वार्षिक प्रतिवेदन

Annual Report

2021-22

icmr | NIN
INDIAN COUNCIL OF MEDICAL RESEARCH | NATIONAL INSTITUTE OF NUTRITION

ICMR - NATIONAL INSTITUTE OF NUTRITION
Indian Council of Medical Research
Hyderabad, Telangana, INDIA

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Research Highlights

ASSESSMENT OF NUTRITIONAL STATUS OF BELOW 12 YEARS CHILDREN OF MUZAFFARPUR DISTRICT, BIHAR - A RAPID NUTRITIONAL ASSESSMENT:

An outbreak of Acute Encephalitis Syndrome (AES) reported in litchi growing rural areas of the Muzaffarpur district of Bihar in 2019, where a total of 149 children were died. A rapid study was carried out by the ICMR-National Institute of Nutrition to assess the nutritional status of the children in litchi growing areas. In general, the rural children were subsist on inadequate diets, both quantitatively and qualitatively. The same was reflected in the nutritional status of the children where the prevalence of underweight (AES: 31.6% & non-AES: 25.1%) and stunting (AES: 47.7% & non-AES: 38.7%) among children under 5 was high. The prevalence of anaemia ranges from a low 47.7% in 3-5 years children to a high 76.4% in children below 3 years. Similarly, the prevalence of B12 deficiency was low 45.4% in children 3-5 years to a high 58.6% in children below 3 years. I general, all the deceased children were from underprivileged or marginalized communities. Most children reportedly consumed Litch fruits and were exposed to the hot sun during the summer. Existing literature shows Litchi fruits contain hypoglycin A or Methylene cyclopropylglycine (MCPG) known to cause hypoglycemia and metabolic derangement. Therefore, parents are sensitised not allowing their children to skip a meal at night time and should not let them play outdoors during the daytime in the hot summer.

DEVELOPMENT OF PREBIOTIC NOODLES CONTAINING GALACTO - OLIGOSACCHARIDES:

Noodles is one of the common instant foods consumed globally for ages and became an important portion in the Asian's diet. The wide variations in the regions, cultures, climate etc have created a large variety of noodles with local variations. The current study was intended to develop a prebiotic formulated noodle (Galacto-oligosaccharides) and to study its physicochemical, rheological and sensory properties etc. Prebiotics are the non-digestible food components which is selectively metabolised by gut microbiota and improve host health by modulating the growth of beneficial bacteria in the colon. Twelve different formulations of noodles were prepared and subjected to nutrient and sensory evaluation. The moisture content varied from 9.47% [Wheat flour (99 g)+1g Micronutrients (MN)] to 12.6% (Maida (94g) + galacto-oligosaccharides (5g)+1g Micronutrients (MN)), and the percent of protein ranged from 8.21% (100% Wheat flour noodles)to 11.64% [Maida (74%) +DFBG (20%) + galacto-oligosaccharides (5%) +1g Micronutrients (MN)]. The percent of ash content in noodles tested ranged from 0.41% [Maida (99g) +1g Micronutrients (MN)] to 1.46% in [Maida (75%) +DFBG (20%) + galacto-oligosaccharides (5%)], and the fat content was 2.01% [Maida (95g) + galacto-oligosaccharides (5g)]to 3.58% [Wheat flour (99 g)+1g Micronutrients (MN)]. The insoluble dietary fibre of the noodles ranged from 0.23% (Maida

(95g) + galacto-oligosaccharides (5g)) to 8.36 [Wheat flour (75%) +DFBG (20%) + galacto-oligosaccharides (5%)] and soluble dietary fibre from 0.42% [Maida (75%) +DFBG (20%) + galacto-oligosaccharides (5%)] to 3.8% (100% Wheat flour). The percent of carbohydrates ranged between 64.07% [Wheat flour (75%) +DFBG (20%) + galacto-oligosaccharides (5%)] to 79.71% [Maida (95g) + galacto-oligosaccharides (5g)].Formulations containing Maida (74%) + DFBG (20%) + galacto-oligosaccharides (5%) +1g Micronutrients (MN); and Wheat (74%) + DFBG (20%) + galacto-oligosaccharides (5%) +1g Micronutrients (MN) showed an increase in protein with 11.64 g% and 10.07 g% respectively and was acceptable during sensory evaluation indicating that Bengal gram along with galacto-oligosaccharides increases the protein and fiber content in noodles.

CONSUMPTION PATTERN OF ARTIFICIAL SWEETENERS USED IN FOOD PRODUCTS AND AS TABLE TOP SWEETENERS AMONG NORMAL, OVERWEIGHT, OBESE AND TYPE II DIABETES INDIVIDUALS LOCATED IN MAJOR METROPOLITAN CITIES OF INDIA:

Artificial sweeteners are synthetic non-caloric sweeteners characterized by a strong sweetening flavour without calories, mainly found in soft drinks, snack foods, sugar-free candies, and dairy products. Since their introduction, the mass media have reported about potential cancer risks, which has contributed to undermine the public's sense of security. Hence this study proposed to examine consumers knowledge of artificial sweeteners and quantifying levels of high intensive sugars present in various food products available in a particular region. The survey on the consumption pattern of artificial sweeteners among type II diabetic subjects indicated that 86% of individuals consumed table top sweeteners. The preference for these artificial sweeteners were 27% saccharine, 25% sucralose, 23% aspartame, 10% stevia, and 4% acesulfame K. 74% of dieticians did not recommend using artificial sweeteners, while 26% recommended AS for weight management and glycemic control. Among the Artificial sweeteners, stevia (28%) and sucralose (15%) were most recommended by the dieticians. The content of sweeteners in various food products quantified using HPLC and TLC indicated that they were found to be preferable for human consumption and was according to the amount recommended by Acceptable daily intake (ADI).

STUDIES ON RESISTANT STARCH OF PLANT FOODS AND ITS HYPOGLYCEMIC EFFECT IN HUMAN:

Resistant starch is the starch portion that cannot be digested in the small intestine, but may be fermented in the large intestine. When resistant starch reaches the bowel, it is broken down (fermented) by the resident bacteria, generating a range of beneficial changes like increasing stool bulk, giving a mild laxative effect which promotes regularity. The ingestion of resistant starch will decrease the post prandial glucose levels. As we know there are limited food choices for people with Diabetes thus there will be a monotony of foods. Therefore, we would make an attempt to diversify the food choices. Therefore, this study was aimed to

determine resistant starch content in commonly consumed foods and to develop certain low GI/GL food products which may be useful for people with Diabetes. Twenty one different samples were analysed including, cereals, pulses, roots and tubers and vegetables The results of the study showed that, in cereals, high resistant starch content is present in jowar (5.717 g/100g), in pulses, red gram dal (29.4 g/100g), in roots and tubers, Colocasia (45.7 g/100g) and in vegetables, plantain (39.88 g/100g).Effect of different processing methods increased the resistant starch in cereals due to retro gradation and reduced RS content in pulses as well as in roots and tubers due to starch gelatinisation and solubilisation and starch lipid complexes respectively. Dry heat- reduced RS content due to dextrinization. Moist heat-increased RS content due to gelatinisation. The food product developed by using different ratios of wheat and plantain sensory acceptability score of 22% for 50:50, 26% for 60:40 and 52% for 70:30. The glycemic index study showed low glycemic index value of 51.5 for food product developed from 50:50 wheat-plantain ratio compared to 60:40 and 70:30. Hence, this low glycemic index food product can be considered as a potential food alternative to use in control of Type 2 diabetes.

EFFECT OF MATERNAL PROTEIN RESTRICTION ON BODY COMPOSITION AND PROTEIN QUALITY CONTROL PROCESSES IN THE SKELETAL MUSCLE OF THE OFFSPRING:

Several studies suggest that maternal protein content and source can affect offspring health. However, chronic impact of maternal quality and quantity protein restriction, and reversible changes upon rehabilitation on body composition, and protein quality control (PQC) processes in skeletal muscle in offspring is not known. This study examined the effects of maternal low-quality protein (LQP) and low-protein (LP) intake from preconception to post-weaning, followed by rehabilitation from weaning on body composition, glucose-homeostasis, metabolic factors, skeletal muscle (SM) proteolysis, ER stress, autophagy and ubiquitin -proteasome system (UPS) in rat offspring. Wistar rats were exposed to LQP or LP isocaloric diets for 7 weeks before pregnancy to lactation. After weaning, offspring were either continued on these diets or rehabilitated with normal protein (NP) for 16 weeks. LQP and LP offspring had lower body weight, fat and lean mass, insulin and HOMA-IR than NP. LQP-offspring had higher cholesterol, T3, T4, lower triacylglycerides, glucose, while unaltered in LP than NP. Interestingly, LP-offspring showed augmented PQC processes and increased SM protein degradation than NP-offspring. Majority of above outcomes were reversed upon rehabilitation. These results suggest that chronic exposure of rats to a maternal LQP and LP diets induced differential adverse effects by influencing body composition, and skeletal muscle proteolysis which were reversed upon rehabilitation.

DEVELOPMENT OF A-CRYSTALLIN MINI CHAPERONE PEPTIDES AS THERAPEUTIC MOLECULES FOR DIABETIC OCULAR DISEASES:

α-Crystallin is composed of αA and αB subunits in a 3:1 ratio in the mammalian eye lens. They show chaperone-like activity and protect cells from multiple stressful events. The

specific sequence within the αA (70KFVIFLDVKHFSPEDLTVK88) and in αB (73DRFSV NLDVKHFSPEEL KVK92) has been reported to have chaperone and anti-apoptotic effects. Here, we investigated the protective and therapeutic effects of individual α-crystallin peptides and their combination in a 3:1 ratio in diabetic cataract and retinopathy rat model and cell lines. Even though systemically administered α-crystallin peptides did not prevent hyperglycemia, they delayed cataract progression and preserved retinal function in the diabetic rats. Furthermore, α-crystallin peptide administration reduced the aggregation and insolubilization of protein. Additionally, hyperglycemia-induced oxidative and ER stress were also attenuated upon α-crystallin peptides administration. α-Crystallin peptides alleviated the hyperglycemia-induced apoptosis by reducing the caspase-3 activity and Bax levels. Additionally, α-crystallin peptides attenuated ER stress and oxidative stress in HeLa cells. α-Crystallin peptides preserved the retinal function, delayed the progression of diabetic cataract by attenuating the protein aggregation, oxidative stress, ER stress, and apoptosis. These studies will likely help in developing the α-crystallin peptides as therapeutic agents.

EFFECT OF DIETARY ZINC DEFICIENCY ON SKELETAL MUSCLE PROTEOSTASIS AND MITOCHONDRIAL BIOLOGY IN GROWING RATS:

Zinc is a crucial trace element for the growth and development of all living organisms. Nearly 60% of the total body zinc content is present in the skeletal muscle and zinc deficiency leads to reduced growth, mass, and work capacity of skeletal muscle. However, the underlying mechanisms in connection with skeletal muscle proteostasis and mitochondrial biology are not clear, and hence, we investigated aspect using a rat model. Results indicate a decreased mean muscle fiber cross-sectional area and increased apoptosis in the muscle of zinc-deficient rats. Activation of the ubiquitin-proteasome system as indicated by increased levels of the E1 enzyme, MuRF1 (muscle-specific E3 ligase; muscle atrophy marker), and proteasomal activity was observed in the zinc-deficient rats. Declined autophagy (Beclin1, ATG5, and LC3), and increased ER stress markers were observed. Zinc deficiency also affected mitochondrial biology including fission, fusion, transcription, and oxidative phosphorylation components. Thus, zinc deficiency appears disturb skeletal muscle proteostasis, and mitochondrial biology, causing muscle atrophy.

ANTICANCER EFFECT OF CINNAMON EXTRACT AND ITS ACTIVE COMPONENT PROCYANIDIN B2 IN A RAT MODEL OF PROSTATE CANCER:

Cinnamon is a wonder spice and is known to possess many health beneficial activities such as anti-diabetic, anti-inflammatory, anti-oxidant and anti-cancer. Our recent work demonstrated the proteasome-inhibitory and anti-cancer effect of cinnamon and its active compounds in human prostate cancer cell lines. In this project, we studied the chemopreventive efficacy of cinnamon and its bioactive compounds in a rat model of prostate cancer. Histopathological changes such as hyperplasia and Prostate Intraepithelial Neoplasia [PIN] induced by the combination of chemical carcinogen and testosterone in the prostate were reversed by cinnamon and its bioactive compounds. Similar to chemopreventive drugs, cinnamon and its

bioactive compounds led to inhibition of cell proliferation, induction of apoptosis, inhibition of oxidative stress and angiogenesis, proteasome inhibition and inhibition of metastasis in prostate tissue. In conclusion, our data demonstrates that cinnamon and its bioactive compounds have a beneficial effect against carcinogen-induced prostate carcinogenesis. Hence, cinnamon may act as a potential chemopreventive agent against prostate cancer.

EFFECT OF TRADITIONAL COOKING ON PHYTONUTRIENT CONTENT AND RADICAL SCAVENGING ACTIVITY IN CEREALS AND MILLETS:

In the present investigation, traditionally processed sorghum, pearl millet and finger millet (viz. cooking, fermentation with curd, fermentation without curd, addition of curd to fermented millets) were analysed for their nutritional and anti-nutritional properties. Protein content was significantly higher in the cooked and fermented with curd in sorghum (20.57±0.37 g/100g) and pearl millet (20.27±0.27 g/100g). Phytic acid content in millet flours ranged from 4.77±0.07 (pearl millet) to 8.6±0.15 mg/g (sorghum) and it was observed a sharp reduction (3.28±0.09 mg/g) in the sorghum after cooked and fermented overnight and mixed with curd. Nutrient retention of water soluble vitamins was increased in all the traditionally processed millets. The traditionally cooked, overnight fermentation then added with curd was found to decrease phytic acid to a greater extent (62.9%) and increase Fe and Zn content which may enhance the bioavailability of both the micronutrients.

EXPLORING THE PROTECTIVE EFFECT OF GAMMA ORYZANOL ON DIET-INDUCED MODEL OF NON-ALCOHOLIC STEATO-HEPATITIS:

Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of liver diseases ranging from simple accumulation of fat (fatty liver or steatosis), nonalcoholic steatohepatitis (NASH) which potentially progress to advanced liver disease, cirrhosis and hepatocellular carcinoma. Currently there are no FDA approved drugs that are specifically tailored for treating patients with NAFLD. Hence dietary and lifestyle management are the only option for NAFLD. The dietary supplementation of phytochemicals has proven beneficial in managing the features of NAFLD. Gamma oryzanol, a phytochemical from rice bran oil has gained worldwide attention as nutraceutical in recent years. Gamma oryzanol is known to have anti-inflammatory and antioxidant properties, and as inflammation and oxidative stress play a crucial role in the progression of NAFLD, the present study was done to investigate the protective effect of gamma oryzanol in western diet-induced animal model NAFLD. The results of the present study demonstrated that gamma oryzanol at a dose of 300mg/kg body wt could ameliorate western diet- induced NAFLD. In addition, gamma oryzanol supplementation also reduced visceral adiposity. Given the high prevalence of NAFLD and non-availability of pharmacological interventions for their management, gamma oryzanol appears as a promising dietary intervention with potential beneficial effects in the management of NAFLD.

IMPACT OF SALMONELLA KILLING LYTIC BACTERIOPHAGES ON PROBIOTIC MICROBIOTA:

The studies on the impact of Salmonella killing lytic bacteriophages on probiotic microbiota (Lactobacillus lactis, Lactobacillus rhamnosus, Lactobacillus acidophilus, Streptococcus thermophilus, and Bifidobacterium breve) showed no spots and inhibition zone both in spot test assay and agar well diffusion assays. The results of the turbidometric assay showed that even after incubation for up to 24h, the growth of probiotic microbiota remained unaffected. The results of this study clearly showed that the administration of lytic bacteriophages will not harm the probiotic microbiota and are likely to be safe for use in food preservation.

DOSE-RESPONSE OF SALMONELLA SURVIVAL AND INFECTION IN AN *IN-VITRO* MODEL OF THE HUMAN INTESTINAL TRACT AS A PROXY FOR FOODBORNE PATHOGENS:

Studies on Salmonella survival in simulated gastric fluid (SGF) showed a reduction (0.3 log) in Salmonella after 30 min of incubation time while studies on Salmonella survival in simulated intestinal fluid (SIF) showed an increase in (0.8 log) in Salmonella population after 2h of incubation time. The results of this study clearly showed the survival potentiality of Salmonella in simulated gastric and intestinal fluid. The study also provided data on the Infective dose of Salmonella spp. to cause infection.

ASSOCIATION BETWEEN PESTICIDE RESIDUE CONCENTRATION IN TISSUES AND WITH THE LYMPHOMA, LEUKAEMIA AND BREAST CANCERS:

Sample size: 360; Subjects – farmers with history of pesticide exposure (cases) and non-farmers (controls) with no history of exposure – diagnosed with lymphoma, leukaemia and breast cancers and healthy controls belonging to the family of cases with neither history of exposure nor diagnosed with cancers. Of the 360 plasma samples analysed for pesticide residues, only 66 cases (18%) showed 17 pesticide residues (0.05 – 97.12ng/mL) while no residues were detected both in the control and healthy control groups. Similarly, of the 270 tissue samples analysed, only 98 cases (36%) were detected with 14 pesticide residues (1.5 to 493.5 ng/mL). About 17 subjects were detected with two pesticide residues (0.05 – 16.85 ng/mL) among the control group diagnosed with breast cancer (n=30). Genetic polymorphism in GSTM1/TT1 and CYP2E1 was higher among the cases as compared to the controls and healthy controls ($p<0.05$), while, 8-OHdG levels were also significantly higher among the cases as compared to the controls and healthy controls ($p<0.05$). However, there found no significant association between the levels of pesticide residues in plasma/tissues, serum 8-OHdG levels/genetic polymorphism in the three genes studied ($p>0.05$).

DERMAL PENETRATION OF PESTICIDE RESIDUES IN FARMWOMEN WORKERS: ASSESSMENT OF COST-EFFECTIVE PROTECTIVE GEARS A PREVENTIVE MEASURE:

Analysis of pesticide residues/residual metabolites/ haematological parameters was carried out in dermal washings (hand/wipe/patch), blood and urine samples among 360 farm-workers (farm men and farm women) who are not using PPE (n=180) /using commercially available PPE (n=60) provided and PPE prepared using available resources (n=120) and provided for free-of-cost. Unsafe pesticide handling practices among the study subjects were observed. The mean concentration of ten pesticide residues detected ranged from 0.002 - 246 µg/mL in hand washings, 0.002 - 198.3 ng/cm2 in patch and 0.0001 - 1740 ng/cm2 in wipe samples among the subjects not using PPE. The same were reduced in hand washings (0.01 - 16 and 0.0001 - 11.44 □g/mL); patch (0.001 - 6.57 and 0.0001 - 1.82 ng/cm2); and wipe samples (0.003 - 72.9 and 0.0008 - 39.7 ng/cm2) after using commercially available and PPE prepared and provided to them respectively. Further, Dimethyl phosphate, Diethyl thio-phosphate and Diethyl dithio-phosphate were detected in plasma (1.9 – 936.1 ng/mL) and urine (0.45 – 535.03 ng/mL) samples among the subjects not using PPE; while there was a reduction in plasma (0.25 – 15.7) and urine (0 – 68.99) after using commercially available/PPE prepared and provided to them. Further, increased inflammation (CRP, IL-6, IL-1β, Cortisol, TNF-α) and AChE inhibition was observed when compared to their respective normal ranges among those not using PPE, while the same was reduced after using commercially available and PPE prepared and provided to them. A Local Programme Advisory Committee (LPAC) on Demo and Awareness Training program conducted to assess the field level operations revealed their keen interest on adopting the safety measures such as using PPE, while engaged in farming activities.

IMPACT OF LONG-TERM USE OF DOUBLE FORTIFIED SALT WHEN USED PRENATALLY, ON MATERNAL IRON AND IODINE STATUS AND COGNITIVE DEVELOPMENT OF INFANTS IN RURAL MEGHALAYA - A PILOT STUDY:

The study was conducted in two blocks of the East Garo district of Meghalaya. A qualitative research study was conducted to understand the food habits and health-seeking behaviours of Garo women during pregnancy. It was found that no special care or diet was taken during pregnancy, as it is a natural phase it does not require special attention. However, antenatal check-ups were considered important. Pregnant women were unaware of the need for iron-folic acid tablets during pregnancy. Consumption was not regular either due to non-compliance or non-availability. For the pilot study, a total of n=151 pregnant women (in the second or third trimester) were enrolled in the Double Fortified Salt group (n=57) or iodised salt group (n=68). Supplementation was carried out for 8±1 months. There were no baseline differences between the two groups. At end line (three months infants age) haemoglobin, C-reactive protein, ferritin, and vitamin B12 were similar in both groups. Serum transferrin (43.33±27.47 (DFS) 44.29±33.16 (Iodised) (nmol/mL)) was higher (p=0.045) in the iodized

salt group. The infant development at three months showed no significant difference between the groups.

IMPACT OF INTEGRATED COGNITIVE BEHAVIOUR THERAPY AND PRANAYAMA ON SLEEP QUALITY OF WOMEN LIVING IN WELFARE HOSTELS:

Sleep is an essential natural behavioural process of the body to rest after the day's wakeful activities. Without sleep, optimum functioning is not possible. Integrated Cognitive Behaviour Therapy (CBT) was used for sleep disturbances, self-imposed sleep deprivation, and irregular sleep patterns among women, along with sleep psycho-education and pranayama this was compared to intervention based on only CBT and only Pranayama. The study was conducted among women living in welfare hostels, and more than 50% showed sleep disturbances. The intervention was for three months (3 to 4 group sessions). Significant mean differences between pre-and post-intervention were found. Statistically significant differences indicated that the three interventions resulted in improved sleep quality after the interventions. Although psychological well-being improved in all three groups, the mean difference was significantly higher for the integrated intervention group. The same trend was seen for psychological distress as well but not for perceived stress which showed all the groups benefitted similarly from the intervention. Integrated interventions are more effective however, CBT and Pranayama alone can also be beneficial.

ADAPTATION OF THE FOOD AND AGRICULTURAL ORGANIZATION'S, EDUCATION FOR EFFECTIVE NUTRITION IN ACTION (ENACT) AND FOOD SYSTEMS' COURSES FOR NUTRITION EDUCATION IN INDIA:

This project had two outputs output -1 was the Indian adaptation of the ENACT course material. The ENACT (developed by the FAO for Africa) is a comprehensive hands-on Nutrition Education course that has immense applicability in community nutrition. The ENACT course material was adapted to the Indian context. The course material included a resource book, students book and tutors guide. As part of this project, the 11 units of the resource book and student's book were adapted to the Indian context. This includes contextualizing the case studies and also the pictures and graphics to Indian settings.

Under output-2, scientific video scripts were developed and produced. Six FAO's e-learning modules on "Nutrition and Food Systems" on the following topics were developed.

1.) Nutrition, Food Security and Livelihoods: Basic Concepts.
2.) Why does nutrition matter?
3.) How does the food system influence nutrition?
4.) Making agriculture and food system nutrition-sensitive: key principles.
5.) Making agriculture and food systems nutrition-sensitive - key interventions.
6.) A conducive international environment for nutrition.

Each of the above e-learning modules runs for 45 minutes duration. These videos are meant for use in face-to-face learning for students of post-graduation in nutrition & dietetics in different universities in India. Six universities have agreed to adopt the study material and e-learning modules as Add-on courses in their PG curriculum of nutrition & dietetics.

I. Public Health Nutrition

1. ASSESSMENT OF NUTRITIONAL STATUS OF BELOW 12 YEARS CHILDREN OF MUZAFFARPUR DISTRICT, BIHAR- A RAPID NUTRITIONAL ASSESSMENT

An outbreak of Acute Encephalitis Syndrome (AES), with high incidence of morbidity and mortality was reported among children residing in some of the litchi growing rural areas of Muzaffarpur district of Bihar in 2019. A total of 628 children with signs and symptoms of AES were admitted in Sri Krishna Medical College Hospital (SKMCH) (436) and Kejriwal Hospital (162); of them, 149 children died due to AES (23.7%).

The first case of AES in Muzaffarpur was recorded in the year 1995 when it was thought that the deaths were due to heat stroke. However, outbreaks of AES have been occurring since 2004. Over a period, many aetiologies have been proposed for the "mystery disease" causing seasonal deaths in the area.

Keeping in view the magnitude of the problem, the Ministry of Health, GOI, constituted a central team comprising of experts from ICMR institutes, AIIMS, New Delhi & Patna, NIMHANS, Bangalore and CMC, Vellore to study the magnitude of AES outbreak as well as the etiology for the same. Accordingly, the central team has visited the area, including hospitals during June 2019 and submitted a comprehensive report to DG, ICMR. The following are the salient observations of the report. The study team observed a correlation between the AES and history of consumption of litchi fruit and also a majority of the children suffering from AES were found to be undernourished. The health infrastructure in the areas was observed to be inadequate.

A survey was carried out to assess the nutritional status of the children residing in AES-affected areas and non-affected villages located in litchi growing areas of Muzaffarpur district as control areas with the following objectives:

General Objective

To assess the comprehensive nutritional status of the children residing in AES-affected and non-affected control villages in the litchi growing areas of Muzaffarpur district, Bihar.

Specific Objectives

- To assess the prevalence of stunting, wasting and underweight among children under 12 years of age.

- To assess food and nutrient intakes of children by one-day 24 hour dietary recall and frequency of consumption of various food groups.
- To assess the prevalence of anaemia among children.
- To assess the micronutrient deficiencies among children.
- To assess the knowledge and practices of IYCF, health-seeking behaviour and the extent of utilisation of national nutrition intervention programs among mothers of young children.
- Qualitative assessment to evaluate food habits and taboos in the community using Focussed Group Discussions (FGDs).
- To asses uptake of nutrition programs

METHODOLOGY

Study Design: A community-based cross-sectional survey was carried out in Muzaffarpur district of Bihar adopting a multi-stage random sampling procedure to assess the nutritional status of the children.

***Children <5 years*:** Considering the prevalence of wasting among under 5-year-old children in Muzaffarpur as 18% (NFHS-4) and the absolute precision of 4%, non-response rate of 10% and design effect of 1.5, the sample size arrived at was 747. However, a sample size of 750 each was covered for children of below 3 years and 3-5 years from the district of Muzaffarpur.

***Children of 6-11 years*:** Considering the prevalence of thinness as 40% (NNMB: 2011-12), with an absolute precession of 5%, non-response rate of 10% and design effect of 1.5, the sample size arrived at was 608. However, a sample size of 650 children was covered for this age group from the district of Muzaffarpur.

***Inclusion criteria*:** Children of below 12 years residing in litchi cultivated villages in the district of Muzaffarpur were selected for the study.

***Exclusion criteria*:** Non-litchi cultivated areas and children above 12 years were excluded in the study.

INVESTIGATIONS

- Household Socio-demographic particulars
- Measurement of height and weight of children below 12 years.
- Clinical examination for nutritional deficiency signs.
- Morbidity pattern during preceding fortnight was collected
- A one-day 24-hour dietary re-call in a sub-sample of households.
- A food frequency questionnaire (FFQ) survey in subsample of children
- Estimation of haemoglobin from an intravenous blood sample by Cyanmethaemoglobin method among all children
- Estimation of nutritional biomarkers like vitamin A, folic acid, vitamin B_1 & B_{12}, serum ferritin, CRP and HbA1c.

- To assess the knowledge and practices of mothers of young children regarding IYCF, health-seeking behavior and the extent of utilization of national nutrition intervention programs.
- Focus group discussion - Qualitative assessment.

RESULTS

A total of 937 households (HHs) were covered for the present study. The average family size was 6.2. All the samples characteristics observed in both the areas were more or less same. In about two-thirds of the HHs, the family size was 5-8; in about 13% of HHs, the family size was 8 and over. In general, about 65% of the fathers and 55% of the mothers of the children covered were literates. In three fourths of HHs major occupation (73.5%) was non-agricultural labour and a majority of the HHs (74.5%) were landless. The average per capita annual income of HHs covered for the present study was Rs.16,291/- whereas the annual income of the HHs in AES-affected villages was lower (Rs.15,123) as compared to Non-AES affected villages (Rs.18,444).

More than one third of the families were residing either in *kutcha* houses (36.8%), while half of the HHs were living in *semi-pucca* houses (49%). In a majority (85%) of HHs, the source of drinking water was a tube/ bore well. Nearly 81% of the HHs covered were using firewood as cooking fuel and only 19% of HHs were using LPG. About a half of HHs (46%) had the facility of a sanitary latrine.

Prevalence of (%) child deaths and percent of HHs participation in national programmes is presented in Table-1. In general, the proportion of child deaths reported in the surveyed villages during the preceding year was 1.7%. Of them, a higher proportion was from AES villages (2.5%) as compared to non-AES villages (0.3%).

A majority of HHs in both areas availed the national programmes such as the public distribution system (63.5%), ICCDS (48.8%), MDM (39.8%) and Annapurna scheme (12.3%). Practices of mothers of < 3 Yr children by IYCF practices is presented in Table-2. About 67% (AES: 68.3% & non- AES: 64%) mothers reported that they initiated breastfeeding to the newborn within one hour of delivery. Only 37.5% (AES: 37.8% & non- AES: 36.8%) mothers initiated complementary food to their infants soon after completion of 6 months.

CONSUMPTION OF LITCHI FRUITS

3-5 years children

History of consumption of litchi fruits during the previous summer (season and consumption) was obtained from the mothers of children 3-5 years. About 95% of mothers (AES: 94.7% & non-AES: 94.2%) reported that their children consumed litchi fruits during the previous litchi season. Of them, about 53% (AES: 54.4% & non- AES: 51.7%) of mothers reported that their children used to consume litchi fruits daily and used to consume 2-4 times in a day and an average of 148.3g (AES: 158.1g & non- AES: 131g) per days. About 8% of children generally complained of adverse effects after eating litchi fruits; they include pain abdomen, nausea, vomiting, headache, etc.

Prevalence (%) of surveyed HHs and percent of HHs availing NNPs

Table 1. Distribution (%) of HHs by Child Deaths and Participation in National Programmes

Particulars		AES (n=608)	Non-AES (n=329)	Pooled (n=937)
Number of (%) Child Deaths		2.5	0.3	1.7
Age Group of Child (Death)	<3 yrs	1.2	0.3	0.9
	3-5 yrs	1.2	0.0	0.7
	6-12 yrs	0.1	0.0	0.1
Cause of Child Death	AES Deaths	1.5	0.0	0.9
	Haemetological Problems	0.0	0.3	0.1
	Diarrhoea	0.2	0.0	0.1
	Pneumonia	0.3	0.0	0.3
	Don't known	0.5	0.0	0.3
Availing National Programmes	MGNREGA	19.2	18.5	19.0
	PDS	65.8	59.3	63.5
	Annapurna Scheme	12.0	12.8	12.3
	ICDS	42.9	59.6	48.8
	MDM	42.6	34.7	39.8

IYCF Practices among mothers of < 3 Yr children

Table 2. Distribution (%) of mothers of < 3 Yr children by IYCF Practices

Particulars		AES	Non-AES	Pooled (N=602)
Time of initiation of Breast Feeding (hrs)	<1 hr	68.3	64.0	66.7
	1-3 hrs	18.5	21.3	19.5
	3-12 hrs	5.6	6.6	5.9
	12-24 hrs	3.4	4.3	3.7
	24-36 hrs	1.9	0.5	1.4
	36-48 hrs	1.3	1.4	1.4
	≥48 hrs	1.1	1.9	1.4
Pre-Lacteals given to New born		28.6	17.1	24.4
Type of Pre Lacteal feed given to the child	Plain water	4.5	4.3	4.4
	Glucose water	8.2	1.9	5.9
	Honey	4.2	0.5	2.9
	Animal Milk	2.9	4.3	3.4
	Human milk	0.5	0.5	0.5
	Others	5.3	1.9	7.3
First Secretion (Colostrum) of Breast milk given to the baby		74.9	78.7	76.2

6-12 year Children

History of consumption of litchi fruits during the previous summer, i.e. litchi season was also obtained from the mothers of children 6-12 years. About 85% of mothers (AES: 82.5% & non-AES: 90%) reported that their children consumed litchi fruits during the previous litchi season. Of them, about 61% (AES: 61.4% & non- AES: 59.6%) of mothers reported that their children used to consume litchi fruits daily and used to consume 2-4 times a day and the average of 215.8g (AES: 218.8g & non- AES: 211g) per days. In general, about 13% of children complained of adverse effects after eating litchi fruits; they include pain abdomen, nausea & vomiting, headache, etc.

Dietary Intakes

In general, all the food groups except for other vegetables and roots & tubers among 1-3 years children, pulses & legumes, other vegetables and roots & tubers among 4-6 year children were below the RDIs. Similarly, the intakes of all the nutrients except for protein and thiamine among 1-3 years and protein, thiamine, vitamin C and folic acid among 4-6 year children were below the RDAs.

Nutritional Status in terms of Anthropometry

Table 3. The prevalence of undernutrition among children by age group

Age group	**Number**	**Undernutrition**		
		Underweight (weight/age)		
		AES	Non-AES	
0-5 Yrs	N	percent	N	Percent
Boys	393	31.8	225	23.6
Girls	336	31.3	181	27.1
Pooled	729	31.6	406	25.1
	Stunting (height/age)			
Boys	393	45.3	225	38.2
Girls	336	50.6	181	39.2
Pooled	729	47.7	406	38.7
	Wasting (weight for height)			
Boys	393	11.7	225	9.3
Girls	336	8.3	181	11.1
Pooled	729	10.2	406	10.1
6-12 Yrs	Thinness			
Boys	181	11.1	101	21.8
Girls	163	7.9	117	16.3
	Stunting			
Boys	181	37.1	101	22.8
Girls	165	26.7	119	18.5
Mothers	Undernutrition (CED) 18.5 BMI Kg/m^2			
	761	30.9	418	31.9

Undernutrition among children under 5 years

In general, the prevalence of underweight among under 5 children was 29.1% (AES: 31.6% & non- AES: 25.1%), and the proportion of children underweight was significantly higher in AES villages as compared to Non-AES villages. A similar trend was observed with respect to stunting (AES: 47.7% & non- AES: 38.7%). While the prevalence of wasting was comparable between the areas. In general, the overall prevalence of undernutrition in the Muzaffarpur district was lower compared to figures reported by the NFHS-4 for Muzaffarpur.

Undernutrition among children under 5-12 years

The overall prevalence of thinness among boys of school-age children (6-12 years) was reportedly higher (21.8%) in non-AES villages as compared to children in AES villages (11.1%). A similar trend was observed among girls (AES: 16.3% & non-AES: 7.9%).

