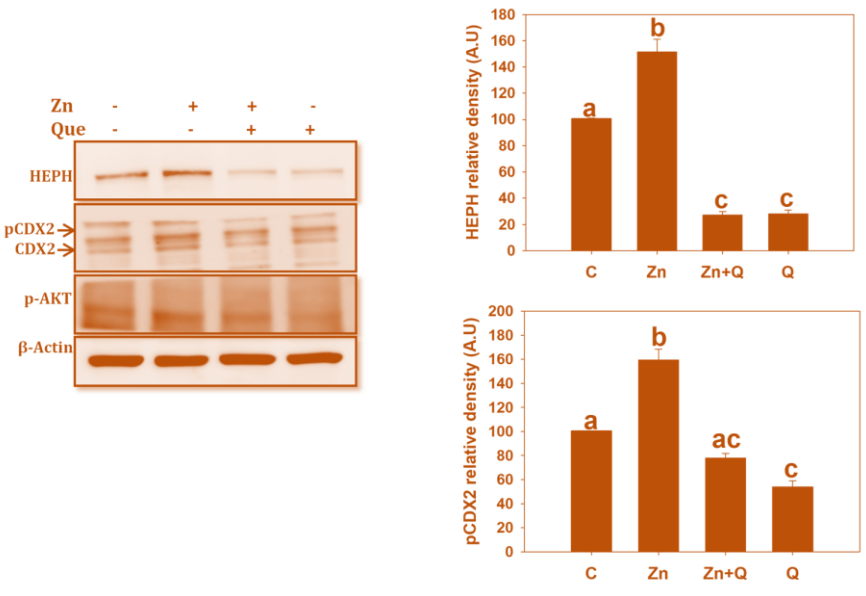


Figure-2: Effect of quercetin on expression of iron transporters in Caco-2 cells: Differentiated Caco-2 cells were treated with quercetin (100 µmol/L for 0, 12 and 24 h), after which the protein (**A**) and mRNA (24 h), (**B**,**C**) expression of HEPH (~130 kDa), FPN1 (~62.5 KDa), CDX2 (~34 KDa), DMT1 (~62 KDa), IRP2 (90 kDa) were measured by western blotting or qPCR. The densities were normalized to the β-actin, loading control. The bars (mean ± SEM) without common superscripts differ significantly ($p < 0.005$); one way ANOVA, post hoc Tukey's test.

Figure 3. Effect of Zinc and/or Quercetin on expression of hephaestin and CDX2 in Caco-2 cells: Differentiated Caco-2 cells were treated with quercetin (Q, 100 µmol/L) and/or Zn (100 µmole/L) 12 h, after which the protein expression of HEPH (~130 kDa), CDX2 (~34 kDa) and pAkt (p-Ser 4 ~60 kDa) were measured by western blotting. Wherever present quercetin was added 2 h prior to the Zn. The densities were normalized to the β-actin, loading control. The bars (mean ± SEM) without common superscripts differ significantly ($p < 0.05$); one way ANOVA, post hoc Tukey's test.



INFERENCE AND CONCLUSION

In summary, the findings presented here strongly suggest that quercetin exerts its inhibitory effect on iron transport from intestinal cells through the down-regulation of HEPH, in addition to FPN1 expression within these cells. This regulation may be achieved, at least in part, by the inhibition of the PI3K-CDX2 pathway. These results open the door to the intriguing possibility of utilizing the PI3K pathway to either enhance or reduce iron absorption, offering new avenues for the modulation of iron homeostasis within the body.

8. Effects of Bisphenol-A on lipid accumulation in hepatocytes and adipocytes

Bisphenols are a large family of chemicals that are used mainly to produce polycarbonates plastics and epoxy resins. As per USFDA and the Environmental Protection Agency current human acceptable daily intake dose (ADI) is 4 μg/Kg of bodyweight/day. Bisphenol-A is a model xenoestrogen. Despite possessing only modest estrogenic activity compared with 17ß-estradiol (E2), over the last decade BPA has been shown to produce a range of adverse effects in laboratory animals especially targeting the reproductive system. BPA binds to estrogen receptors and is likely involved in the onset of many of its adverse effects, and several studies in animal models have shown that such effects are observed after exposure to low doses. Recently, a study showed that, perinatal exposure to BPA altered early adipogenesis in the rat, which is mediated by PPAR-γ, a nuclear hormone receptor whose deregulation is involved in the onset of diabetes and obesity. In our earlier studies, when rats fed with BPA at 50 and 100 μg/kg/day, we found elevated lipid levels (20 to 40% increase) in BPA groups. Therefore, we were interested to understand the mechanism and the effect of BPA treatment on the expression of genes involved in lipid synthesis in hepatocytes and adipocytes and whether curcumin can protect from such adverse effects. Therefore, the study was planned with the following objective.

OBJECTIVE

Whether curcumin counteracts BPA modulated gene expression in hepatocytes (HepG2) and adipocytes (3T3-L1).

METHODS

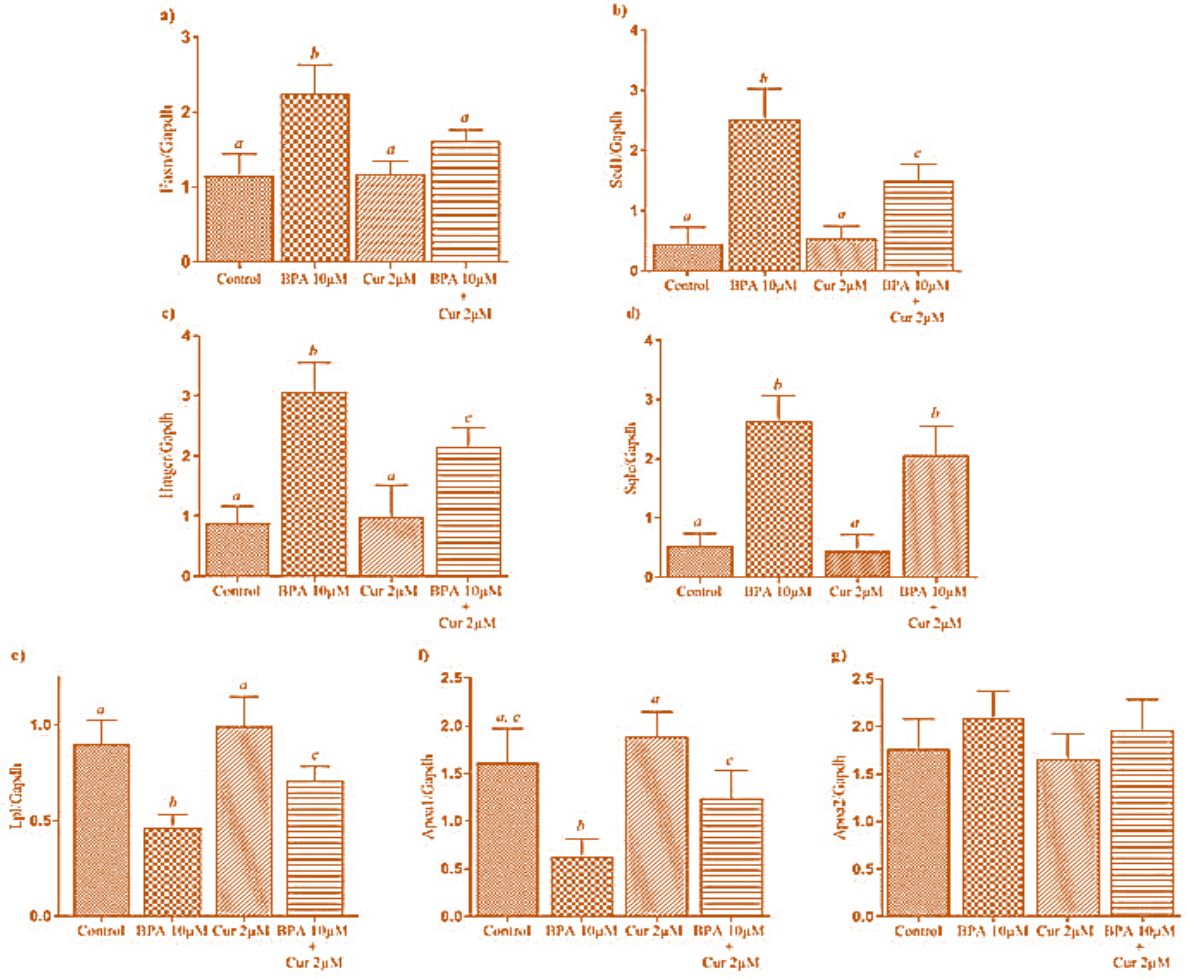
- Cell viability assay (MTT assay) was performed to determine the dose range for BPA and curcumin for treating the cells for gene expression study. Different concentrations of BPA (0, 2, 5, 10, 20, 40, 80 & 100 μM) and Curcumin (0, 2, 5, 10 μM) were used in HepG2 and 3T3-L1 cells.
- Hepatocytes, HepG2 cell culture Human hepatocyte cells (HepG2) were seeded in 6 well plates containing DMEM media free from serum and antibiotic. Next day, the cells were treated with BPA (10μM) with or without curcumin (2μM) or DMSO as a control for 24 hours.
- Adipocytes, 3T3-L1 cell culture Two days after confluence 3T3-L1 preadipocytes cells were stimulated with DMEM supplemented with 10% FBS and DMI cocktail and BPA (10μM) with and without curcumin (2μM) for 24 hrs and till Day 8.
- Quantitative PCR (qPCR) following various treatments, total RNA was isolated from the treated cells using TRIzol following manufacturer's instructions. Following cDNA synthesis, expression levels of Fasn, Scd1,

Hmgcr, Sqle, Apoa1, Apoa2, Srebf2, Srebf2, aP2, C/EBPα, PPAR-γ and GAPDH were analysed using CFX-96 (Bio-Rad, USA) and SYBR green (Takara, Japan). Gene expression changes were calculated using relative standard curve method. qPCR was performed in duplicates for each biological replicate. All the gene expression data were normalized to the corresponding GAPDH level for each sample and are represented as the mean±SD.

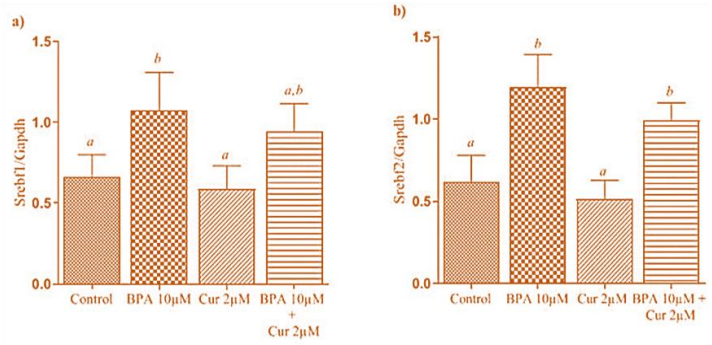
- Statistical Analysis GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA) was used to analyse the data by one-way ANOVA. Pairwise comparisons were performed when results proved significant, applying the Tukey's test to control for error due to multiple comparisons. P<0.05 was considered significant.