In contrast, the overall prevalence of stunting among boys of school-age children (6-12 years) was reportedly higher (37.1%) in AES villages as compared to children in non-AES villages (22.8%). A similar trend was found among girls (AES: 26.7% & non-AES: 18.5%).

Nutritional status of the Mothers

Chronic energy deficiency (CED = BMI<18.5 Kg/m^2) among mothers of children covered in this present survey was 31.2% (AES: 30.9% & non- AES: 31.9%) and the CED ranges from a low 25% among mothers of 40-49 years age group to a high 44.4% among the mothers of younger age group, i.e. 15-19 years.

BIOCHEMICAL PARAMETERS

Children below 3 years

The prevalence of anaemia among below 3 year children was 69.5% and significantly higher among children in AES villages (76.4%) compared to their counterparts in non-AES villages (59.8%). Prevalence of B12 deficiency in AES villages was 54.7%, and the corresponding figure in their counterparts in non-AES villages was 58.6%. While the prevalence of Folic acid deficiency was relatively lower in AES villages (23.8%) as compared to non-AES villages (26.5%). The proportion of children with riboflavin (Vitamin B1) deficiency among children below 3 years in AES villages was 17.4%, against 19.8% in non-AES villages. Similarly, the prevalence of sub-clinical vitamin A deficiency was 7.5% in AES villages as against 7.1% in non-AES villages.

Children 3-5 years

The prevalence of anaemia among 3-5 years children was relatively higher among children in AES villages (56%) as compared to their counterparts in non-AES villages (47.7%). In general, the prevalence of anaemia was higher among boys as compared to girls. The HBA1C indicating the average blood Glucose level during the last 2-3 months among the children in AES villages was 7.2% and the corresponding figure in non-AES villages was 2.8%. Likewise, the prevalence of B12 deficiency among 3-5 year children residing in AES villages was 54.9% and the corresponding figure in their counterparts in non-AES villages 45.4%. While the prevalence folic acid deficiency was relatively lower in AES villages (25.4%) as

compared to non-AES villages (26.9%). The proportion of children with riboflavin (vitamin B1) deficiency in children 3-5 years in AES villages was 27.4%, against 37.3% in non-AES villages. Similarly, the prevalence of sub-clinical vitamin A deficiency was 9% in AES villages as against 4.7% in non-AES villages.

Children 6-12 years

The prevalence of anaemia among 6-12 years children was relatively higher among children in AES villages (85.9%) as compared to their counterparts in non-AES villages (84.9%). In general, the prevalence of anaemia was higher among girls as compared to boys. The HbA1c indicating the average blood glucose level during last 2-3 months among the children in AES villages was 6.8% and the corresponding figure in non-AES villages was 15.1%. The prevalence of B12 deficiency among 6-12 year children residing in AES villages was 71.1% and the corresponding figure in their counterparts in non-AES villages was 59.8%. While the prevalence folic acid deficiency was relatively lower in AES villages (23.5%) as compared to non-AES villages (27.12%). The proportion of children with riboflavin (vitamin B1) deficiency among children 6-12 years in AES villages was 34.5% as against 23.1% in non-AES villages. Similarly, the prevalence of sub-clinical vitamin A deficiency was 8.5% in AES villages as against 3.4% in non-AES villages.

Verbal Autopsy Report

A total of 13 verbal autopsies were conducted from 13 different villages where child deaths occurred. Eleven out of 13 deceased children were girls, and two were boys. All the deceased children were from underprivileged or marginalized communities (SC/ST/OBC) except one. Clinical presentation in the children was classic, with a similar course of events before the culmination to death. An apparently normal child with no history of any injury suddenly develops fits, usually during the middle of the night, towards the dawn, followed by loss of consciousness. Prolonged fasting before the onset of symptoms was reported in 6 out of 13 children, and long sun exposure was also reported in 6 out of 13 children. The progress of the disease was rapid and gave little time for the parents to act. In most cases (10/13), the symptoms progressed to loss of consciousness within 12 hours of the onset of the first symptom.

Survivors' Case Studies Report

A total of 14 survivors' Case Studies were conducted in 12 different villages. Of them, six were girls and eight were boys, while six children were below 6 years and the rest above 6 years of age. Clinical presentation in the children was classic, with a similar course of events. An apparently normal child with no history of injury suddenly develops fits, usually during the middle of the night, towards the dawn, followed by loss of consciousness. Some children had associated symptoms. Fever was present in 13 out of 14 children, mostly of duration of 2 to 24 hours before the onset of loss of consciousness. Chills and profuse sweating were frequently found among those who had fever (9 out of 13). Seizures was absent in 4 out of 14 children. Profuse sweating, nausea/vomiting were observed in 9 and neck stiffness was complained in 11 out of 14 cases. The hospital stay would be brief. Infrequent symptoms included fast breathing (2/14), jaundice (1/14) and diarrhoea (1/14). Prolonged fasting before the onset of

symptoms was reported in 5 out of 14 children, and prolonged sun exposure was reported in 9 children. All fourteen children were shifted to the hospital and received medical care. The stay in hospital ranged from 1 day to 6 days, with an average stay of 2.5 days.

Focused Group Discussions (FGDs) with mothers

Focused group discussions were conducted with mothers of under 12 years children in 15 villages of Muzaffarpur; of them, 13 were from the AES affected villages. Most of the mothers opined that litchi is not to be blamed for AES fatalities and felt that chronic exposure to heat and poor hygiene were the aetiologies for AES deaths in their respective villages. They felt that most of the deaths happen among children who play a lot under the sun so heat must have been the cause of AES. Mothers also reported that kids generally drink a lot of water after consuming litchi fruits and sleep without dinner. They also mentioned, that the consumption of litchi fruits was more among school age children. After vigorous IEC campaigns, the mothers now know that the child should be fed before sleeping and should not be left to sleep empty stomach, though they sometimes say due to lack of food, they may eat less some days. Mothers felt that hospital facilities were lacking, so they preferred private clinics over government setup, and the coverage of children for IFA tablets/ syrup and vitamin A supplementation (VAS) was poor.

CONCLUSIONS

In general, the rural children of the Muzaffarpur district were subsisting on inadequate diets, both quantitatively and qualitatively. Similarly, the intakes of a majority of the nutrients among the children were below the suggested levels. The overall intakes of foods and nutrients were relatively higher among children residing in AES-affected villages than those in non-AES villages. However, a higher proportion of the children living in AES villages had undernutrition than those in non-AES villages. The prevalence of anaemia was also significantly higher among the children residing in AES-affected villages than those in non-AES villages.

All the deceased children were from underprivileged or marginalized communities (SC/ST/OBC) except one. The mothers reported prolonged fasting before the onset of symptoms in 6 of 13 deceased children, and long exposure to the sun was reported in 6 of 13 children. Most children reportedly consumed Litch fruits and were exposed to the hot sun during the summer. Existing literature shows Litchi fruits contain hypoglycin A or Methylene cyclopropylglycine (MCPG) known to cause hypoglycemia and metabolic derangement. Therefore, parents are sensitised not allowing their children to skip a meal at night time and should not let them play outdoors during the daytime in hot summer. However, infections origin cannot be ruled out as the episodes were seasonal and were associated with acute onset of fever.

2. COMMUNITY BASED SERO-SURVEILLANCE OF SARS-COV-2 INFECTION IN TELANGANA STATE, JANUARY 2022

The Indian Council of Medical Research (ICMR) conducts National Level Sero-Surveillance studies longitudinally as repeat cross sectional studies during May, August, December 2020 and June 2021 in 70 districts of 21 states across India. In the fourth round of serosurvey a high SARS-CoV-2 sero-prevalence was reported in the state of Telangana. However, the samples were collected only from 3 out of 32 districts of the state, as it was for the estimation of national level prevalence, so, it is not a representative sample for the entire state. In January 2021, a large epidemiological survey was carried out among thirty urban municipal wards of Hyderabad population (>10 years and above age) in a geographically representative population of 9500 and the study revealed that the sero-prevalence of SARS COV-2 antibodies was about 54%. The Telangana state government's media bulletin dated 18/03/2021 (during the pre-survey planning period) showed that the positivity rate was 3.2%, case fatality rate was 0.55% and recovery rate was 98.7%. A geographically representative data (district level) on the extent of spread of SARS COV 2 infection is not available for the State of Telangana as available for other states like Tamil Nadu, Karnataka and Maharashtra for taking district/region specific containment measures for prevention and control of Covid-19 infection in the state. Therefore, the present study was carried out with an objective to estimate the sero-prevalence of SARS COV-2 antibodies among the population aged 6 years and above and among healthcare workers, at each district level of Telangana State. The study also aimed to determine the socio-demographic and other risk factors for SARS-CoV-2 infection in the community.

METHODS

Study Design

A cross-sectional community-based survey was carried out among males and females aged 6years and above and healthcare workers by adopting multistage random sampling procedures, with a plan to repeat these surveys at periodic intervals in these districts, if required.

Sample size and Sampling procedure

Assuming a sero-prevalence of 20% (8), absolute precision of 5%, confidence interval of 95%, and design effect of 1.75 and 15% non-response rate, sample size arrived at was 496 (rounded to 500) for each district. Of these, 400 samples were collected from the public and 100 from health care professionals i.e., from doctors, nurses, laboratory technicians from the PHC/District hospital. In each district 10 clusters (9 villages and 1 urban ward) were selected by simple random sampling. The village/ward was divided into 4 segments using natural geographical demarcations like lanes or by-lanes. Ten Subjects were selected from each of the four segments. In each segment, the survey team chose a random start to select the first household and covered the consecutive households until 10 individuals in the age group of 6

years and above were included after taking consent/assent. Currently infected SARS-CoV-2 cases, who were in home isolation, pregnant or lactating women, severely bedridden were excluded in the survey.

DATA COLLECTION AND SAMPLE TESTING

The data and blood sample collection were done during the period between 4th January to 2nd February, 2022. For the purpose, twenty field teams (consisting of one Scientist, one Technician and one Phlebotomist) and four laboratory teams (consisting of one Technician, 2 Laboratory Assistants), were involved in data and sample collection. All the teams were thoroughly trained and standardised about the study methodology and questionnaire.

After obtaining individual informed consent, information on basic socio-demographic particulars, Covid-19 exposure history including RT-PCR/RAT test status, other clinical and vaccination history were collected by using a Computer-Assisted Personal Interviews (CAPI) through Open Data Kit software. The anthropometric measurements of all the subjects were collected using SECA® digital weighing scale (100 g accuracy) to weigh body weight in Kg and SECA® stadio-meter (1 mm accuracy) to measure height in centimeters.

Trained phlebotomists in each of the survey team collected 3 to 4 ml of venous blood from each participant by taking appropriate aseptic conditions. The collected samples were sent to the identified district laboratory in cold chain where serum separation was done by using centrifuge within 4 to 6 hours of sample collection. The serum aliquots were shifted to ICMR-NIN through cold chain for estimating SARS-CoV-2 specific IgG antibodies. SARS-CoV-2 specific IgG antibodies were estimated using Abbott Architect i1000 SR automated analyser with Abbott SARS-CoV-2 IgG assay, which has a sensitivity of 100% and specificity of 99.6%. Testing procedures were followed as per the manufacturer's instructions. For quality assurance, one per cent of the negative sera were re-tested and observed for any variation in the result.

DATA ANALYSIS

The data was checked for its consistencies and prepared a master database with anonymized sample IDs. Statistical analysis was performed by using Microsoft Excel. The distributions and sero-positivity are given in percentages. The sero-prevalence was presented by age group, gender, social strata, area of residence, and COVID-19-related characteristics of study participants.

RESULTS

A total of 7,686 households were contacted across the state, out of which, 57 households were locked, and 39 households refused to participate. From the 7,590 HHs (98.8%) that agreed to participate in the study, a total of 17,010 participants were found eligible, of which 2,469 were not available at the time of interview and 172 participants refused to take part in the study. Thus, a total of 14,369 participants from the general population could be interviewed. Among these, blood samples could not be collected from 55 participants and the tests were indeterminate in 135 number of samples. So, sero-prevalence for general population has been estimated from a total of 14,179 samples (83.4%). Concurrently, 3,419 health care workers were contacted of whom except for one participant, all the other 3,418 HCWs agreed to take part in the study. Among them, blood sample could not be collected from 3 HCWs and results

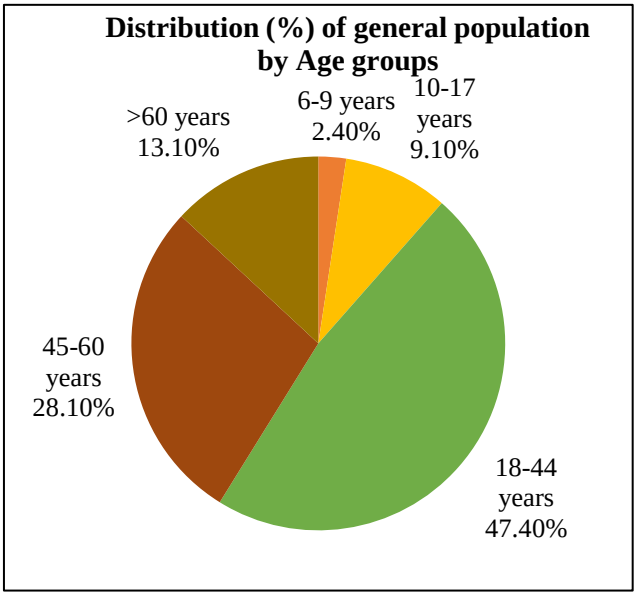
were indeterminate in 31 number of samples. So, sero-prevalence for HCWs has been estimated from a total of 3384 samples (98.9%).

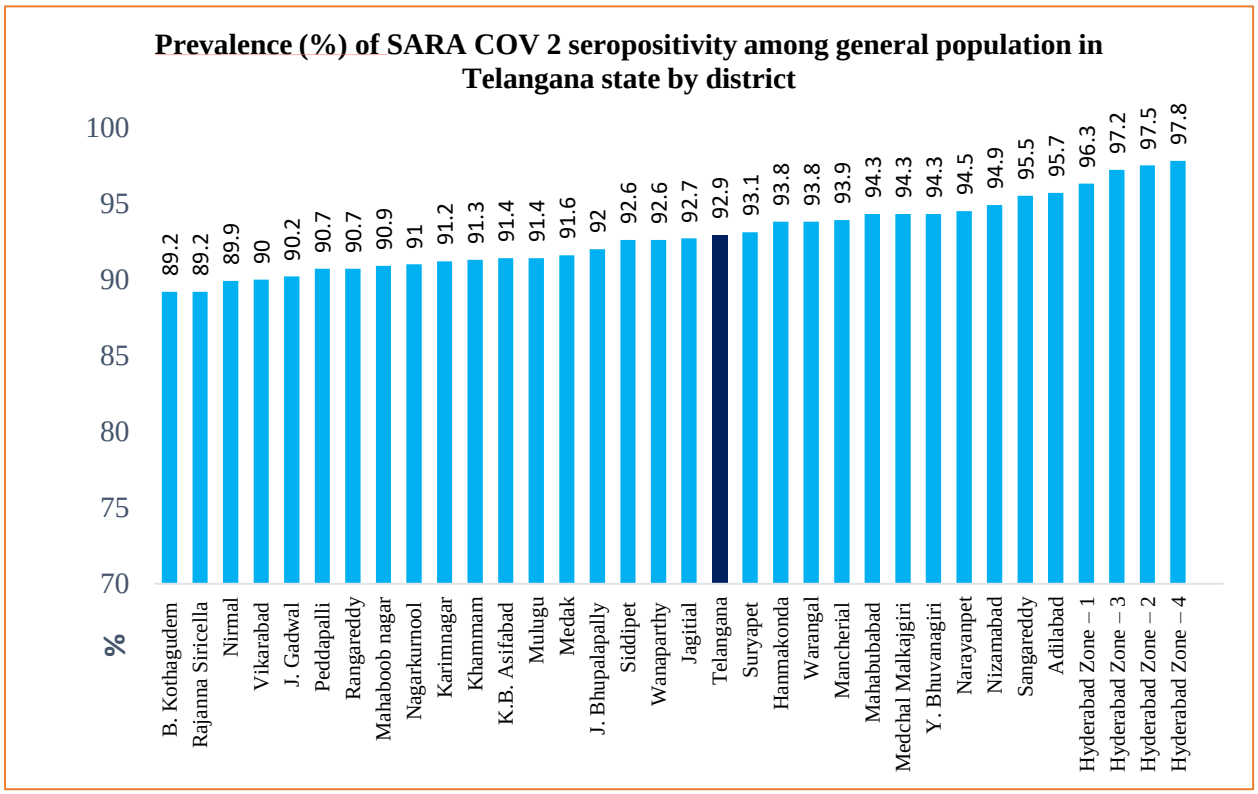
Majority were among the age group 18-44 years (47.4% in general population and 68.76% of HCWs) More than half the participants (56%) among general population and two-third of health care workers (67.2%) were females. Nearly 78% of the participants were from rural background while rest belonged to urban areas. Among the HCWs interviewed, nearly 39% were nurses, 36% were paramedics, 15% were laboratory personnel and nearly 10% were doctors. About 15% of the population and 16% of the HCWs reported themselves as suffering either from hypertensive or diabetics. Nearly two-thirds (66.4%) of the total interviewed participants reported that they had never performed screening tests for SARS-COV-2 infection earlier, and only 7% reported to have ever been positive. About 69% of the HCWs reported to have never tested positive. Less than 1% in general population and nearly 6% of HCWs have reported to have been positive more than once.

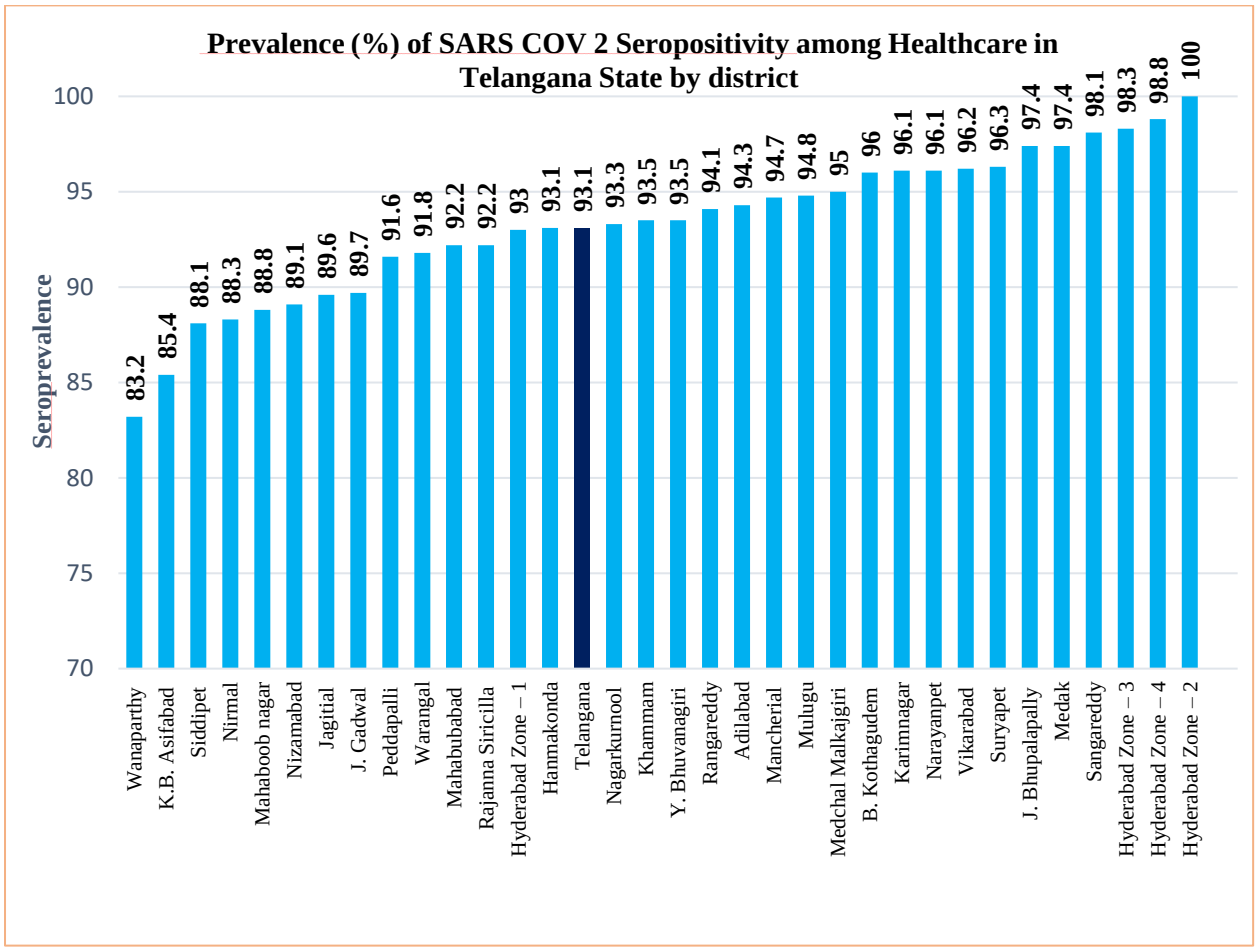
Almost all (97%) the eligible general population and 99% of the HCWs had received at least one dose of SARS-COV-2 vaccination and three fourth of general population (72%) and nearly 94% of HCWs had received two doses of vaccine. More than 92% of the vaccinated participants received Covishield. Nearly 2% of the general population and 12% of HCWs reportedly said that they had covid-19 infection even after vaccination. Among the general population, 84.8% of those who received Covishield and 83.3% of those who received Covaxin had completed two doses of vaccination. The similar proportion was 96.1% for Covishield and 90.1% for Covaxin among healthcare workers.

Sero-prevalence and its associated factors

Overall, the sero-prevalence of SARS-COV-2 among the general population and health care workers was 92.9% and 93.1% respectively in the Telangana State. The sero-prevalence was higher among 18-60 year adults (95%) as compared to children of 6-9 years (67.3%) and adolescents aged 10-17 years (77.5%). There were no significant differentials observed in gender, community, urban-rural, religion, education status, occupation, morbidity, covid-19 contact history. However, the prevalence of sero-positivity was significantly higher in participants (99%) who had previous history of covid-19 infection compared to who had never been positive for covid-19 (93%). Importantly, the prevalence of sero-positivity was very low among non-vaccinated participants (77%) as compared to vaccinated (1 dose: 91.4%; 2nd dose: 96%). Overall, the sero-positivity among the general population ranged between 89.2% in Bhadradri Kothagudem and Rajanna Siricilla and highest in Hyderabad 97%, while among the healthcare workers it ranged from 83.2% in Wanaparthy district to 98% in Hyderabad.




Prevalence (%) of SARA COV 2 seropositivity among general population in Telangana state by district
%
100
95
90
85
80
75
70
B. Kothagudem 89.2
Rajanna Siricella 89.2
Nirmal 89.9
Vikarabad 90
J. Gadwal 90.2
Peddapalli 90.7
Rangareddy 90.7
Mahaboob nagar 90.9
Nagarkurnool 91
Karimnagar 91.2
Khammam 91.3
K.B. Asifabad 91.4
Mulugu 91.4
Medak 91.6
J. Bhupalapally 92
Siddipet 92.6
Wanaparthy 92.6
Jagitial 92.7
Telangana 92.9
Suryapet 93.1
Hanmakonda 93.8
Warangal 93.8
Mancherial 93.9
Mahabubabad 94.3
Medchal Malkajgiri 94.3
Y. Bhuvanagiri 94.3
Narayanpet 94.5
Nizamabad 94.9
Sangareddy 95.5
Adilabad 95.7
Hyderabad Zone – 1 96.3
Hyderabad Zone – 3 97.2
Hyderabad Zone – 2 97.5
Hyderabad Zone – 4 97.8


Prevalence (%) of SARS COV 2 Seropositivity among Healthcare in Telangana State by district
Seroprevalence
100
95
90
85
80
75
70
Wanaparthy 83.2
K.B. Asifabad 85.4
Siddipet 88.1
Nirmal 88.3
Mahaboob nagar 88.8
Nizamabad 89.1
Jagitial 89.6
J. Gadwal 89.7
Peddapalli 91.6
Warangal 91.8
Mahabubabad 92.2
Rajanna Siricilla 92.2
Hyderabad Zone – 1 93
Hanmakonda 93.1
Telangana 93.1
Nagarkurnool 93.3
Khammam 93.5
Y. Bhuvanagiri 93.5
Rangareddy 94.1
Adilabad 94.3
Mancherial 94.7
Mulugu 94.8
Medchal Malkajgiri 95
B. Kothagudem 96
Karimnagar 96.1
Narayanpet 96.1
Vikarabad 96.2
Suryapet 96.3
J. Bhupalapally 97.4
Medak 97.4
Sangareddy 98.1
Hyderabad Zone – 3 98.3
Hyderabad Zone – 4 98.8
Hyderabad Zone – 2 100

INFERENCE AND CONCLUSION

SARS-COV-2 sero-surveillance can be helpful in predicting future course of pandemic and to take containment measures. Prior to the second wave of COVID-19 in India, about 75% of the population was seronegative and majority were non-vaccinated. Subsequently, a huge surge of 2nd and 3rd waves were experienced in India. The present study carried out during January 2022 has shown that the prevalence of SARS-COV-2 sero-positivity is 93% and vaccination coverage is also about 98%. Therefore, the future COVID-19 surges are likely to be non-significant. However, about one-fourth of the child and adolescent population in Telangana did not have detectable antibodies against SARS-CoV-2 by January 2022. This group of population may significantly be vulnerable to infection.

A high SARS COV-2 sero-prevalence observed in the study population should not be a reason for complacency. Therefore, few covid-19 appropriate behaviours like wearing masks and maintaining hand hygiene should be continued in public places and huge gatherings may be avoided for some time. Continued surveillance for COVID-19 infection is necessary to detect an upsurge as early as possible. The state should aim for 100% coverage of 2nd and 3rd (precautionary dose) doses of vaccination for all eligible groups. Vaccination coverage for children, especially among the 12-17 years needs to be intensified and initiation of vaccination may be considered for 6-11 year-old children as well.

3. POPULATION BASED SERO-SURVEILLANCE FOR SARS-COV-2 INFECTION TRANSMISSION IN INDIA: FOURTH SURVEY, JUNE-JULY, 2021

India began COVID-19 vaccination in January 2021, initially targeting healthcare and frontline workers. The vaccination strategy was expanded in a phased manner and currently covers all individuals aged 18 years and above. India experienced a severe second wave of COVID-19 during March–June 2021. We conducted a fourth nationwide serosurvey to estimate prevalence of SARS-CoV-2 antibodies in the general population aged over 6 years and healthcare workers (HCWs). The objectives of the fourth survey are:

1. Estimate the prevalence of SARS-C0V-2 antibodies among (a) children aged 6-17 y and (b) adults in the general population, at the national level.
2. Estimate the prevalence of SARS-C0V-2 antibodies among healthcare workers at the national level.

METHODS

We did a cross-sectional study between 14 June and 6 July 2021 in the same 70 districts across 20 states and 1 union territory where 3 previous rounds of sero-surveys were conducted.

From each district, 10 clusters (villages in rural areas and wards in urban areas) were selected by the probability proportional to population size method. From each district, a minimum of 400 individuals aged >6 years from the general population (40 individuals from each cluster) and 100 HCWs from the district public health facilities were included. The serum samples were tested for the presence of IgG antibodies against S1-RBD and nucleocapsid protein of SARS-CoV-2 using chemiluminescence immunoassay. We estimated the weighted and test-adjusted sero-prevalence of IgG antibodies against SARS-CoV-2, along with 95% CIs, based on the presence of antibodies to S1-RBD and/or nucleocapsid protein. Our institute was nodal for the state of Telangana where 3 districts namely Kamareddy, Nalgonda and Jangaon were a part of the study.

RESULTS

Of the 28,975 individuals who participated in the survey, 2,892 (10%) were aged 6–9 years, 5,798 (20%) were aged 10–17 years, and 20,285 (70%) were aged >18 years; 15,160 (52.3%) participants were female, and 21,794 (75.2%) resided in rural areas. The weighted and test-adjusted prevalence of IgG antibodies against S1-RBD and/or nucleocapsid protein among the general population aged >6 years was 67.6% (95% CI 66.4% to 68.7%). Seroprevalence increased with age ($p < 0.001$) and was not different in rural and urban areas ($p = 0.822$). Compared to unvaccinated adults (62.3%, 95% CI 60.9% to 63.7%), seroprevalence was significantly higher among individuals who had received 1 vaccine dose (81.0%, 95% CI 79.6% to 82.3%, $p < 0.001$) and 2 vaccine doses (89.8%, 95% CI 88.4% to 91.1%, $p < 0.001$). The sero-prevalence of IgG antibodies among 7,252 HCWs was 85.2% (95% CI 83.5% to 86.7%). Important limitations of the study include the survey design, which was aimed to estimate sero-prevalence at the national level and not at a sub-national level, and the non-participation of 19% of eligible individuals in the survey.

INFERENCE AND CONCLUSION

Nearly, two-thirds of individuals aged > 6 years from the general population and 85% of HCWs had antibodies against SARS-CoV-2 by June–July 2021 in India. As one-third of the population is still seronegative, it is necessary to accelerate the coverage of COVID-19 vaccination among adults and continue adherence to non-pharmaceutical interventions.

Sero-prevalence (%) of IgG antibodies in India against SARS-CoV-2, India, (June–July 2021)

Measure	General population aged ≥6 years			Healthcare workers		
	Anti-N antibodies	Anti-S1-RBD antibodies	Anti-N and/or anti-S-RBD antibodies	Anti-N antibodies	Anti-S1-RBD antibodies	Anti-N and/or anti-S-RBD antibodies
Number of individuals tested	28,975	28,975	28,975	7,252	7,252	7,252
Number positive	11,289	18,388	19,336	2,305	6,112	6,186
Unweighted prevalence*, percent (95% CI)	38.9 (37.9–40.1)	63.5 (62.3–64.6)	66.7 (65.6–67.8)	31.8 (29.7–34.0)	84.3 (82.5–85.9)	85.3 (83.6–86.8)
Weighted prevalence**, percent (95% CI)	38.5 (37.3–39.7)	64.4 (63.2–65.6)	66.6 (65.3–67.9)	—	—	—
Adjusted prevalence***, percent (95% CI)	38.3 (37.0–39.5)	66.8 (65.5–68.0)	67.6 (66.4–68.7)	31.5 (29.4–33.7)	87.4 (85.6–89.1)	85.2 (83.5–86.7)

*Adjusted for clustering.
**Weighted for design weights.
***Adjusted for test performance.
N, nucleocapsid protein.

Sero-prevalence of IgG antibodies against SARS-CoV-2 in India by selected characteristics (June–July 2021)

Characteristic	General population			Healthcare workers		
	Number tested	Number positive (anti-N and/or anti-S1-RBD antibodies)	Weighted and test-performance-adjusted seroprevalence, percent (95% CI)	Number tested	Number positive (anti-N and/or anti-S1-RBD antibodies)	Test-performance-adjusted seroprevalence, percent (95% CI)
Age						
6–9 years	2,892	1,635	57.2 (55.0–59.4)	—	—	—
10–17 years	5,798	3,584	61.6 (59.8–63.3)	—	—	—
18–44 years	12,522	8,245	66.7 (65.3–68.0)	5,133	4,401	86.5 (84.9–88.0)
45–60 years	5,545	4,217	77.6 (76.1–79.0)	1,997	1,686	85.1 (83.0–87.1)
>60 years	2,218	1,655	76.7 (74.6–78.7)	122	99	80.4 (71.9–86.8)
Gender						
Male	13,783	9,018	65.8 (64.4–67.1)	3,523	3,024	86.2 (84.4–87.8)
Female	15,160	10,295	69.2 (67.9–70.5)	3,722	3,157	85.9 (84.1–87.6)
Other	32	23	83.4 (59.1–94.6)	7	5	66.4 (26.9–91.3)
Area of residence						
Rural	21,794	14,398	66.7 (65.4–68.1)	—	—	—
Urban non-slum	5,266	3,587	69.1 (66.6–71.6)	—	—	—
Urban slum	1,915	1,351	71.0 (66.8–74.7)	—	—	—
History of COVID-19-related symptoms since 1 January 2021						
Yes	1,748	1,262	76.8 (74.4–79.0)	925	838	85.2 (83.6–86.8)
No	27,227	18,074	66.9 (65.7–68.1)	6,327	5,348	91.5 (89.2–93.2)
Previously tested for COVID-19						
Yes	4,372	3,196	78.7 (77.1–80.2)	4,892	4,194	86.9 (85.2–88.4)
No	24,584	16,127	65.6 (64.4–66.9)	2,360	1,992	84.2 (81.9–86.3)
Previous COVID-19 test result						
Reported positive for COVID-19	782	674	88.9 (86.8–90.8)	1,354	1,275	94.8 (93.4–96.0)
Reported negative for COVID-19	3,419	2,425	75.2 (73.2–77.0)	3,395	2,789	83.3 (81.1–85.3)
Don't know	171	97	71.1 (59.2–80.5)	143	130	90.1 (80.6–95.2)
COVID-19 vaccination status among adults						
0 dose	12,599	7,758	62.3 (60.9–63.7)	759	507	64.8 (60.1–69.2)
1 dose	5,038	4,016	81.0 (79.6–82.3)	972	834	87.7 (85.0–89.9)
2 doses	2,631	2,331	89.8 (88.4–91.1)	5,521	4,845	88.6 (87.1–90.1)
Timing of blood sample collection						
Less than 21 days after first dose	1,711	1,242	73.5 (70.6–76.2)	191	151	78.0 (70.7–83.9)
21 days or more after first dose	3,327	2,774	85.9 (84.3–87.4)	781	683	89.8 (87.2–92.1)
7 days or more after second dose	2,630	2,330	90.4 (88.9–91.7)	5,513	4,837	88.6 (87.1–90.1)
Vaccine type						
Covaxin	587	473	80.2 (76.1–83.8)	498	428	86.5 (82.7–89.5)
Covishield	6,945	5,751	85.2 (83.8–86.5)	5,973	5,229	88.6 (87.0–90.1)
Previously positive for COVID-19, by vaccination status						
0 dose	487	402	88.0 (83.0–91.8)	140	116	83.6 (76.0–89.2)
1 dose	145	134	95.0 (90.6–97.4)	154	146	95.2 (90.4–97.7)
2 doses	150	138	94.0 (88.2–97.1)	1,060	1,013	96.1 (94.4–97.4)

N, nucleocapsid protein.