RESULTS

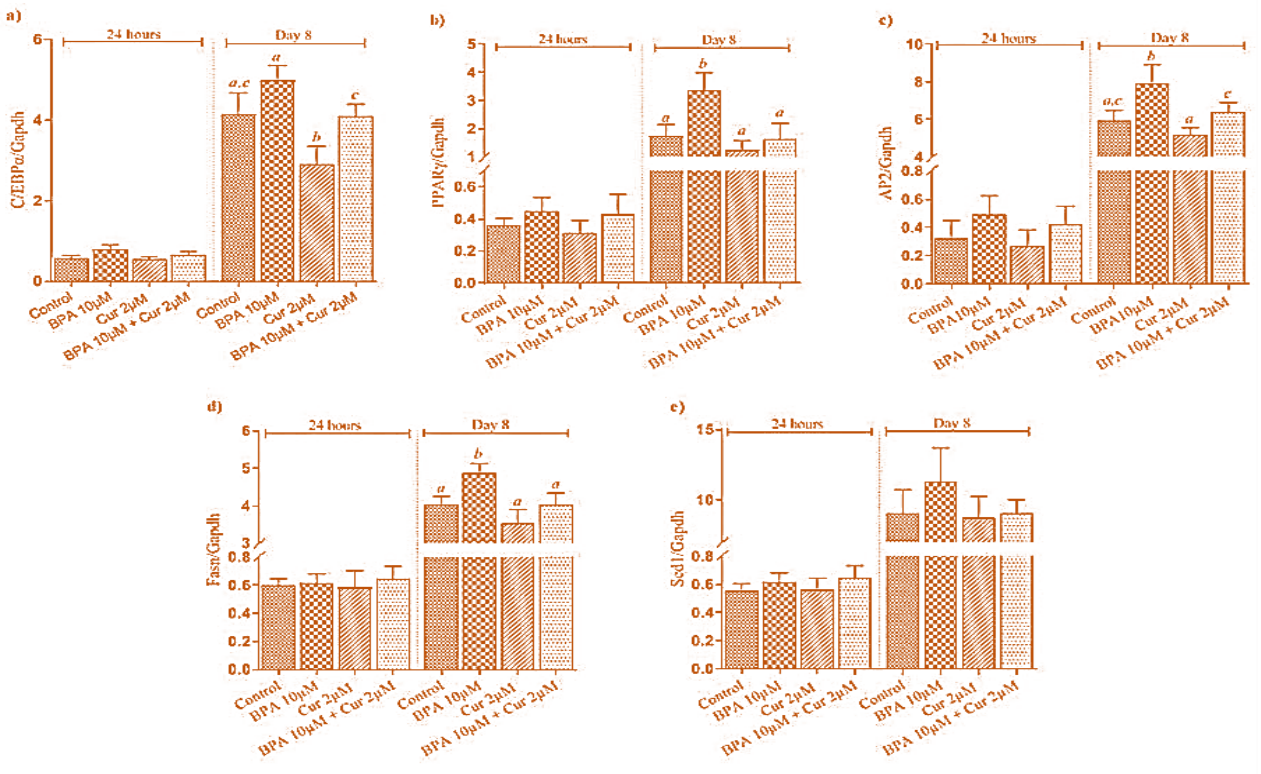
Effect of curcumin on expression of genes involved in lipid synthesis in BPA treated HepG2 cells: We observed that, Fasn, Scd1, Hmgcr and Sqle expression were significantly upregulated in BPA treated cells as compared to those of control cells (p<0.05). However, when BPA-treated cells were exposed to 2 μM curcumin, the levels were significantly downregulated as compared to those treated with BPA alone (Figure 1a-1d). On the other hand, BPA treated cells showed significant downregulated levels of expression of Lpl and Apoa1 compared to control untreated cells (p<0.05) and co-treatment with 2μM curcumin along with 10 μM BPA showed upregulated expression when compared with 10 μM BPA (p<0.05) (Figure 1e-1f). However, expression levels of Apoa2 due to BPA treatment was slightly increased but it was not found to be significant (Figure 1g). Curcumin alone did not have any significant effect on the expression of these genes (Figure 1).



Effect of curcumin on the expression of transcription factors Srebf1 and Srebf2 in BPA treated HepG2 cells: Sterol regulatory element binding proteins (SREBPs) can regulate the lipid homeostasis by regulating its target genes, which are crucial for the cholesterol and fatty acid metabolism. Therefore, we examined whether BPA targets the genes srebf1 and srebf2 which encodes SREBP1c and SREBP2. It was found that BPA treated cells had higher levels of Srebf1 and Srebf2 expression. Treatment of BPA-treated cells resulted with curcumin resulted in significant reduction of srebf1 and srebf2 expression (Figure 2a & Figure 2b).



Curcumin inhibits the effect of BPA on the genes involved in lipid metabolism genes and transcription factors in differentiating adipocytes: BPA is a known obesogen and is found to enhance adipogenic signaling in human mesenchymal stem cells by stimulating the activity of lipoprotein lipase (LPL), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and peroxisome proliferator-activated receptor-γ (PPARγ) which are all engaged in lipid metabolism and storage. Adipogenic transcription regulators like C/EBPα and PPARγ and an adipogenic marker aP2 were found to be significantly ($p<0.05$) upregulated in BPA treatment compared to control on day 8 post induction of differentiation. However, treatment of curcumin at 2μM alone was found to suppress BPA-induced expression of these adipogenic regulators. In addition, fatty acid synthase (Fasn) mRNA expression levels were significantly upregulated at day 8 during adipocyte differentiation in 10 μM BPA treated 3T3L-1 cell-lines. In addition, in the presence of curcumin, the expression of Fasn was down regulated in BPA-treated cells (Figure 3d). However, expression of Scd1, was not found to be significantly altered in the 3T3-L1 adipocytes in the presence or absence of BPA at day 8 post-differentiation (Figure 3e).



INFERENCE & CONCLUSION

The data presented herein suggest that, BPA (10μM) altered the expression levels of genes involved in fatty acid production, cholesterol synthesis, lipid metabolism-associated transcription factors and lipoprotein-apolipoprotein metabolism. Curcumin (2μM) could suppress the enhanced expression of BPA-induced of genes responsible lipid metabolism in HepG2 cell lines. BPA being an obesogen, upregulated several genes involved in adipogenesis including PPARγ considered as master transcriptional regulator of adipogenesis. Treatment of curcumin was found to inhibit BPA-induced expression of the genes involved adipogenesis. Therefore, inclusion of curcumin in diet can prevent the toxic effects of BPA on adipogenesis and lipid metabolism.

VI. PATHOLOGY AND MICROBIOLOGY

1. Oral toxicity study of a new salmonella killing bio-control agent NINMB 13076 bacteriophage

Bacteriophages are bacterial viruses that infect and multiply only within their specific hosts. There are many thousands of different strains of these phages, each one is very specific infecting only individual species of bacteria. The special characteristics of lytic bacteriophages, in particular their target specificity, rapid bacterial killing, and ability to self-replicate, make them especially appropriate for food protection applications. Lytic bacteriophages target and adhere to the surface of the susceptible bacteria and inject their DNA (or RNA) into the cell. The new viruses then go on to infect even more bacteria. Virus-infecting bacteria are harmless to humans, animals, and plants. There is a growing concern among producers, regulatory agencies, and consumers about the microbiological safety of food. Disinfection methods such as treatment with organic acids, water vapor or both have been used to minimize microbial contamination but alter food's organoleptic properties. Salmonella, killing lytic bacteriophages were isolated and these lytic bacteriophages will be used as a bio-control agent to kill Salmonella in Foods. Since these lytic bacteriophages are used along with the food, their safety efficacy study has been studied in animals before their application in food.

OBJECTIVES

1. To observe for toxicity effects in animals for morbidity and mortality.
2. To study the various parameters like body weight, histopathological changes in the stomach, intestine, spleen, pancreas, and liver of test animals.

METHODS

Oral toxicity of lytic bacteriophages in mice

To perform this study, ethical permission was obtained from the Institutional Animal Ethical Committee of the National Institute of Nutrition (Ref No. ICMR-NIN/IAEC). A total of twelve healthy BALB/c mice were used for this experiment which was conducted for 28 days. Out of 12, six of them were used as test animals and the remaining six were used as control animals. The 6 control/test animals were further divided into two categories that are three male and three female animals. The mice were housed in a row in standard polycarbonated open cages with a top grill that has a facility for feeding and drinking water in polycarbonate bottles with stainless steel sipper tubes. Purified bacteriophage NINP13076 and SM buffer were prepared for the oral dosage. The phages were aliquoted into 14 tubes (1ml each) and were stored at 40C throughout the experiment. The control animals were administered with 100µl of SM buffer every day while the test animals were administered with 100µl of phage filtrate. The oral dosage was given to the mice using a 16-gauge ball-tipped feeding needle. During the entire experiment, animals were examined every day for any sign of morbidity or mortality. After the set time period of the experiment, animals were euthanized by using carbon dioxide asphyxiation. Different organs like kidneys, liver,

spleen, intestines, thymus, and pancreas of all the control and test animals were collected and organ weights were measured (Table 2). Monitoring of activities Monitoring of activities such as mortality, water consumption, feed consumption, and general observation of the mice was done bi-weekly. The body weights of all the mice were recorded before starting the experiment and were also monitored every week to compare the same (Table 1).

Table 1.Urine test analysis of test and control Balb/c mice

Investigation	Test	Control
Physical exam		
Colour	Acidic	Acidic
Appearance	Clear	Clear
pH	6.5	6.5
Nitrate	Positive	Negative
Specific gravity	1.03c	1.03c
Chemical exam		
Protein	3+	2+
Glucose	1+	Negative
Bilirubin	2+	1+
Urobillinogen	1 EU/dL	1 EU/dL
Ketones	1+	1+
Leukocytes	Negative	Negative
Microscopic exam		
Blood	Negative	Negative

Hematoxylin and eosin staining

Three adjoining sections were taken from each tissue and staining was done using haemotoxylin and eosin stains. The slides were then observed in an inverted microscope to evaluate the histopathology. Isolation of probiotic microbiota from the gut of BALB/c mice Saline was injected into the segments and this procedure was repeated multiple times until all the contents of the segment were collected in the centrifuge tube. The centrifuge tubes were intestinal contents were subjected to centrifugation for 2 minutes at 12000 rpm. The supernatant was collected and serial dilution upto 10-4 was done using PBS (Phosphate buffered saline). After serial dilution, 100µl of the diluent was plated on MRS (De Man, Rogosa and Sharpe agar) and Bifidobacterium selective agar for Lactobacillus and Bifidobacterium species respectively. The media plates were then incubated for 48 hours at 370C and then observed for the growth of probiotic microbiota. Bifidobacterium selective agar plates were incubated in anaerobic conditions to support the growth of Bifidobacterium spp. Isolation of Salmonella spp from the gut of BALB/c mice. The diluent of the intestinal contents was plated (100µl) on Salmonella-Shigella agar and the plates were incubated at 370C for 24-48 hours. After the incubation period, the plates were observed for the growth of Salmonella.

Table 2. Analysis of caecum samples for probiotic microbiota

	Lactobacillus spp. (cfu/gm)	*Bifidobacterium* spp. (cfu/gm)
Control		
CM1	190	300
CM2	300	300
CM3	180	290
CF1	210	300
CF2	80	290
CF3	210	300
Test		
TM1	240	280
TM2	250	250
TM3	150	280
TF1	250	300
TF2	110	300
TF3	230	310

CM – Control male, CF – Control female, TM – Test male, TF – Test female

RESULTS

1. It was found that the phage isolated from sewage was specific to S. enteritidis obtained from the American Type Collection Center (ATCC).The isolated phage produced distinct lytic zones on the lawns of S. enteritidis in a spot test assay, indicating its capacity to kill Salmonella.
2. After giving phages (high dose) to male and female BALB/c mice for twenty-eight consecutive days, no significant adverse effects were noticed. No mortality was noted over the 28-day study period, either in the test group or the control group. During the duration of the study, no mice's physical characteristics and diarrheal symptoms, were noticed. After the study period, there was no noticeable difference between the animals in the control and test groups in terms of mean body weight.