II. Dietetics Studies

1. DEVELOPMENT OF PREBIOTIC NOODLES CONTAINING GALACTO OLIGOSACCHARIDES

Noodles are one of the staple foods consumed in many Asian countries. Instant noodles have become internationally recognized food, and worldwide consumption is on the rise. Noodle is a food product which is consumed in a great amount in Indian scenario. Food safety regulator Food Safety Standards Authority of India (FSSAI) is setting new regulation for quality standards especially for instant noodles in order to bring clarity in quality standards. Many researchers are exploring the potential of noodle fortification as an effective public health intervention and improve its nutritional properties. Prebiotics are indigestible compounds which selectively fermented and result in certain changes in composition and/or activity of gut's natural microflora and provide health benefits. In food industry these compounds are commonly used for improving nutritional value and/or meeting technological goals. One of the most widely used types of prebiotics is inulin which is a linear polysaccharide. Another group of carbohydrate based indigestible prebiotics are galacto oligosaccharides (GOS) which are structurally more stable at high temperatures. Additionally it has been proved that a synergistic effect may exist between long and short chain prebiotics (such as GOS) from nutritional and rheological aspects. However, up to now, many researchers studied on application of prebiotics in different types of food products. But there seems to be no published research about prebiotic noodles.

OBJECTIVE

The main objective is to study the physicochemical, rheological and sensory properties of prebiotic noodles formulated by long chain inulin, GOS.

METHODS

Preparation of balanced diet prebiotic incorporated noodles: Twelve different formulations of noodles were prepared by using standard formulations with defatted bengal gram flour as protein source, galacto-oligosaccharides incorporated with micronutrient powder (MNP) from UNISEF. Noodles were prepared using Kent Noodle & Pasta Maker. The nutritional composition of all the formulation was carried out using standard AOAC methods.

Estimation of proximate composition of the noodles: The powdered samples of different noodles were subjected to analyse fat, moisture, crude fiber, and ash using AOAC-2006 methodology (934.01, 942.05, 962.09, and 920.39), and crude protein by Kjeldahl method (AOAC 984.13). Whereas, available carbohydrate was estimated by using modified anthrone method (Devindra et al. 2017).

SENSORY EVALUATION

Sensory attributes were evaluated by a group of semi-trained panellists [both men and women (n=36)] using a 9-point Hedonic scale. The contents of the different noodles were described to individuals; those who were sensitive to wheat were excluded from the study. The average age of the panellists was 28±4 years. The resistant starch rich plantain flour incorporated noodles was evaluated for its acceptability with reference to appearance, colour, aroma, texture, taste, flavour and overall palatability.

RESULTS

The twelve different formulations of prebiotic noodles are represented in Table 1. The results of the macronutrients on different noodles are represented in Table 1. The percent of the moisture content was ranged from 9.09% (100% Maida noodle) to 12.6% (Maida (94g) + galactooligosaccharides (5g)+1g Micronutrients (MN)), and the percent of protein ranged from 8.21% in 100% Wheat flour noodles to 11.64% in Maida (74%) +DFBG (20%) + galactooligo-saccharides (5%) +1g Micronutrients (MN). The percent of ash content in noodles tested ranged from 0.41% in Maida (99g) +1g Micronutrients (MN) to 1.46% in Maida (75%) +DFBG (20%) + galacto oligosaccharides (5%), and the fat content was 2.01% in Maida (95g) + galacto oligosaccharides (5g) and 3.58% in Wheat flour (99 g)+1g Micronutrients (MN). The insoluble dietary fibre of the noodles ranged from 0.23% (Maida (95g) + galacto oligosaccharides (5g)) to 7.37% (Wheat (74%) +DFBG (20%) + galacto oligosaccharides (5%) +1g Micronutrients (MN)) and soluble dietary fibre from 0.42% (Maida (75%) +DFBG (20%) + galacto oligosaccharides (5%)) to 3.8% (100% Wheat flour).

Table 1. Formulation of Prebiotic Noodles

Formulations	Ingredients
Formulation 1	Wheat flour (100g)
Formulation 2	Maida (100g)
Formulation 3	Maida (95g) + galacto oligosaccharides (5g)
Formulation 4	Wheat (95g) + galacto oligosaccharides (5g)
Formulation 5	Maida (75%) +DFBG (20%) + galacto oligosaccharides (5%)
Formulation 6	Wheat flour (75%) +DFBG (20%) + galacto oligosaccharides (5%)
Formulation 7	Wheat flour (99 g)+1g Micronutrients (MN)
Formulation 8	Maida (99g) +1g Micronutrients (MN)
Formulation 9	Maida (94g) + galacto oligosaccharides (5g)+1g Micronutrients (MN)
Formulation 10	Wheat (94g) + galacto oligosaccharides (5g) +1g Micronutrients (MN)
Formulation 11	Maida (74%) +DFBG (20%) + galacto oligosaccharides (5%) +1g Micronutrients (MN)
Formulation 12	Wheat (74%) +DFBG (20%) + galacto oligosaccharides (5%) +1g Micronutrients (MN)

DFBG= Defatted Bengal gram

The percent of carbohydrates ranged between 64.07% (Wheat flour (75%) +DFBG (20%)+ galacto oligosaccharides (5%)) to 79.71% (Maida (95g) + galacto oligosaccharides (5g)). The sensory acceptability of developed noodle products (Maida (74%) +DFBG (20%) + galacto oligosaccharides (5%) +1g Micronutrients (MN) and Wheat (74%) +DFBG (20%) + galacto oligosaccharides (5%) +1g Micronutrients (MN) was 52%, 36% respectively.

Table 2. The proximate composition of the different formulated prebiotic noodles

No	Sample ID	Moisture	Protein	Ash	Total fat	DF		Ave CHO	Total
		G/100g	G/100g	G/100g	G/100g	IDF G/100g	SDF G/100g	G/100g	Total
1	Formulation 1	9.68	8.21	1.16	3.18	6.99	3.80	71.44	104.46
2	Formulation 2	10.09	9.04	0.45	2.22	0.51	1.66	77.13	101.11
3	Formulation 3	10.50	8.95	0.44	2.01	0.23	0.65	79.71	102.50
4	Formulation 4	9.34	8.50	1.12	3.13	6.68	3.55	72.79	105.10
5	Formulation 5	9.64	10.67	0.74	2.31	2.32	0.42	73.30	99.40
6	Formulation 6	9.87	10.75	1.46	3.16	8.36	1.11	64.07	98.78
7	Formulation 7	9.47	9.36	1.22	3.58	5.61	1.45	66.84	97.53
8	Formulation 8	12.48	8.22	0.41	2.27	0.97	0.43	72.99	97.77
9	Formulation 9	12.60	7.66	0.46	2.15	0.47	0.78	74.14	98.26
10	Formulation 10	9.68	9.14	1.11	3.13	4.98	1.51	75.17	104.74
11	Formulation 11	10.17	11.64	0.86	2.25	1.61	1.12	70.96	98.60
12	Formulation 12	9.91	10.07	1.44	3.12	7.37	1.67	70.67	104.25

CONCLUSION

A nutritious and balance diet noodles can be prepared by incorporation of defatted bengal gram, galaco oligosaccharides and micronutrients in such a way that, it could increase the protein fiber and micronutrient content up to required optimum level. Bengal gram founds rich source of protein and rice bran founds rich source of fibers, so it could be incorporated in noodles.

2. CONSUMPTION PATTERN OF ARTIFICIAL SWEETENERS USED IN FOOD PRODUCTS AND AS TABLE TOP SWEETENERS AMONG NORMAL, OVERWEIGHT, OBESE AND TYPE II DIABETES INDIVIDUALS LOCATED IN MAJOR METRO-POLITAN CITIES OF INDIA

Artificial sweeteners are synthesized carbohydrate or protein derivatives, which are 200-1300 folds, sweeter than sugars and therefore are used as a sweetening agent in food and dairy industries. Most of these sweeteners are excreted from the body without undergoing metabolization (sucralose and acesulfame potassium), however, some of it may be metabolized to respective amino acids with the liberation of a negligible amount of methanol as in case of Aspartame. Consumption of artificial sweeteners is gradually increasing in India due to the availability of sugar-free products and tabletop low-calorie sweeteners, which are known to regulate the patient's glycemic levels to achieve diabetic management. However, there are many studies that have depicted the adverse effect of artificial sweeteners on gut health and neurological imbalances at the doses slightly higher than recommended levels in animal and human studies. Therefore, the levels of these in food products are to be restricted within the recommended levels, so that people achieve below ADI levels for longer durations. In the current study, the risk assessment of artificial sweeteners were analyzed based on consumer's knowledge on artificial sweeteners and quantifying levels of high intensive sugars present in various food products available in a particular region.

AIMS AND OBJECTIVES

1. To collect and analyze the artificial sweeteners content in various sugar-free or zero calorie food and other products available in various metropolitan cities of India.
2. To assess the knowledge and intake levels of various artificial sweeteners in normal, overweight, obese and type II diabetic individuals located in various metropolitan cities of India using food frequency questionnaires.
3. To examine the opinion, practices, and recommendations of artificial sweeteners by registered dieticians working in the various metropolitan cities of India.

METHODS

Materials

Different branded varieties of products such as Mouthwash, Beverages, Sweets, Chocolates, and Chewing gums were procured from local markets of twin cities of Hyderabad and Secunderabad, Telangana State, India. Standard artificial sweeteners such as Aspartame, Saccharine, Acesulfame-k, Neotame and Sucralose were procured from (Sigma- Aldrich) all other chemicals are analytical grade. The LC C18 column was procured from Supelco (25cmx4.6mm,5µm), and TLC plates from Merck (TLC Silica gel 60G F254 Glass Plates).

The analysis of artificial sweeteners such as Aspartame, Saccharine, Acesulfame-k and Neotame was carried out by using High Performance Liquid Chromatography (HPLC) (Dionex UltiMate 3000 U-HPLC) and Sucralose by thin layer chromatography (TLC).

HPLC Operating Conditions: HPLC analysis of artificial sweeteners such Aspartame, Saccharine, Acesulfame-k and Neotame was carried out by the procedure described by Vallvey (2004). The chromatographic system consisted of a Dionex HPLC-DAD system chromatograph equipped with Chromeleon software an integrator stainless steel Supelco C-18 column (25 cmx 4.6 mm, 5µm particle size). The flow rate was kept at 1 mL/min and the injection volume is 20 µl. The detection wavelength for Aspartame is 208 nm for 10 min run time, Neotame 210 nm for 16 min run time and Acesulfame-k and Saccharine 229 nm for 3 min run time respectively. The HPLC was calibrated daily by injecting 20 µl standard solutions of individual artificial sweeteners, the concentration of each artificial sweeteners was 2.5mg/ml and 0.5 mg/ml for sucralose.

Estimation of sucralose by TLC: Sucralose was estimated by following the method of 41st Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1993) with slight modification. The commercially available TLC plates (TLC Silica gel 60G F254 Glass Plates) were used for the analysis of Sucralose. The estimation of sucralose was carried out by using the modified method of anthrone.

SURVEY PROCEDURES

The pilot research survey was conducted during February-March 2020 through an online portal using the platform docs.google.com. It was a cross-sectional study, within a cohort group, representative of the targeted population of the general public including only patients with type II diabetes. The demographic details of the participants were collected through questions provided in the questionnaire. The questionnaire was a semi-structured questionnaire including closed and a few open-ended questions. Further, the proforma for examining the current recommendations, opinions of dieticians on artificial sweeteners was also collected through an online survey.

RESULTS

Of all the beverages studied, Monster (combination) shows lowest (0.15g/100g) level of sweetner, Acesulfame Potassium and highest level was found in Coke (0.29g/100gm) and sucralose is lowest (0.42g/100gm) in Monster (combination) and highest (2.70g/100gm) in Monster (plain). In chewing gums, Centerfresh-2 has lowest (0.17g/100gm) Aspartame sweetener and highest level (3.86g/100gm) in mint and sucralose is lowest (0.60g/100gm) in S mint and highest 0.82g/100gm in centerfresh. Saccharine sweetener is lowest (0.49g/100gm) in Cadbury and highest (2.80g/100gm) in Closeup. Neotame could not be detected in mouthwash, beverages, sweets, chocolates, chewing gums and other food products.

The survey on the consumption pattern of artificial sweeteners among type II diabetic subjects indicated that 86% of individuals consumed table top sweeteners. The preference for these artificial sweeteners were 27% saccharine, 25% sucralose, 23% aspartame, 10% stevia, and 4% acesulfame K, and among the consumers, 41% preferred having it daily once.

Table 1. Artificial sweetener content in Beverages (g/100g)

Sample name	Aspartame	Saccharine	Acesulfame Potassium	Sucralose	Neotame
Coke	0.22 ± 0.000[d]	-	0.29 ± 0.115[a]	-	-
Red Bull	-	-	0.16 ± 0.006[b]	0.52 ± 0.003[a]	-
Monster (combi)	-	-	0.15 ± 0.006[c]	0.42 ± 0.000[d]	-
Monster (plain)	-	-	-	2.70 ± 0.003[c]	-
Pepsi Diet	-	-	0.22 ± 0.000[d]	-	-
Pepsi Black	-	-	0.21 ± 0.000[d]	-	-
Mirinda	-	-	-	-	-
Mountain Dew	-	-	-	-	-
Pepsi	-	-	-	-	-

Values are expressed as Mean ± SD of triplicates values. Different alphabets within a column indicate significant difference at $p<0.05$.

Table 2. Artificial sweetener content in Chewing gums (g/100g)

Sample name	Aspartame	Saccharine	Acesulfame Potassium	Sucralose	Neotame
Happy dent (sugarfree)	2.42 ± 0.01a	-	-	-	-
Mentos	2.62 ± 0.01[b]	-	-	-	-
Mint	3.86 ± 0.00[c]	-	-	-	-
Center fresh 2	0.17 ± 0.01[d]	-	-	-	-
Orbit 2	0.19 ± 0.00[e]	-	-	-	-
Orbit 1	-	-	2.37 ± 0.01[d]	-	-
Center fresh	-	-	-	0.82 ± 0.02[a]	-
Smint	-	-	-	0.60 ± 0.35[b]	-
Double mint	-	-	-	0.80 ± 0.02[a]	-

Values are expressed as Mean ± SD of triplicates values. Different alphabets within a column indicate significant difference at $p<0.05$.

The study indicated that 74% of dieticians did not recommend using artificial sweeteners, while 26% recommended AS for weight management and glycemic control and suggested to the patients suffering from discomfort to discontinue consumption of AS. Among the AS, stevia (28%) and sucralose (15%) were recommended by the dieticians. About 19% of dieticians have consumed Natura sugar-free AS. Majority of the dieticians (72%) felt there is insufficient research regarding the safety and side effects of AS. Dieticians strongly agreed to educate patients to consume low-calorie glycemic foods/beverages for weight management and glycemic control over the use of AS foods and beverages. Most agreed that AS increases one's

sweetness threshold and might cause future health complications while few had a strong opinion of its link to various cancers.

CONCLUSION

In conclusion, the quantity of all sweetener in various food products quantified were found to be within the recommended level of ADI. Some of the food products were found to contain combination of more than one artificial sweetener, for example among soft drinks, Red Bull was found to contain combination of Acesulfame Potassium and Sucralose. The ranges of artificial sweeteners in beverages were found to be within 0.2-0.5 grams per 100 grams, for chewing gums it is around 0.17-2.4 grams. Likewise, the ranges of artificial sweeteners in other food varieties were found to be in preferable range for human consumption.

It can be concluded from the study that there is uncertainty and difference in the opinions about the uses and consumption of artificial sweeteners among dieticians and type II diabetic patients, which is mainly due to the diversity in the health information available among different public networks and media.

3. STUDIES ON RESISTANT STARCH OF PLANT FOODS AND ITS HYPOGLYCEMIC EFFECT IN HUMAN

Diabetes mellitus is characterized by chronic hyperglycemia and altered carbohydrate, protein and lipid metabolism due to the insufficient insulin secretion or insulin action. Diet as one of the modifiable aspect of the type 2 DM thus it can be corrected by making some changes in the dietary pattern. Dietary guidelines for Indians recommend that a balanced diet should provide around 50-60% of total calories from carbohydrate, 10-15% from proteins, 20-30% from both visible and invisible fat. Most of our dietary portion is composed by carbohydrates in which sugar, starches and fibers are found. Starch is classified into rapidly digestible (RDS), slowly digestible (SDS) and resistant starch (RS). Resistant starch is the starch that cannot be digested in the small intestine, but may be fermented in the large intestine. So, the ingestion of resistant starch will decrease the post prandial glucose levels.

The glycemic index (GI), which characterizes the carbohydrate in different foods. Foods are classified into three categories of Glycemic Index i.e., low GI:≤55, medium GI= 55-69, high GI:≥70. The low GI foods are beneficial in management of disease conditions such as diabetes mellitus and cardio vascular disease.

Application of processes that optimize and stabilize resistant starch and its utilization as an ingredient in functional food product development will greatly contribute to its availability for consumption. There is a need of many recipes/foods for people with diabetes. Therefore a systematic study will be conducted to determine resistant starch in commonly consumed foods

and various strategies will be developed to bring out certain low GI/GL food products which may be useful for people with Diabetes.

OBJECTIVES

- Determination of Resistant Starch in commonly consumed foods.
- Effect of Domestic Processing on Resistant Starch in commonly consumed foods.
- Determination of hypoglycaemic effect of high resistant starch foods in animal model

METHODS

Materials: Different varieties of food samples such as cereals (market rice, bajra, ragi, korra, wheat, jowar, low GI rice, barley and maize), pulses (red gram dhal, Bengal gram dhal, bengal gram whole, green gram whole, green gram dhal, lentil, red kidney bean and cowpea), roots and tubers (colocasia, potato, yam), vegetables (plantain) were procured from local markets of twin cities i.e., Secunderabad and Hyderabad. The food sample was subjected for cleaning and made free of dust.

Preparation of samples: All the cereals and pulses were milled in UDY cyclone mill and made into fine powder which can be sieved by 60 mesh sieve. Roots and tubers and vegetable were wiped with a muslin cloth and peeled and edible portion was sliced into 1-2 cm and immediately surface sterilized by absolute ethanol followed by drying at 40-60^{o}C for 24-36 hours in hot air oven. Dried samples were ground to fine powder and stored in air tight containers for further analysis.

Estimation of proximate composition: The powdered samples of different noodles were subjected to analyse fat, moisture, crude fiber, and ash using AOAC-2006 methodology (934.01, 942.05, 962.09, and 920.39), and crude protein by Kjeldahl method (AOAC 984.13). Whereas available carbohydrate was estimated by using modified anthrone method and the resistant starch was determined by commercially available kit (Megazyme kit method 2004).

Effect of processing methods on resistant starch: Effect of different domestic processing methods such as cooking, boiling, pressure cooking, microwave, sautéing, shallow frying and deep frying on resistant starch and storage at room temperature and refrigerator at different time intervals (0h, 4h, 8h, 12h) on resistant starch was carried out.

Development of low glycemic index food: The low GI food was developed by using wheat and plantain flour in combination (70:30, 60:40, 50:50) and the sensory valuation of the food products was were evaluated by a group of semi-trained panellists [both men and women (n=36)] using a 9-point Hedonic scale for acceptability. *In Vivo* GI Study was carried out as per the method described by FAO/WHO 1998.

RESULTS

The results of the resistant starch content of commonly consumed cereals ranged from 0.17% (Foxtail millet) to 5.72% (Jowar or Sorgham) in pulses from 1.86% (Bengal gram) to 29.4% (red gram) in roots and tubers from 28.41% (Potato) to 45.7% (Colocassia) and in vegetables plantain showed 39.88% of resistant starches. The results of database on resistant starch content could be applicable for the use of raw foods as an ingredient in the new functional food product development for the dietary management of chronic diseases.

Table 1. Determination of Resistant starch, Non-Resistant starch and Total starch (g/100g DW)

	Sample name	RS	NRS	TS
Cereals				
1	Market rice	1.211±0.13	73.18±3.275	74.39±3.391
2	Bajra	1.449±0.333	57.84±0.64	59.29±0.805
3	Ragi	1.498±0.073	55.36±0.977	56.85±1.037
4	Foxtail millet	0.167±0.054	65.55±2.373	65.72±2.411
5	Wheat	3.005±0.512	49.87±1.899	52.88±1.402
6	Jowar	5.717±0.082	49.82±1.716	55.54±1.779
7	Low GI Rice	3.539±0.295	66.61±5.626	70.15±5.462
8	Barley	1.067±0.01	62.9±0.207	63.97±0.207
9	Maize	3.464±0.338	46.63±0.686	50.1±0.786
Pulses				
10	Red Gram Dhal	29.4±0.967	5.05±0.086	34.43±0.949
11	Bengal Gram Dhal	1.859±0.072	26.18±0.652	28.04±0.706
12	Bengal Gram whole	4.638±0.248	24.51±0.914	29.15±0.974
13	Green Gram Whole	4.371±0.229	19.59±3.803	23.97±3.907
14	Green Gram dhal	3.49±0.156	35.98±2.455	39.47±2.472
15	Lentil	4.65±0.401	32.57±0.613	37.22±0.704
16	red kidney bean	27.85±0.745	1.173±0.215	29.03±0.878
17	Cowpea	2.671±0.137	29.91±1.398	32.58±1.277
Roots and tubers				
18	Colocasia	45.7±4.227	2.883±0.237	48.58±4.424
19	Potato flour	28.41±2.358	19.31±0.249	47.73±2.588
20	yam	31.19±1.379	2.431±0.376	33.62±1.08
Vegetables				
21	Plantain	39.88±2.764	17.03±0.702	56.91±2.906

[a] Each value is the average of triplicate determinations.
±, One SD.

Effect of different processing methods on resistant starch in cereals- increased RS content due to retro gradation. Pulses- reduced RS content due to starch gelatinisation and solubilisation, thus accessible to digestive enzymes. Roots and tubers- reduced RS content due to starch lipid complexes (lack of availability of starch for retro gradation). Dry heat- reduced RS content due to dextrinization. Moist heat- increased RS content due to gelatinisation.

The results of the sensory acceptability of developed products (wheat: plantain) (50:50, 60:40, 70:30) was showed 22% for (50:50) products, 26% for (60:40) product and 52% for (70:30) ratio product respectively. The GI of developed food product resulted (wheat: plantain) (50:50, 60:40, 70:30) 51.5 for (50:50) products, 57.6 for (60:40) product and 60.75 (70:30) ratio product) respectively.

Table 2. The ratio of the product and the glycemic index/load of the different products

Developed product variation (Wheat: Unripe plantain flour)	Glycemic Index of the food product	Glycemic load of the food products
50:50	51.50±5.8	25.75±2.38
60:40	57.6±505	28.8±7.8
70:30	60.75±5.6	22.78±3.0
100:0	62.43±6.1	28.37±5.3

[a] Each value is the average of ten participants

±, One SD.

CONCLUSION

The resistant starch (RS) content in cereals was low when compared with pulses. In pulses red kidney bean and red gram dhal was found to have high amount of RS than other pulses. In roots and tubers, colocasia, yam and potato observed high amount of resistant starch. In vegetable, plantain was rich in resistant starch. Effect of different processing methods on the resistant starch, cereals showed increased in RS content whereas, pulses showed reduction of RS content. Roots and tubers also showed reduced RS content. Low GI product showed the sensory acceptability of (wheat: plantain) (50:50, 60:40, 70:30) was 22%, 26%, 52% respectively. The low GI product can be explored for the control of type 2 diabetes.

III. Basic Studies

1. INVESTIGATING THE EFFECT OF DIETARY ZINC DEFICIENCY ON SKELETAL MUSCLE PROTEOSTASIS AND MITOCHONDRIAL BIOLOGY IN GROWING RATS

Zinc is an important trace element in the body and is crucial for the growth and development of living organisms. Zinc has an essential role in preserving human health, especially regarding antioxidants, anti-inflammation, immunity, proliferation, proteostasis, apoptosis, and so on. Hence, a significant decrease in zinc levels could be detrimental to the organism. According to World Health Organization evaluations, around 8,00,000 deaths per year are linked to zinc deficiency, and 50% of them are children <5 y of age. Hence, a sufficient dietary supplement is essential to avoid zinc deficiency, which is characterized by growth retardation, reduced food intake, impaired energy homeostasis, alopecia, keratosis, infertility, and delayed wound healing.

Nearly 60% of the total body zinc content is present in the skeletal muscle. Skeletal muscle proteostasis is vital to the organ that serves as a regulator of inter-organ crosstalk for the organism's protein metabolism. Zinc deficiency is associated with reduced muscle growth, cell mass, and total work capacity of skeletal muscle. Zinc deficiency was shown to be an independent predictor of sarcopenia (age-dependent progressive decline of muscle mass and function) in patients with chronic diseases. Zinc was also shown to be involved in both muscle contraction and neuromuscular transmission. However, the effect of zinc deficiency on skeletal muscle proteostasis and mitochondrial biology has not been understood clearly. Hence, the consequences of dietary zinc deficiency on skeletal muscle proteostasis and mitochondrial biology was investigated in growing rats.

METHODOLOGY

Animal experimentation: Three-week-old male Wistar/ Kyoto weanling rats were obtained from and maintained at the Institute's Animal Facility. After 1 wk of acclimatization, the rats were fed either a zinc-deficient diet (ZnD group, n = 8, <1 mg of zinc/kg diet; ad libitum) or zinc sufficient (CON group; n = 9, AIN-93 G) diet pair-fed with ZnD group (47.5 mg of zinc/kg diet) for a period of 7 wk. Skeletal muscle (gastrocnemius) tissue was collected at the end of the animal experiment (7 wk) from overnight fasted rats and snap-frozen in liquid nitrogen and stored at -80°C. At least four samples from each group were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) solution for histopathologic analysis. All the experimental protocols and procedures were approved and in accordance with the institutional animal ethical committee.

Analysis of the cross-sectional area of muscle fibers: The rat skeletal muscle fiber cross-sectional area was analyzed on hematoxylin and eosin (H&E) stained transverse sections (0.4 mm). The sections were observed under a bright-field microscope (Leica Microsystems; Wetzlar, Germany), and images were captured with a digital camera at 40X magnification. The individual area of 75 adjacent muscle fibers in each section was measured for four rats in a group by means of ImageJ software (NIH, Bethesda, MD, USA), and the mean value of muscle fiber cross-sectional areas was calculated.

Real-time quantitative reverse transcription-polymerase chain reaction and immune-blotting were performed to study the target gene and protein expression, respectively. The chymotrypsin-like proteasomal activity was analyzed by the fluorescence method.

STATISTICAL ANALYSIS

All the data were expressed as mean § SEM. The Student's t-test was used for the comparison between two experimental groups. $P < 0.05$ was considered as significant.

RESULTS

Zinc deficiency affects skeletal muscle morphology: The mean cross-sectional area of the gastrocnemius muscle fiber was significantly decreased in ZnD rats relative to the area in the pair-fed control group (as shown in Fig. 1). The distribution of fiber sizes in ZnD rats was shifted toward smaller fibers in zinc deficiency. More numbers of small myofibers and a smaller number of large fibers were observed in ZnD rats than in CON rats.

Zinc deficiency disturbs muscle proteolytic systems: The ubiquitin-proteasome system (UPS) has a major stake in eukaryotic cellular protein turnover and is responsible for the degradation of >80% of intracellular proteins. Ubiquitin-activating enzyme E1, the first enzyme to initiate ubiquitin-based protein targeting is increased in the muscle of ZnD rats. The ubiquitin-ligase enzymes (E3s) have a central role in determining the selectivity and specificity of the UPS. The two muscle-specific E3s; muscle RING finger-1 (MuRF1) and muscle atrophy F-box (MAFbx/Atrogin1), have been shown to be implicated in the regulation of skeletal muscle atrophy. When we examined the status of these two atrogenes, MuRF1 was upregulated whereas Atrogin1 showed an increasing trend at the transcription level. The chymotrypsin-like proteasomal activity was significantly increased.

The autophagy-lysosome system is another important proteostasis network warranting cell metabolism and cell component turnover. Results showed decreased protein expression of crucial autophagy markers: Beclin1, ATG5, and LC3 in ZnD rats. The decreased p62 protein expression was also observed, indicating increased lysosomal activity in zinc deficiency.

Zinc deficiency activates muscle unfolded protein response: Another proteostasis mechanism exists in the endoplasmic reticulum (ER), known as unfolded protein response (UPR) which monitors and maintains proteostasis in the ER. We investigated the status of UPR/ER stress markers in the skeletal muscle of rats fed on zinc deficiency. We observed increased pIRE1/IRE1 ratio, CHOP, and cleaved caspase-12 protein in ZnD confirming the activated UPR or ER stress.

Zinc deficiency affects muscle mitochondrial protein expression: We observed decreased expression of both fission proteins (FIS1 and DRP1) and fusion proteins (MFN2 and OPA1) during zinc deficiency. There was a significant decline in the mitochondrial transcription factor (TFAM) protein expression which is a key activator of mitochondrial transcription and replication. The protein expression of mitochondrial brown fat uncoupling protein 1 (UCP1) was decreased in zinc deficiency. Mitochondrial oxidative phosphorylation system complex proteins succinate dehydrogenase and ubiquinol-cytochrome C oxidoreductase (UQCRC2) were decreased in ZnD rats. SIRT3 is a nicotine adenine dinucleotide-dependent protein deacetylase localized to the mitochondrial matrix that regulates the cellular energy metabolism and was found to be decreased in expression during zinc deficiency.

Zinc deficiency promotes muscle cell apoptosis: We observed a significant increase in the BAX protein and BAX-to-BCL2 ratio as well (as shown in Fig. 2). Cleaved caspase-3, which is an apoptotic marker and proteolytic enzyme, also showed increased tendency but with no statistical significance. Furthermore, ER stress-induced apoptotic markers CHOP and cleaved caspase-12 were also increased in ZnD rats.

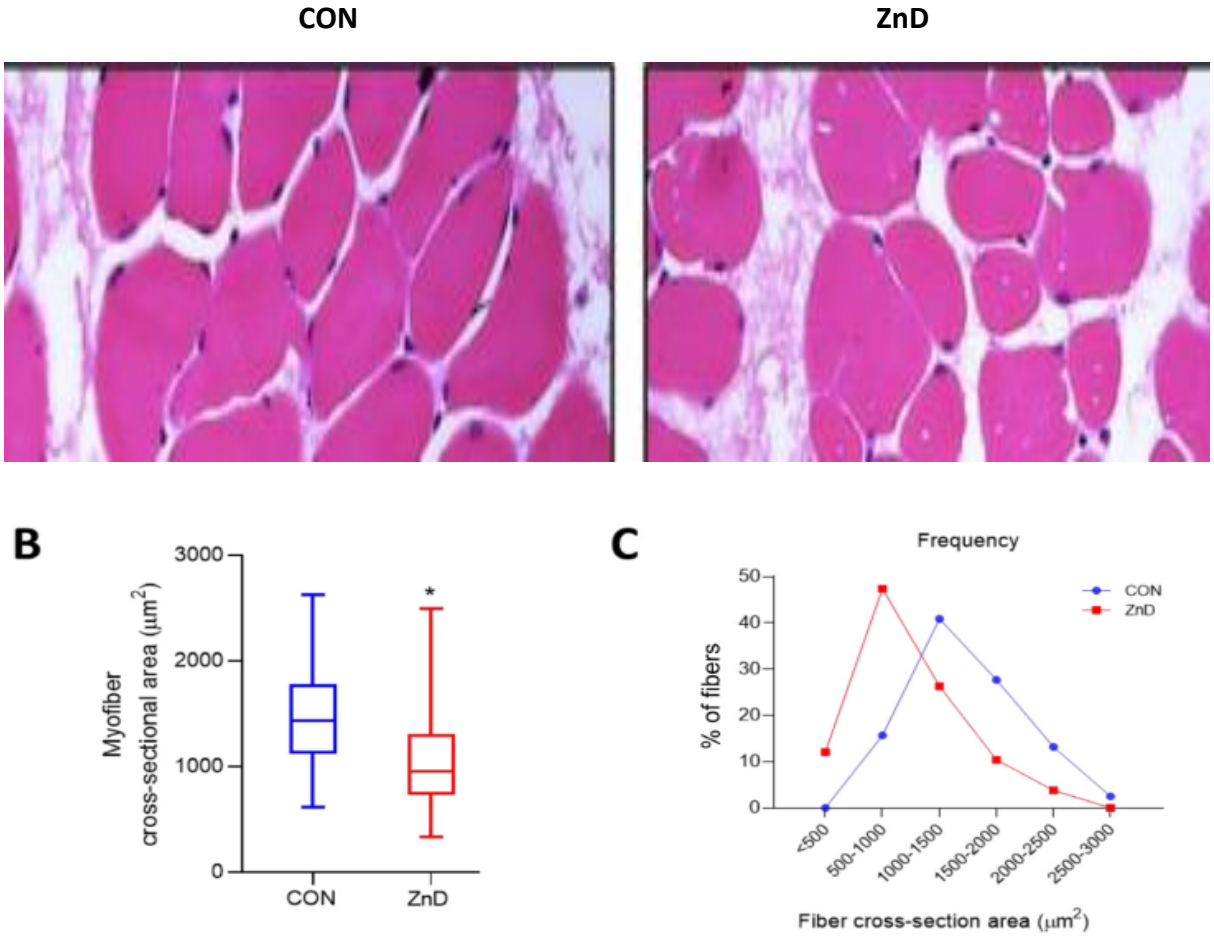


Fig. 1. Effect of Zinc deficiency on skeletal muscle histology. (**A**) Representative bright-field microscopic images of H&E-stained transverse gastrocnemius muscle sections of ZnD and CON rats. Scale bar = 100 µm, Magnification = 40X. (**B**) Quantification data of mean myofiber cross-sectional area. Box and Violin plot showing minimum to maximum values and horizontal line at the mean. (**C**) Frequency of myofibers of increasing cross-sectional area. Data is mean ± SEM. CON group (zinc-sufficient) was pair-fed with ZnD group. *Significant difference from the CON group; ($P < 0.05$). CON, control group; ZnD, zinc-deficient group.