3. The organs of all the animals in both the control and experimental groups were normal histologically. During the macroscopic examination, all the organs of the phage-treated mice had colors that were comparable to those of the untreated animals. Both treated and untreated mice had no gross lesions on their skin, bladder, pancreas, lungs, kidney, heart, thymus, intestines, or caecum. Hepatocytes and portal tracts in the liver sections of the phage-administered mice were normal and resembled those in the livers of control mice, according to a microphotograph of the liver sections. However, only one animal in the experimental group showed mild portal tract and parenchymal inflammation in the liver.
4. Both control and experimental animals' stomachs had normal gastric glands and lining epithelium. The kidneys of the experimental and control mice both had normal glomeruli and tubules. Normal intestinal lining epithelium with crypts and goblet cells was visible in the large intestine of the experimental animal and the control animal. The small intestine displayed villi with normal villi composition and normal lining epithelial cells. Normal islet and acini cells could be seen in the pancreas of control and experimental mice. The experimental animal's spleen displayed typical red and white pulp. Both experimental and control animals' testicles displayed typical spermatic tubules filled with mature sperm. Comparatively to the thymus of control animals, experimental animals' thymus displayed normal thymic cells and Hassall's corpuscles.
5. Studies on probiotic microbiota, particularly Lactobacillus and Bifdobacterium species, isolated from the caecum of treated and untreated mice found no significant variations in their population. In treated mice, the mean log CFU/ml of Lactobacillus, ranged from 2.0 to 2.3 log, while in untreated animals, it ranged from 1.9 to 2.4 log. For Bifdobacterium spp., the mean log CFU/ml for treated mice increased from 2.3 to 2.4 log, whereas the values for untreated animals changed from 2.4 to 2.5 log. No statistically significant differences were found between the mean values of the test and control mice samples which were done in triplicates.
6. The results of the effect of lytic bacteriophages on Salmonella present in the caecum samples indicated that Salmonella was not detected in the caecum samples of treated mice, however, Salmonella was detected in one caecum sample of untreated mice.

INFERENCE & CONCLUSION

The results of this study clearly showed that oral administration of the lytic Salmonella phage did not have any significant adverse effects on the animals, may not harm the probiotic gut microbiota, and are likely to be safe for use in food preservation.

1. Evaluation and role of isolated compound from amla fruits on volproic acid induced autism spectrum disorder (ASD) in experimental BALB/C mice

Autism is a neurodevelopmental disorder characterized by impaired social behavior, poor communication, stereotypic behavior, abnormal sensitivity to sensory stimuli and self-injurious behavior. Autism is usually seen in before three years of age. The exact cause of Autism is unclear but increased oxidative stress; hyperserotonemia and loss of Purkinje cell integrity in cerebellum are some of the pathological findings. The Amla (*Emblica officinalis*) fruit contain vitamin C, minerals, amino acids, tannins, flavonoids, and other important phytochemicals which are believed to possess diverse pharmacological and biological effects. The fruit also contains Phyllemblin, as one of the major constituents, and it is an ester by chemical composition. Phyllemblin from the fruit pulp is identified as Ethyl gallate. It contains mainly antioxidant properties and mild CNS depressant action and Antiserotonin.

METHODS

The study was reviewed and approved by the Institutional Animal Ethics Committee (ICMR-NIN/IAEC/02/007/2019). NCLAS, Tarnaka, Hyderabad, India. BALB/C mice were monitored and from the date of birth and recorded as post-natal day (PND) 0. On the PND 13 animals were divided into three different groups. First group considered as negative control. Group II was considered as VPA treated group and received VPA on PND 14 followed by water for injection oral administration thereafter up to PND 40, Group III were VPA treated mice which received 100 mg/kg of amla extract daily from PND 13 to PND40 by the oral route. All the animals except group I were injected subcutaneously with sodium valproate at a single dose of 400mg/kg on PND 14. At the end of behavioral testing, animals were sacrificed; brain was isolated for biochemical estimations and histopathological examination, immuno-histochemical studies, determination of protein expression of 5-HT2A, D2, and iNOS by Western blot, all objectives of this project have been completed.

RESULTS

Immuno-histochemical studies

Effect of EAFA on serotonin (5-HT1D and 5-HT2A) receptor protein in postnatal VPA exposed mice in the current study, we first observed the levels of 5-HT1D and 5-HT2A receptor protein expressions in the postnatal mice treated with VPA by immune histochemical analysis. Compared to control animals, VPA-treated mice had more positive expression of 5-HT1D and 5-HT2A receptor proteins in the Purkinje and granular cells of the cerebellum ($p<0.001$; $p<0.001$), cerebral cortex ($p<0.003$; $p<0.002$), and the hippocampus ($p<0.002$; $p<0.004$). Furthermore, treatment with EAFA for VPA-exposed mice showed expression of these serotonin receptor proteins was significantly decreased in the Purkinje and granular cells of the cerebellum ($p < 0.026$; $p < 0.005$), cerebral cortex ($p < 0.016$; $p < 0.006$), and the hippocampus ($p < 0.019$; $p < 0.016$) compared to VPA-treated mice. These findings indicate that EAFA treatment (100 mg/kg, orally) given to VPA-exposed mice could attenuate the 5-HT1D and 5-HT2A receptor proteins (Figures 1A & B).

Figure 1A

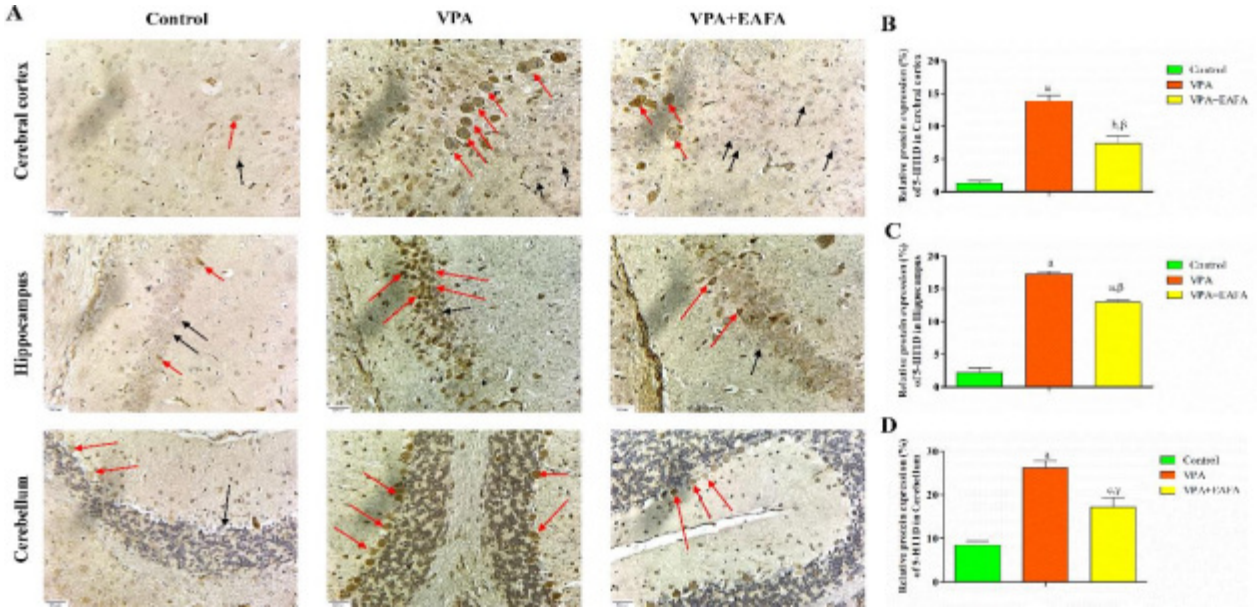
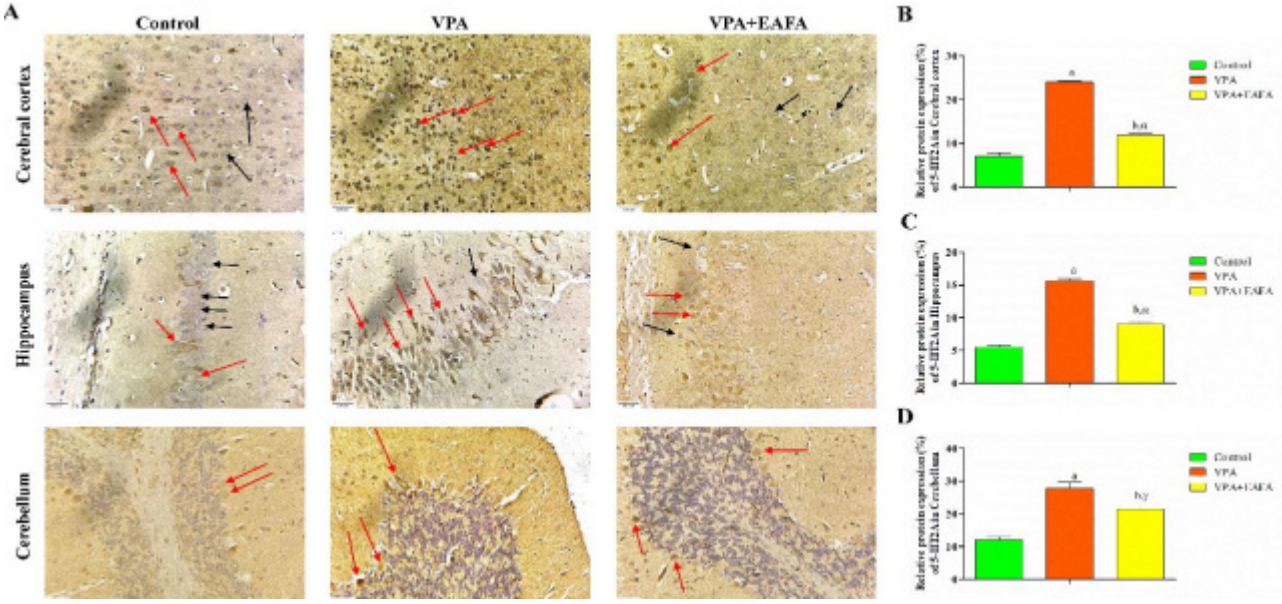


Figure 1B

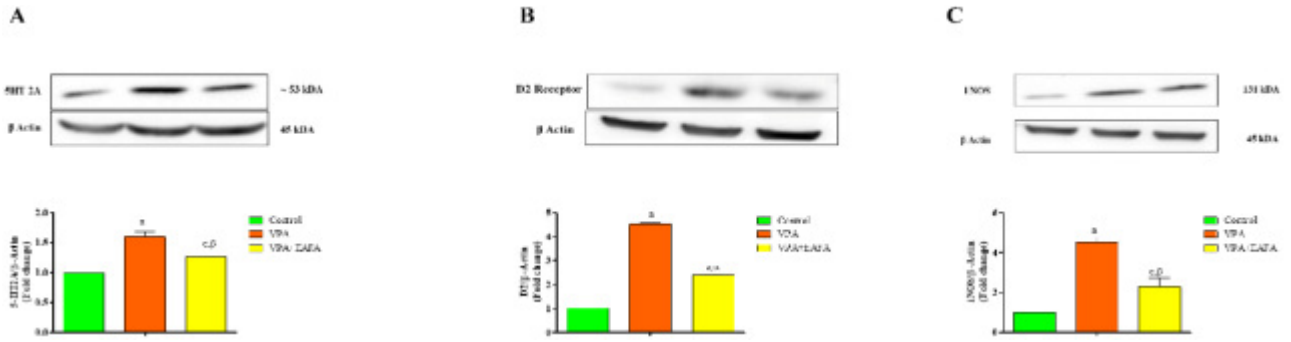


Western blot Analysis Effect of EAFA on 5-HT2A in the cerebellum of pups postnatally exposed to VPA