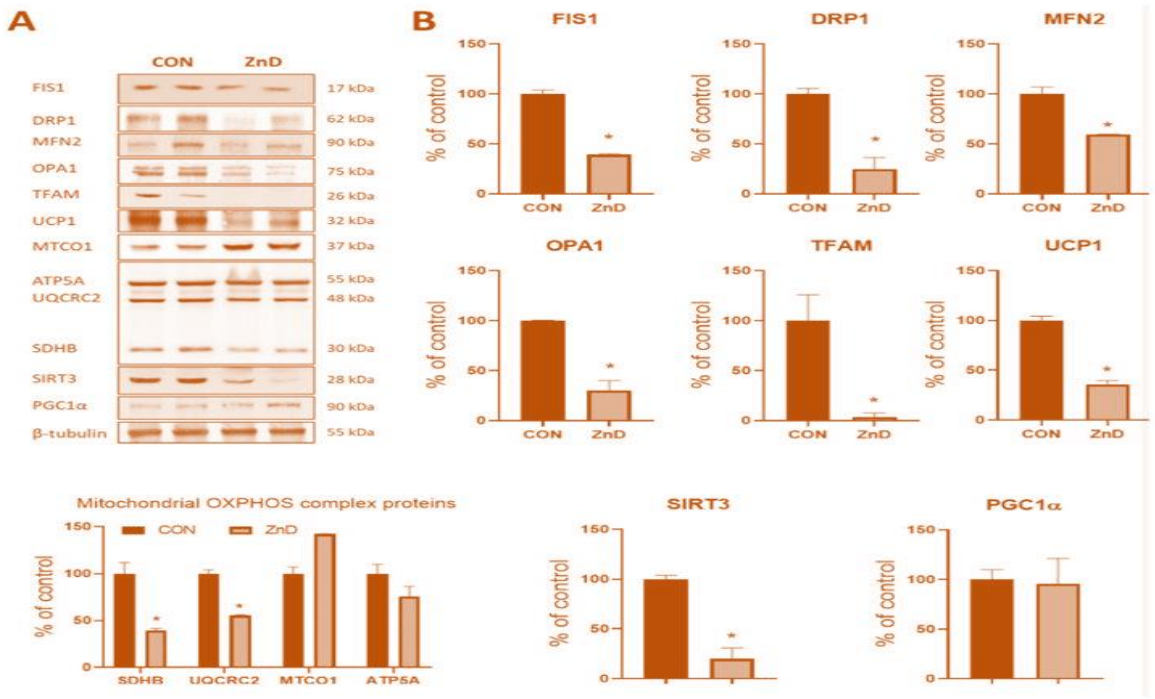


Fig. 2 Effect of zinc deficiency on skeletal muscle mitochondrial markers. (**A**) Representative images of immunoblots for mitochondrial proteins in ZnD and CON rats. (**B**) Quantification data of immunoblots. The protein expression was normalized to the β-tubulin and was represented as percent of control. Data are mean ± SEM (n = 3). CON group was pair-fed control (zinc-sufficient) group. *Significant difference from the CON group; $P < 0.05$. CON, control group; ZnD, zinc-deficient group.

INFERENCE & CONCLUSION

Zinc deficiency in growing rats negatively affected skeletal muscle proteostasis mechanisms and mitochondrial biology. The muscle is a reservoir of whole-body protein and hence, proteostasis is indispensable for not only the muscle mass and function but also whole-body protein metabolism. Furthermore, the zinc deficiency-induced mitochondrial dysfunction could yield energy imbalance, excessive free radical production, and apoptosis. However, the mechanism by which zinc deficiency affected each of the abovementioned processes and their interlinking, if any, is the subject of future studies.

2. DEVELOPMENT OF α-CRYSTALLIN MINI CHAPERONE PEPTIDES AS THERAPEUTIC MOLECULES FOR DIABETIC OCULAR DISEASES (ECR/2017/00027)

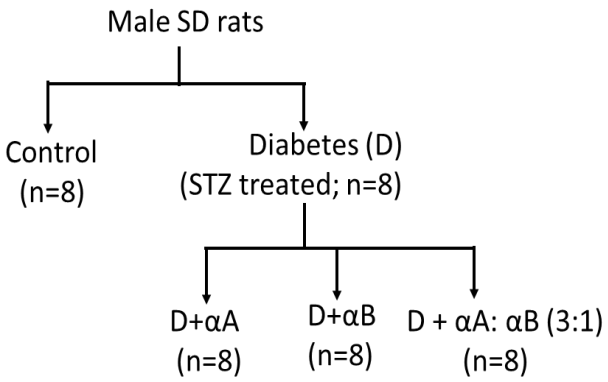
Diabetes is a leading cause of blindness due to cataract and retinopathy. Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes. Cataract, including diabetic cataract (DC), is responsible for 80% blindness of the world, is associated with protein aggregation due to protein modifications. Despite available surgeries and recent progress in treatments, new and improved therapeutic interventions are much needed to tackle the blindness. Alpha-crystallin are soluble proteins of the lens and composed of αA-crystallin (αAC) and αB-crystallin (αBC) subunits in 3:1 ratio. They show chaperone like

activity and prevents the aggregation of proteins, and cell death. Specific sequence within the αAC from 70-88 and in αBC from 73-92 has effective chaperone and protective activity under *in vitro* and *in vivo*. However, protective effect of α-crystallin chaperone peptides on diabetic ocular diseases remain unexplored. However, optimal dose, route, time of injection, combination of peptides, and molecular mechanisms involved in ameliorating cataract and retinopathy by α-crystallin peptide needs to be explored. The goal of this proposal is to investigate the protective effect of α-crystallin chaperone peptides with respect to the above points on diabetic ocular diseases using diabetic rat model.

OBJECTIVES

1. To optimize the dose, route of administration, combination of peptides, timing of injection (preventive/therapeutic) of α-crystallin chaperone peptide on DC and DR.
2. To investigate the effect of α-crystallin administration on molecular mechanisms of cataract and retinopathy.
3. To validate the effect of α-crystallin chaperone peptide on molecular mechanisms of protein quality control.

METHODS



A 19-mer of αAC, a 20-mer of αBC was custom synthesized. Five-month old male SD rats were obtained from animal facility of National Institute of Nutrition, Hyderabad and maintained at a temperature of 22±2 ^{0}C, 50% humidity and 12 h light/dark cycle. Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the Institutional Animal Ethical Committee (IAEC) of the ICMR-National Institute of Nutrition. The control rats (n=8) received 0.1 M sodium citrate buffer, pH 4.5 as a vehicle whereas the experimental rats received a single intraperitoneal (i.p) injection of STZ (35 mg/kg) in the same buffer. At 72 h after STZ injection, fasting blood glucose levels were monitored. Animals with blood glucose levels >150 mg/dL were considered for the experiment and divided into four groups. A group of animals in STZ induced diabetes received intraperitoneal (I.P) injection PBS as vehicle (Diabetic, D; n=8). Remaining animals received IP injection (route of administration) of 50µg (dose) of either αAC (D+αA; n=8) or αBC (D+αB; n=8) or αAC and αBC in 3:1 ratio (D + αA:αB; combination of peptides) mini chaperone peptides in PBS on weekly basis with two injections from the onset of cataract. All animals were fed with AIN-93 diet *ad libitum*. Body weight and blood glucose concentration of each animal were measured weekly. At the end of the experiment, rats were fasted overnight and sacrificed by CO_2 asphyxiation. Onset, progression and maturation of cataract was assessed by slit-lamp biomicroscope while retinal function by electroretinography. Molecular mechanisms of delay/ prevention of cataract was be studied by crystallin aggregation, oxidative

stress, cell death, UPR in the lens was investigated. Finally, the effect of chaperone peptides was validated using *in vitro* cell culture systems (HeLa and HEK-293 cells). HeLa cells were used to study the effect of α-crystallin peptides on tunicamycin (TM) induced ER stress and chaperone assays while HeLa cells were used to study the impact of α-crystallin peptides on TM-induced ER stress and TBHP-induced mitochondrial oxidative damage. In brief, HEK-293 and HeLa cells were grown in DMEM supplemented with 4.5 g/L glucose, 10% heat-inactivated FBS, 100 units/ml penicillin and 100 µg/ml streptomycin, 1% glutamax and cultured in 100 mm culture plates. For inducing ER stress, cells were co-treated with TM (10µg/ml) and either scrambled peptide (40µg) or αAC peptide (40µg) or αBC peptide (40µg) or combined αAC and αBC peptide in 3:1 ratio for 6 h. After 6 h, media was removed and washed with chilled PBS twice, and cells were collected for protein extraction. We used TBHP induced oxidative stress model to test the protective effect of α-crystallin peptides against oxidative stress. Cells were co-treated with TBHP (200µM) and either scrambled peptide (40µg) or αAC peptide (40µg) or αBC peptide (40µg) or combined αA and αBC peptide in 3:1 ratio for 4 h. ER stress, Formation of reactive oxygen species (ROS), antioxidant enzymes, mitochondrial membrane potential (MMP) and biogenesis**,** ubiquitinated proteins, chaperone assays were investigated.

RESULTS

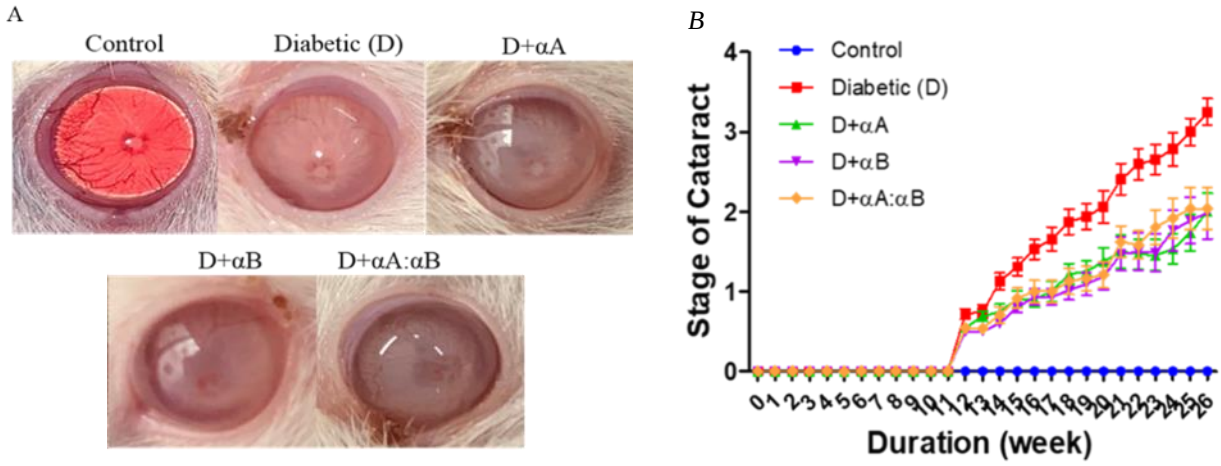
Body weight and fasting blood glucose levels: The body weight of diabetic animals was significantly reduced compared to control. However, α-crystallin peptides did not show any effect on body weights. The FBG levels were significantly increased in diabetic animals and maintained for the entire experimental period when compared with control rats. Nevertheless, α-crystallin peptides administration did not show any significant effect on FBG levels.

α-Crystallin peptides delay the cataract progression in diabetic rats: While the onset of cataract due to hyperglycemia was observed in diabetic animals after 11 weeks of STZ injection and progressed to mature cataract by 26 weeks in untreated diabetic lenses, systemically administered α-crystallin peptides delayed the progression of cataract in diabetic rats. At the end of the experiment, the severity of cataract was significantly lower in peptide treated groups, than in untreated diabetic rats (Fig 1). All the lenses in the control group were clear throughout the experimental period without any opacification. The data suggest that progression and maturation were delayed with α-crystallin peptide treatment. Furthermore, it seems that α-crystallin peptide administration reduced the aggregation and insolubilization of protein. Additionally, hyperglycemia-induced oxidative and ER stress were also attenuated upon α-crystallin peptides administration. Moreover, α-crystallin peptides alleviated the hyperglycemia-induced apoptosis by reducing the caspase-3 activity and Bax levels. It appears that individual peptides and their combination are equally effective against diabetic cataract.

α-Crystallin peptides preserve the retinal function in diabetic rats: ERG was performed to determine the effect of α-crystallin peptides on retinal function. We evaluated the retinal responsiveness by monitoring the a-wave, b-wave and oscillatory potentials (OPs), which primarily reflects photoreceptor cells (outer retinal function), ON bipolar and muller cells, amacrine cells activity (inner retinal function) respectively. The dark adopted or scotopic a-wave (Fig 2A) and b-wave amplitudes (Fig 2B), OPs (Fig 2C & D) were significantly decreased

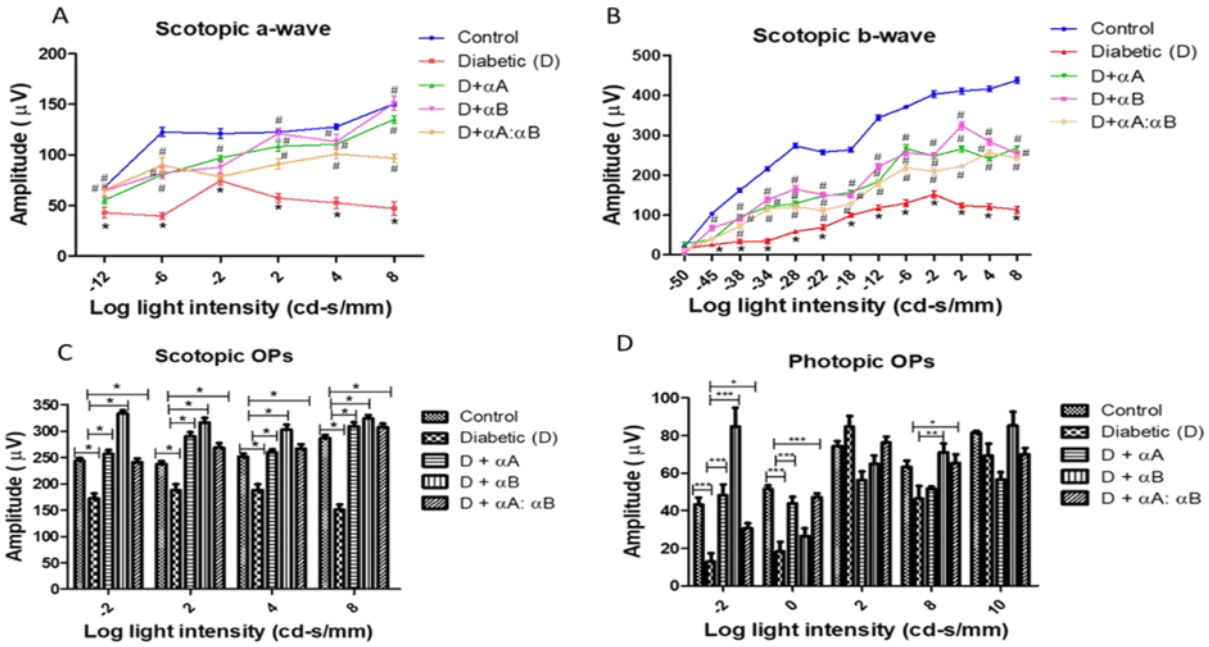
in diabetic group compared to control. Interestingly, the decrease in scotopic a-wave and b-wave amplitudes and OPs were significantly attenuated in αA, αB, and αA: αB-crystallin treated diabetic rats compared to untreated diabetic rats. However, there was no significant difference in a-wave and b-wave implicit times between the groups.

Fig 1: **Exogenous administration of α-crystallin peptides delay the diabetic cataract in rats**.



(***Panel A***) Representative photographs of lens from each group at the end of the experiment. (***Panel B***) Quantitative representation of cataract progression in each group with time. Stage of cataract in each group was averaged at a given time and the average stage of cataract along with standard error was plotted as a function of time.

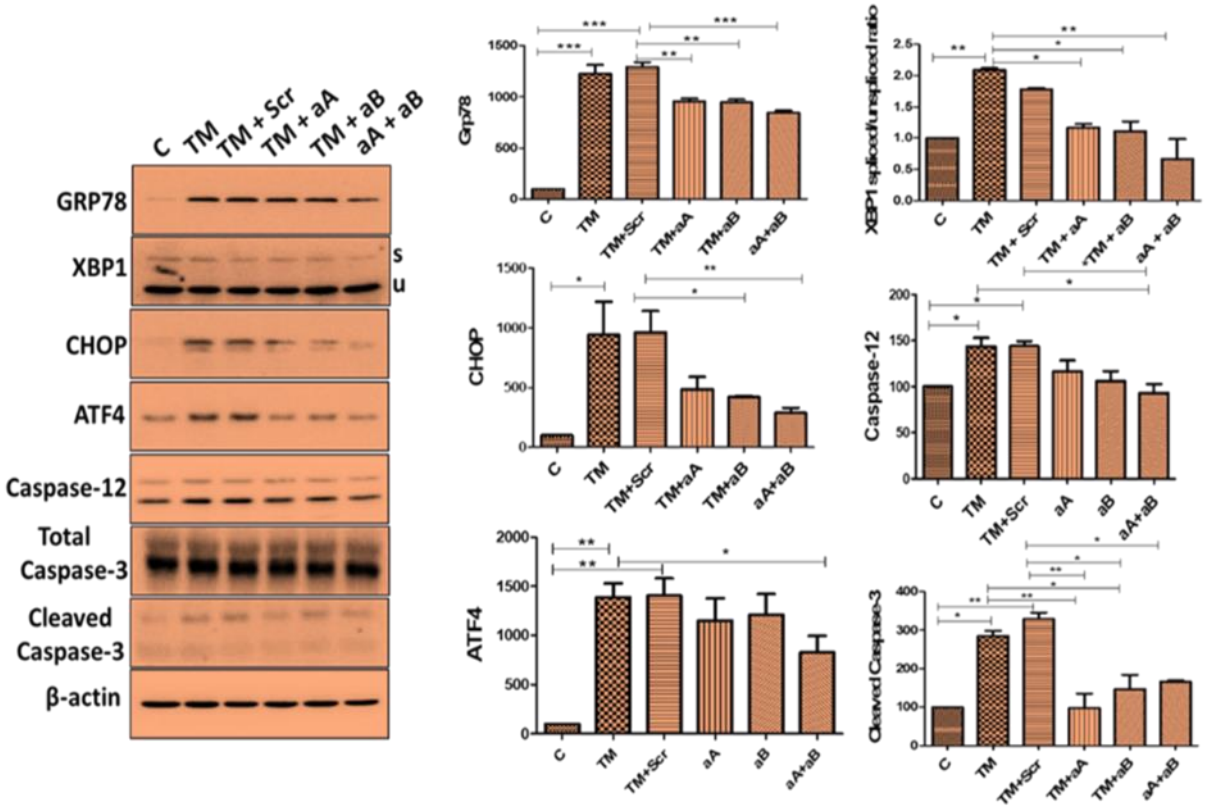
Fig 2. Electroretinogram recordings in control and STZ induced diabetic rats



(***Panel A***) Scotopic a-wave (***Panel B***) Scotopic b-wave; *significantly different from control, #significantly different from diabetic (D) group. (***Panel C***) Scotopic oscillatory potentials (OPs), (***Panel D***) Photopic OPs. Data are represented as mean ± SEM (n=6 for each group). * p<0.05, **p<0.01, ***p<0.001

Impact of α-crystallin peptide against TM-induced ER stress and TBHP-induced oxidative stress: We have chosen HeLa and HEK-293 cells for fathoming the impact of α-crystallin peptides against cellular stress since they do not express sHsps except Hsp27. Interestingly, αAC and αBC peptides inhibited tunicamycin (TM) induced ER stress (Fig 3), apoptotic cell death, and accumulation of ubiquitinated proteins in HeLa and HEK-293 cells. Likewise, αAC and αBC peptides reduced the loss of antioxidant enzyme activities, suppressed the reactive oxygen species formation, protected the cells from apoptosis against TBHP-induced oxidative stress. Moreover, αAC and αBC peptides preserved the mitochondrial membrane potential and improved the mitochondrial biogenesis and dynamics against TBHP induced oxidative stress. The combination of peptides appears to be more effective than individual peptides in thwarting TM-induced ER stress markers and protecting the cells and mitochondrial biogenesis from TBHP-induced oxidative stress.

Fig 3: Attenuation of TM-induced ER stress by α-crystallin peptides



HeLa cells are co-treated with TM, Scramble peptide, αAC, αBC mini chaperone peptides and their combination for 6 hrs and investigated the ER stress and apoptosis markers (n=3, *p<0.05, **p<0.01, and ***p<0.001).

INFERENCE AND CONCLUSION

The major findings of the study are that systemic administration of α-crystallin peptides, including αAC, and αBC peptides and their combination delayed the progression of cataract and preserved the retinal function in diabetic rats, decreased the insolubilization of protein, and formation of protein cross-linking. Furthermore, α-crystallin peptides inhibited the hyperglycemia-induced oxidative stress and attenuated the ER stress in the rat lens.

Additionally, α-crystallin peptides protected the lens from hyperglycemia-induced apoptotic cell death. Further, αAC and αBC peptides inhibited the TM induced ER stress and apoptotic cell death, reduced the ubiquitinated protein levels, protected the antioxidant enzyme activities and inhibited the TBHP induced ROS formation and oxidative stress-induced apoptosis, preserved the MMP, mitochondrial biogenesis, and dynamics against TBHP induced oxidative stress in cell lines.

Overall, we show that systemically administered α-crystallin peptides delayed the progression of diabetic cataract by attenuating the protein aggregation, oxidative stress, ER stress, apoptosis and preserved the retinal function in rats. Further, α-crystallin peptides inhibit cellular stress and could be developed as therapeutic agents or pharmacological chaperones against diseases where ER and oxidative stress are causative factors.

3. EFFECT OF MATERNAL PROTEIN RESTRICTION (QUANTITY AND QUALITY OF PROTEIN) ON BODY COMPOSITION AND PROTEIN QUALITY CONTROL PROCESSES IN THE MUSCLE OF THE OFFSPRING

Extensive epidemiological reports and studies in animal models have shown that a maternal low protein (MLP) diets during fetal and early life development influences the adulthood risk of metabolic diseases. Along with the protein content in the diet, maternal low-quality protein (MLQP) diet influenced the BWs and speculated to impact the risk of developing metabolic diseases in the offspring. Chaperones, unfolded protein response, ubiquitin proteasome system, and autophagy predominantly constitutes the protein quality control (PQC) system of the cell. The PQC have shown to play key role in skeletal muscle atrophy. Experimental maternal protein restriction (MPR) studies solely focused on either gestation or lactation alone or combined periods of gestation and lactation and do not emulate the human condition, where undernutrition is not only confined to fetal life but, rather, is a chronic condition.

Therefore, in the present study, we evaluated the chronic effect of MLQP and MLP diets from combined periods of preconception to post-weaning on body composition, metabolic factors, PQC components and proteolysis in skeletal muscle (SM) and brain of the offspring and examined the impact of rehabilitation.

AIMS AND OBJECTIVES

i). To determine the impact of MLP and MLQP diet on body composition and body growth of the offspring.

ii). To investigate the effect of MLP and MLQP diet on morphology and cellular protein quality control processes in the brain and skeletal muscle of the offspring.
iii). To evaluate the impact of MLP and MLQP diet on epigenetic changes associated with protein quality control processes.

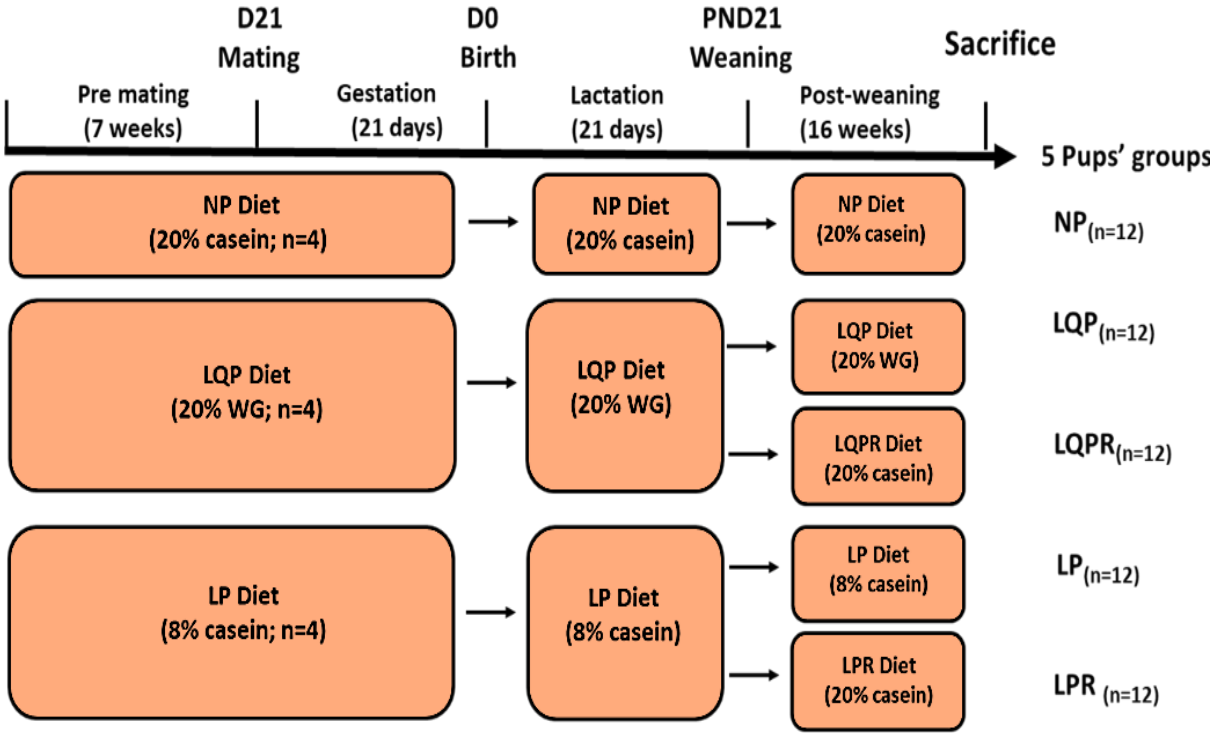
METHODS

All procedures involving animal experiments were approved by the Institutional Animal Ethical Committee (ref # ICMR-NIN/IAEC/01/001/2019) of the ICMR-National Institute of Nutrition (ICMR-NIN). 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 ^{o}C, 50% humidity). After one week of acclimatization, animals were randomly assigned to be fed an AIN93-based isocaloric diet containing either NP (20% casein, n=4) or LQP (20% WG, n=4), or restricted protein or low-protein (LP; 8% casein, n=4) for 7 weeks. We have chosen 8% of casein as LP diet as it was considered as a moderate protein restriction. After 7 weeks, female rats were mated with male rats in the proportion of 2:1, and the presence of mating plug was checked early in the morning. The appearance of mating plug designated as day 0 of pregnancy. Dams were housed in individual cages and provided with food and water ad libitum and continued on their respective diets throughout the experiment.

All diets were formulated as per AIN-93G guidelines. At birth, litter size, sex, and pup weight were recorded, and the day of delivery was designated as day 0 of postnatal life. To ensure homogeneity, the litters were normalized to nine pups wherever possible. At weaning (postnatal day 21), both male and female offspring weight was recorded. After weaning, offspring divided into the following groups: offspring born to mothers fed with an isocaloric diet containing either NP (n=12), or LQP (n=12), or a group of animals from LQP group rehabilitated with NP diet for recovery (LQPR; n=12), or LP diet (LP; n=12), or a group of animals from LP group rehabilitated with NP diet for recovery (LPR; n=12) and maintained on their respective diets for 16 weeks. The food intake of mothers and offspring were recorded daily. The whole animal experiment design was depicted in (Fig 1). Diets were isocaloric on metabolizable energy. The animal's whole-body composition including total body surface area, bone mineral content (BMC), bone mineral density (BMD), total mass, lean mass, fat mass, percent lean body mass (% LBM), and percent body fat (% BF) were assessed using DEXA scanning. DEXA was conducted 10 weeks after weaning in the offspring. Maternal and offspring body weights (BWs) were recorded weekly from the start of the experiment. Offspring were sacrificed after maintaining their respective diets for 16 weeks after weaning.

Animals fasted overnight, and a fasting blood sample was collected via retro-orbital plexus for plasma analysis. Body organs were collected, weighed, and snap-frozen in liquid nitrogen and stored at -80°C for further analysis. Fasting glucose (FG), Insulin, triacylglycerides, cholesterol, alkaline phosphatase (ALP), hemoglobin (Hb), T3 and T4, cortisol and leptin levels were estimated using the commercially available kits. Skeletal muscle histology was determined by H&E staining. Total protein degradation, urinary 3-methyl histidine, UPS components, proteasomal activity, autophagy, caspase-3 activity and muscle atrophy markers were determined in SM of the offspring.

Fig 1. Experimental design: Female WNIN rats were alimented with either a normal protein (NP) diet having 20% casein (n=4) or low-quality protein (LQP) having wheat gluten (WG) diet (n=4) or a low-protein (LP) diet having 8% casein (n=4) for 7 weeks followed by mating with males. All animals received the same diet throughout gestation and lactation. At the weaning, offspring were assigned into the following groups: NP diet (n=12), LQP diet (n=12), LQPR diet (a group of offspring from LQP group rehabilitated with NP diet; n=12), LP diet (n=12), LPR diet (a group of offspring from LP group rehabilitated with NP diet; n=12).



RESULTS

***Body weight and food intake*:** Body weight of the pups were significantly lower in LQP and LP at birth, LP and at weaning than NP-offspring respectively (Fig 2A-B). Absolute food and energy intake of LQP and LP was significantly lower than NP-offspring (Fig 2D-E). Relative food intake was significantly greater in LQP followed by LP than the NP-offspring across the first few weeks. But, this difference decreased across subsequent weeks and was comparable between the groups at the end of the experiment (Fig 2F). At the end of the experiment, the BWs were drastically lower in LQP (75%) and LP (52%) than NP-offspring. However, BWs were significantly higher in LQPR (44%) and LPR (29%) than LQP and LP-offspring, respectively (Fig 2G). Body length at weaning and at the end of the experiment was significantly lower in the LQP and LP than NP-offspring (Fig 2C & 2H). However, body length was significantly higher in the LQPR and LPR than LQP and LP-offspring at the end of the experiment (Fig 2H).

***Whole-body composition of the offspring*:** Whole-body composition parameters at 10 weeks post-weaning were recorded using DEXA (Table 1). Percent lean body mass was significantly higher in the LQP and LP than NP-offspring. LQP and LP-offspring had significantly lower total body surface area, BMC, BMD, total, lean, and fat mass than in NP-offspring at 10 weeks after weaning. Percent LBM was significantly higher in the LQP and remained unchanged in

LP than NP-offspring. Percent BF was significantly lower in the LQP than NP-offspring. Total body surface area, BMC, BMD, total mass, and lean mass, was significantly higher in the LQPR and LPR than LQP and LP-offspring, respectively.

Table 1: Effect of maternal protein restriction followed by rehabilitation on whole-body composition and metabolic factors in offspring at 10 weeks after weaning

	NP (n=6)	LQP (n=6)	LQPR (n=6)	LP (n=6)	LPR (n=6)
Area (cm^2)	57.48 ± 8.33^{a}	15.02 ± 3.39^{b}	44.29 ± 5.12^{c}	30.81 ± 2.57^{d}	46.92 ± 8.28^{c}
BMC(g)	8.43 ± 1.32^{a}	1.51 ± 0.38^{b}	6.05 ± 0.92^{c}	3.33 ± 0.42^{d}	6.5 ± 1.16^{c}
BMD (g/cm^2)	0.14 ± 0.007^{a}	0.10 ± 0.01^{b}	0.13 ± 0.006^{a}	0.10 ± 0.006^{b}	0.13 ± 0.007^{a}
Total mass (g)	311.58 ± 64.93^{a}	52.2 ± 9.38^{b}	205.75 ± 49.25^{c}	119.45 ± 18.03^{b}	234.11 ± 55.01^{cd}
Lean mass (g)	233.74 ± 49.05^{a}	45.48 ± 8.06^{b}	164.06 ± 39.9^{c}	87.48 ± 12.2^{b}	180.71 ± 43.53^{ac}
% LBM	77.76 ± 2.77^{a}	90.13 ± 4.49^{b}	82.83 ± 4.07^{ac}	76.19 ± 2.21^{ac}	79.92 ± 1.85^{ac}
Fat mass (g)	69.41 ± 18.36^{a}	5.21 ± 2.85^{b}	35.63 ± 12.52^{c}	28.65 ± 5.98^{c}	46.88 ± 11.37^{c}
% BF	22.23 ± 2.75^{a}	9.91 ± 4.42^{b}	17.16 ± 4.05^{ac}	23.81 ± 2.1^{ac}	20.08 ± 1.87^{ac}

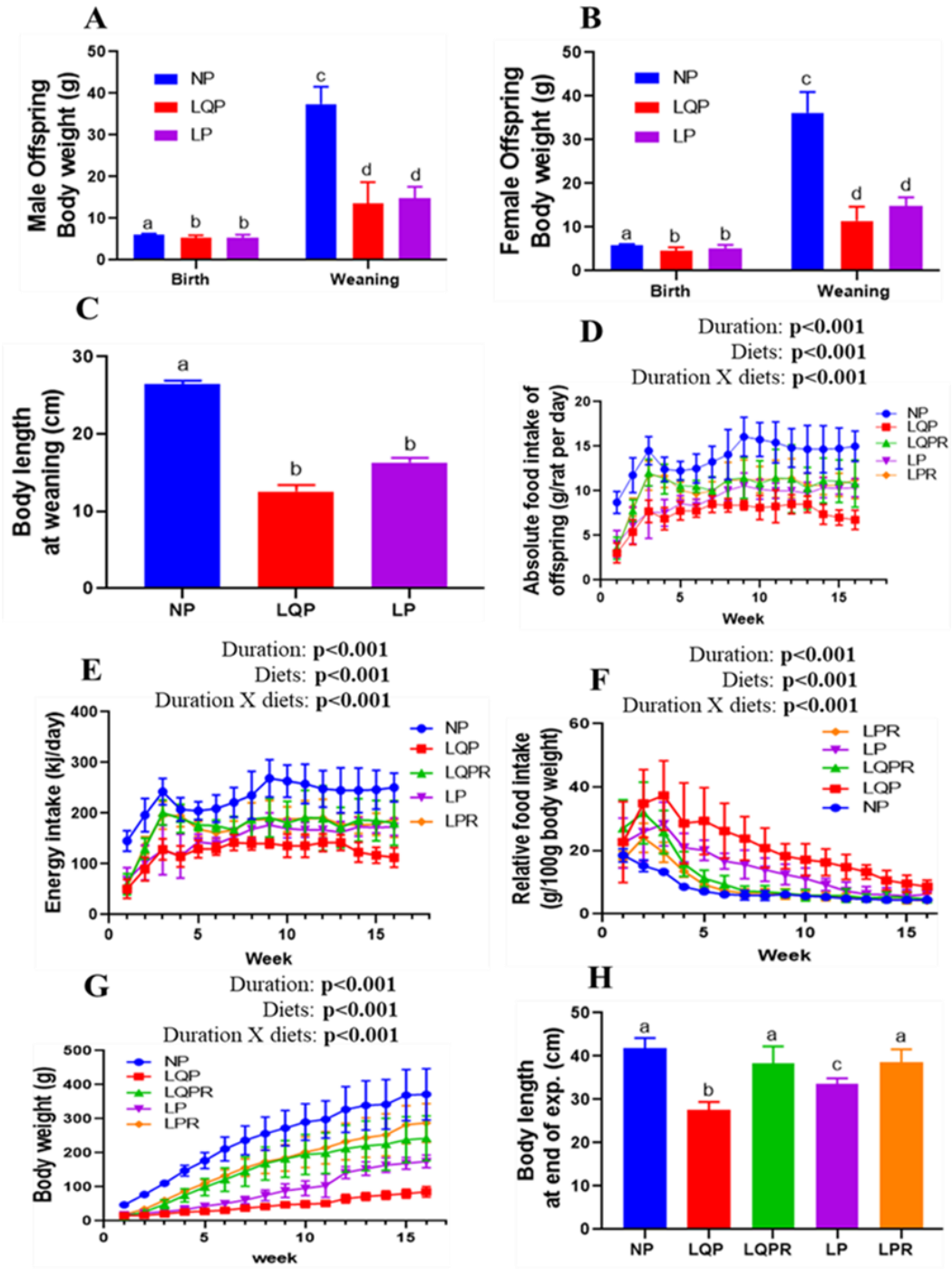
Whole-body composition of the offspring was assessed by dual-energy x-ray absorptiometry scanning at 10 weeks after weaning. Data represented as the mean ±SD. (n=6 per group). Mean values that share different superscript letters differ significantly from each other ($p<0.05$).NP, normal protein; LQP, low-quality protein; LQPR; low-quality protein group rehabilitated with NP diet, LP, low-protein; LPR, low-protein group rehabilitated with NP diet.