We first observed that the postnatal mice treated with VPA showed an obvious increase in the level of and 5-HT2A ($p<0.001$) receptor proteins expression in the cerebellum compared with control mice. Furthermore, when VPA-exposed mice were given EAFA at a dose of 100 mg/kg, and 5-HT2A levels significantly decreased ($p < 0.007$) compared to VPA alone exposed mice (Figure 8A). Moreover, the expression of 5-HT2A receptor protein in the cerebral cortex, hippocampus, and cerebellum of VPA and VPA+EAFA mice was also investigated by immunohistochemistry (Figure 2A), which was quite similar to the results of Western blotting. Effect of EAFA on D2 in the cerebellum of pups postnatally exposed to VPA: Dopamine (DA) is a key neurotransmitter involved in social behaviour, social cognition, and movement regulation. Compared to the control mice, postnatal VPA-exposed mice showed significantly increased levels of D2 receptor protein in the cerebellum ($p<0.001$) while treatment with EAFA at a dose of 100 mg/kg considerably decreased the levels of D2 receptor protein ($p<0.001$)

compared to the VPA group (Figure 2B). Effect of EAFA on iNOS in the cerebellum of pups postnatally exposed to VPA: As compared to the control mice, VPA-exposed mice showed significantly increased ($p<0.001$) levels of the inflammatory biomarker iNOS in the cerebellum. When EAFA was given to VPA-exposed mice at a dose of 100 mg/kg, iNOS levels decreased ($p<0.004$) when compared to VPA-exposed mice. Thus, indicating a reduction in the inflammatory marker iNOS by the administration of EAFA to the VPA-exposed animals (Figure 2C).

Figure 2



INFERENCE & CONCLUSION

In conclusion, this is the first demonstration that EAFA plays a neuroprotective role in ameliorating social deficits, learning disabilities, loss of motor coordination, anxiety, nociception, and locomotion activity in the VPA-induced postnatal autism animal model. A total of 5 compounds were quantified in the ethyl acetate fraction of amla extract using UPLC-MS/MS. Moreover, EAFA has also been shown to significantly improve protection of neuronal injury, have anti-oxidant properties, and reduce the up-regulated protein expression of 5-HT1D, 5-HT2A, and D2 in various brain regions in a VPA-induced postnatal autism mouse model. Additionally, alopecia is one of the significant findings from both our previous study [7] and the current study revealed that the EAFA treatment prevented hair loss. Furthermore, due to its low side effect profile, amla may be a viable candidate because it is an effective herbal medicine for the long-term treatment of ASD compared to currently marketed drugs.

VIII. NUTRITION INFORMATION COMMUNICATION AND HEALTH EDUCATION (NICHE)

1. Assessing effectiveness of front-of-pack nutrition labels (FoPNL) for processed food products in India – A study on formats, acceptability and potential use

Front of Pack Nutrition Labelling (FoPNL) could be one of the several strategic communication methods which could generate awareness and motivate consumers to make healthy choices. But the decision on which of the FoPNL will be suitable in the Indian context should be based on data on consumer acceptability, and understandability of the different label formats. Different formats of FOPNL are being used globally either voluntarily or by mandatory implementation. In India, the Food Safety Standards Authority of India (FSSAI) is considering to implement a symbol-based FOPNL. However, context specific evidence on the effectiveness of FOPNL is needed to inform ongoing advocacy and regulatory processes in India. The decision to which type of FOPNL should be used in a country should be based on the local research, along with regional and global evidence, and in consideration of each country's specific objectives for developing a FOPNL policy. Given this background, the current study was carried out with the following objectives: 1. To test the consumer acceptability, likeability, reliability and understandability of five FOPNL formats - namely Nutri-score (NS), Health Star Rating (HSR), Warning Labels (WL), Multiple Traffic Lights (MTL) and Nutri-star (NSR). 2. To compare the different formats of FOPNL on attributes such as noticeability, comprehensive and cognitive workload, speed, informativeness and objective understanding, legibility and purchase intention to identify the most suitable FOPNL for India.

METHODOLOGY

A cross-sectional study was conducted among 3231 participants (Adults – 2616, and adolescents – 615) in the age group 10-60 years in five regions of India. The data collection was completed in a single contact with the participated using a validated questionnaire. The questionnaire had three distinct parts. In part one, socio demographic and history of health condition, food label reading habits, frequency of purchasing packaged foods were recorded. Part two of the questionnaire assessed the perception of the participants on likeability, attractiveness and perceived cognitive workload of the five different formats of the FOPNL i.e. Nutri-Score (NS), Health Star Rating (HSR), Warning label (WL), Multiple traffic lights (MTL), and Nutri-Star (NSR). In the part three 1/5th of the participants was randomised to one of the five FoPNL format and asked questions which assessed the objective understanding, perceived product healthfulness, purchase intention and willingness to change purchase behaviour.

RESULTS

Most of the respondents reported that they checked expiry date (74.2), Over 60percent of the participants also read the brand name and 57.7 percent saw the manufacturing date. A small proportion of participants reported that they also considered nutrition information. Most commonly checked nutrients were calories, total fat, sugar, salt and protein. Over 39 percent of the participants reported that they look for the symbols indicating vegetarian and non-vegetarian foods. Among the quality symbols ISI symbol was the most checked one.

NS was ranked as the preferred first choice (41.3) of FoPNL by most respondents and also scored highest in terms of likeability while warning label was the least preferred choice. In terms of identifying healthy and unhealthy foods NS, HSR and NSR scored better than WL and MTL. However, with regard to reliability (which assessed whether the participant trusted the information provided by the FOPNL), nutrient specific labels MTL (78.3) and WL (70.5) scored better than NS, HSR and NSR.

In terms of objective understanding and perceived product healthfulness there was not much difference in identifying the healthiness of healthiest variants between the formats studied except for WL. With respect to healthiest shown in WL format participants got confused since there was no symbol to prompt them about the healthiness of the product. With regard to moderately healthy foods, the HSR and NSR had higher percentage identifying it as healthy than other labels, this could due to positive effect of presence of star known as 'halo effect'. With regard to the purchase intention of healthiest and Least healthy (extremes) there was not much difference in participants' responses across the FOPNL formats. However for the healthy, moderately healthy, and unhealthy products there was a clear difference in participants' responses. Among the summary indicators, NS and nutrient specific warnings, the WL are performing better in altering the purchase intention of participants. The amber colour in MTL was not fully understood by the participants. Similarly, the summary indicators NS and in nutrient-specific WL format performed better in terms of reducing the consumption, choosing another healthier option and even stopping to eat the product of unhealthy variants.

Table 1. Food Label information reading practices among the participants

Particulars	Frequency (Percent) (N=3231)		
	Always	Sometimes	No
1. Brand name	1969 (60.9)	519 (16.1)	743 (23)
2. Manufacturing date	1863 (57.7)	548 (17)	820 (25.4)
3. Expiry date	2396 (74.2)	418 (12.9)	417 (12.9)
4. Ingredients	476 (14.7)	879 (27.2)	1875 (58)
5. Storage	443 (13.7)	747 (23.1)	2041 (63.2)
6. **Nutrient Information**			
a) Calories	307 (9.5)	956 (29.6)	1968 (60.9)
b) Total Fat	307 (9.5)	967 (29.9)	1957 (60.6)
c) Saturated Fat	256 (7.9)	887 (27.5)	2088 (64.6)
d) Transfat	255 (7.9)	865 (26.8)	2111 (65.3)
e) Carbohydrates	295 (9.1)	879 (27.2)	2052 (63.7)
f) Sugar	331 (10.2)	903 (27.9)	1997 (61.8)
g) Protein	314 (9.7)	881 (27.3)	2036 (63)
h) Salt	299 (9.3)	881 (27.3)	2051 (63.5)
i) Cholesterol	288 (8.9)	836 (25.9)	2107 (65.2)
7. Allergen	242 (7.5)1111	661 (20.5)	2328 (72.1)
8. Veg and Non veg symbol	656 (20.3)	617 (19.1)	1958 (60.6)
9. **Quality symbols**			
a) ISI	785 (24.3)	658 (20.4)	1788 (55.3)
b)AGMARK	642 (19.9)	531 (16.4)	2058 (63.7)
10. FSSAI License	740 (22.9)	614 (19)	1877 (58.1)

INFERENCE & CONCLUSION

The choice of the FOPNL format for Indian scenario should not base only on wider acceptability and appeal but on its ability to influence food choice. If the purpose of FOPNL is to promote healthy food choices (based on the relative healthiness of the foods or the available variants of similar foods) then summary labels may be useful. Alternatively, in the context of growing overweight, obesity and non-communicable diseases if the FOPNL has to serve as a preventive tool and deter the consumers from consumption of nutrients of concern, then warning indicator labels (like WL and NSR) could be helpful.

Table 2. Comparative likeability, attractiveness and perceived cognitive workload of the five FoPNL format/subjective Understanding of five FoPNL format

	Likeability			Attractiveness			Perceived Cognitive Workload			
FoPNL	Easy to judge the nutrient quality	Want to see this label	Label preference (1st preference)	Identify healthy food	Identify unhealthy foods	Reliability	Easy to understand	Complex to understand	Think about food	Helps quickly decide what to buy
Nutri score	92.1	86.2	41.3	94.9	91.7	50.4	97.3	2.3	85.3	87.5
Health star rating	89.5	76.2	17.1	90.9	88.7	47.3	96.9	2.5	77.7	81.9
Warning Labels	77.2	61.2	6.7	76.9	76.9	70.5	82.8	13.3	66.4	65.3
Multiple Traffic Lights	85.1	70.2	16.1	85	83.4	78.3	89.9	7.8	73.9	73.4
Nutri Star	90.7	80.8	19.4	91.6	90.4	51.3	97.4	2.8	81.8	84.2

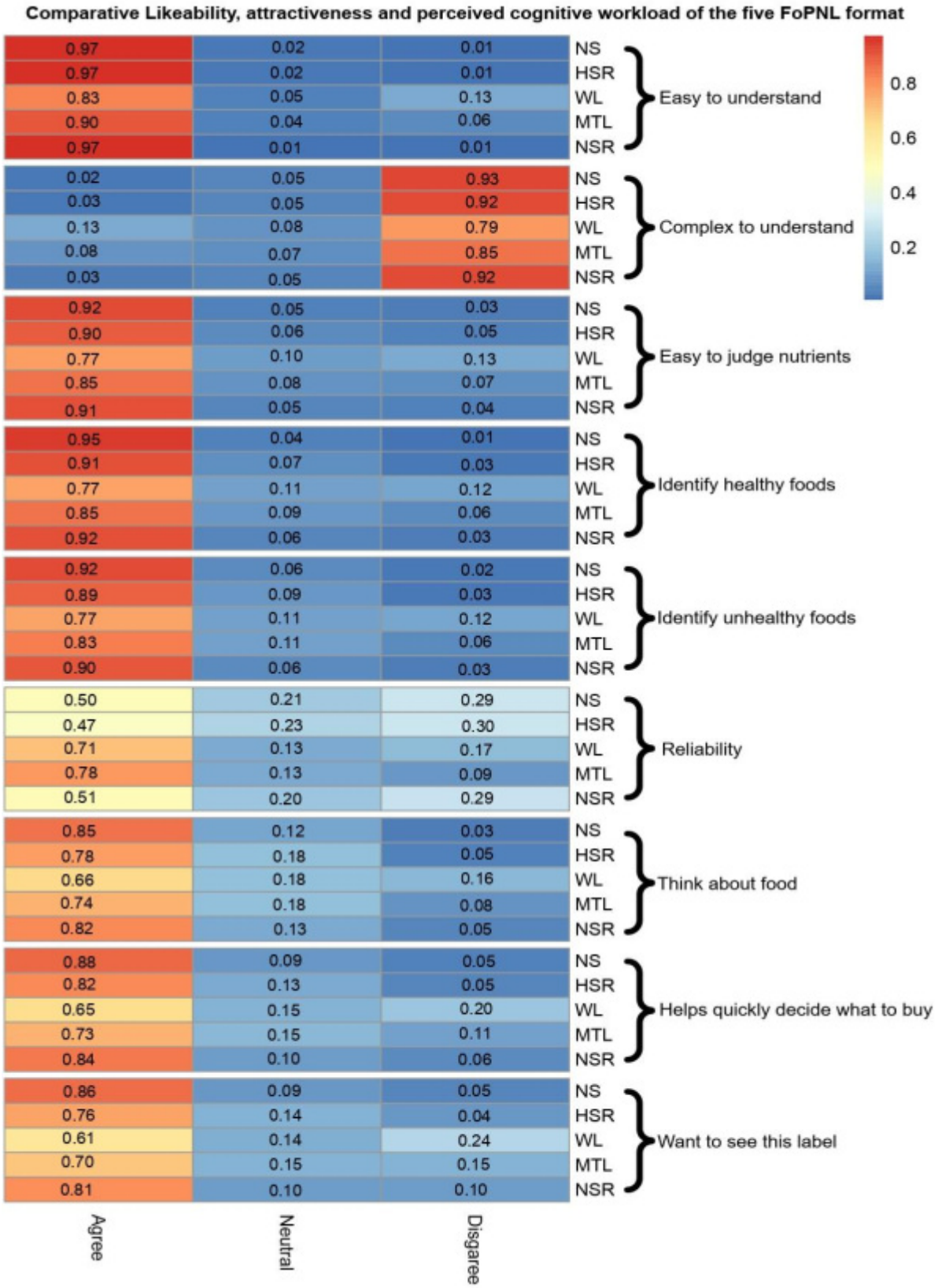
*The percentage of participants who said they 'strongly agree' or 'agree' for the statement are combined

Table 3. Objective understanding, purchase intention, food choice and behaviour of the participants (N=3231)

FoPNL Attributes	Nutri score (n=644)					Health Star Rating (n=632)					Warning labels (n=631)					Multiple Traffic lights (n=659)					Nutri Star (n=665)				
	VH	H	MH	UH	VUH	VH	H	MH	UH	VUH	VH	H	MH	UH	VUH	VH	H	MH	UH	VUH	VH	H	MH	UH	VUH
Product Healthfulness	92.2	76.2	28.4	60.6	60.2	93.4	51.1	67.7	58.7	57	78.4	42.5	17.7	41.7	53.7	90.1	59.2	48.4	49.9	61.8	89.6	47.8	61.6	67.4	55.9
Buying Intention																									
Will buy the product	95	86.3	28.4	6.8	3.9	95.5	90.8	66.7	12.6	4.4	84.5	43.1	19.2	8.1	4.3	93.5	84.2	43.7	9.7	3.9	89.2	90.4	60.3	9.8	6.9
Will not buy the product	2.9	9.6	49.1	88.2	93.5	3.2	6.3	24.7	77.6	91.5	6.8	48.8	73.7	87.5	90.5	3.5	9.9	40	79.4	92.3	9.4	6.3	28.4	85	89.8
Consumption pattern																									
Reduce the consumption of the product	2.8	7.6	45	84	92.4	3.7	7.1	25.3	74.9	87.8	5.6	40.7	69.1	84.4	86.7	3.3	7.7	39	77.3	90.1	9.3	8.3	29.7	82.9	85.1
Will not reduce the consumption of the product	95.1	88.2	37.7	11.6	5.6	93.5	89	66.7	14.4	8.2	86.5	52.6	24.4	10.8	8.4	93	82.4	46.4	10.9	5.9	89.2	87.7	59.1	12.8	11.3
Product Choice																									
Will choose another product	2.3	6.5	49.2	84.7	90.9	2.6	5.2	23.4	73.5	86.8	5	40.9	66.5	86	87.3	2.3	7.5	34.5	76.6	87.6	8.9	6.8	30.1	81.1	85.3
Will not choose another product	94.9	89	34.9	9.8	5.6	94.3	90	66.7	14.1	6.8	87.2	51.4	26.8	8.6	6.8	93.8	83.6	48.9	11.2	7.2	89.2	88.8	59.2	13.4	11.2
Eating Behaviour																									
Will stop eating the product	2.5	4.8	44	83.7	90.2	2.4	4.3	19.8	72	86.5	4.5	36.3	55.3	80.3	83.4	2	5.9	33.7	74.8	88.9	7.3	5.6	23.4	80.8	85.7
Will not stop eating the product	95.2	90.1	38.4	9.9	4.7	94.7	89.7	68.2	14.2	6.3	88.6	51	34.4	11.7	7.9	93.9	83.8	50.3	11.7	4.8	90.7	88.6	63.4	13.6	9.5

*Values in the table indicate percentage. VH- Very Healthy, H- Healthy, MH- Moderately Healthy, UH- Unhealthy, VUH- Very Unhealthy. **Nutri score** – VH-A, H-B, MH-C, UH-D, VUH-E; **Health star rating**= VH-5 stars, H=4 stars, MH=3.5 stars, UH=2.5 stars, VUH=1 star; **Warning Labels**= VH-No octagon, H-1 octagon, MH- 2 octagon, UH-3 octagon, VUH-4 octagon, **Multiple traffic lights**= VH-4 green, H-3 green and 1 orange, MH-2 green, 1 red and 1 orange, UH-2 red and 2 orange, VUH-4 red; **Nutri star**= VH-5 golden star, H-4 golden star, MH-3 golden star, UH-2 golden star, VUH-1 golden star

Figure 1. A heat map of the Comparative likeability, attractiveness and perceived cognitive workload of the five FoPNL format



2. Promoting nutrition and health of corporate employees with workplace intervention-A study using communication for behavioural impact (COMBI) approach

Around 4.14 million Indians are employed in the largest private employment sector of the India-the Information Technology (IT) and Business Process Outsourcing (BPO). However, the work style of the employees in this sector is dominantly sedentary and exposes them to detrimental obesogenic environment, work stress, unhealthy lifestyle contributing to the growing rate of Non-Communicable Diseases (NCDs) and Metabolic Syndrome (MetS). Consequently, the economic costs of NCDs in terms of absenteeism, lack of productivity and health care claims is very high, forming a major barrier to economic growth of the country. Workplace Wellness Programs (WWPs) have been recognized internationally as one of the best ways of promoting health and reducing NCD risk factors among employees. Unfortunately, the concept WWPs as an important part of occupational health is not yet widely accepted in India. Among those present, WWPs orientated towards nutrition and lifestyle are rare. Therefore, this study aimed to develop and implement a flexible model of a nutrition-based WWP with strategic interventions tailored to Indian IT workplace environments.

The specific objectives of this study were:

1. To assess the food, physical activity environment (FPAE) of a workplace, diet, lifestyle and nutritional status of corporate IT employees, and prevalence of MetS in the population
2. To develop and implement a multi-component nutrition intervention model to promote the lifestyle and nutrition patterns of employees
3. Assessing the impact of intervention in terms of the utility of the programme, organizational changes, health and nutritional status of employees

METHODOLOGY

The study was conducted in IT organizations of varied sizes located within the city of Hyderabad, India. The study sites and participants were recruited through multi-stage sampling technique. The IT organizations of varied operational levels (small, medium, big), with no ongoing WWPs, were selected purposively in the first stage. In the second stage, each included worksite was treated as a cluster and all apparently healthy employees, voluntarily agreeing to participate in the study were recruited through cluster sampling. the study was ethically approved by the institutional ethical committee of ICMR-National Institute of Nutrition, Hyderabad, India (Protocol. No. 09/I/2017).

A mixed method approach was used in the study as follows:

(i) To assess Food and Physical Activity Environment (FPAE) of the workplace and employee and employers' perspectives on the scopes of initiating the WWP, qualitative methods-Focus Group Discussions (FGDs), Ground Truthing using a workplace FPAE checklist were used. Cross-sectional data were collected on employee health and nutrition status using anthropometry (height, weight, waist circumference), clinical (blood pressure), biochemical (fasting blood glucose, HbA1c, lipid profile) and

biomarker levels (Homocysteine, malondialdehyde, Interleikin-4 and Interleukin-6). A health, food, and lifestyle questionnaire (HFLQ) was developed and used to assess the employees' knowledge, attitude and practices (KAP). The Harmonized Criteria (2009) have been used to quantify the prevalence of MetS in the study population.

(ii) A systematic exploratory research design was adopted to develop the conceptual framework of the workplace intervention model. It involved an extensive review of the literature, selection of theoretical base, contextualization and modification of the plan, creating intervention plan and instruments and developing an evaluation plan.

(iii) The impact of the intervention was evaluated using a pre-post longitudinal method by repeating the baseline survey methods. Employee attendance and feedback were collected to assess programme retention and utility.

RESULTS

A total of 3 IT organizations (Big-Worksite A (>5000 employees, Medium-Worksite B >500 employees and Small-Worksite C, <200 employees) were purposively recruited. A total of 359 employees (219 employees-Worksite A, 78 employees-Worksite B, 62 employees-Worksite C) had voluntarily enrolled in the study.

A total of 7 FGDs conducted separately with the senior and junior employees (3 in Worksite A, 2 each in Worksite B and C) revealed striking differences between senior and junior employees in terms of concerns about their health and efforts to take precautionary measures to prevent NCDs. The seniors were against making WWPs a part of workplace policy, as it might be misused by the employees to stay away from desks for a longer duration and thus may result in less productivity. Whereas junior employees were concerned that voluntary participation in WWPs might impact their upgradation as they may be perceived to be whiling away time by the senior management. The data gathered from 'ground truthing' showed that although worksite A had better workplace FPAE facilities like gym and walking tracks, utilization remained poor. In worksites B and C there were limited physical activity facilities, and consumption or distribution of unhealthier food items prevailed.