MLP diet is associated with increased muscle protein degradation in the offspring: The total protein degradation (TPD) was significantly higher in the LP offspring compared to NP (Fig 3A). However, TPD tended to be lower in LPR offspring than in the LP. It has been reported that 3-MH is a reliable index for skeletal muscle protein degradation. 3-MH was also significantly higher in the LP compared to NP offspring (Fig 3B). Moreover, excretion of 3-MH was significantly lower ($p<0.001$) in LPR offspring compared to LP offspring (Fig 3B).

***MLP diets associated with increased activity of UPS in the muscle of offspring*:** We found that prolonged MLP diets significantly increased the E1, atrogin1, UCHL5, ubiquitin-conjugates in LP offspring than the NP (Fig 3C-H). In the same line, chymotrypsin-like activity of proteasome was also significantly decreased in LP offspring compared to NP (Fig 3I). But, E1 levels were significantly increased while UCHL5, ubiquitinated proteins tended to decrease in LPR compared to LP offspring.

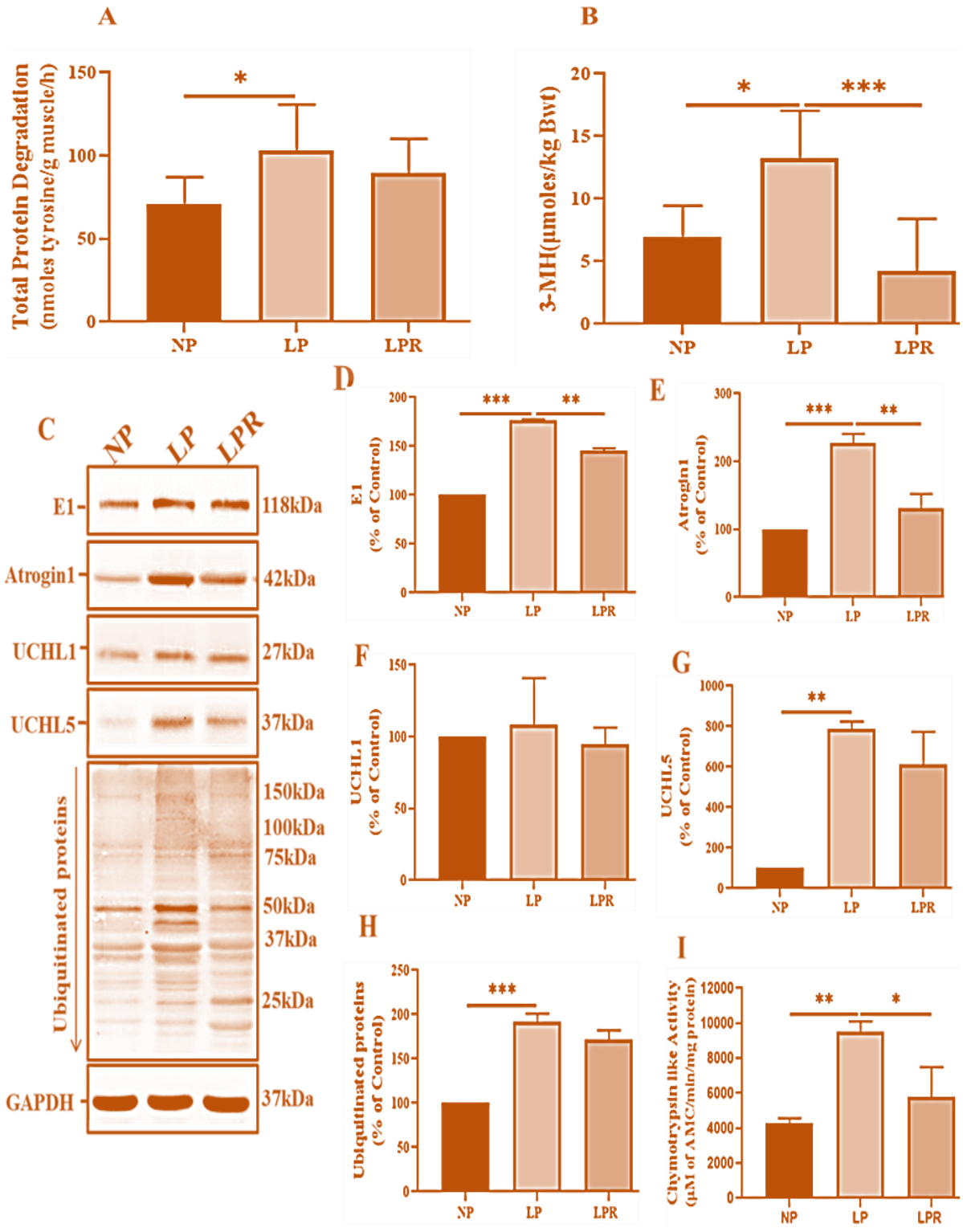
***MLP diets lead to altered expression of muscle-specific E3 ubiquitin ligases in the SM of offspring*:** We investigated the mRNA expression of E3 ubiquitin ligases (*atrogin1/MAFbx*, *MuRF1*, *TRIM72*), *MSTN*, *MYOG* using qRT-PCR and found that MLP diets caused a significant increase in the expression of the *atrogin1*, *MuRF1* (1.5-fold), *TRIM72* (6-fold), and *MSTN* (17-fold) in the muscle of the LP offspring compared to NP (Fig 3J-M). On the other hand, expression of *MYOG* was significantly decreased in LP offspring compared to NP (Fig 3N).

Fig 2. Effect of maternal protein restriction followed by rehabilitation on body weight, food intake, and body length in the offspring



(A) body weight of the male pups at birth and weaning. (B) BWs of the female pups at birth and weaning (C) body length of the pups at weaning (D) absolute food intake (E) energy intake (F) relative food intake (G) weekly BWs of the offspring from weaning to end of the experiment (H) body length of the offspring at the end of the experiment. Data were tested using two-way ANOVA and one-way ANOVA. The bars indicate the mean ± SD, and the bars that share different superscript letters differ significantly (n=10 -12 per group; $p < 0.05$). NP, normal protein; LQP, low-quality protein diet; LQPR; low-quality protein group rehabilitated with NP diet, LP, low-protein; LPR, low-protein group rehabilitated with NP diet.

Fig 3. Chronic MLP diets increases skeletal muscle proteolysis and UPS components in the offspring



(Panel A) the total protein degradation TPD was measured by the amount of free tyrosine released into the medium ***(Panel B)*** estimation of 3-MH excreted in the urine. Values are the mean ± SD, *n* = 6-8; *p<0.05, and ***p<0.001. (***Panel C***) representative western blots of UPS components, (***Panel D-E***) quantification of western blots NP, normal protein; LP, low-protein; LPR, low-protein group rehabilitated with NP diet.

INFERENCE AND CONCLUSION

The important findings of the study are as follows: chronic MLQP and MLP diets i)profoundly reduced the BWs by lowering LBM, fat mass, ii) enhanced the insulin sensitivity by promoting hypoglycemia and hypoinsulinemia in the offspring, iii) rehabilitating of LQP and LP-offspring with NP diet from weaning induced the catch-up growth by completely or partially reversing the above outcomes, iv) Thyroid profile, lipid profile, FG, and relative weight of few organs have shown the differential effect in MLQP and MLP diets, v) increased the skeletal muscle protein degradation, vi) activated the major proteolytic systems; UPS, and autophagy, vii) increased the expression of genes associated with muscle-specific E3 ubiquitin ligases, myostatin and decreased the myogenin and viii) rehabilitation with NP diet from weaning restored the above outcomes partially or completely to NP offspring.

In conclusion, we report that chronic MLP and MLQP diets induce differential adverse effects by programming the body composition and metabolism followed by reversible changes upon rehabilitation with NP, which may have implications in infant and young child supplementary nutrition programs in developing countries. Prolonged MLP diets induced muscle atrophy and accelerated SM proteolysis by augmenting the PQC components and reversed by rehabilitation.

4. ANTI-CANCER EFFECT OF CINNAMON EXTRACT AND ITS ACTIVE COMPONENT PROCYANIDIN B2 IN A RAT MODEL OF PROSTATE CANCER

Cinnamon is a wonder spice and is known to possess many health beneficial activities such as anti-diabetic, anti-inflammatory, anti-microbial, anti-oxidant and anti-cancer. Cinnamon extract (CE) is reported to induce tumor cell death in cancer cell lines, by mechanism(s) such as NFkB and AP1 inhibition or alteration of mitochondrial membrane potential. Procyanidin B2 (PCB2) was recently isolated and characterized as the active component from C.*zeylanicum* and was demonstrated to ameliorate cataract and nephropathy in a diabetic rat model. Our recent work, demonstrated that cinnamon extract and its active component PCB2 inhibited proteasome activity, both in a purified 20S system and cellular 26S proteasome from cancer cells. Furthermore, we also reported the anti-cancer potential of cinnamon extract and its PCB2-enriched fraction in human prostate cancer cells. Interestingly, both CE and PCB2 had a minimal effect on the viability in normal lung cells. Hence, in the present study we have assessed the proteasome-inhibitory and anti-cancer potential of cinnamon and its active components cinnamaldehyde [CNMD] and the PCB2-enriched fraction *in vivo* in a rat model of prostate cancer.

The following are the broad objectives:

1) To induce prostate cancer in a rat model using a chemical carcinogen.
2) To assess the efficacy of cinnamon and its active components CNMD and PCB2 as chemo preventive agents *in vivo.*

METHODOLOGY

Animal experiment was conducted using adult rats [3 months old] with the following experimental protocol. Control (CONT) was administered only the vehicle (sesame oil); Cancer Induced (CI) were given MNU (*N-methyl N-nitroso urea*) + TP (*Testosterone propionate*); CI+LCN were given MNU + TP + Cinnamon powder (1.2g/kg BW); CI+ HCN were given MNU + TP + Cinnamon powder (2.4g/kg BW); CI + PCB2 were given MNU + TP + PCB2-enriched fraction (750ug/kg BW); CI + CNMD were given MNU + TP + CNMD (150 mg/kg BW); CI + MG-132 were given MNU + TP + MG-132 (500 ug/kg BW through IV route).Animals were fed on the respective diets and sacrificed at the age of 8 months. Histopathological analysis of the prostate tissue was carried out using hematoxylin and eosin staining method. Thiobarbituric acid reactive substances [TBARS], the activities of catalase (CAT) and superoxide dismutase (SOD) were measured using standard methods. DNA oxidation was assessed by measuring the amount of 8(OH)dG using a kit and expressed as 8OHdG%.mRNA levels of different genes were studied by qPCR, while the expression of the proteins was done by western blotting using specific antibodies.

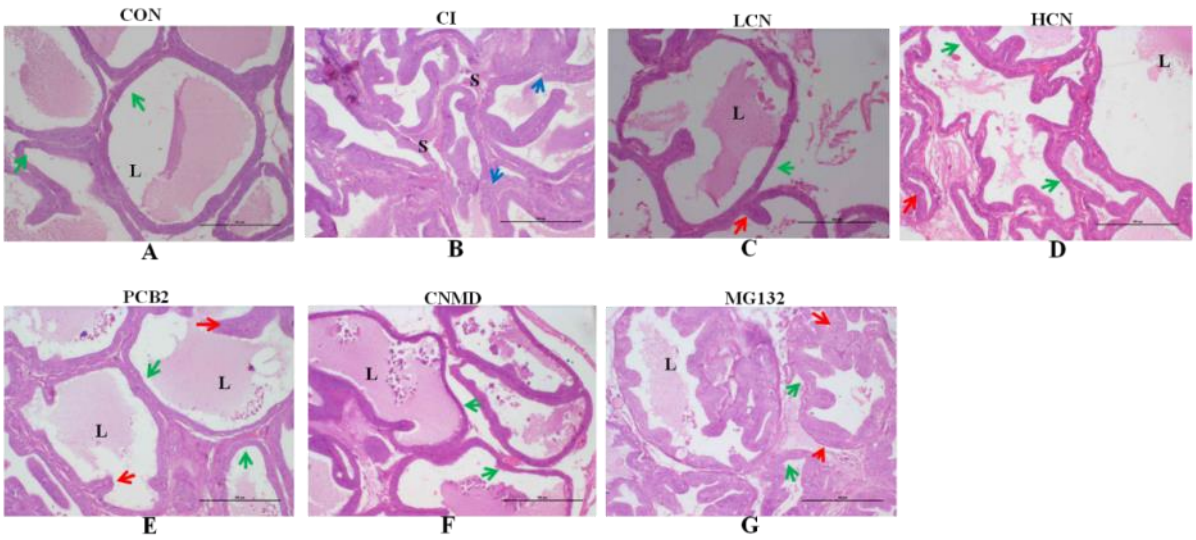
RESULTS

- Histopathological analysis was done by staining prostate sections from the different experimental groups using H&E. In control group [Fig1A], all animals (10/10) in the control group exhibited normal prostate with large lumina with some filled with prostatic epithelial secretions and were lined by a single layer of cuboidal to columnar epithelial cells. As expected in the *CI* group [Fig 1B], majority of the animals (8/10) showed Prostate Intraepithelial Neoplasia (PIN) changes with epithelium lining these glands showing mild dysplasia and stratification ranging from 2-5 layers with crowding and disorientation of the cells with narrowing of the lumen. In the *LCN* [Fig 1C] group animals displayed predominantly hyperplastic changes in the epithelium while, 4/10 had normal prostate. In the *HCN* & *CNMD* groups [Fig 1D & 1E] animals showed hyperplastic changes in the epithelium and 7/10 animals showed histologically normal prostatic epithelium. In the *PCB2* [Fig 1F] group 4/10 animals showed hyperplastic changes while the remaining 6/10 animals showed normal prostatic epithelium with absence of dysplastic epithelium. In *MG132* [Fig 1G] group, 1/10 animals showed features of low-grade PIN, 5/10 animals showed hyperplastic prostate and 4/10 animals (40%) were normal. Representative pictures of H&E stained prostate sections are shown in Fig 1.

- TBARS [as a measure of lipid peroxidation] and the anti-oxidant enzymes [SOD & CAT] were estimated in prostate tissue lysates from the control, CI and treatment groups. The CI group showed significantly ($p<0.01$) higher amount of TBARS compared to control group [Fig 2A]. Treatment with either cinnamon, PCB2. CNMD and MG132 appeared to decrease the lipid peroxidation in the prostate tissue. The SOD activity appeared to be lower in the

CI group than control group. Treatment with either cinnamon, PCB2, CNMD and MG132 appeared to increase the SOD activity [Fig2B]. On the other hand, the activity of catalase enzyme was significantly ($p<0.001$) higher in the CI group compared to controls. Treatment with either cinnamon, PCB2. CNMD and MG132 appeared to decrease the catalase activity [Fig 2C].The 8OHdG levels were significantly ($p\leq0.01$) higher in the CI group compared to control group [Fig 2D]. Treatment with cinnamon, its bioactive compounds and MG132 significantly ($p\leq0.05$ or 0.01) decreased the 8OHdG levels, except for PCB2 group which was not different from the CI group. There was a significant ($p\leq0.01$) decrease in mRNA levels of GSTP1 in the *CI* group compared to control. Treatment with either cinnamon, its bioactive components significantly ($p \leq0.001$) increased the levels of GSTP1 mRNA [Fig 2E]. The results are shown in Fig. 2.

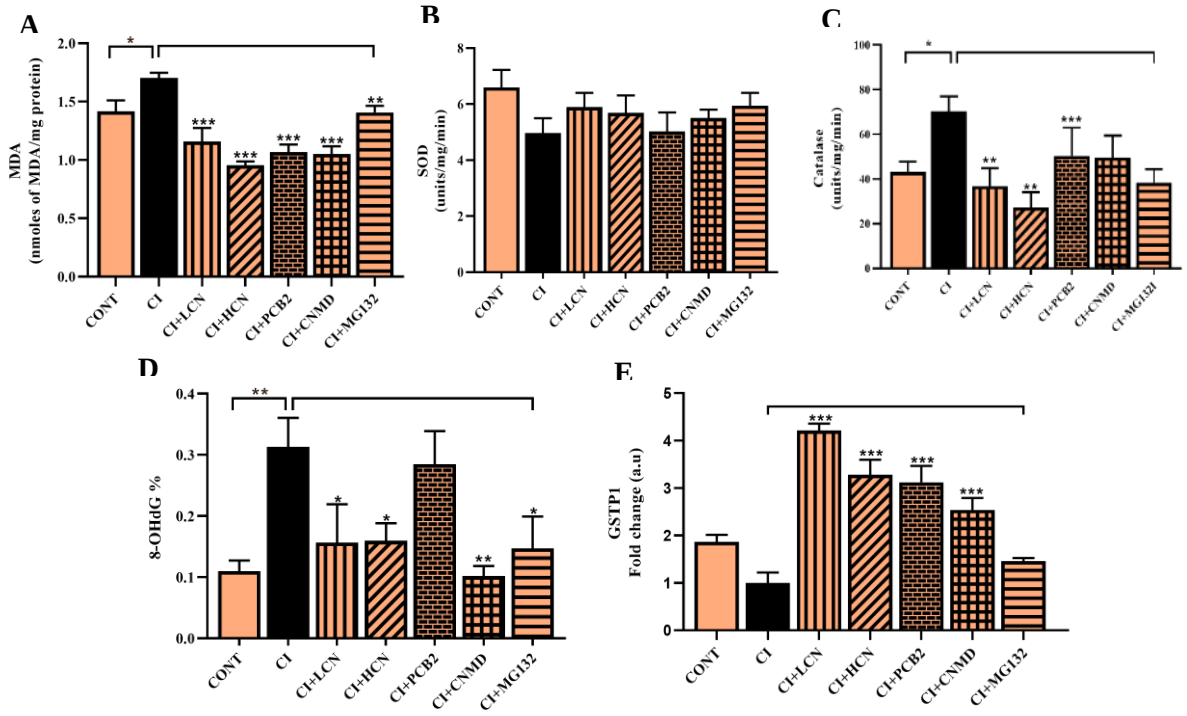
Fig 1. Cinnamon and its bioactive compounds reverted the histological changes induced by the carcinogen in the prostate tissue



Microphotograph of rat prostate stained with Hematoxylin and Eosin stain at 20X magnification. (A) Control (*CONT*) group (B) Cancer Induced (*CI*) group(C) CI+LCN group. (D) CI + HCN group (E) CI+PCB2 group. (F) CI+CNMD group (G) CI+MG132 group.

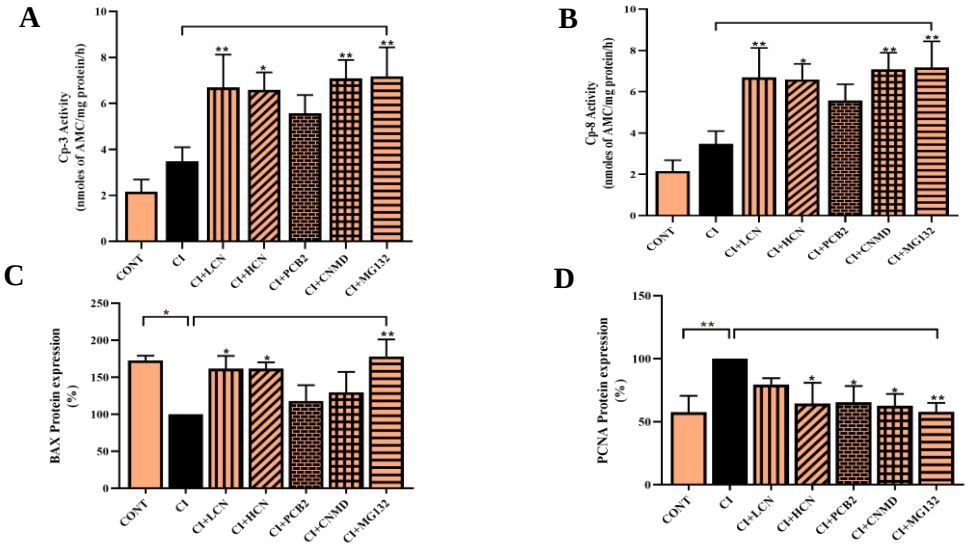
- The levels of caspase-3, caspase-8 activity [markers for apoptosis] and the protein levels of pro-apoptotic protein [Bax], proliferative cell nuclear antigen [PCNA] – a marker for cell proliferation were assessed in prostate tissue of all groups. The caspase-3 and caspase-8 activities were significantly ($p\leq0.01$) higher in the treatment groups compared to control group [Fig 3A & 3B]. The protein levels of Bax were significantly ($p\leq0.05$) decreased in the *CI* group compared to control and there was an increase in the expression of Bax in the treated groups compared to control group [Fig 3C]. PCNA levels were significantly ($p\leq0.01$) higher in the *CI* group compared to control group. Treatment with cinnamon or its bioactive components or MG132 significantly ($p\leq0.05$ or 0.01) decreased the PCNA levels [Fig 3D].

Fig 2. Cinnamon and its bioactive compounds alter the levels of oxidative stress parameters and decreases proteasomal activity in the prostate tissue



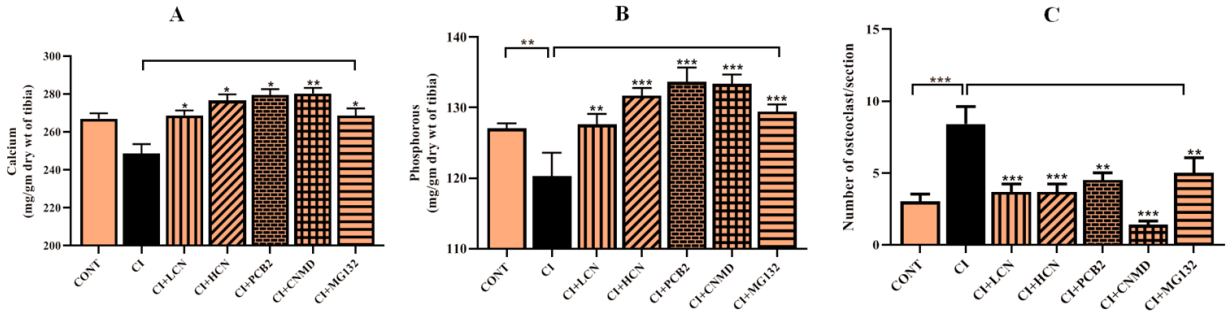
MDA content (A), SOD activity (B) Catalase activity (C), 8OHdG levels (D) and mRNA levels of GSTP1 (E) in the prostate tissue. Data is presented as mean ± SEM of 6 animals in each group. Data are considered statistically significant at a probability level of $p \leq 0.05$ (*); $p \leq 0.01$(**); $p \leq 0.001$ (***). Data with red asterisk shows Cancer Induced (CI) is significantly different from the control group and data with black asterisk shows the treatment groups are significantly different from CI group.

Fig. 3. Cinnamon and its bioactive compounds induce apoptosis and decrease proliferation of the prostate tissue



(A) caspase 3 activity; (B) caspase-8 activity, (C & D) depicts the quantification of protein expression of Bax& PCNA. Data is presented as mean ± SEM of 6 animals in each group. Data are considered statistically significant at a probability level of $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***).

Fig 4. Cinnamon and its bioactive compounds increase bone mineral content and decrease osteoclasts



A and B shows the calcium and phosphorous levels in tibia bone in cancer induced and treated groups. C depicts the number of osteoclasts observed in H&E stained sections of tibia bone. Data is presented as mean ± SEM of 6 animals in each group. Data are considered statistically significant at a probability level of $p \leq 0.05$ (*); $p \leq 0.01$(**); $p \leq 0.001$ (***).

INFERENCE AND CONCLUSION

In this study we assessed the chemopreventive efficacy of cinnamon and its bioactive compounds in a rat model of prostate cancer. We demonstrate that cinnamon and its bioactive compounds were able to reverse the histological changes such as hyperplasia and PIN observed in the cancer-induced group. Cinnamon and its active compounds also acted as anti-oxidant, anti-angiogenic, anti-metastatic and pro-apoptotic agents in this rat model. Hence, cinnamon and its bioactive compounds act as chemopreventive agents in this pre-clinical model. Further this work warrants testing the efficacy of cinnamon and its compounds in clinical trials.

5. EXPLORING THE PROTECTIVE EFFECT OF GAMMA ORYZANOL ON DIET-INDUCED MODEL OF NON-ALCOHOLIC STEATOHEPATITIS

Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of liver diseases ranging from simple accumulation of fat, nonalcoholic steatohepatitis (NASH) which progresses to cirrhosis and hepatocellular carcinoma. Despite the significant burden on the public health system, currently there are no FDA-approved drugs for the treatment of NAFLD. Hence dietary and lifestyle management is the only option for the management of NAFLD. The dietary supplementation of phytochemicals has proven beneficial in managing the features of NAFLD. Gamma oryzanol (GO), a phytochemical from rice bran oil has gained worldwide attention as a nutraceutical in recent years. GO is a potent antioxidant and more effective than vitamin E in reducing oxidative stress induced degenerative diseases. It is known to reduce plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride levels thereby

preventing atherosclerosis through decreased cholesterol absorption, increased fecal excretion of cholesterol and bile acids, and decreased hepatic biosynthesis of cholesterol. Since GO is known to have anti-inflammatory and antioxidant effects, and as inflammation and oxidative stress play a crucial role in the progression of NAFLD, it is of interest to study the protective effect of GO on the prevention of NAFLD.

AIMS AND OBJECTIVES

1. To establish Western diet (High calorie diet containing high saturated fat, cholesterol and sucrose) induced NASH with fibrosis model in C57BL/6 mice.
2. To study the impact of GO on lipid metabolism, oxidative stress, and inflammation in Western diet induced NASH model.

METHODOLOGY

To establish diet-induced model of NASH, weanling male C57/BL6 mice (n=36) were fed chow diet for one month and after that they were randomly divided into two groups (n=18 per group) – Control and Western diet (WD) groups. The control group was fed on standard diet (containing 10 % fat) and normal drinking water whereas the WD group was fed on diet (containing 21 % milk fat, 42% sucrose and 1.25 % cholesterol) and the drinking water was supplemented with high fructose/glucose (55% fructose and 45% glucose) mimicking High Fructose Corn Syrup (HFCS) consumption for a period of 16 weeks. At 8, 12 and 16 weeks, a subgroup of animals (n=6) from each group fasted for 6 hrs and blood samples were collected, plasma was separated and analyzed for glucose, alanine aminotransferase (ALT) activity, triglycerides and cholesterol levels. The animals were euthanized by CO_2 asphyxiation. Liver samples fixed in formalin, embedded in paraffin wax and cut into 5 µm sections for hematoxylin and eosin (H and E) staining to study steatotic and inflammatory changes. Hepatic fibrosis was assessed by Masson's trichrome staining.

After establishing the diet-induced model of NASH, an interventional study was done to investigate the impact of GO supplementation on WD-induced NASH. Male C57/BL6 mice were divided into five groups consisting of 8 animals in each group. The various groups were as follows: Control group – Mice were fed a chow diet and sunflower oil was administered orally as a vehicle. WD group – Mice were fed WD and sunflower oil was administered orally as a vehicle. WD + GO low dose group – Mice were fed WD and GO (100mg/kg body weight) dissolved in sunflower oil was administered orally. WD + GO mid-dose group - Mice were fed WD and GO (300mg/kg body weight) dissolved in sunflower oil was administered orally. WD + GO high dose group - Mice were fed WD and GO (600mg/kg body weight) dissolved in sunflower oil was administered orally. Control mice were given normal drinking water whereas WD groups were provided drinking water containing 55% fructose and 45% glucose. At the end of the study (12weeks), animals fasted for six hours, blood was collected through cardiac puncture in EDTA tubes. Plasma was separated and stored at -80^{o}C for the analysis of biochemical parameters. The animals were euthanized by CO_2 asphyxiation. Liver, kidney, retroperitoneal fat (RP) and epididymal fat (EP) and mesenteric fat (MS) were excised quickly, rinsed with saline, weighed and snap frozen in liquid nitrogen and stored at -80 °C for further analysis. A small piece of the liver was stored in RNA Later for gene expression analysis and

another portion of the liver was immediately immersed in neutral buffered 10% formalin for histopathological examination. Adiposity index was computed as the sum of visceral adipose tissue (RP + EP + MS) weights and expressed as a percentage of total body weight.

RESULTS

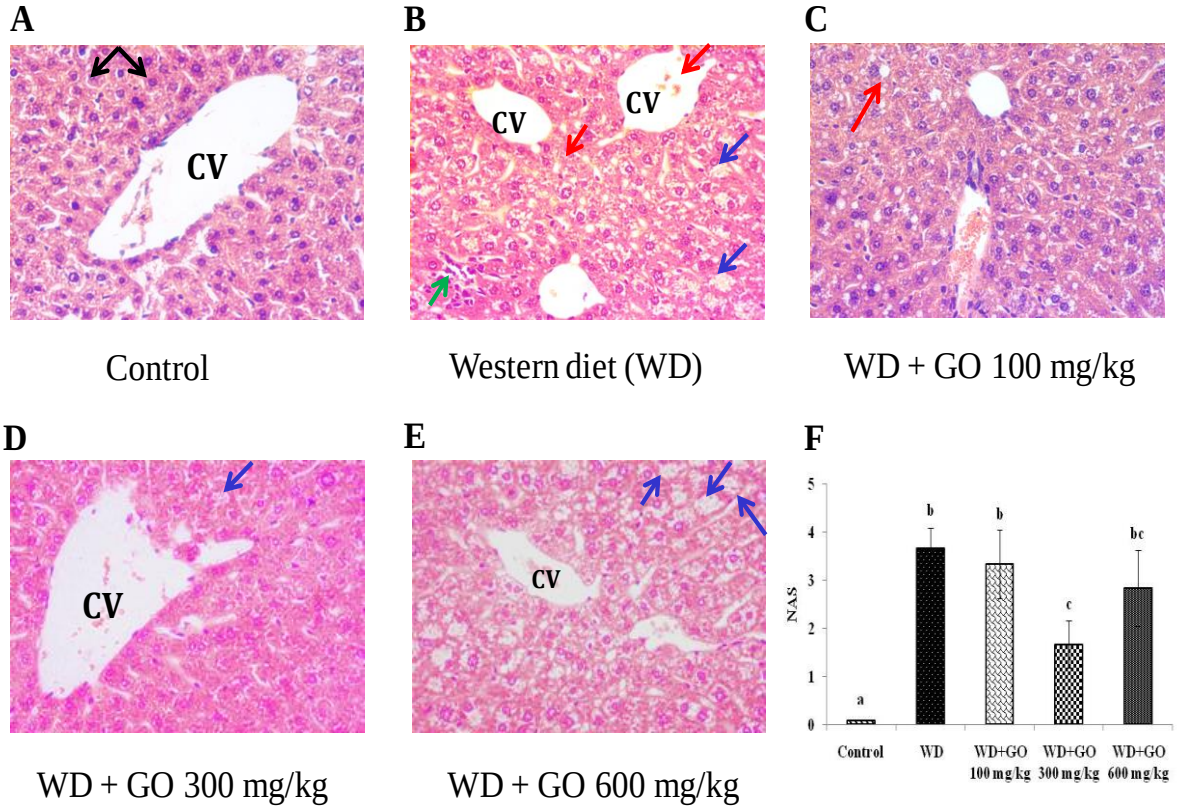
Mice fed WD for a period of 12 weeks developed NASH with fibrosis. Interventional studies with GO showed that WD feeding significantly increased the body weight as compared to mice reared on the control diet. The groups treated with GO at 100 mg/kg body wt. and 300 mg/kg body wt. normalized the body weight with respect to control. Liver weights were significantly increased in WD fed group as compared to the control group. However, groups treated with GO irrespective of the dosage showed a significant decrease in relative liver weights as compared to animals reared on the WD diet alone. The visceral adiposity was significantly increased in WD fed group as compared to the control group.

However, GO supplementation reversed WD induced visceral adiposity at 100 mg/kg body wt and 300 mg/kg body wt. Fasting plasma glucose levels were significantly higher in WD fed group as compared to the control group. However, irrespective of dosage, groups treated with GO significantly reduced plasma glucose levels. Plasma total cholesterol level was significantly increased in WD fed group as compared to the control group and supplementation with GO at 300 mg/kg body wt. significantly reduced plasma cholesterol levels. Plasma ALT level was significantly increased in WD fed group as compared to the control group. However, treatment with GO at 100 mg/kg and 300 mg/kg body wt., normalized the ALT. The liver triglyceride and cholesterol levels were significantly increased in WD fed group as compared to the control group. However, irrespective of the dosage, GO treatment significantly reduced the liver triglyceride and cholesterol levels. Mice fed WD significantly decreased plasma adiponectin and increased leptin levels as compared to control diet fed mice.