Assessment of health and nutrition status and behavioral risk factors of the employees pointed out that though the median age of the employees was 30 years (26-35) years, 44.02% were overweight, 16. 85% were obese, 48.21% of female employees had waist circumference >80cm, 3.89% were diabetic. Low HDL-C level was the most frequently present metabolic risk factor. In all, 29.87% of the study population were considered to have metabolic syndrome since they had ≥3 metabolic risk scores (Figure 1). Those with metabolic syndrome were significantly elder (p=0.00) and the level of MDA (p=0.003), homocysteine (p=0.001), IL-6 (p=0.017), IL-4 (p=0.000) were significantly higher among them. Among the employees >30 years although MetS risk was significantly lower their lifestyle risk factors such as skipping of meals and frequent eating out were significantly higher.

After baseline situation analysis, the following prototype of the intervention with targeted interventions at different levels was developed (Figure 2). A complex nutrition-based intervention model prototype based on socio-ecological theory was developed and intervention components were delivered

using Communication for Behavioral Impact (COMBI) approach at individual, interpersonal and organisation levels. The interventions targeted at reducing the risk factors of MetS were implemented over 6 months through health screening, one-to-one and telephonic consultations at individual level. At interpersonal level, 12 group awareness sessions, demonstrations and events were conducted. At organisation level, enabling environment was created through modification of workplace food and physical activity environment (FPAE).

Post-intervention data revealed high employee engagement, positive changes in employee health behaviour and workplace FPAE (Table 1). A mild but significant reduction occurred in body weight ($z=-3.59$, $p<0.01$), LDL-cholesterol ($z=-4.75$, $p<0.01$), triglyceride ($z=-4.63$, $p<0.01$) (Table 2).

Figure 1. Clustering of metabolic risk factors among the employees

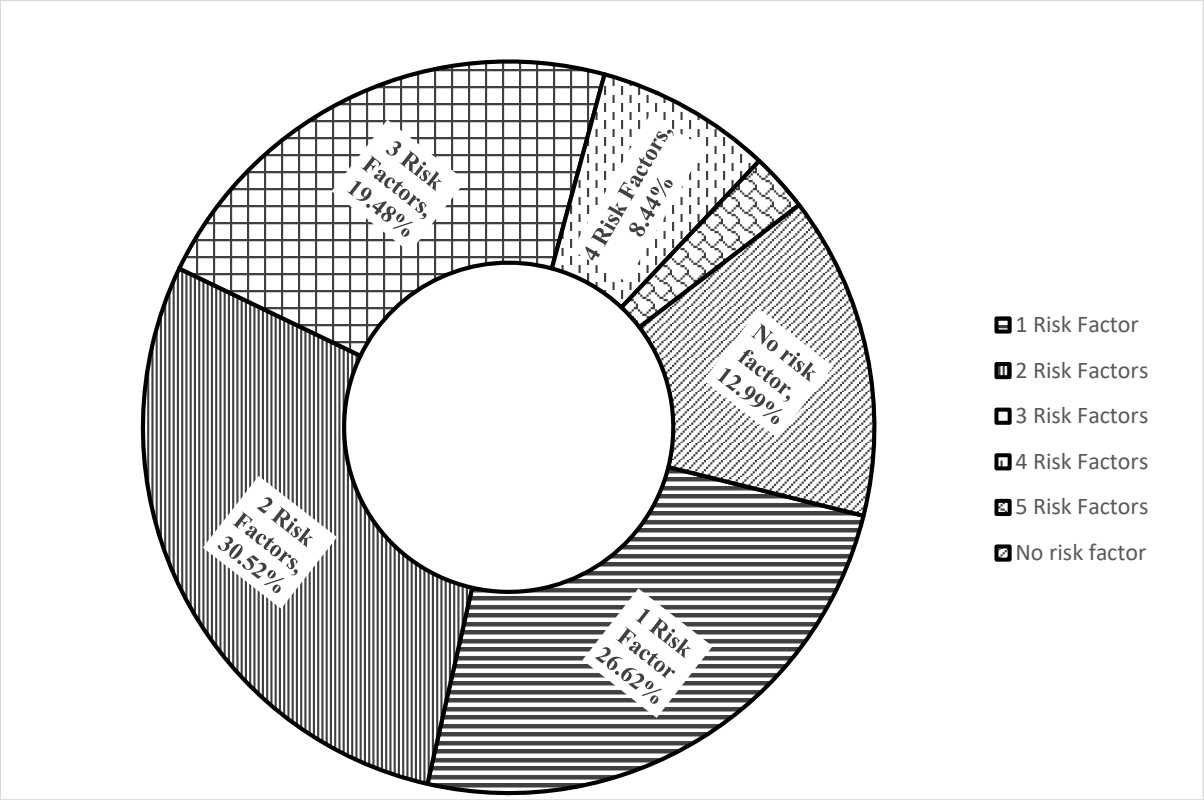


Figure 2. The intervention model prototype designed for the study

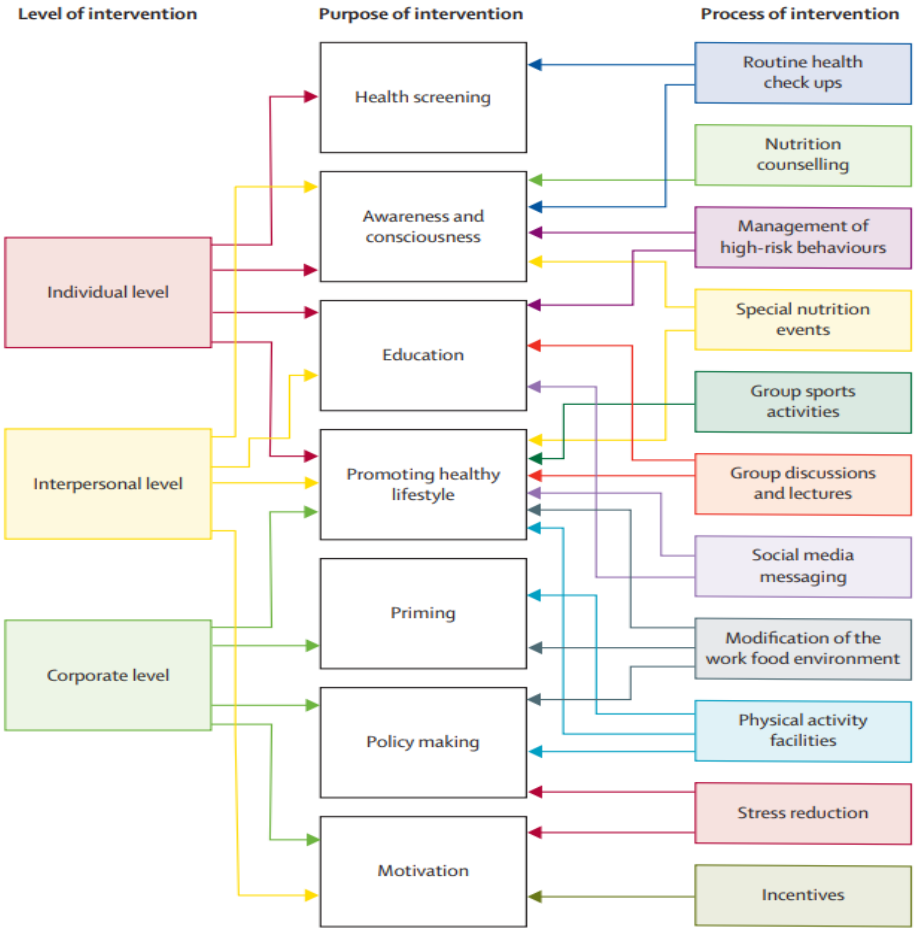


Table 1. The impact of the intervention at the organizational level

Category of change	Modifications undertaken
Workplace policy changes	• The top management accepted the need of a WWP and endorsed initiation of 'Revitalize' in the organization. • Human Resource Manager was delegated to facilitate all the study activities. • Involvement of management and admin for motivating employees, giving away prizes. • Appointed designated a wellness coordinator to initiate an active culture within the organization. • Management allowed designated time for regular physical activity within the work hours (as per employee's convenience)
Organizational Initiatives to promote a healthy work culture	• Post lunch walk-breaks, promoting usage of stairs, walk meetings were initiated to promote an active culture at the workplace.

	• Nutrition and health digital posters designed by the research team were sent out via intercom as pop-up alerts at regular intervals to the employees (16 messages sent through intercom over 8 weeks)
Modifications in Food and physical Activity Environment (FPAE) of the workplace	• Replacement of vending machines dispensing sweetened beverages with fresh fruit juices, coconut water and buttermilk. Sugar sachets were provided separately. • Partial modification of cafeteria menu with priming of fresh fruits and salads counters in cafeteria (worksite B had shared cafeteria) • Nutrient content information of the commonly consumed items of the cafeteria menu was provided to the employees to help them make informed choices. • Monthly physically active events like Zumba and '5-K' running sessions were initiated. • Tie up with local badminton club to engage employees in regular physical activity

Table 2. Comparison of baseline and post-intervention data anthropometric, clinical, and biochemical parameters of the employees

Parameters	Baseline (n=67) Median (P_{25}-P_{75})	Post-Intervention (n=67) Median (P_{25}-P_{75})	Wilcoxon test	Significance
Weight (kgs)	70.00 (61.9-78.70)	68.80 (58.1-76.2)	Z=-3.595	p<0.01*
BMI (kg/$m^{2)}$	24.58 (22.8-26.0)	23.73 (22.5-25.41)	Z=-3.676	p=0.82
Waist Circumference (cm) Male Female	 89.2 (80.1-94.8) 89.2 (79.5-95.3)	 85.9 (77.9-92.7) 86.9 (77.4-92.95)	 Z=-5.61 Z=-4.93	 p<0.01* p=0.84
Systolic Blood Pressure (mmHg)	118	114	Z=-1.027	p=0.305
Diastolic Blood Pressure (mmHg)	80	78	Z= -2.755	p=.006
Fasting Blood Glucose (FBG) mg/dL	92 (90-99)	93.5 (88-94)	Z=-3.694	p=0.241

Glycosylated Haemoglobin (HbA1c) %	5.56 (5.53-5.8)	5.32 (5.5.55)	Z=-4.751	p=0.802
Total Cholesterol (TC) mg/dL	193 (160.6-211.9)	180 (157.1-209.8)	Z=-2.011	p<0.05*
High Density Lipoprotein (HDL) mg/dL Male Female	 42 (34-47.5) 42 (34-48)	 48 (30-45) 43.5 (38-46)	 Z=-1.584 Z=-2.675	 p=0.013* p=0.532
Low Density Lipoprotein (LDL) mg/dL	109 (95-141)	104 (90-138)	Z=-4.751	p<0.01*
Triglycerides (TG) mg/dL	149 (118-169)	134 (106-160)	Z=-4.632	p<0.01*

INFERENCE AND CONCLUSION

Overall, this intensive WWP was found to be partially effective in promoting the health and nutrition status and health behaviour of IT employees working in a medium-sized organization. Although it did not lead to a significant change in all aspects but modest improvements were noted in physical functioning, diet pattern, supplemented by near significant reduction in anthropometric and biochemical parameters. This comprehensive nutrition-based WWP model showed efficacy in improving employees' nutrition knowledge, health behavior and FPAE and can be adapted to contexts and sectors where sedentary work continues to increase MetS.

VIII. NIN ANIMAL FACILITY

1. Effect of paternal calorie restriction of diet induced obesity on metabolism of their offspring

Obesity is a multifactorial disorder caused due to a chronic imbalance of energy intake versus expenditure. Parent's obesity influences the development of obesity in children which has been studied by several laboratories. Research studies have shown that paternal obesity affects childhood obesity and the potential role of epigenetic regulation in its trans-generational transmission. However, it is not clear whether the calorie restriction (CR) of obese father has any effect on their offspring and if so, the effect of trans-generational transmission and its epigenetic regulation in offspring remains to be addressed. Does calorie restriction promote progression of altered glucose tolerance, induces insulin resistance and chronic inflammation, needs to be studied. Will a calorie restriction epigenetically modulate the gene expression in a diet induced obese male rat strain and their offspring is the key question? Rationale: It has been proposed that interactions between genotypes and diet restricted environment in fathers will synergistically increase the susceptibility to obesity in their offspring. It can be speculated that CR in fathers could modulate the gene expression in various tissues of offspring through epigenetic mechanism and may lead to obesity. Does the paternal CR have an impact on offspring's metabolisms for the development of obesity?

OBJECTIVES

- To evaluate the effect of paternal calorie restriction on development of obesity in offspring by modulating physical and physiological parameters.
- To determine the differential expression of m-RNA in various tissues
- To decipher the promoter DNA methylation of the differentially expressed genes
- The correlation analysis of gene expression to the phenotype

METHODS

After obtaining IAEC clearance, Male Wistar Rats (WNIN) at the age of 21 day used for the study. Obesity induced by High fat diet (60%) for 8-12 weeks. After inducing obesity animals divided into HFD, HFD-CR1(Shifted to control diet which is equal to 40% CR) and HFD-CR2 (Shifted to control diet which is equal to 50% CR) for 6-8 weeks. All the four groups Control (control diet since inception), HFD, HFD-CR1, HFD-CR2 (N=8 per group, F0) will be mated to healthy age matched females fed ad-libitum on control diet to raise F1. The pups born to these animals will be divided into two groups Control and HFD, fed on respective diets for 8 weeks from age of 21 days. Comparative analysis was performed, pups born to Con, HFD, HFD-CR fathers. F0 euthanized after successful mating, whereas F1 euthanized after 8 weeks of Control or HFD feeding. Methods: Anthropometric Measurements such as Energy intake, Body Mass Index (BMI) and Feed Efficiency Ratio (FER) were calculated as per the standard procedures. Biochemical parameters Plasma adiponectin and insulin was assessed by sandwich ELISA, the levels of fasting glucose, triglycerides, total cholesterol, and HDL cholesterol were determined (Biosystems S.A, Spain). Leptin, a rat adipocytokine, was quantified using Milliplex Rat cytokine immunoassay kit (Millipore, USA). Gene expression by using RT-PCR method. Data statistically analyzed.

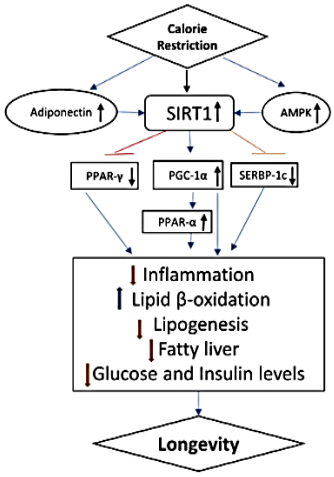
RESULTS

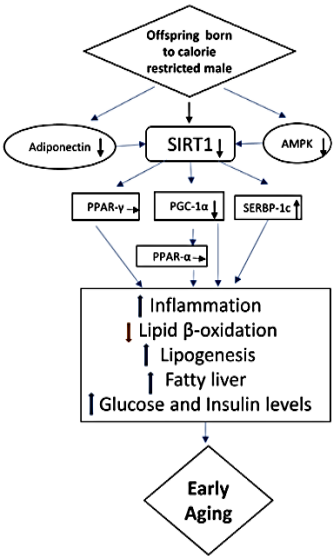
Significant increase in Energy intake per day with significant decrease in Feed Efficiency Ratio (FER) in the high fat group when compared to their respective controls. It was also observed significant rise in Body weight, Body Mass Index (BMI) and Total Body Fat percent (%) compared to their controls in high fat groups. After 12 weeks the high fat fed animals (Obese) were divided into 3 groups, High Fat (continued with high fat diet), CR-I (50% calorie restriction) and CR-II (40% calorie restriction). After 8 weeks of calorie restriction a significant decrease in Energy intake per day in calorie restricted animals and increase in FER in CR-I & CR-II, but significant increase only in CR-I noticed when compared to control and high fat groups. CR-I and CR-II males were mated with age matched females fed with control diet ad-libitum and the pups born were divided after weaning into different groups based on the diet fed (control or high fat) for 10 weeks. Male pups born to CR-I male parents fed control diet (CR-I-CDM) & High fat diet (CR-I-HFDM) and female pups fed control diet (CR-I-CDFm) & High fat diet (CR-I-HFDFm). Male Pups born to CR-II male parents fed Control Diet (CR-II-CDM) & High fat diet (CR-II-HFDM) and females pups fed Control Diet (CR-II-CDFm), High fat diet (CR-II-HFDFm). It was observed that the energy intake of the pups born to calorie restricted groups have been increased, there was significant increase in CR-I-CDM, CR-I-HFDM, CR-II-HFDM, CR-I-CDFm and CR-I-HFDFm compared to their respective age and gender matched controls. BMI and Total body fat percent of the pups born to CR groups have increased but significant increase was observed in pups born to CR-I group compared to their respective controls. The WAT weights and AI of the pups born to calorie restricted groups have increased but significant increase was observed only in pups born to CR-I group compared to their respective controls. The TAC was significantly decreased and catalase activity significantly increased in the pups born to calorie restricted groups compared to their respective controls. The lipid profiles of the calorie restricted animals have shown an increase in Total cholesterol, Triglycerides and HDL-cholesterol, but significant increase was observed only in CR-I compared to their controls. The mRNA expression analysis of hepatic lipid metabolism, lipogenesis genes have shown an increase in levels of Fatty acid synthase (FAS) and Stearoyl CoA desaturase 1 (SCD-1) in calorie restricted groups, but significant increase was observed in CR-I compared to the control and high fat groups. Additionally, the expression of the β-oxidation of lipids genes i.e., Acyl-CoA oxidase 2 (ACOX2) and carnitine palmitoyl transferase 1 (CPT1) have decreased in calorie restricted groups, but significant decrease of ACOX2 was observed in CR-I and CPT1 in CR-I and CR-II groups compared to the control and high fat groups. The relative mRNA expression in liver of glycolysis enzymes Pyruvatekinase (PK); Glucokinase (GK) were significantly downregulated in both CR-I and CR-II whereas the gluconeogenesis rate limiting enzymes Phosphoenolpyruvate Carboxykinase (PEPCK) and Glucose-6-phosphatase (G6Pase) upregulated in CR-I and CR-II but the significant increase of PEPCK and G6Pase was observed in CR-I group compared to the controls. The circulatory glucose levels are found to be significantly increased in the pups born to calorie restricted groups except CR-II-CDFm compared to their respective controls. The PEPCK and G6P expression was upregulated in pups born to calorie restricted groups but significant increase was seen in pups born to CR-I compared to their respective controls. The over expression of DNA methyltransferases genes such as DNMT1, DNMT3a and DNMT3b in calorie restricted male parents' testis was observed but significant more expression was seen in CR-I.

INFERENCE & CONCLUSION

Calorie restriction in male rats have shown decreased anthropometric measures, decreased inflammation, increased lipid profiles, lipid synthesis & decreased β-oxidation and decreased blood glucose, insulin, increased gluconeogenesis, and decreased glycolysis which in future may progress to obesity, insulin resistance and hyperglycemia. The intensity of the above effects was more in CR-I than CR-II. This proves that CR-I has many deterioration effects then CR-II so it can be inferred that the mild CR with gradual increase of calories might be more beneficial as weight loss regimen. These effects of CR on the male parent might cause diet-gene interactions which lead to epigenetic reprogramming that might be imprinted to the sperm and causes the intergenerational transmission. The differential expression of genes involved in the lipid and glucose metabolism in calorie restricted (CR-I and CR-II) male parents is due to the DNA methylation of the promoter regions clearly indicated by the over

expression of the DNA methylases such as DNMT1, DNMT3a and DNMT3b found in their testis. The study demonstrates the importance of paternal diet and its transgenerational impact in the development of metabolic syndrome. Further pave way in future to provide fathers with optimal dietary recommendations.





PARAMETER	CR MALE PARENTS	OFFSPRINGS BORN TO CR PARENTS
ENERGY INTAKE	DECREASED	INCREASED
BMI	DECREASED	INCREASED
BODY WEIGHTS	DECREASED	INCREASED
BODY Fat %	DECREASED	INCREASED
WAT weights	DECREASED	INCREASED

The schematic representation of the metabolic pathways that are effected in paternal CR and their offspring

PhD SCHOLARS

S.No	Name of Student	Name of Guide	Thesis Title
1	Harshavardhana H E	Dr. G Bhanuprakash Reddy	Profibrotic mechanisms in diabetic complications: Role of dietary agents.
2	S. Udaykanth		Role of vitamin B12 in diabetic neurodegerneration
3	Santhoshi vani Akkenapally		Studies on Th2 Cytokines and Micronutrients in Asthma
4	K. Krishna Kalyan		Studies on a functional food formulation for diabetes and its complications
5	G. Soumya		Role of non-enzymatic protein glycation in multi-organ fibrosis during aging and age-related disorders
6	V.V.Vineela		Evaluation of micronutrient status and biomarkers of aging in frailty
7	James Thomas	Dr. Bharati Kulkarni	Role of Maternal stress and Iron status on Cognition in Infants
8	U. V. Ramakrishna	Dr. S. N. Sinha	Oxidation, Characterisation and Anti-cancerous activity of Epigallocatechin Gallate from Camellia Sinensis
9	Balaji Gouda		Evaluation and role of isolated compound from Amla fruit on valproic acid induced autism spectrum disorder (ASD) in expremental albino mice
10	Dileshwar Kumar		Neurobehavioral and Biogenic amines manifestations of the agricultural population exposed to organophosphate insecticides: a study in Telengana region, India.
11	Priyanka Raju Chougule	Dr. Sudip Ghosh	Effect of Ethyl gallate and Propyl gallate on apoptosis related protein and gene expression in DSS induced colitis in C57BL/6J mice
13	Sandip Kumar Kotturu		Role of microRNAs in the development of obesity and diabetes
14	Divya Kumari		Understanding molecular cross-talks among functionally contrasting cell lines during zinc deficiency
15	Arnab Chatterjee		Tanscriptomic analyses of functionally contrasting tissues involved in zinc homeostasis
16	Madhumanti Dhua		Amelioration of insulin resistance by TLR2 ligands from Mycobacterium tuberculosis
17	Summaiya Alam Lari	Dr. J. Padmaja	Assessment of pesticide residues penetration into the skin using protective gear in field conditions
18	Arun Pandiyan		Association between pesticide residues concentration in tissues and with the Lymphoma, Leukemia and Breast cancers
19	Srividya G	Dr. Ayesha Ismail	Anticancer potential of Cinnamon and its bioactive component(s) in prostate cancer: *In vitro* & *In vivo* Studies
20	Ramesh G		Molecular mechanism(s) involved in Vitamin D deficiency induced Muscle Atrophy