However, treatment with GO at 100 mg/kg body wt and 300 mg/kg body wt. did not alter plasma adiponectin. At a higher dose of GO (600mg/kg). Adiponectin level was further decreased. GO supplementation at 100 mg/kg body wt and 300 mg/kg body wt., normalized the leptin level but not at 600 mg/kg body wt. Histopathological evaluation of the liver (H and E Staining) showed normal hepatic architecture in the control diet fed group (Fig -1). However, the WD fed group showed steatosis predominantly of microvesicular type and focal collection of lymphocytes in the parenchyma. The group treated with GO at 100 mg/kg body wt., showed a moderate number of hepatocytes with macro and microvacuoles and GO at 600 mg/kg body wt. showed numerous hepatocytes with microvacuoles.

However, GO at 300 mg/kg body wt. showed few scattered hepatocytes with microvacuoles. The NAFLD activity score was significantly increased in WD fed group as compared to the control group indicating borderline NASH. The groups treated with GO at 100 mg/kg and 600 mg/kg body wt., were comparable with the WD fed group. However, GO at a dose of 300 mg/kg body wt significantly reduced the NAFLD activity score compared to WD fed group.

Fig 1. Representative liver histological image with H&E staining (20X magnification)



(A) Control diet fed animal showing normal hepatocytes with the central vein (CV) (B) Western diet fed animal showing many hepatocytes with microvacuoles and focal collection of lymphocytes in the parenchyma (C) Western diet fed and gamma oryzanol 100 mg/kg body wt., treated animal showing moderate number of hepatocytes with macro and microvacuoles (D) Western diet fed and gamma oryzanol 300 mg/kg body wt., treated animal showing few scattered hepatocytes with microvacuoles (E) Western diet fed and gamma oryzanol 600 mg/kg body wt., treated animal showing numerous hepatocytes with microvacuoles, (F) NAFLD activity score (NAS), n = 6 per group. Black arrows indicate normal hepatocytes, blue arrows indicate microvacuolation; Red arrows indicate macrovacuolation; Green arrows indicate focal collection of lymphocytes.

CONCLUSION

The results of the present study demonstrated that GO at a dose of 300mg/kg body wt could ameliorate WD induced NAFLD. In addition, GO supplementation also reduced visceral adiposity. Given the high prevalence of NAFLD and non-availability of pharmacological interventions for their management, GO appears as a promising dietary intervention with potential beneficial effects in the management of NAFLD.

IV. Food Composition and Nutrient Availability

EFFECT OF TRADITIONAL COOKING ON PHYTONUTRIENT CONTENT AND RADICAL SCAVENGING ACTIVITY IN CEREALS AND MILLETS

Cereals and grains not only provide more than 50% of world's caloric intake and protein intake but are also good source of other micronutrients. Whole grains are rich sources of fibre, vitamins, minerals and phytochemicals such as phenolics, lignans, B-glucan, inulin, resistant starch, sterols and phytates. Millets especially have high protein, essential fatty acids, dietary fibre, B-vitamins and minerals and help in rendering health benefits like reduction in blood sugar, blood pressure regulation, thyroid, cardiovascular and celiac diseases and many other age related chronic diseases. Millets are rich in several phytochemicals possessing a number of bioactivities that could impart beneficial health effects to prevent and delay the occurrence of non-communicable diseases (NCDs). The consumption of millets as food has significantly declined over the past three decades.

Phenolic compounds are one of the most highly diversified groups of phytochemicals found in all plant organs and are therefore an integral part of the human diet. A unique array of free phenolic compounds along with their glycosides and esters are present in cereal and millet. Therefore, quantifying them individually is important. Phenolic compounds are concentrated in the bran layers and are liable to losses during the separation of seed coat in the milling process. Phenolic compounds such as ferulic acids and dehydrodiferulates in whole grains shows unique presence in cereal grains. Recent studies also have demonstrated that the form of phenolic compounds as well as their proportions varies with the type of millet. Phenolic compounds of finger millets are concentrated in the seed coat and their content in the seed coat and flour fraction were 6.2% and 0.8%, respectively.

A number of studies have reported the antioxidant activity of the extracts of several millet grains. In these studies antioxidant activity of grain extracts displayed through their free radical scavenging, reducing power, ferrous chelating properties and inhibition of reactive oxygen species was demonstrated, all of which play a major role in the inhibition of oxidation of lipids. Hence, millets are beneficial as radical scavengers and can inhibit a number of oxidative reactive species. Due to their antioxidant power, a number of intervention studies were also carried out to demonstrate their beneficial role of millets in health and disease.

However, millets and grains undergo through various processing before consumption. In India, traditional processing methods include soaking, fermentation, cooking or heat treatment, malting, all of which causes changes in the phenolic content present in the millets. However,

there is limited information on the phenolic content and antioxidant activities of different types of millets and grains from India and especially on millets after undergoing the traditional cooking methods followed by Indians. Therefore, the study will be carried out with the following objectives.

OBJECTIVES

1. To determine total and individual polyphenol content in raw and cooked cereals & millets.
2. To determine free and bounded polyphenols in raw and cooked cereals & millets.
3. To determine total flavonoids in raw and cooked cereals & millets.
4. To determine antioxidant activity (DPPH, FRAP, ABTS) in raw and cooked cereals & millets.

Work done during the period: Three millets, namely sorghum (*Sorghum vulgare*), pearl millet (*Pennisetum typhoideum*), and finger millet (*Eleusine coracana*), were purchased, 2 kilogram (kg) each from three different markets of Secunderabad and Hyderabad, Telangana and composited into single (totally six kilogram) sample for the subsequent studies. Traditionally prepared curd was purchased from a local shop, Secunderabad, Telangana.

SAMPLE PREPARATION

All the millets were cleaned by removing unwanted foreign particles if any (Fig 1). The whole sorghum and pearl millet were soaked in water for four hours, and then the water was drained. The soaked millets were spread on a blotting paper at room temperature for 30 mins. They were coarsely ground using a domestic mixer grinder. The pearl and powder were separated using a sieve (60 mesh, 0.25 mm). Initially, the pearl was added to the boiling water and cooked until it turned soft, and then the powder was added and allowed to cook further. Finger millet was made into fine flour using a commercial flour mill. The flour was added to boiling water (in the ratio of 1:2 w/v) and stirred continuously to avoid the formation of lumps. The sample was cooked until it turns into a thick paste. Then the paste was made into small round balls (dumplings). All the three cooked millets were allowed to bring to room temperature then divided into four equal parts. The first part was stored as such at -20°C in air-tight containers until further analysis. The second part was mixed with an equal amount of curd and kept for overnight fermentation. Water was added (upto submersible level) to the third and fourth part of the cooked millet and stored at room temperature overnight for fermentation. After overnight fermentation, the fourth part was mixed with an equal quantity of curd. All the samples were homogenized using a domestic mixer grinder and stored at -20°C in air-tight containers till further analysis. All the cooking and subsequent processing were carried out using stainless steel vessels.

Determination of phytic acid, minerals, and phytate mineral molar ratio: Total phytic acid (IP6) was analyzed by the anion exchange method (AOAC, 986.11). Phytic acid was extracted using 2.4% HCl. First, columns were prepared by adding 0.5 g AG 1-X4 resin, then 5 ml of distilled water was added for resin bed formed. Then 15 ml of 0.1 M NaCl was added to remove any contaminating phosphate ions; again, 15 ml of water was added to wash columns. Samples

were prepared with EDTA-NaOH reagent, poured into columns, and let stand for 20 minutes, then washed with 15 ml water and 0.1 M NaCl to remove unbound foreign materials and lower inositol phosphates, respectively. The resin was eluted with 15 ml 0.7 M NaCl to release the bound inositol hexaphosphate (phytate) and collected into 100 ml Kjeldahl flasks. Then glass beads (3 no.), 3.0 ml HNO_3 and 0.5 ml H_2SO_4 were added to the Kjeldahl flasks and digested under the hood over medium heat until the cloud of thick yellow vapor fills the neck of the flask. Then flasks were cooled, and salts formed were dissolved in water then transferred into 50 ml volumetric flasks. Ammonium molybdate solution (2.0 ml) and sulfonic acid reagent (1.0 ml) were added, mixed well, and made up the volume with distilled water. The mixture was incubated for 15 min at room temperature. The absorbance of sample solutions was measured at 640 nm using a UV visible spectrophotometer.

The phytate concentration in food samples calculate (phytate contains 28.2% phosphorus):

$$\text{Phytate (mg/g sample)} = \frac{\text{make up volume} \times \text{mean K} \times \text{absorbance}}{\text{weight of sample} \times 0.282 \times 1000}$$

Where, mean k is the mean of concentrations of standard divided by absorbance of standard.

Homogenized and/or ground samples (~1g) digested using Suprapure nitric acid (67% v/v) and Hydrogen peroxide (30% v/v) in the ratio of 2:1 (v/v), in a microwave Mars Xpress CEM. Samples were allowed to cool to room temperature, then made up to 25 ml using volumetric flasks and analysed for minerals such as Fe and Zn content. The mineral was determined using Atomic Absorption Spectrophotometer (Analytikjena ContrAA 700) operated with Aspect CS 2.2.1.0 tech software. Absorbance for Fe was taken at 248.32 nm and for Zn at 213.86 nm.

The molar ratios between phytic acid and minerals were calculated by dividing the mole of phytate with a mole of mineral content using the following formula (FAO/IZiNCG, 2018).

$$\text{Phytic acid : Mineral Molar ratio} = \frac{\frac{\text{PA}}{\text{MW (PA)}}}{\frac{\text{Min}}{\text{MW(Min)}}}$$

Where, PA = Phytic acid analysed; MW (PA) = Phytic acid molecular weight (660.06 Da); Min= Mineral content (Zn/Fe); MW (Min) = Mineral molecular weight (Fe = 55.845 Da; Zn = 65.38 Da.

DETERMINATION OF WATER-SOLUBLE VITAMINS

DIONEX (make) Ultimate 3000 (model) Ultra High-Performance Liquid Chromatographic system (UHPLC) with Chromeleon software was used for the analysis and quantification of water soluble vitamins in food samples. After extraction, tubes were centrifuged at 4000 rpm at 10°C for 10 minutes. The supernatant was collected and filtered into amber coloured HPLC autosampler vials with 0.45 µm PVDF syringe filter. Vitamins were separated on a reverse-phase chromatographic column. Thermo-scientific BDS hypersil C18 column 250x4.6mm, 5µ particle size was used for vitamins except for Vitamin B_9 with the flow

rate adjusted to 1 ml/min except for B_6 (0.8 ml/min) and B_9 (0.5 ml/min). Phenomenex Luna 3 μm C18 (2) 150x2mm LC column was used for B_9.

For the determination of vitamins B_2 and B_3, a sample (1 gram) was extracted with 0.1M HCl. The column temperature was maintained at 40°C. Phosphate buffer (0.05 M, pH 3.2) and acetonitrile were used as mobile phase in the ratio 70:30. Diode Array Detector (DAD) was used for quantification of B_2 with excitation λ at 445 nm, emission λ at 522 nm. While, Phosphate buffer (25 mM, pH 3.02) and acetonitrile were used as mobile phase in the ratio 95:5, and fluorescence detector (FLD) was used for B_3 at 260 nm. B_5 was extracted with 3% acetic acid. The mobile phase was phosphate buffer (0.1M, pH 2.25) and acetonitrile (95:5). The column temperature was maintained at 40°C. B_5 was quantified at 260 nm using a fluorescence detector. B_6 extraction was performed using 5% metaphosphoric acid. 25mM phosphate buffer with pH 3.2 and acetonitrile (70:30) was used as the mobile phase. The column temperature was 35°C for vitamin B_6. Diode Array Detector (DAD) was used for quantification of B_6 with excitation λ at 290 nm, emission at λ 395 nm. For B_9 samples were extracted by K_2HPO_4, ascorbic acid, sodium azide, and 2-mercapto ethanol along with enzymes α–amylase, protease, deconjugase, where SEP-PAK cartridge was used for sample purification. Phosphate buffer (pH 2.2) and acetonitrile were used as mobile phase in the ratio 95:5. Vitamin B_9 was estimated using Diode Array detector excitation λ at 220 nm and emission λ at 440 nm. Vitamin C was extracted with 3% metaphosphoric acid, 8% glacial acetic acid for analysis. The column temperature was maintained at 30°C for vitamin C. Mobile phase was phosphate buffer (0.05M, pH 3.2) and acetonitrile (90:10). Vitamin C estimation was performed at 260 nm using a fluorescence detector.

RESULTS

Proximate principles: The proximate principles (moisture, protein, ash, fat and dietary fibre) were analysed in the raw pearl millet (PMR), finger millet (FMR), sorghum millet (SMR), and traditionally processing its counterparts, and the results are given in Table 1. All proximate values are expressed as g in 100 g of the edible portion on dry weight basis. The protein content of raw millets such as PMR, FMR, and SMR were 9.45±0.37, 7.18±0.42, and 10.60±0.26 g/100g, respectively**.** Among traditionally processed millet foods, the significantly highest (p<0.05) amount of protein was found in SMFWC (20.57±0.37 g/100g) and PMFWC (20.27±0.27 g/100g), followed by FMFWC (16.92±0.07 g/100g). The lowest protein was found in SMC (9.91±0.18), PMC (9.30±0.06), and FMFW (7.88±0.12 g/100g).

Raw finger millet (FMR) contains the highest amount of ash (2.14±0.04 g/100g) compared to PMR (1.24 g/100 g) and SMR (1.35 g/100 g). Amidst different processed foods, the millet cooked, fermented overnight, and added curd had the highest ash content in all the three millets studied (SMFWC-2.56±0.06, PMFWC-2.61±0.14, and FMFWC-3.81±0.05 g/100g). The fat content of raw pearl millet was the highest (4.3 g/100g) among all raw and processed millets. However, raw sorghum (SMR) and finger millet (FMR) was higher than their cooked forms (1.40 and 1.36 g/100 g, respectively). Among the different processing, there was a significant reduction observed in the cooked millet fermented overnight then added with curd in pearl millet (0.64 g/100 g). In contrast, the lowest fat content among different processed finger millet was observed in the cooked finger millet (FMC-0.58 g/100g). Dietary fiber (both

insoluble and soluble) analysed in the raw, and cooked millets are represented in Table-1. The total dietary fiber content of sorghum was between 9.33 and 9.97 g/100g, while it was 10.13 and 11.4 g/100g in pearl millet and finger millet. Among the two dietary fiber fractions, more than 80% are from insoluble dietary fibre. Among three millets, carbohydrate content was observed to be higher in both finger millet and sorghum (around 77%) than pearl millet (75%). The cooked millets added with curd either before or after fermentation found to have lesser carbohydrate than other counterparts.

TOTAL PHYTIC ACID CONTENT

The total phytic acid content and its retention in traditionally processed sorghum, pearl millet, and finger millet is summarized in Figure 2. The total phytic acid content in raw millet flours was found to be 8.6±0.15 mg/g (SMR), 5.69±0.19 mg/g (FMR), and 4.77±0.07 mg/g (PMR). Reduction of phytic acid was observed in all the millets after the cooking (minimum of 16.14% and maximum of 49.18%) and in subsequent processing where samples were fermented with curd overnight also reduced phytic acid content (minimum of 20.96% and maximum of 54.53%). Among the different processing, the maximum reduction in total phytate was found in the millets fermented overnight, followed by added curd (FMFWC-3.75±0.06; PMFWC-3.37±0.17; SMFWC-3.28±0.09 mg/g).

MINERAL COMPOSITION

The iron (Fe) and zinc (Zn) content of raw and domestically processed sorghum, pearl millet, and finger millet are presented in Table 2. Amongst the three raw millets analysed, pearl millet flour had the highest zinc (3.32±0.15 mg/100g), followed by finger millet (2.09±0.09 mg/100g) and sorghum (1.95±0.01 mg/100g). Between processed millet samples, zinc content was found to increase in the fermented sorghum (with or without curd) samples. The raw sorghum contains 1.95 mg/100g, which was increased up to 2.72 mg/100g in SMFW. However, no significant ($p<0.05$) change was observed among the raw and processed pearl millet and finger millet (Table 2). Determination of iron content in three different millets revealed that raw finger millet contained the highest of iron (5.15±0.75 mg/100g) followed by sorghum flour (3.32±0.12 mg/100g) and pearl millet flour (3.29±0.19 mg/100g). Amid traditionally processed millet foods, the elevated Fe content was distinguished in the millets fermented overnight after traditional cooking (SMFW-7.24±0.52; PMFW-8.40±0.334 and FMFW-8.95±0.63 mg/100 g). The cooked millet fermented along with curd seems the second-highest Fe content. However, all the different processed millet foods appear with an increased iron concentration in all the three millets studied here.

ESTIMATION OF PHYTATE: MINERAL MOLAR RATIO

Phytate: mineral molar ratios (zinc and iron) were determined and given in Table 2. Raw millets were found to have higher ratio values than the processed. The highest molar ratio between phytate: Zn and phytate: Fe was recorded in the raw sorghum (43.79 and 21. 94, respectively). The molar ratio between phytate and Zn was 14.25 in the raw pearl millet and 26.98 in finger millet.

Table 1. Proximate principles of traditionally processed sorghum, pearl millet, and finger millet and their respective flours (g/100g)

Sample Name	Protein	Ash	Fat	Dietary fibre			Carbo hydrate	Moisture
				IDF	SDF	TDF		
	g/100g							
Sorghum								
SMR	10.60± 0.26^{c}	1.35± 0.05^{c}	1.40± 0.04^{a}	8.33± 0.02^{a}	1.09± 0.03^{a}	9.42± 0.1^{a}	77.23± 0.21^{a}	7.87± 0.07^{d}
SMC	9.91± 0.18^{d}	1.25± 0.04^{c}	1.24± 0.05^{b}	8.05± 0.14^{a}	1.28± 0.01^{a}	9.33± 0.14^{a}	78.28± 0.43^{a}	75.38± 0.5^{c}
SMFC	15.60± 0.07^{b}	2.36± 0.03^{b}	0.90± 0.09^{c}	7.96± 0.01^{a}	1.46± 0.02^{a}	9.42± 0.02^{a}	71.71± 0.90^{c}	83.61± 0.56^{b}
SMFW	10.13± 0.09^{cd}	1.22± 0.00^{c}	0.62± 0.02^{e}	8.00± 0.01^{a}	1.96± 0.01^{a}	9.97± 0.01^{a}	78.05± 0.49^{b}	87.86± 1.56^{a}
SMFWC	20.57± 0.37^{a}	2.56± 0.06^{a}	0.78± 0.01^{d}	8.01± 0.04^{a}	1.58± 0.41^{a}	9.60± 0.46^{a}	66.44± 0.81^{d}	88.80± 0.34^{a}
Pearl millet								
PMR	9.45± 0.37^{d}	1.24± 0.2^{b}	4.30± 0.02^{a}	9.02± 0.01^{a}	1.09± 0.02^{c}	10.11± 0.02^{c}	74.88± 0.43^{a}	7.87± 0.09^{d}
PMC	9.30± 0.06^{d}	1.35± 0.04^{b}	2.04± 0.12^{b}	8.34± 0.01^{c}	1.79± 0.03^{b}	10.13± 0.05^{c}	77.14± 0.25^{a}	72.97± 0.93^{c}
PMFC	16.46± 0.18^{b}	2.56± 0.04^{a}	1.43± 0.00^{c}	8.42± 0.03^{c}	1.85± 0.04^{ba}	10.28± 0.02^{b}	69.26± 0.68^{c}	81.80± 0.44^{b}
PMFW	10.49± 0.11^{c}	1.41± 0.48^{b}	0.91± 0.03^{d}	8.70± 0.03^{b}	1.93± 0.01^{ba}	10.64± 0.04^{a}	76.52± 0.79^{b}	87.81± 0.20^{a}
PMFWC	20.27± 0.27^{a}	2.61± 0.14^{a}	0.64± 0.02^{e}	8.11± 0.02^{d}	2.02± 0.02^{a}	10.13± 0.04^{c}	66.32± 0.38^{d}	88.24± 0.34^{a}
Finger millet								
FMR	7.18± 0.42^{d}	2.14± 0.04^{b}	1.36± 0.03^{a}	9.42± 0.13^{a}	2.08± 0.04^{a}	11.41± 0.10^{a}	77.91± 1.31^{a}	10.50± 0.00^{e}
FMC	8.09± 0.08^{c}	2.27± 0.09^{b}	0.58± 0.06^{d}	9.04± 0.03^{ab}	2.00± 0.02^{a}	11.13± 0.07^{ab}	77.95± 0.48^{a}	68.03± 0.51^{d}
FMFC	15.68± 0.09^{b}	3.70± 0.04^{a}	0.98± 0.01^{b}	8.66± 0.04^{bc}	1.79± 0.14^{a}	10.45± 0.10^{cb}	69.10± 1.03^{b}	77.88± 0.47^{c}
FMFW	7.88± 0.12^{c}	2.41± 0.07^{b}	0.64± 0.02^{d}	8.42± 0.02^{c}	1.85± 0.01^{a}	10.27± 0.03^{c}	78.79± 0.72^{a}	82.55± 0.79^{b}
FMFWC	16.92± 0.07^{a}	3.81± 0.05^{a}	0.83± 0.01^{c}	9.05± 0.01^{ab}	1.81± 0.02^{a}	10.86± 0.03^{b}	67.68± 0.72^{b}	84.12± 0.38^{a}

*Values represent mean ± standard deviation of triplicate analyses and values with same superscript within the column doesn't differ significantly at $p<0.05$ at one way ANOVA.

The Zn molar ratio was significantly reduced in all the processed millets after fermenting, followed by the addition of curd (SMFWC-13.60; PMFWC-9.48; FMFWC-10.48). The molar ratio between phytate and Fe in raw sorghum was 21.94 followed by raw pearl millet (12.26) and finger millet (9.34). Cooking and processing of millet were found to reduce the phytate: Fe molar ratios. The lowest Fe molar ratio was found in the cooked millets subsequently fermenting overnight in the case of pearl millet and sorghum (4.12 and 5.97, respectively). Whereas the cooked finger millet then fermented and added curd seems to be better for the lowest phytate: Fe molar ratio (4.85).

Table 2. Mineral content and phytic acid: mineral molar ratio of traditionally processed sorghum, pearl millet and finger millet samples

Sample Name	Zinc	Iron	Phytic acid mineral molar ratio	
	mg/100g		PA/Zn	PA/Fe
Sorghum				
SMR	1.95±0.01^{b}	3.32±0.12^{b}	43.79	21.94
SMC	2.19±0.11^{b}	3.88±0.12^{b}	19.80	9.50
SMFC	2.55±0.05^{a}	6.09±0.91^{a}	15.22	5.81
SMFW	2.72±0.05^{a}	7.24±0.52^{a}	18.61	5.97
SMFWC	2.59±0.02^{a}	4.16±0.04^{b}	13.60	7.24
Pearl millet				
PMR	3.32±0.15^{a}	3.29±0.19^{c}	14.25	12.26
PMC	3.34±0.13^{a}	5.77±0.34^{b}	11.86	5.87
PMFC	3.51±0.10^{a}	6.75±0.616^{b}	10.56	4.73
PMFW	3.54±0.04^{a}	8.40±0.334^{a}	11.46	4.12
PMFWC	3.52±0.02^{a}	5.71±0.215^{b}	9.48	5.00
Finger millet				
FMR	2.09±0.09^{a}	5.15±0.75^{b}	26.98	9.34
FMC	2.21±0.04^{a}	6.87±0.46^{ba}	21.35	5.85
FMFC	3.19±0.10^{a}	7.81±0.91^{a}	14.19	4.95
FMFW	3.39±0.738^{a}	8.95±0.63^{a}	21.53	5.21
FMFWC	3.54±0.231^{a}	6.55±0.64^{ba}	10.48	4.85

*Values represent mean ± standard deviation of triplicate and values with same superscript within the column doesn't differ significantly at $p<0.05$ at one way ANOVA.

Sorghum: Raw (SMR), Cooked with water (SMC), Cooked and fermented with curd overnight (SMFC), Cooked and fermented with water overnight (SMFW), Cooked fermented with water overnight added curd (SMFWC). Pearl Millet: Raw (PMR), Cooked with water (PMC), Cooked and fermented with curd overnight (PMFC), Cooked and fermented with water overnight (PMFW), Cooked fermented with water overnight added curd (PMFWC). Finger Millet: Raw (FMR), Cooked with water (FMC), Cooked and fermented with curd overnight (FMFC), Cooked and fermented with water overnight (FMFW), Cooked fermented with water overnight added curd (FMFWC).

Figure 2. Raw, cooked and fermented millet foods

A1 to A5 - Sorghum [A1-Raw (SMR), A2-Cooked (SMC); A3-Cooked and fermented with curd overnight (SMFC), A4- Cooked and fermented with water overnight (SMFW), A5-Cooked fermented with water overnight added curd (SMFWC)].

B1 to B5 - Pearl millet [B1-Raw (PMR), B2-Cooked (PMC); B3-Cooked and fermented with curd overnight (PMFC), B4- Cooked and fermented with water overnight (PMFW), B5-Cooked fermented with water overnight added curd (PMFWC)].

C1 to C5 - Finger millet [C1-Raw (FMR), C2-Cooked (FMC); C3-Cooked and fermented with curd overnight (FMFC), C4- Cooked and fermented with water overnight (FMFW), C5-Cooked fermented with water overnight added curd (FMFWC)].

SUMMARY AND FINDINGS OF THE STUDY

- In the present investigation traditionally processed sorghum, pearl millet and finger millet (*viz*. cooking, fermentation with curd, fermentation without curd, addition of curd to fermented millet) were analysed for their nutritional and anti-nutritional properties.
- Protein was found significantly highest in SFWC (20.57±0.37 g/100g) and PMFWC (20.27±0.27 g/100g). Fat content was highest in pearl millet flour (4.30±0.02 g/100g). Whereas, among traditionally processed PMC (2.04±0.12 g/100g) found to have maximum fat content. Meanwhile, traditional processing reduced fat content drastically in all the samples.
- Finger millet flour contain considerable amount of IDF (9.42±0.13 g/100g), SDF (2.08±0.04 g/100g) and TDF (11.41±0.10 g/100g). FMFW (78.79±0.72 g/100g) found with highest amount of carbohydrate.
- Phytic acid content in millet flours ranged from 8.6±0.15 to 4.77±0.07 mg/g. Sorghum flour contains highest amount of phytic acid i.e. 8.6±0.15 mg/g and it reduced sharply in SFWC (3.28±0.09 mg/g).
- Estimation of minerals showed that FMFWC (3.54±0.23 mg/100g) contain highest amount of zinc and FMFW (8.95±0.63 mg/100g) have highest amount of iron among all the processed samples. Phytic acid: zinc and iron molar ratio was observed lowest in PMFWC (9.48) and PMFW (4.12) respectively.
- Among the water soluble vitamins, B_2 was found highest in PMFC (0.173±0.002), B_3 in SFWC (1.806±0.132), B_5 in PMFWC (0.411±0.011), B_6 in PMC (0.083±0.004), B_9 and vitamin C in FMFWC (19.549±1.08 and1.96±0.18).
- Nutrient retention for water soluble vitamins in traditionally processed samples was found highest for B_2 in SFW (64.4%), B_3 in SFWC (58.7%), B_5 and B_6 in FMFWC (67.9% and 18.3%), B_9 in FMFW (43%), vitamin C in SFW (67.3%).
- Nutrient and anti-nutrient retention of raw and traditionally processed millets was investigated and found phytic acid content significantly reduced by 62.9% in traditionally processed sorghum. Retention of water soluble vitamins showed that Vitamin-C (63%), B_2 (64%), B_3 (58%) and B_5 (68%) in the processed millets.
- The traditionally cooked, overnight fermentation then added with curd was found to reduce phytic acid to a greater extent (62.9%) which may enhance the bioavailability of minerals especially iron and zinc.

V. Pathology and Microbiology

DOSE RESPONSE OF *SALMONELLA* SURVIVAL AND INFECTION IN AN *IN-VITRO* MODEL OF THE HUMAN INTESTINAL TRACT AS A PROXY FOR FOODBORNE PATHOGENS

Quantitative Microbial Risk Assessment (QMRA) is a process of estimating human health risks due to exposures to microbial pathogens. Dose-Response modeling is the key to QMRA as it provides a link between exposure dose and the probability of infection. The role of exposure in the epidemiology of *Salmonella* can be explained using dose-response assessment both for infection and acute enteric illness. A microbiological criterion defines the "acceptability of food product" based on the presence or absence of microorganism, its toxin or metabolites per unit of a lot, volume, or mass. A microbiological criterion setting is very useful for both regulatory authorities and food business operators with special reference to food safety.

Salmonella is one of the four global causes of diarrhoeal diseases in the world. CDC estimates *Salmonella* causes 1 million foodborne illnesses every year in the United States (CDC, 2018). CDC estimates *Salmonella* causes about 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths in the United States every year. The limit of *Salmonella* spp. in foods should be as low as one CFU/g, and *Staphylococcus aureus*– 10^6cfu/g)-Ref: USFDA-BAM (Bacteriological Analytical Manual).

A part of quantitative microbial risk assessment is "dose-response (DR) assessment" in which exposure to pathogenic microorganisms is translated into a human health risk. To set up microbiological criteria for Indian conditions dose-responses relations for food pathogens have to be carried out. Risk assessment studies help in developing public health policies and identifying appropriate food safety measures in order to reduce the burden of foodborne diseases (WHO, 2017). Dose-response assessment based on the human clinical experiment is more common. Animal data have also been used for pathogens that cannot be tested in humans because they cause severe or long-term health effects. Due to the specificity of the host-pathogen relation, translation of animal results to the human host is difficult and the use of an animal proxy may be problematic.

The dose-response of *Salmonella* infection in an *in vitro* model provides insight into the relative risk for human health. In most disease outbreaks, the number of pathogens in the food is rarely reported. In vitro, human gastrointestinal passage can be used to study these barriers as a partial model for infection dose-response in humans.

OBJECTIVES

1. To develop a static, sequential gastric and small intestinal model system for dose-response study.
2. To assess the attachment and invasion of *Salmonella* into the human small intestinal epithelium.

METHODOLOGY

Bacterial strain and culture conditions: For this study, we have used the strain of *S. enteritidis* (ATCC-13076) procured from the American Type Culture Collection. This is the most common food pathogen causing foodborne diseases in the world. For investigation in the model system, the bacteria will be cultured on XLD (Xylose Lysine Deoxycholate) Agar at 37ºC and from there the colony will be cultured on Brain Heart Infusion broth (BHI).

Simulated Gastrointestinal fluids: The composition of simulated gastrointestinal fluid (SGF) and simulated intestinal fluid (SIF) was based on previously described methods.

The pH was set at the desired value with hydrochloric acid (1.0 mol/L). Mucin and pepsin, both radiation sterilized, was added after filter sterilization, and the final SGF was mixed overnight at room temperature. In regular experiments, the pH of SGF was set at 2.5 with hydrochloric acid (1.0 mol/L).

SIF-complete consisted of two solutions: SIF-basic and bile solution. SIF- complete was prepared by mixing 3 parts SIF-basic with 1 part bile solution.

Chemicals	Quantity
Sodium chloride	175.0 g/L
Sodium dihydrogen phosphate	88.8 g/L
Potassium chloride	89.6 g/L
Calcium chloride	22.2 g/L
Ammonium chloride	30.6 g/L
Glucose	65.0 g/L
Glucuronic acid	2.0 g/L
Urea	25.0 g/L
Glucosamine	33.0 g/L
Bovine serum albumin fraction V	1.0 g/L
Mucin (Type II from porcine stomach)	3.0 g/L
Pepsin	1.3 g/L

Simulated intestinal fluid (SIF), SIF-Basic

Chemicals	Quantity
Sodium chloride	175.3 g/L
Sodium bicarbonate	84.7 g/L
Potassium dihydrogen phosphate	8.0 g/L
Potassium chloride	89.6 g/L
Magnesium chloride	5.0 g/L
Urea	25.0 g/L
Calcium chloride dehydrate	29.8 g/L
Bovine serum albumin fraction V	1.0 g/L
Lipase	0.5 g/L
Pancreatin	3.0 g/L

CACO-2 CELL CULTURE

Bile solution

Chemicals	Quantity
Sodium chloride	175.3 g/L
Sodium bicarbonate	84.7 g/L
Potassium chloride	89.6 g/L
Urea	25.0 g/L
Calcium chloride dehydrate	29.8 g/L
Bovine serum albumin fraction V	1.8 g/L
Bile	6.0 g/L

Caco-2 cells was cultured and differentiated, essentially as described before. In more detail, Caco-2 cells, obtained from the American Type Culture Collection (ATCC, HTB-37, USA), will be routinely maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Scotland) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, Scotland), 1% non-essential amino acids (Gibco), 1% glutamine 100× (Gibco) and 0.1% gentamicin (50.0 mg/mL, Gibco) in 75 cm2 flasks (Corning Inc., USA). The cells will be grown to confluence (ca. 1.0 × 106 cells mL−1, 7 days) at 37◦C in a humidified atmosphere of 95% air and 5% CO2. Differentiation of the Caco-2 cells into cells simulating the small intestinal epithelium was achieved by culturing the cells in monolayers in 12-well tissue culture plates (Corning Inc., USA). For this, Caco-2 cells was seeded at a density of 1.6×10^5 cells/mL, and growth medium was changed every 2 or 3 days. These cells are known to be fully differentiated after being cultured for 14 days.

Simulated Gastrointestinal Passage: Before the start of the experiment, the pH of the SGF was rechecked and reset at 2.5 ± 0.1 using hydrochloric acid (1.0 mol/L). From an overnight bacterial culture (ON), 1 ml was mixed with 9 ml SGF, and incubated for 30 min at 37◦C in a humidified atmosphere of 95% air and 5% CO2. Subsequently, 4 ml SGF/strain-mixture was mixed with 40 ml SIF-complete, and incubated for 2 h at 37◦C in micro-aerophilic conditions (6% O2) on an orbital shaker at 50 rpm. Further interaction of the SIF/strain-mixture with intestinal cells is described below (attachment and invasion assay). From each step in the gastrointestinal passage, an aliquot was used for enumerating the number of surviving bacteria.

Attachment and Invasion Assay: The method used for studying the rate of epithelial attachment (ATT) and invasion (INV) was based on previous work by Berk (2008). Prior to attachment and invasion assays, Caco-2 cells will be washed three times with sterile phosphate-buffered saline to remove traces of antibiotics. After the final washing, 1 mL pre-warmed DMEM without FBS and gentamicin (ECM, experimental culture medium) was added to each well. Afterward, each well of the plates is inoculated with a 40 µL SGF/SIF/strain mixture per well (per strain all wells of a 12-well plate will be inoculated). The plates were incubated at 37◦C in a humidified atmosphere of 95% air and 5% CO2 during 1 hr for attachment assay. After incubation, the medium was aspirated and the monolayers are rinsed three times with PBS in order to remove non-attached/loosely attached bacteria. Subsequently, the cells were used for two purposes, namely either determination of the number of attached and invaded bacteria, or determination of the number of invaded bacteria. For enumerating the number of attached and invaded bacteria, Caco-2 cells in 6 of the 12 wells is lysed (in order to liberate the bacteria) with 1 mL 1% (v/v) Triton-X100 (Merck) in PBS, for 5 min at room temperature. Twice, the Triton lysate from three wells was combined and the two lysates are named ATT1

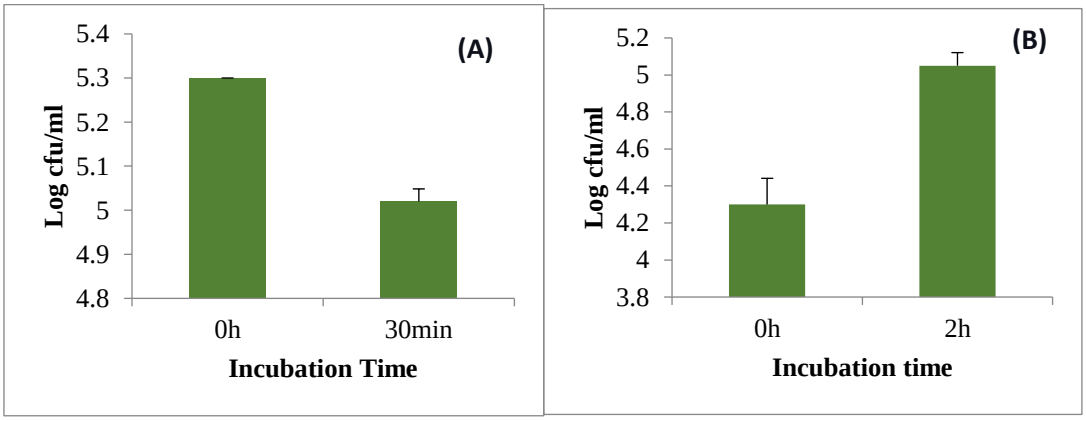
and ATT2. For quantifying the number of invading bacteria, the cells in the other six 6 wells of the 12-well plate were treated with ECM supplemented with 0.3% gentamicin (50 mg/mL, Gibco). The plates were incubated for 3 h at 37◦C in a humidified atmosphere of 95% air and 5% CO2. After incubation, the cells were washed three times with PBS to remove excess antibiotic and lysed with 1% (v/v) Triton-X100 to liberate invaded bacteria. Twice, the Triton lysate of three wells was used for determining the number of *Salmonella* that invaded the Caco-2 cells.

Enumeration of Bacteria in the Stages of the GIT-System: From the overnight (ON), SGF, and SIF stages of the GIT system, a single sample was investigated to determine the bacterial load. At the attachment and invasion stages, two samples are investigated for their bacterial load. After appropriate 10-fold serial dilutions, each sample was plated in duplicate on TSA. The output of the GIT simulation is from the first three stages (ON, SGF, and SIF) duplicate counts for each appropriate dilution. The output for the attachment and invasion stages is duplicate counts for each appropriate dilution for ATT1, ATT2, INV1, and INV2, respectively.

RESULTS

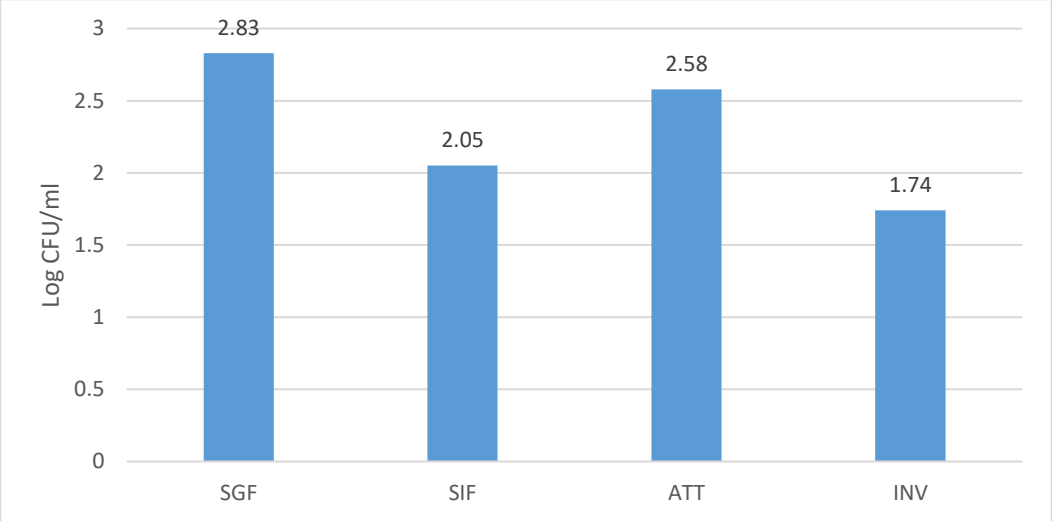
1. SGF and SIF model was prepared successfully *in vitro* to conduct Salmonella survival and dose-response study
2. The studies on *Salmonella* survival in SGF show that there was a reduction in (0.3 log) in *Salmonella* observed after 30 min of incubation time.
3. The studies on *Salmonella* survival in SIF show that there was an increase in (0.8 log) in the *Salmonella* population observed after 2h of incubation time.
4. The relative population size of the Salmonella strain that attached to the Caco-2 cells was significantly higher (0.5 log) when compared to the number of cells that survived in simulated intestinal fluid.
5. The relative population size of the Salmonella strain that invaded the Caco-2 cells was significantly lower (0.8 log) than the number of cells attached to the cells.
6. The study also showed that all the cells that survived and attached could not enter Caco-2 cells.
7. The infective dose of Salmonella strain was found to be 1.7 log CFU/ml

Fig 1. Salmonella survival in simulated gastric fluid (A) and simulated intestinal fluid (B)



* Mean ± SD values of *Salmonella* population

Fig 2. The potentiality of Salmonella strain during attachment and invasion of Caco-2 cells



INFERENCE AND CONCLUSION

The results of this study clearly showed that the survival potentiality of *Salmonella* in simulated gastric and intestinal fluid. The study also provided the data on the infective dose of *Salmonella* spp. to cause infection.

2. IMPACT OF *SALMONELLA* KILLING LYTIC BACTERIOPHAGES ON PROBIOTIC MICROFLORA

Bacteriophages are bacterial viruses that have great potential to use as biocontrol agents in foods. Lytic bacteriophages offer the number of desired properties like specificity for target bacteria, self-replication, and self-limiting and ubiquitous presence in nature making bacteriophages an excellent tool for food safety.

A healthy human intestinal microbiota is composed of symbionts, commensals, and some pathobionts. Generally, gut microflora is comprised of several bacterial communities that are involved in many functions such as metabolic barrier effect, trophic function etc. In dysbiosis conditions, the composition of the intestinal microbiota is altered resulting in the reduction of symbionts, commensals, or an increase in the number of pathobionts. Dysbiosis in gut microflora leads to Irritable bowel syndrome (IBS), ulcerative colitis, and Crohn's disease.

Recently we have isolated *Salmonella* killing lytic bacteriophages from sewage and tested its effectiveness as a biocontrol agent against *Salmonella* sp. Since these lytic bacteriophages are consumed along with the food, its effect on selective gut microflora has to

be studied. Hence this study is proposed to see the impact of *Salmonella* killing lytic bacteriophages on selective probiotic microflora.

OBJECTIVES

1. To see the effect of *Salmonella* killing lytic bacteriophages on probiotic gut microflora.
2. To see the effect of probiotics and *Salmonella* killing lytic bacteriophages on *Salmonella* spp.

METHODOLOGY

- ***Reference cultures:*** The reference gut microbial organisms like *Lactobacillus acidophilus (Moro) Hansen and Mocquot (ATCC-314), Lacobacilluslactis subsp. lactis (ATCC-11454), Lactobacillus rhamnoses Hansen and Lessel (ATCC-9595), Streptococcus thermophilusOrla-Jensen (ATCC-19258), and Bifidobacteriumbreve Reuter (ATCC-15700) were* procured from American Type Culture Collection (ATCC).
- ***In vitro growth assay (Turbidity assay):*** This assay was carried out to observe the effect of *Salmonella*-specific phages on selective probiotic microbiota**.** Probiotic gut microbiota (500µl) grown in MRS broth (4.5ml) was mixed with 100µl of lytic bacteriophages suspended in SM buffer. For control, instead of lytic bacteriophages, 100µl of SM buffer was used. Both the control and test tubes were incubated at 37ºC for 24h. Absorbance values were measured in a spectrophotometer at 600nm at 3, 6, and 24h post-incubation.
- ***Spot test Assay:*** This assay was performed to examine the bactericidal ability of lytic bacteriophages against probiotic microbiota on nutrient agar plates. One ml of probiotic microbiota was spread on the plates followed by the removal of excess inoculum and the plates were air-dried at room temperature. Filtrated supernatant containing the phages (1µl) was dropped on the agar and allowed to dry. After incubation at 37°C for overnight, the plates were observed for the appearance of a transparent clear zone.
- ***Agar Well Diffusion Test:*** Agar well diffusion assay was also carried out to observe the effect of lytic phages on probiotic microbiota. A known quantity of probiotic culture was added to the plates by the spread plate method and small wells were made with the help of a cork borer. A known amount (20 µl) of lytic bacteriophages was inoculated onto the wells. Plates were kept for incubation at 37^0C for 24hr. After incubation, the plates were observed for the zone of inhibition.
- ***Synergistic effect of probiotics on phages:*** For this experiment, five individual exponential phase cultures of probiotic microbiota (100 µl each) were mixed with 4.5 ml of MRS broth and taken in a test tube. SM buffer alone was used as a control. Both the control and the test tubes were incubated at 37ºC for 24hr. The turbidometric assay was performed in a spectrophotometer by recording absorbance values at 600nm at 3, 6, and 24hrs post-incubation.

STATISTICAL ANALYSIS

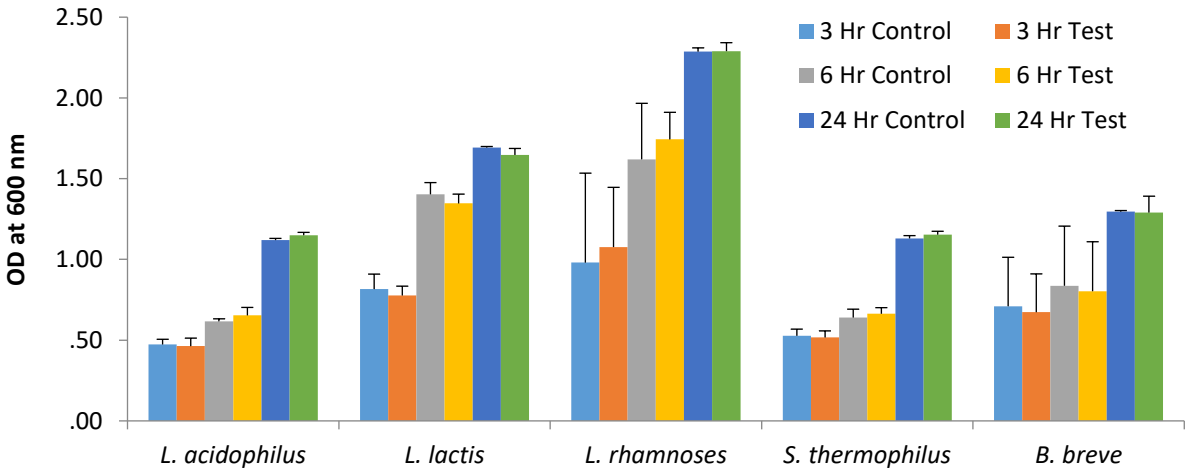
T-test was used to compare phage-treated samples and their controls at different time points. The same data were analyzed using univariate ANOVA with repeated measurements

under each probiotic microbiota at three-time points under the control and experiment groups. Data were analyzed using SPSS 19.0 software.

RESULTS

1. Spot test assay and Agar well Diffusion assay were performed using the lytic phage for five different probiotic organism species. No spots and inhibition zone were observed both in spot test assay and agar well diffusion assays (Fig.1). All the experiments were performed in triplicates.

Fig 1. Effect of *Salmonella* killing lytic bacteriophage on probiotic microbiota *In vitro*



(a) *L. acidiphilus* (b) *L. lactis* (c) *L. rhamnoses* (d) *S. thermophilus* (e) *B. breve* for 3, 6, and 24h by turbidometric assay. Triplicate OD values of both control (MRS broth and SM buffer) and test (MRS broth and phage) samples are expressed as mean ± SD. All the values were found to be non-significant at the 5% level.

2. Inoculation of all the five probiotic cultures individually with the lytic phage did not significantly affect their growth even after incubation up to 24hr.

3. Each phage-treated sample was compared to control for each probiotic microbiota and the effect on growth was found to be non-significant at a 5% level.

4. The readings under the control and test group were statistically similar at a given time point. The readings under probiotic microbiota were significantly different at different time points (P<0.01).

5. Similarly, the effect of *Salmonella* killing lytic bacteriophage on the growth of mixed probiotic microbiota for 3, 6, and 24hr was also found to be non-significant.

INFERENCE AND CONCLUSION

The study provided data on the effect of lytic bacteriophages on probiotic microbiota. The study also provided data on the synergistic effect of probiotics on lytic bacteriophages. The isolated NINP13076 *Salmonella* killing lytic bacteriophages are proved to be generally regarded as safe against probiotic microbiota and can be used for bio food preservation.

VI. Food Safety

1. DERMAL PENETRATION OF PESTICIDE RESIDUES IN FARM WOMEN WORKERS: ASSESSMENT OF COST-EFFECTIVE PROTECTIVE GEARS - A PREVENTIVE MEASURE

Pesticide exposure among farm workers may occur directly while during mixing up of the formulations and indirectly while engaged in the agricultural farming activities such as planting, pruning, weeding, picking, and/or harvesting etc. The exposure to pesticides during manipulation, preparation, and field application may occur by ingestion, inhalation, or dermal contact. It is well established fact that, of the different ways of pesticides entry into human body, the dermal exposure is the most relevant route of exposure for pesticide applications. Therefore, assessment of dermal exposure is an important component of risk assessment, as the skin is the largest organ of human body and represents an important route of exposure. However, the protection afforded through Personal Protective Equipment (PPE) may be considered as an essential tool for the pesticide applicators in minimizing the dermal exposure. Therefore, the present study proposes to assess the risk factors (dermal exposures through exposure to pesticide residues) among the farming community of Rangareddy district, Telangana State in order to develop a tool to assess the dermal exposure rate to pesticides among farm workers engaged in agricultural operations at risk of contamination.

OBJECTIVES

1. To assess the exposure to commonly used pesticides among the farm workers while engaged in different agricultural activities by collecting the information from selected/identified farm workers using/not using any protective gear and other relevant information using a pre-tested questionnaire.
2. To assess the extent of dermal exposure of farm workers cultivating paddy/ vegetable/ commercial crops by collecting the dermal washings/ surface wipes/patches of exposed dermal regions with/ without using any of the protective gear.
3. To estimate the extent of residues penetration in the blood and the amount that gets excreted through urine from the three different crop cultivators (farm men/women), with/ without using any of the protective gear for predominantly used pesticides and their common metabolites.
4. To estimate hematological parameters such as inflammatory markers (CRP, IL-6, IL-1β, Cortisol, TNF-α), micronutrient status (Vitamins A and E) and enzymatic alterations (AChE activity) due to exposure.

METHODOLOGY

***Study design*:** A community-based follow-up study

Study area: Rangareddy district, Telangana

Selection of subjects: The farm men and women with an age group of 18 to 60 years who are engaged in different farming activities while cultivating the paddy/ vegetable/ commercial crops will be selected. While, the subjects who found to be suffering from degenerative disorders such as cardiovascular, diabetics, hyper/ hypo tension, or any dermatological allergy, fungal infection or pregnant women, etc. will be eliminated from the study.

Sample size: A total of 360 subjects (about 60 of farm men and farm women each from paddy/ vegetable/ cotton cultivators) were selected, who are engaged in agricultural field activities with/without using any of the PPEs available either commercially or in any form.

Survey and selection/identification of the subjects and collection of relevant information using a pre-tested questionnaire/samples: The pretested-questionnaire consisting of 172 variables was administered to 249 farm workers. With the exclusion of 32 farm workers, a total of 217 farm workers were only included as study subjects. Out of 217 farm workers (farm women/men), 180 farm workers were randomly selected as study subjects for the purpose of collection of samples. Out of this 180 study subjects, randomly selected subjects (n=60) were provided with commercially available PPE for free of cost, followed by collection and analysis of samples. The same subjects (n=60) were then provided with the cost-effective PPE prepared using the polypropylene material which normally used for keeping the urea. Further, again from 180 subjects 60 more subjects who have not used any PPE were chosen to provide with the cost-effective PPE for free of cost.

Standard Operating Procedures were followed for collection of hand washings, patch, wipe, blood and urine samples (USEPA 1996) and OECD 1997): All the samples collected were kept in ice box containing ice packs and transported to the laboratory immediately and were stored at -20^{o}C till analyzed. All the extractions were completed not later than seven days after the collection of samples.

Total number of analyses per subject in duplicates: (Hand washings: 1, Wipe: 1, Patches: 1, Blood for pesticide residual metabolites: 1, Blood for estimation of inflammatory markers: 1, Urine-1 and = 6 x 2): 12 numbers. About 360 study subjects (180 who are not using any PPE, 60 using commercially available PPE provided to them and 120 using cost-effective PPE made by us and provided to them) were completed with respect to the pesticide residues analysis in washings of hand/patch/wipe, residual metabolites analysis in blood/urine samples and inflammatory markers, liver function tests, micronutrient status and AChE activity which results in 4320 (360 x 12) numbers of analyses.

Bio-Chemical analysis of the samples: After the collection and the extraction of hand washings, patches, wipes, blood and urine samples from the subjects, analysis was carried out simultaneously using Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS). The methods followed for analysis of pesticide residues/residual metabolites/ inflammatory markers/ micronutrient status/ enzymatic alteration in the above samples were standardized and validated in the laboratory prior to their analysis.

Optimized conditions of the LC-MS/MS: Instrument: 4000-QTRAP triple quadrupole mass spectrometer (Applied Biosystems MDS Sciex, USA); Ultra-fast liquid chromatography apparatus (UFLC, Shimadzu, LC 20 AD, and binary pump). Column: Zorbax SB-C18 HPLC Column (4.6 X 150mm and 5-Micron). Column Temperature: 25°C. Mobile phase A: water with 0.1% formic acid. Mobile phase B: Methanol with 0.1% formic acid. Flow rate: 0.8mL/min. Injection Volume: 35µL. Run time: 32 min. Program: Gradient. Mass source parameters: Interface heater temperature: 500˚C. Ion-spray voltage: 5500 eV; Gradient program follows 0.0-1min, 90% A; 1-20 min, 2% A; 20-25min, 2% A; 25-25.10min, 90% A; 25.10-30min, 90% A.

RESULTS

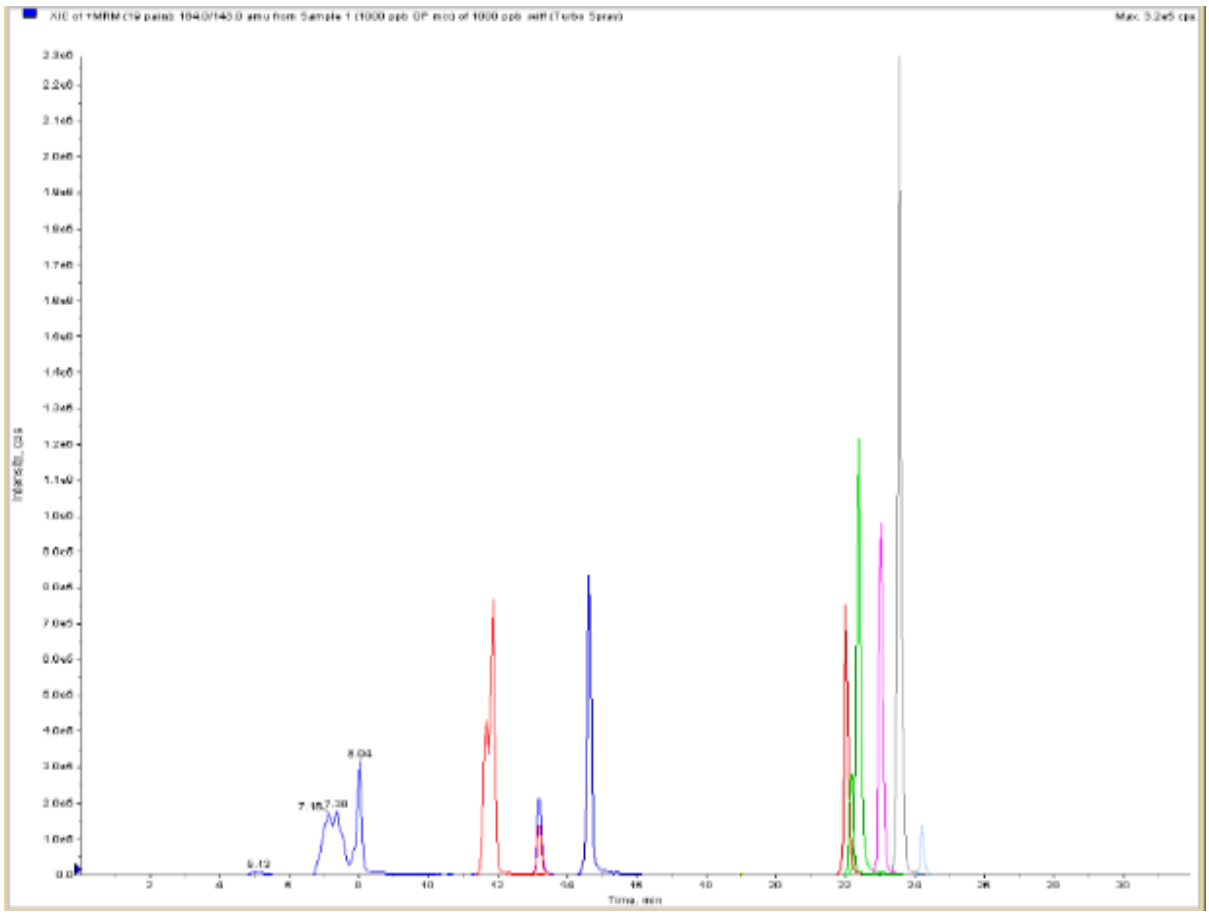
Standardization of the method for the estimation of pesticide residues in washings of hand / wipe/ patch, and common urinary/ residual metabolites of organophosphorous pesticide residues: The method for pesticide residues in hand washings samples was found to be linear from 0.5 to 1000 ng/mL with the square of the regression (R^2) ranged from 0.987 to 0.999. The LOD and LOQ for pesticides in hand washings samples were Acephate (0.5; 5), Monocrotophos (1; 2), Quinalphos (0.5; 1), Profenophos (0.5; 1), Chlorpyrifos (1; 2), Phorate (0.5; 1), Dimethoate (1; 2), Emamectin Benzoate (1; 5), Imidacloprid (1; 5) and Phenthoate (1; 2). The relative standard deviation (RSD) in terms of repeatability (intra-day) and reproducibility (inter-day) was found to be <15%. Further, the method for pesticide residues in patch/wipe samples was found to be linear from 0.2 to 1000 ng/mL with R^2 ranged from 0.991 to 0.999. The LOD and LOQ for pesticides in hand washings samples were Acephate (0.2; 0.5), Monocrotophos (0.5; 5), Quinalphos (0.2; 0.5), Profenophos (0.5; 5), Chlorpyrifos (0.5; 1), Phorate (0.5; 5), Dimethoate (0.2; 0.5), Emamectin Benzoate (0.5; 20), Imidacloprid (0.5; 20) and Phenthoate (0.5; 1). RSD in terms of intra-day and inter-day prescision was also found to be within acceptable criteria of <15%. Further, the method for four Dialkyl phosphates was found to be linear from 1 to 1000 ng/mL. The LOD of DMP (0.165), DETP (0.018), DEDTP (0.116), and DEP (0.0216), while the LOQ were 0.552, 0.061, 0.386 and 0.071 respectively.

Knowledge, Attitude and Practice (KAP): The mean age (years) of farm men (n=133, 61%) was found to be 38.99 years while it was 33.89 years for farm women (n=84, 39%). Most of the subjects (92%) reported that their houses were located away from the farms while 11% (n=24) work as agricultural laborers and 82% have their own agricultural farms. The average extent of land holdings calculated was found to be 3.88 acres. The status of education of the study participants revealed that one third (34%) of them (n=73) are illiterates and two-thirds (66%) have completed their secondary level of education. Overall it was found that, despite the awareness of the participants on potential health risks on pesticide handling practices, their attitude toward the safe implementation practices was found to be poor.

Estimation of pesticide residues in washings of hand, patches and wipes collected from farm workers not using any PPE (n=180), using commercially available PPE (n=60) and cost-effective PPE (n=120): Ten pesticide residues were detected from hand washings among subjects who are not using any PPE (n=180), of which Quinalphos (N=118), Profenophos (111), Monocrotophos (N=106), and Imidacloprid (104) were found among more number of subjects and Imidacloprid was detected at a highest mean ± SD concentration (18.89 ± 38.23 □g/mL) as

compared to the other residues. Further, the analysis of hand washings samples among subjects who are using commercially available PPE (n=60) and subjects using cost-effective PPE (n=120) provided to them, revealed that the concentration values (mean ± SD) were low for the detected pesticide residues in comparison with the subjects not using any PPE. As regards the patch washings, of the ten pesticide residues detected, Monocrotophos and Quinalphos each were detected in more number of subjects (N=114), while Acephate being detected at a higher mean ± SD concentration (15.11 ± 44.84 ng/cm2) among subjects who are not using any PPE. Further, it was found that the mean concentration of Monocrotophos was highest at 1.37 ng/cm^2 among the subjects who are using commercially available PPE, while it was for Emamectin Benzoate (0.283 ng/cm^2) among those using cost-effective PPE provided to them. Further, about ten pesticide residues were detected in the washings of wipes collected from the subjects, of which Monocrotophos (N=108) was detected at highest mean ± SD concentration (43.53 ± 142.2 ng/cm^2) among subjects who are not using any PPE. The results of analysis for wipe samples among the subjects who are using commercially available PPE provided to them revealed that Monocrotophos (N=55) was detected in more number of samples with highest mean concentration (15.96 ng/cm^2), whereas among subjects using cost-effective PPE, Chlorpyrifos (N=56) was detected in more number of samples, while Quinalphos (N=20) was detected at highest mean concentration (2.71 ng/cm^2).

Figure 1. Chromatogram showing selected organophosphorous compounds with an internal standard (TPP) in blank patch sample spiked at a concentration of 1000 ng/mL.



Estimation of residual metabolites in blood and urine samples: To assess the extent of absorption of pesticide residues in the blood and the amount that gets excreted through urine, the estimation of Dialkyl phosphates viz., Dimethyl phosphate (DMP), Diethyl phosphate (DEP), Diethyl thio-phosphate (DETP) and Diethyl dithio-phosphate (DEDTP) was done. It was found that DMP (90%), DETP (63%) and DEDTP (31%) were detected in blood samples and the levels of DETP were found to be relatively high (28.08 ng/mL) as compared to the other detected metabolites. While the levels of DMP, DETP and DEDTP detected were relatively at a lower levels in the blood among those using PPE in any form as compared to those who were not using any PPE. The assessment of urinary metabolites of commonly used organo-phosphorous compounds revealed that DMP was detected in all the samples and also at a highest concentration (63.88 ng/mL), while DETP and DEDTP were detected in 91 and 87% respectively in the urine samples. However, their levels were reduced in case of subjects who have used commercially available PPE and cost effective PPE provided to them for free of cost.

Estimation of various hematological parameters: There found an increase in the levels of IL-6, IL-1□□□ cortisol among those subjects who are not using any PPE (n=180) as compared to their respective normal values. While there found a reduction in their levels among subjects using PPE provided in any form (commercially available and cost-effective) when compared with those who are not using any PPE at all, implying that PPE offered partial protection to them. Similarly, the enzymatic alteration of AChE was found to be more among the subjects who were not using any PPE. However, the levels of micronutrients such as vitamins A, E, serum total protein and A/G ratio were low or marginal among all the subjects irrespective of they using / not using any PPE and it was attributed to their poor nutritional status

Training Program: As part of the Local Programme Advisory Committee program, a Demo and Awareness Training Program was imparted in the local language to nearly 120 subjects who include farm women, men and their family members. Subjects were given extensive education and training on self-health monitoring, hazards of exposure, benefits of using protective devices, hygienic practices to be followed, Good Agricultural Practices to be adopted, etc. This might bring change in their attitude slowly, in the interest of protecting their own health, as they are the key persons, backbone and strength of the Nation in enhancing the food security.

Education and training of farm men and women (with LPAC member being part of the programme) on the safe methods of adoption of Personal Protective Equipment (PPE)

INFERENCE AND CONCLUSION

Based on the study findings, the pesticide residues in dermal washings (hand, patch and wipe samples) and residual metabolites in blood and urine samples were detected among those who are not using any PPE followed by inhibition of AChE activity, increased levels of inflammation in serum IL-6, IL-1β, TNF–α and cortisol and lower levels of vitamins A, E, and total protein, with a decrease in the same after using PPE (commercially available or cost-effective PPE provided to them for free for cost). It is interesting to note in the present investigation that the infrastructure support was provided to the farm workers by the distribution of cost-effective PPE which was prepared and designed by us for free of cost in order to protect themselves from the adverse health effects due to exposure to pesticides is on par with the commercially available PPE provided to the farming community in providing the protection among the subjects; necessitating the need and recommending them to use protective gears by utilizing the available resources suitably with them. This will not only protect them from exposure by minimizing the morbidity burden due to pesticides exposure and thus out of pocket health expenditure can be curtailed but also provide a source of revenue/income generation and empower themselves with minimum investment. Furthermore, education and training on the safety measures such as use of PPE and adoption of GAPs, and designing their own PPE using available resources is being imparted among the farm workers during LPAC training programmes.

Overall, the findings of the present study have confirmed the adsorption capability of the designed PPE and in providing a high degree of protection for the predominantly and commonly used pesticides for the crops cultivated in the field area. The data on the exposure assessment-analysis among the farming community using the PPE have highlighted the impact of benefits of use of PPE in minimizing the dermal penetration of pesticide residues and their absorption into the blood and excretion through urine. This data generated would also be facilitating the policy makers and Government Agencies not only to strengthen the guidelines by revising and updating the same so as to make them accessible as user friendly to the farming community and further to adopt the safe handling, hygienic protective measures in order to safeguard their health through exposure.

2. ASSOCIATION BETWEEN PESTICIDE RESIDUE CONCENTRATION IN TISSUES AND LYMPHOMA, LEUKAEMIA AND BREAST CANCERS

Occupational exposure to pesticides occurs during the preparation and handling of pesticides. In addition, exposure among spouses of farmers occur, when engaged in helping their spouses in mixing up of the formulations. Pesticide exposures can cause acute as well as long-term chronic adverse health effects. Various other mechanisms have been attributed to the

carcinogenesis due to pesticide toxicity. Some of the common mechanisms include oxidative stress, chromosomal aberrations, genotoxicity, genetic polymorphism, endocrine disruption and DNA damage etc. The present study aims to study the association between lymphoma, leukaemia and breast cancers among the farmers with history of exposure (cases/experimental group) and non-farmers (control group) with no history of exposure and also the healthy control group with neither history of exposure nor diagnosed with any type of cancers but belonging to the family of farmers. Further, it also aimed to assess the triggering mechanism of action by way of evaluating the genetic polymorphism in candidate genes viz., CYP2E1, GSTT1, GSTM1 and DNA adduct (8-OHdG) formation among the farmers and non-farmers visiting/admitted and diagnosed with three types of cancers mentioned.

AIMS AND OBJECTIVES

i. To estimate the pesticide residues in blood samples and tumour tissues and to assess the DNA adduct formation (8-OHdg) in the cases and control subjects.

ii. Also, to study the association of polymorphism in candidate genes like CYP2E1, GSTT1 and GSTM1 in cases and control subjects.

iii. To assess the cancer type and grade using histopathological analysis.

METHODOLOGY

***Study design and area*:** It is a hospital-based case control study. The study was conducted in MNJ Institute of Oncology & Regional Cancer Centre, Hyderabad.

Sample size: All the 360 subjects belong to the cases/ experimental (from the farming community with lymphoma, leukemia and breast cancers) and control group (non-farming community with similar types of cancers) are the patients visiting MNJ Institute of Oncology and Regional Cancer Centre situated in Hyderabad, Govt. of Telangana.

Questionnaire: The questionnaire was originally prepared by the statisticians and epidemiologists from ICMR-NIN based on studies conducted previously on pesticide exposure among farming communities. Subsequently, the questionnaire was also pre-tested and validated followed by collecting the data by administering the same to about 247 variables through personal one on one interview mode.

Sample collection: Blood (2ml), tumour tissue samples and tissue (50mg) surrounding the tumour from both cases and controls was collected for the purpose of estimation of pesticide residues and to assess the genetic polymorphism and DNA adduct formation.

ANALYSIS OF PESTICIDE RESIDUES

Plasma: The plasma samples (200µl) collected from both cases and control subjects were extracted using 200µl of acetonitrile followed by addition of internal standard (TPP) and mixed thoroughly for about 15 seconds using vortex mixture. Subsequently, the extract was concentrated using a speed vacuum concentrator and the contents were reconstituted with 200µl of ACN followed by analysis using LC-MS/MS.

***Tissue*:** The tissue samples (10-15 milligrams) were taken and spiked with 40µl of internal standard (TPP) to which 1000µl of acetonitrile was added followed by addition of 20µl of

nonidet-P-40 substitute (tergitol). The mixture was thoroughly homogenized and was centrifuged at 10,000 rpm for 15 minutes. The separated supernatant was evaporated using vacuum concentrator for 12 hours (overnight) and was reconstituted with 400µl of acetonitrile for the purpose of analysis using LC-MS/MS.