21	Athira AS	Dr. Ayesha Ismail	Vitamin D deficiency induced cardiomyopathy: Role of ubiquitin proteasome and signal transduction pathways
22	Soumam Dutta		Efficacy of vitamin D2 vs D3 in the classical and non classical functions in rat models
23	Jovis Jacob	Dr. Samarasimha Reddy N	-
24	Hanuma Naik	Dr. P. Raghu	Mechanism of Iron and Zinc interactions in intestinal cells
25	Puneeta Singh Yaduvanshi		Modulation of Iron storage and regulation by Zinc in Hepatocytes
26	Konda Venu		Role of Zinc in erythropoeisis
27	Suresh Kondeti	Dr. K. Rajender Rao	Studies on the regulation of glucose homeostasis by fibroblast growth factor 21 in a pre-diabetic obese rat model
28	Anuradha R		Effect of paternal calorie restriction of diet induced obese on metabolism of their offspring
29	V. Sai Kanth	Dr. Sanjay Basak	Maternal exposure of endocrine disrupting chemicals during reproductive development: impact on reprodructive and metabolic programing in the offspring
30	Swetha Boddula	Dr. M. S. Radhika	Etiology of severe anemia and efficacy of treatment in school going children
31	Thenarangam Sangita		Optimization and testing of nutrient dense no sugar and low sugar complementary food mixes for infants and young children - A comprehensive study
32	Shrunga Shree S		-
33	Shally Vishnoi		-
34	Pavithra R C		-
35	Aruna Talari	Dr. Devindra S.	Nutritional quality, prebiotic potential and health benefits of raffinose family oligosaccharides of Pigeonpea (Canjanus Cajan, L)
36	Deepika T		Studies on resistant starch of some plant foods and development of low glycemic index food products
37	Soumya Ranjan Pradhan		Development of database on macronutrients, minerals, glycemic index and glycemic load of commonly consumed ready-to-eat meals
38	Shreya Elma Mathew		Nutritional quality, prebiotic potential and health benefits of raffinose family oligosaccharides of chickpea (*Cicer arietinum*))
39	Sumi MS		Nutritional quality, prebiotic potential and health benefits of raffinose family oligosaccharides of green gram (*Vigna radiata (L.) Wilczek*))
40	Dr. Alekhya G		Identification and Characterization of Anti-Glycating compounds from natural sources for the control of post-diabetic complications.
41	Pallabika Gogoi	Dr. Paras Sharma	Nutritional characterization and bioaccessibilty studies of polyphenols and nutrients from pigmented rice and maize

42	Anwesha Mahajan	Dr. Paras Sharma	Characterization Encapsulation and bioaccessibility studies of polyphenols extracted from fruits and vegetables by products
43	S. Gomathi	Dr. S. Sreenivasa Reddy	Unraveling the essence of riboflavin in retinal function and development
44	Shrabani Das	Dr. Gargi Meur	Impact pf hypoxia on placenta and neonate development
Newly Enrolled			
45	Navya Sree Boga	Dr. Sanjay Basak	-
46	Hridayanka		-
47	Arunima Das		-
48	Laxmi Jaya madhuri	Dr. Suresh C	-
49	Lokesh M		-
50	Prita Ghosh		-
51	Naresh Kumar		-
52	Sumitra Gorain	Dr. Vakdevi V	-
53	Nishita Tiwari	Dr. Satish M	-
54	Dilkash Ara		-
55	Venkatesh N	Dr. R. Ananthan	-
56	VJ Sangeetha		-
57	Pooja Meur		-
58	Julia Sebastian		-
59	Chadrama Barua		-
60	Kavanshree NM	Dr Sudip Ghosh	-
61	Parth Sarin	Dr. Devraj JP	-
62	Nikhita BR		-
63	Anjusha Bhasker	Dr. Shobi Veleri	-
64	B Sai Dheeraja	Dr. N. Arlappa	-

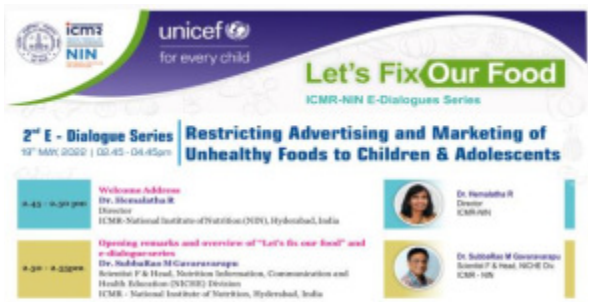
MEETINGS/CONFERENCES/ TRAINING PROGRAMMES HELD AT NIN

Knowledge sharing Workshop was organized for National Media Health Journalists by Ministry of Health and Family Welfare, Govt. of India, ICMR and Press Information Bureau (April 3-6, 2022).

Orientation Training Programme on "Nutritional survey methodology" was organized for the Post Graduate students from different medical colleges of South India (April 18-22, 2022).

Eat Right Mela and Eat Right India Walkathon, was organized by Food Safety and Standards Authority of India, Netprofan, to spread awareness about eating healthy and safe (May 08, 2022).

The 2nd E dialogue in LetsFixOurFood series on "Restricting marketing and advertising of unhealthy foods to children and adolescents was conducted on May 19, 2022.



Ad-hoc hands on training program on "Animal science, breeding, handling and experimental lab animal welfare guidelines" held from May 9-13, 2022.

An awareness program on "Gender sensitization and workplace etiquette" for the staff and students of Institute was organized by the Internal Complaints Committee (ICC) on June 28, 2022.

Laboratory Animal Technician Training Course (LATTC) on "Lab animal science, breeding, handling and experimentation and lab animal welfare" conducted by NIN Animal Facility from June 01-30, 2022.

3rd E-dialogue in LetsFixOurfood series on "Front-of-pack Nutrition labelling (FoPNL)" was held on July 29, 2022.

A workshop for Administrative officers and staff including stores and personal department was conducted on "Competition law and public procurement" on July 23, 2022.

As part of the World Breastfeeding Week celebration, a symposium was conducted on August 4, 2022. Dr.Usha Rani, Prof. & HoD, Dept of Paediatrics, Niloufer Hospital delivered the key note address.

One week training program on "Food based nutritional security for malnourished population of rural HHs by establishing nutrition garden and nutrition education – An intervention research" for the project staff from different state units in the evaluation study of MSSRF was held from August 26, 2022.

The Scientific Advisory Committee (SAC) meeting of ICMR-NIN was held on August 30, 2022.

Dr. Tamilisai Soundararajan, Hon'ble Governor of Telangana and Lt. Governor, Puducherry visited NIN to discuss with the Director and Scientists about tackling malnutrition in Telangana. (Sept. 19, 2022).

ICMR-NIN 105th Foundation Day celebrations was held on Sept. 26, 2022. Prof. Vikas Bhatia, Executive Director, AIIMS, Bibinagar delivered the Foundation Day lecture.

The National Nutrition Month – Poshanmaah 2022 was held from September 1-30, 2022 on the theme "Bacha and Shiksha – Poshan bhi Padhai bhi". Various nutrition education programs and extension activities were conducted in schools and communities during the period.

Dr. Rajiv Bahl, Secretary, Department of Health Research & Director-General, ICMR visited NIN on October 26, 2022 and interacted with the Director and Scientists in a review strategy meeting.

A national webinar was organized on the occasion of World Food Day on October 17, 2022 on the theme of "Leave No One Behind" in pursuit of ensuring nutrition security for everyone.

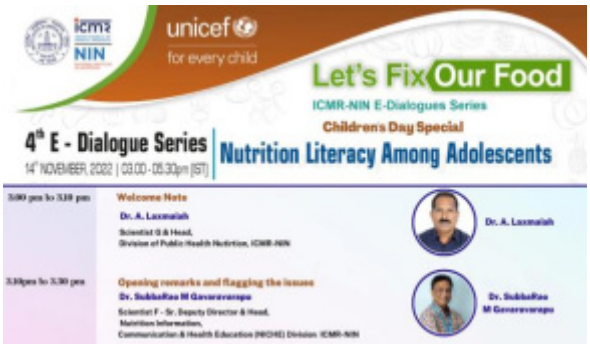
The Academic Council of NIN conducted workshop on "Research methodology and skill sets" from Oct. 27-28, 2022 for scientists and PhD scholars at NIN.

An interactive talk on "Elimination and prevention of violence against women, women empowerment and safety at the workplace" during women safety, security and empowerment week was held on November 25, 2022.

A training program on "Training of Trainers" under National Programme for Prevention and Control of Fluorosis was conducted for State and District Nodal Officers and District Consultants from Andhra Pradesh, Assam, Chhattisgarh, Haryana, Kerala, Telangana and West Bengal (Nov. 22-24, 2022).

A workshop on "Nutrition for the age group of 3-6 and suggested measures for healthy growth" was organized for pre-school teachers (Nov. 03, 2022).

4th E dialogue in LetsFix Our Food series on "Nutrition literacy for adolescents" held on Nov. 14, 2022.



Hon'ble Minister of Health and Family Welfare and Chemicals & Fertilizers, Govt. of India, Dr. Mansukh Mandaviya Ji visited NIN on December 17, 2022 and interacted with Director, Scientists and students. Dr. Rajiv Bahl, Secretary to Govt. of India, DHR and DG, ICMR accompanied the Minister. The Hon'ble Minister

launched the Diet & Biomarkers Survey of India (DABS-I).

54th Annual Conference of Nutrition Society of India (NSI) was held on the theme "Sustainable Healthy Diets – Health for all" from Dec. 22-23, 2022.

A skill development program on "Laboratory Animal Supervisor Training Course (LASTC) was conducted at NIN Animal Facility from Oct. 10 to Dec. 09, 2022.

The University of Hyderabad (UoH) in collaboration with the Department of Science and Technology (DST), Ministry of Science and Technology, organized a Synergistic Capacity Building Training Program STUTI on Jan. 19, 2022.

Hon'ble Governor of Telangana, Dr. Tamilisai Soundararajan inaugurated the Golden Jubilee IAPSMCON 2023, co-organised by AIIMS, Bibinagar on NIN campus on Feb. 02, 2023.

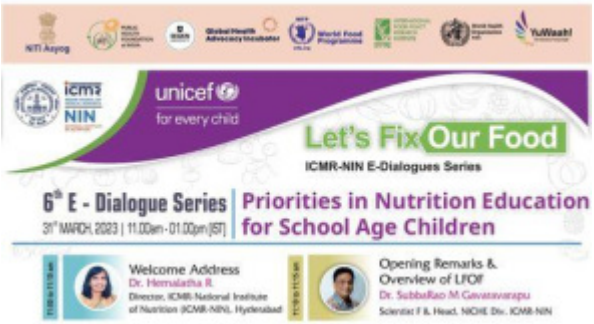
A two day workshop on "Research Proposal Writing" was co-organized by Indian Knowledge Systems (AICTE), MoE, GoI at NIN on 16-17 Feb. 2023.

A five day workshop on "Pre-clinical Research" for AYUSH scientists was organized with Central Council for Research in Ayurvedic Sciences, Ministry of Ayush, Govt. of India. The workshop aimed to bridge the gap between the ancient knowledge and modern science (Feb. 20, 2023).

A five day training program on fluoride for "Laboratory Technicians" was conducted under National Programme for Prevention and Control of Fluorosis (NPPCF). MoHFW, GoI (Feb 06-10, 2023).

Nutrition awareness sessions in different schools was organized and lab visits in NIN as part of the National Science Day celebrations (Feb. 28, 2023).

An E dialogue series on “Lets Fix Our Food” was held with an aim to Advance India’s Young people’s Right to Healthy Foods and Healthy Food Environments on March 31, 2023.



SCIENTIFIC PUBLICATIONS

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

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C. ABSTRACTS

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