Evaluation of DNA adduct: ELISA kits (standardized) were used for evaluating 8-hydroxy-deoxyguanosine levels in plasma samples for all the cases and controls. The method was followed as per the kit protocol and the absorbance was read at 450nm for all the samples.

Genetic polymorphism: The genotyping was done using polymerase chain reaction (PCR) method. The 459bp GSTT1 and 219bp GSTM1 were amplified along with 350bp h-Albumin as internal control. The PCR was carried out with 100ng of template DNA in a 20µl reaction volume. Initially, the primer pool was made by mixing 5µl of both forward and reverse primers of all the three genes, making up with nuclease free water up to 50µl. The PCR cycle conditions were set with initial melting step at 95^{o}Cfor 10 minutes, followed by annealing temperature of 72^{o}C for 30 seconds, thus allowing 35 cycles of amplification and to the final stage where temperature was maintained at 4^{o}C. The amplified product was mixed with 6x gel loading dye and 10µl of the same was loaded on 2% agarose gel and electrophoresis was carried out at 100V for 40 minutes. The ethidium bromide (EtBr) stained gel was later visualized for bands under gel documentation system.

HISTOPATHOLOGICAL ANALYSIS

A standard clinical routine staining (H&E) method was employed to study histopathological changes in tissues and organs. Haematoxylin is the basic dye that has affinity for acid structures of the cell (mostly nucleic acids of the cell nucleus), and eosin is an acidic dye that binds to cytoplasmic structures of the cell. As a result, H&E stains nuclei in blue and cytoplasm in orange-red.

RESULTS

Demographic particulars: Personal demographic particulars such as age, educational status, location of house, type of house, nature of occupation was collected from all the 360 subjects. It was found that more than 80% of the farmers (n=291) visiting MNJ Institute of Oncology and Regional Cancer Centre were from the State of Telangana, while the remaining were from State of Andhra Pradesh. About 95% of the non-farmers were from the State of Telangana. The mean age was found to be around 34 years for both farmers and non-farmers while the mean age of healthy control group was around 27 years. The average land holding by the farmers was found to be 1.3 acres and 67% (n=242) of them reported to have been engaged in cultivating their own land while remaining 33% (n=118) were reported to have worked as agricultural labourers in the present study. The ratio of male to female subjects was found to be 55:45 for the farmers, while it was 50:50 for the non-farmers. The participation of the females among the farmers was found to be predominant in other farming activities viz., cutting, thrashing, weeding and watering while men were actively involved in mixing and spraying of pesticide formulations. More than 80% of the farmers were illiterates, while remaining had only formal primary school education. The major crops cultivated by the farmers was cotton and rice (85%). Further, it was also reported that on an average, 16 ml of pesticide

formulation was used per acre prior to its dilution. The habit of smoking, consumption of tobacco and alcohol among the farmers as found to be higher as compared to the controls and healthy control subjects. Among the smokers, 67% consumed only cigarettes, while around 33% consumed both cigarettes and beedies while the consumption of tobacco was found to be predominant among the women as compared to men.

Knowledge, Attitude and Practices: According to the self - reported information collected from the farmers, only 2% of them have followed safety precautions such as wearing masks and gloves. However, they reported to have used plastic bags as head cap and gloves, towels and handkerchiefs as facemasks as a protective measure to minimize the exposure which however, was not in accordance with International Standards. With respect to the knowledge on symbols present on the pesticide containers and reading of the label information, only 30% (n=61) of the farmers had a rudimentary understanding on the toxicity symbols with green indicating lesser toxicity and red denoting extremely toxic and reported to have no knowledge on the remaining-coloured symbols in yellow and blue. Majority of the farmers were not knowing the technical names of the pesticides used by them. As regards the label information, 74% of the farmers could not read the labels as they were illiterates. Around 7% of the farmers (n=12) reported to be storing them in house along with other items indicating the unsafe practices of pesticide storage. Only 34% of the farmers reported to have taken bath after spraying pesticides, while 29% had washed hands with soap.

PESTICIDE RESIDUES DETECTED IN PLASMA

- Out of the 360 serum samples analysed for pesticide residues, 18% of the farmers (n=66) were detected with thirteen pesticide residues viz., Ethion, Propoxur, Monocrotophos, Chlorpyrifos, Acephate, Pirimiphos, Diazinon, Carbaryl, Dimethoate, Imidacloprid, Anilophos, Fenobucarb and Quinalphos in the range of 0.05 – 97.12ng/mL.
- Among the farmers diagnosed with Leukemia (n=60), 24 showed nine pesticide residues ranging from 0.2 – 97.12ng/mL while 23 farmers diagnosed with lymphoma (n=60) were detected with five pesticide residues viz., Propoxur (n=5), Carbofuran (n=2), Dimethoate (n=10), Imidacloprid (n=5) and Diazinon (n=1) in the range of 1.5 to 43.5 ng/mL.
- As regards the breast cancer among farm women (n=60), only 17 showed seven pesticide residues viz., Acephate, Propoxur, Monocrotophos, Imidacloprid, Malathion, Chlorpyrifos and Ethion in the range of 0.05 – 16.85ng/mL.
- However, the serum samples collected from non-farmers (n=90) and healthy control group (n=90) did not show any pesticide residues.

PESTICIDE RESIDUES IN TISSUES

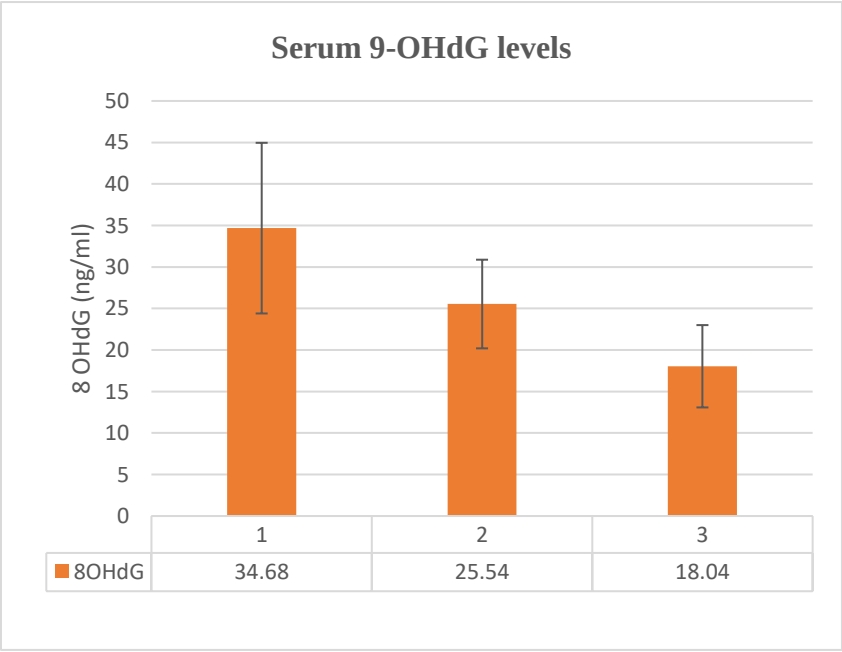
- Of the 270 tissue samples from both farmers and non-farmers, none of the non-farmers who were diagnosed with leukaemia (n=30) and lymphoma (n=30) showed any pesticide residues.
- While the 50% of the non-farm women diagnosed with breast cancers (n=15) were detected with three pesticide residues viz., Propoxur (n=5), Dimethoate (n=13) and Chlorfenvinfos (n=5) in the range of 17 – 342 ng/g in their tissue samples.

- Among the 60 farmers diagnosed with leukemia, 45% of them (n=24) were detected with eight pesticide residues viz., Carbaryl (n=1), Propoxur (n=4), Methiocarb (n=1), Dimethoate (n=2), Diazinon (n=1), Malathion (n=1), Fenobucarb (n=10) and Chlorfenvinfos (n=4) in the range of12.5 ng/g - 1527.9 ng/g.
- It was found that the farmers (n=60) diagnosed with lymphoma, 67% of them (n=41) were detected with four different types of pesticide residues viz., Propoxur (n=22), Dimethoate (n=20), Fenobucarb (n=13) and Chlorfenvinfos (n=17) in the range of 10 to 98.5 ng/ml.
- Out of the 60 farm women diagnosed with breast cancer, 52% (n=33) were detected with six pesticide residues viz., Propoxur (n=5), Fenobucarb (n=12) Carbofuran (n=2), Dimethoate (n=10), Imidacloprid (n=5) and Diazinon (n=1) in the range of 1.5 to 493.5 ng/mL.

SERUM 8-OHDG LEVELS

The serum 8 hydroxy - 2'- deoxyguanosine levels were analysed using ELISA. The standard curve obtained with the r2 value at 0.994. An increase in the levels of 8-OHdG (was found among the farmers as compared to the non-farmers and the healthy control groups ($p<0.05$). Further, there found a significant correlation between the 8-OHdG levels both with respect to the duration of exposure and age among the farmers diagnosed with leukaemia (R=0.6). With respect to lymphoma and breast cancers, independent t-test performed showed no significant difference between the 8-OHdG levels among farm women diagnosed with breast cancer and farmers diagnosed with lymphoma. There found no significant relation to age and duration of exposure.

Fig 2. Levels of serum 8-OHdG in (1) farmers; (2) non-farmers and (3) control subjects
Each bar represents the mean ± standard deviation of serum 8OHdG levels. Significant difference at $p < 0.05$ was observed between the groups.



GENETIC POLYMORPHISM

PCR-RFLP analysis were conducted to assess the genetic polymorphism in the GSTT1, GSTM1 and CYP2E1 genes among farmers, non-farmers and healthy control groups. ANOVA conducted showed a significant difference between the frequencies in the prevalence of genetic polymorphism among the farmers/ farm women and non-farmers diagnosed with three types of cancers ($p \leq 0.05$). Further, the farmers/ farm women diagnosed with leukaemia (n=60), lymphoma (n=60) and breast cancers (n=60) showed higher percentage in the frequencies of genetic polymorphism in all three genes as compared to the non-farmers and healthy controls. The frequencies in genetic polymorphism in GSTT1 and GSTM1 genes among the healthy control group were found to be relatively low (10%) as compared to farmers (39%) and non-farmers (24%) respectively. In addition, the chi-square test performed revealed a significant difference ($p<0.05$) between the frequencies in the prevalence of GSTT1 and GSTM1 polymorphisms within the non-farmers diagnosed with lymphoma, leukaemia and breast cancers.

HISTOPATHOLOGICAL ANALYSIS

Among the farmers and non-farmers diagnosed with lymphoma and breast cancers, the microscopic examination revealed no significant histopathological changes between them, but found to have shown variations in the frequency of grades of cancers among them. Among the 42 farm women diagnosed with breast cancers, 22 were of grade three, while 20 were of grade two. As regards non farmers (n=18), eight were diagnosed with grade three cancer and remaining ten were of grade two. Among the farm women diagnosed with breast cancer, more than 95% (n=41) revealed higher cancer prominence in the right breast as compared to the non-farmers (n=12). Of the 34 farmers diagnosed with lymphoma, 21 were diagnosed with non-Hodgkin's lymphoma and 13 with Hodgkin's lymphoma. While among non-farmers diagnosed with lymphoma (n=26), more than 60% (n=15) were with Hodgkin's lymphoma. With respect to the grade of cancer, 23 farmers diagnosed with lymphoma were of high grade and 4 were large B cell type. A total of 4 non-farmers were found to have been diagnosed B cell low grade lymphoma while 5 were diagnosed with follicular lymphoma. Hence, no distinct or specific interpretations could be made due to the disparity in the cancer grades among the farmers and non-farmers.

INFERENCE AND CONCLUSION

To conclude the study findings, higher prevalence of genetic polymorphism among GSTT1, GSTM1, CYP2E1 genes in the farmers as compared to the non-farmers diagnosed with lymphoma, leukaemia and breast cancers and as well with the healthy controls, who were neither having any exposure nor diagnosed with any type of cancers but belonging to the family of cases. Further, there found increased levels of 8-OHdG among the farmers as compared to both non-farmers and healthy control group ($p<0.05$). A significant correlation between 8-OHdG level with respect to age and duration of exposure was found among the farmers diagnosed with leukemia. However, no such correlation was found among those diagnosed with lymphoma and breast cancers. All the 360 total serum samples analyzed, only 18% (n=66) showed pesticide residues. They were four among the 24 leukemia, six in 23 lymphoma and seven among 17 breast cancer subjects belonging to the farmers group respectively, while only

one pesticide residue viz., pirimiphos was detected in two breast cancer patients of the non-farmers group. However, there were no pesticide residues detected in the plasma samples of the healthy control group. Of the 270 tissue samples subjected to pesticide residue analyses among the farmers and non-farmers groups, 7 pesticide residues in 24 leukemia, 4 in 41 lymphoma and 6 in 33 breast cancer respectively were detected, while only 15 subjects diagnosed with breast cancer belonging to the non-farmers were detected with 3 pesticide residues viz., propoxur, dimethoate and chlorfenvinphos at a lower concentration. Results from the present study can be utilized as a valuable precursor to design future studies with much larger sample size involving other molecular and related parameters for analysis which may give a comprehensive understanding on the triggering mechanism of cancers and association with pesticide residues and for interpretation purpose.

VII. Extension and Training

1. ADAPTATION OF FAOs EDUCATION FOR EFFECTIVE NUTRITION IN ACTION (ENACT) AND E-LEARNING MODULES ON NUTRITION AND FOOD SYSTEMS' COURSES FOR NUTRITION EDUCATION IN INDIA

OBJECTIVES

- To devise suitable ways of adapting and piloting the two courses in the Indian context
- To formulate different approaches to impart need-based training to students by integrating the course in the syllabi of various Universities/ Educational Institutions.
- To develop India-specific FAO e-learning modules on "Nutrition and Food Systems" for use in face-to-face learning for students, after adapting to Indian conditions and piloting.
- To develop appropriate monitoring mechanisms for development and effective usage of both the courses.

METHODS

- Modification and adoption of ENACT study material comprising 10 units (each 150 pages) Total 1500 pages including exercises and e-learning modules meant for students by experts.
- Integrating of ENACT course material and e-learning modules in the existing curriculum of formal nutrition education in different Universities representing various regions of the country.

RESULTS/ ACHIEVEMENTS:

- As per the requirement of the ENACT Project, a local steering committee was constituted.
- The steering committee includes the Vice-Chancellor of Mizoram University as Chairman while the Registrar, Avinashalingam University, representative from FAO and one Scientist from the Extension and Training Division as the members with HoD, E&T Division as Member-Secretary.
- The steering committee meeting was held and discussed the future activities of the ENACT programme.
- A series of nine workshops (online) was conducted in the months of July-August, 2020 with all the faculty members of Food & Nutrition/ Dietetics departments of piloting universities *viz.*, Mizoram University, Delhi University, SNDT University (Mumbai), Avinashalingam University (Coimbatore) and Osmania University (Hyderabad). Officials of FAO

Headquarters (Italy) and Srilanka have acted as resource persons for all the sessions during 9 days of the online workshop.

- Modification of e-learning completed to suit to Indian scenario.
- Modification and adoption of ENACT study material comprising 10 units (each 150 pages) Total 1500 pages including exercises meant for students have been completed.
- Integrating of ENACT course material in the existing curriculum of formal nutrition education in different Universities representing various regions of the country is in progress.

The following publications (study material and e-learning modules) are meant for PG students of Nutrition & Dietetics in Indian Universities as 'Addon' course material.

Sl.No	Titles of Enact course material
1	The need for nutrition education
2	Explaining food, diet, and healthy eating
3	Nutrition education actions
4	How good eating is learnt
5	How Nutrition education is done
6	Theory of nutrition education
7	Analyzing the situation for nutrition education
8	Objectives, messages, motivations for nutrition education
9	Implementation of nutrition education
10	Monitoring and Evaluation
Sl.No	**Titles of E -learning modules**
1.	Nutrition, Food security and Livelihoods: Basic Concepts
2.	Why does nutrition matter?
3.	How does the food system influence nutrition?
4.	Making agriculture and food system nutrition - sensitive: key principles
5.	Making agriculture and food system nutrition - sensitive - key interventions
6.	A conducive international environment for nutrition

INFERENCE AND CONCLUSION

The ENACT material and e-learning modules as mentioned in the above tables are meant to be introduced as Add on course in the universities for the students of Nutrition & Dietetics at PG level. Five universities across the nation *viz.,* Mizoram University, Delhi University, SNDT University (Mumbai), Avinashalingam University (Coimbatore) and Osmania University (Hyderabad) has already consented to introduce this Add on courses.

2. IMPACT OF LONG-TERM USE OF DOUBLE FORTIFIED SALT WHEN USED PRENATALLY ON THE IRON AND IODINE STATUS AND COGNITIVE DEVELOPMENT OF INFANTS IN RURAL MEGHALAYA – A PILOT STUDY

Iron and Iodine are associated with cognitive function. Deficiencies of these nutrients can lead to long-lasting deficits in cognitive performance. The present study is a pilot study to examine the effect of double fortified salt on the iron status and infant development when used from the first trimester of pregnancy to three months of infant's age compared to the use of iodized salt.

AIMS AND OBJECTIVES

- To examine how the use of Double fortified Salt (DFS) during gestation impact infants iron and iodine status and development when compared to the used of iodised salt.

Phase I - A pilot study to assess the feasibility of conducting a Phase II study to assess the impact of long term use of DFS on biomarkers in blood and cognitive development of infants among mothers who consumed iron folic acid tablets for 100days prenatally.

Specific Objectives

1. Conduct formative research to operationalize the use of DFS at the household level including:
   a. Assessing the perceptions of the community to the use of DFS in their households.
   b. Assessing beliefs and behaviors related to food and nutrition during pregnancy.
   c. Assess the prevalent infant feeding practices in the region.
   d. To assess the adherence to, and identify factors associated with the amount of Iron/Folic Acid uptake prenatally (NIPI implementation).
2. To conduct a pilot study to assess the impact of long-term use of DFS on iron status of mothers and infants' cognitive development.

METHODS

Qualitative methods of focus group discussions and in-depth interviews were the methods used for objective no1.

For objective no 2, the sample was divided into two groups: one group was supplied with DFS and the other group was supplied with iodized salt (for approximately 8 months). The baseline and end line measures of iron status parameters and infant development (at three months infant's age) were assessed.

BIOCHEMICAL ASSESSMENTS

Hb was estimated using Cyanmethemoglobin. Serum Ferritin, Vitamin B12 and soluble transferrin receptor (sTfR) were estimated in chemiluminescent immunoassay system (Access 2 from Beckman Coulter, USA). Serum c reactive protein was estimated using Ortho-Clinical diagnostic CRP kits in a dry chemistry Johnson & Johnson Vitros 4600 system. Urinary iodine is estimated by ammonium persulfate method.

RESULTS

Findings related to objective 1 have been reported earlier. Presented below are the findings of Objective 2 work-related to all the objectives of the study is completed. Presently data analysis is in progress. This will be followed by report writing and submission.

Table 1. Biochemical parameters of pregnant women after DFS and Iodized salt supplementation

Parameter	DFS end line	Iodised end line	P value
Hb (g/dl)	10.72±1.98(n=56)	10.77±1.59(n=67)	0.877
CRP (mg/L)	6.10±4.32(n=56)	7.41±6.62(n=67)	0.204
Ferritin (ng/mL)	42.05±51.09(n=56)	63.80±192.13(n=66)	0.412
Serum Transferrin nmol/mL	43.33±27.47(n=55)	44.29±33.16(n=67) *	0.045
VitaminB12 (pg./mL)	238.71±113.53(n=53)	243.68±201.45(n=67)	0.873
Urinary Iodine (150-249 µg/l)	190.30±130.25(n=56)	150.77±78.37(n=67) *	0.040

Haemoglobin, C-reactive protein, Ferritin, Vitamin B12, Serum transferrin were chosen as parameters to study the effect of supplementation of Double fortified salt over iodized salt in pregnant women. The mean haemoglobin values were more or less similar in both the groups with 9.67±3.78 g/dl in DFS group and 10.53±2.44 g/dl in iodised salt group before supplementation. However, the mean values post supplementation were 10.72±1.98 g/dl in DFS group and 10.77±1.59 g/dl in iodised salt group. The mean CRP values were 6.86±4.10 mg/L at baseline in DFS group also did not alter much post supplementation with a value at 6.10±4.32 mg/L. The mean CRP values in iodised salt at baseline and end line are 7. 19±3.84mg/L and 7.41±6.66 mg/L respectively.

Ferritin values (mean values) at baseline were 29.50±35.66 ng/mL in the DFS group and 38.67±42.63ng/mL in iodised salt group which subsequently showed no significant change post supplementation. The Mean Vitamin B12 values were 185.89±140.08 pg/ml and 240.37±182.83 pg/ml at baseline in DFS and iodized salt groups respectively and were not significantly different post supplementation with DFS. Urinary iodine was higher in the DFS group compared to the Iodised salt group post supplementation.

The cognitive development of infants was assessed at three months of infants' age using the Developmental Assessment Scale for Indian infants (DASII).

Table 2. Differences in Chronological age, developmental age and developmental quotient among subjects

Age	DFS (n=57)	IODISED (n=67)	p value
Chronological Age	92.95±18.88	87.64±17.85	0.535
Developmental Age	84.96±17.28	84.57±18.39	0.246
Developmental Quotient	106.21±17.43	104.51±11.19	0.271

There were no significant differences in development among infants in the DFS group and the Iodised salt group. The developmental age was slightly lower than the chronological age in both the groups.

3. IMPACT OF INTEGRATED COGNITIVE BEHAVIOUR THERAPY AND PRANAYAMA ON SLEEP QUALITY OF WOMEN LIVING IN WELFARE HOSTELS

Sleep is an essential natural behavioral process of the body to rest after the day's wakeful activities. Without sleep, one would fail to achieve optimum functioning. Women need to maintain the right amount of sleep, as studies reveal that they report more sleep disturbances on polysomnographic recordings when compared to men.

OBJECTIVES

i) To measure the sleep disturbances and sleep patterns of women by using IOWA Sleep Disturbances Inventory and Sleep Diaries respectively.

ii) To provide integrated psychological interventions namely sleep psycho-education, sleep hygiene education and pranayama and study their impact on sleep disturbances and sleep patterns of the women.

iii) To provide psychological interventions namely sleep psycho education, sleep hygiene education and pranayama separately, and study their impact on sleep.

iv) To assess the psychological well-being of women.

Cognitive Behaviour Therapy is mainly used for anxiety, depression and psychological disorders to dispute the negative assumptions, core beliefs, automatic negative thoughts and maladaptive behaviors. As part of CBT for sleep disturbances, self-imposed sleep deprivation and irregular sleep patterns among women, antecedent thoughts that induce sleep lack, beliefs and behaviors have to be modified.

Sleep Psycho education: Sleep Psycho education implies educating participants about normal and healthy sleep, sleep hygiene, and about maladaptive practices that destabilize sleep. As part of sleep psycho education, participants are advised to follow sleep hygiene regularly for a period of three months to promote the quality of sleep.

Pranayama: Deep breathing excercise such as Pranayama defined as voluntarily controlled respiration process characterized by prolonged and refined inhalation and exhalation in calm and peaceful conditions is widely used as complementary medicine to promote healthy sleep and wellness.

Hypothesis**:** CBT with sleep psycho-education and pranayama together will reduce sleep disturbances and improve sleep patterns among women than CBT with sleep psycho-educationor pranayama alone.

RESEARCH DESIGN

A randomized control design was followed to test the hypothesis. The study was conducted among women in welfare hostels of Hyderabad.

Intervention Duration: 6 months

The research findings were presented objective-wise.

The first objective of the study was to develop an integrated intervention comprising of Cognitive Behaviour Therapy, Pranayama and Dietary Guidelines for enhancing sleep quality and reducing sleep disturbances of women. The method and process of development was reported earlier.

The second objective of the study was to assess the sleep quality, and sleep disturbances of marginalized women pre- and post-intervention. Findings related to this objective are shown in Table 1. Table 1 showed findings of the preliminary screening done for all the willing girls from the marginalized colleges.

Table 1. College wise sleep quality assessment during preliminary screening session

TSWRDCW College Name	Consented	Participated	Invalid response sheets	Valid response sheets	Good sleep quality	Poor sleep quality	Poor sleepers (%)
LB Nagar, Ibrahimpatnam	209	208	18	190	98	92	48.42%
Budvel	238	238	7	231	61	170	73.59%
Mahendra Hills	220	220	12	208	81	127	61.06%
TOTAL	667	666	37	629	243	386	60.50%

Group1=Integrated intervention group, Group 2= Cognitive Behaviour Therapy and Dietary Guidelines group, Group 3= Pranayama and Dietary Guidelines group

All three groups obtained a mean global score of more than 5. Mean global score of 5 and above was indicative of poor sleep according to PSQI norms. Pre-test means and SDs obtained by all the three groups on PSQI were more than 5. This indicates that 100% poor sleepers were recruited for the study as it was a prerequisite of the study to conduct baseline assessment prior to the intervention for poor sleepers. Whereas post intervention, all the three intervention groups showed a mean global score less than 5which indicated improved sleep quality post intervention among the marginalized college girls of all the three intervention groups.

The third objective of the study was to assess psychological wellbeing, psychological distress and perceived stress of marginalized women pre and post intervention. Table 3 showed mean and SD values of the three intervention groups on psychological wellbeing, psychological distress and perceived stress.

Table 2. Mean and SD scores of psychological wellbeing, psychological distress and perceived stress of marginalised college girls pre and post intervention

Variables	Groups	N	Baseline	Endline
			M ± SD	M ± SD
Psychological Wellbeing	1	92	48.1 ± 8.74	70 ± 4.12
	2	86	52.6 ± 9.05	64.9 ± 4.74
	3	86	52 ± 13.54	57.4 ± 9.37
Psychological Distress	1	92	63.8 ± 14.78	43 ± 6.94
	2	86	61.2 ± 18.07	51 ± 11.17
	3	86	62.0 ± 17.11	52 ± 12.20
Perceived Stress	1	92	17.5 ± 4.42	10.8 ± 2.57
	2	86	17.3 ± 5.62	12.1 ± 3.54
	3	86	16.7 ± 5.88	11.5 ± 4.33

Group1=Integrated intervention group, Group 2= Cognitive Behaviour Therapy and Dietary Guidelines group, Group 3= Pranayama and Dietary Guidelines group, M = Mean and SD = Standard Deviations $p<0.05$

According to table 2, psychological wellbeing scores at baseline/pre-intervention were lesser when compared to end line/post-intervention scores which indicated improved psychological wellbeing after the intervention in all the three intervention groups. Psychological distress scores pre-intervention was greater than the post intervention scores which indicated reduction in psychological distress after the intervention in all the three groups. Perceived stress at baseline was greater than that at end line indicating the decreased perceived stress after intervention in all the three intervention groups.

The fourth objective of the study was to analyse the differences among pre and post intervention assessments of sleep quality, sleep disturbances, psychological wellbeing, psychological distress and perceived stress of the three intervention groups namely group 1 which receives an integrated intervention of Cognitive Behaviour therapy, Pranayama and Dietary Guidelines, group 2 which receives Cognitive Behaviour Therapy and dietary

guidelines, and group 3 which receives Pranayama and dietary guidelines. Table 4 showed differences between pre and post intervention assessments of sleep quality, psychological wellbeing, psychological distress and perceived stress of the three intervention groups.

Table 3. Differences between pre and post intervention scores of sleep quality, psychological wellbeing, psychological distress and perceived stress of the marginalized college women of the three intervention groups.

Variables	Groups	N	Increment/ Decrement M ± SD	P
Sleep Quality	1	92	5.6 ± 3.06	
	2	86	4.7 ± 2.36	
	3	86	5.8 ± 2.74	
Psychological Wellbeing	1	92	-21.9 ± 8.11	
	2	86	-12.3 ± 6.50	
	3	86	-5.5 ± 6.50	0.01
Psychological distress	1	92	20.9 ± 10.35	
	2	86	10.2 ± 9.20	
	3	86	10.0 ± 8.20	0.01
Perceived Stress	1	92	6.7 ± 3.37	
	2	86	5.1 ± 3.88	0.01
	3	86	5.2 ± 3.0	

Group1=Integrated intervention group, Group 2= Cognitive Behaviour Therapy and Dietary Guidelines group, Group 3= Pranayama and Dietary Guidelines group, M = Mean and SD = Standard Deviations.

Table 3 showed that there were the significant mean differences between pre and postintegrated interventions of CBT, pranayama and dietary guidelines, group 2which received CBT and dietary guidelines, and group 3 which received pranayama and dietary guidelines. These statistically significant differences indicated that the three interventions resulted in improved sleep quality after the interventions. Although the psychological well-being improved in all the three groups, the mean difference was significantly higher for the integrated intervention group. The same trend was seen for psychological distress as well but not for perceived stress which showed all the groups benefitted similarly from the intervention.

VIII. Nutrition Information, Communication and Health Education

FOOD AND NUTRITION RELATED WEB-SEARCH BEHAVIOUR, FOOD SCARES AND CHANGES IN PERCEPTION AND PRACTICE DURING COVID- 19 SITUATION AMONG INDIANS

Even before the declaration of COVID-19 as a global pandemic by the World Health Organisation, the WHO Director-General, Tedros Adhanom Ghebreyesus had mentioned about another epidemic of misinformation spreading through social media and termed it as 'infodemic'. Along with the awareness generation many misinformation and rumours from unreliable sources about spread and safety or preventive measures of COVID-19 had made its way through the internet and social media creating confusions, panic and concern among common people. Concerns around precautionary measures related to hygienic food handling practices, grocery shopping, use of cleaners and disinfectants to prevent transmission of virus though food have been prevailing. For 'immunity-boosting' as a therapeutic strategy against COVID-19, consumption of certain micronutrients such as Zinc, Vitamin C, vitamin A in form of food, nutraceuticals, herbal traditional medicines had gained importance along with over consumption of carbohydrate and fat dense food owing to quarantine related stress and boredom. Overall, the COVID-19 had impacted general population's food related behaviour. Therefore, this study was designed to understand the food and nutrition related queries, practices of Indian internet users and their reliability on the source of information in the COVID-19 era.

OBJECTIVES

- To understand the web search behaviour of Indian internet users in the area of top trending COVID- 19 associated food and nutrition related news during the different phases of the pandemic.
- To assess the impact of the top trending information on food related topics on the perception and practices of Indian internet users.

METHODOLOGY: The study was being conducted in two distinct phases.

i. In the first phase, the most common media propagated news from print and *e*- news in the area of food associated COVID information were identified. The data on web-search behaviour of Indian internet users on those topics were obtained from the online search traffic data using tool- Google Trends. The top trending search terms were

divided into 4 broad categories such as “Immunity”, “Eating behaviour”, “Food safety” and “Covid scare”. Data was collected from the date of first confirmed case of COVID-19 in India (27th January 2020) till 30th June 2021.

ii. In the second a closed ended KAP questionnaire was developed. Content validity was done by 5 experts from different fields and the approved questionnaire was pre-tested on 30 individuals.

iii. The questionnaire was uploaded on different social media platforms to obtain cross-sectional information from active internet users from different parts of India.

iv. pre-coded, closed-ended,

RESULTS

The association between the change in Relative Search Volume (RSV) on Google Trends (GT) of 34 popularly searched keywords classified by the researchers under 5 different categories - “Immunity”, “Eating behavior”, “Food safety”, “Food scares and concerns” and “Covid scare” showed a steep rise in search for immunity boosters, vitamin supplement brands “ayush kadha (ayurvedic decoction) during the first wave (April- August 2020). With a brief period of decline in the search trend, it again hiked correspondingly with the growing number of positive cases during the second wave in India (Figure 1). An online survey conducted on adult Indian internet users (n=572) reported high (71.9%) consumption of Vitamin C rich fruits as well as Vitamin C (68.2%) and Zinc (61.4%) supplements to boost immunity. Traditional Indian spices like ginger and garlic were used by 62.9% and 50.9% respondents respectively (Figure 2). Most respondents reported to rely on social media for gathering COVID-19 associated tips for boosting immunity, however those with history of COVID-19 infection reported to rely more on doctors and health professionals for information.

Figure 1. A positive correlation was observed between the number of COVID cases and search terms like “vitamin D”, “Limcee”, “grocery delivery”, “vegetable sanitizers”, “alcohol” etc. whereas, search terms like “street food”, “junk food”, “food delivery” etc. were negatively correlated to the number of cases.

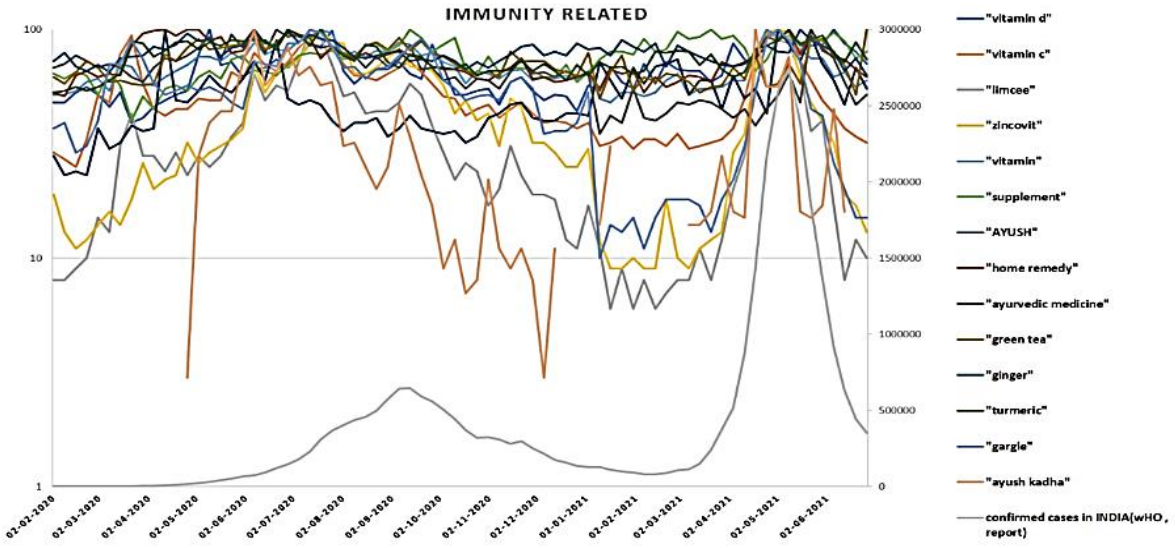
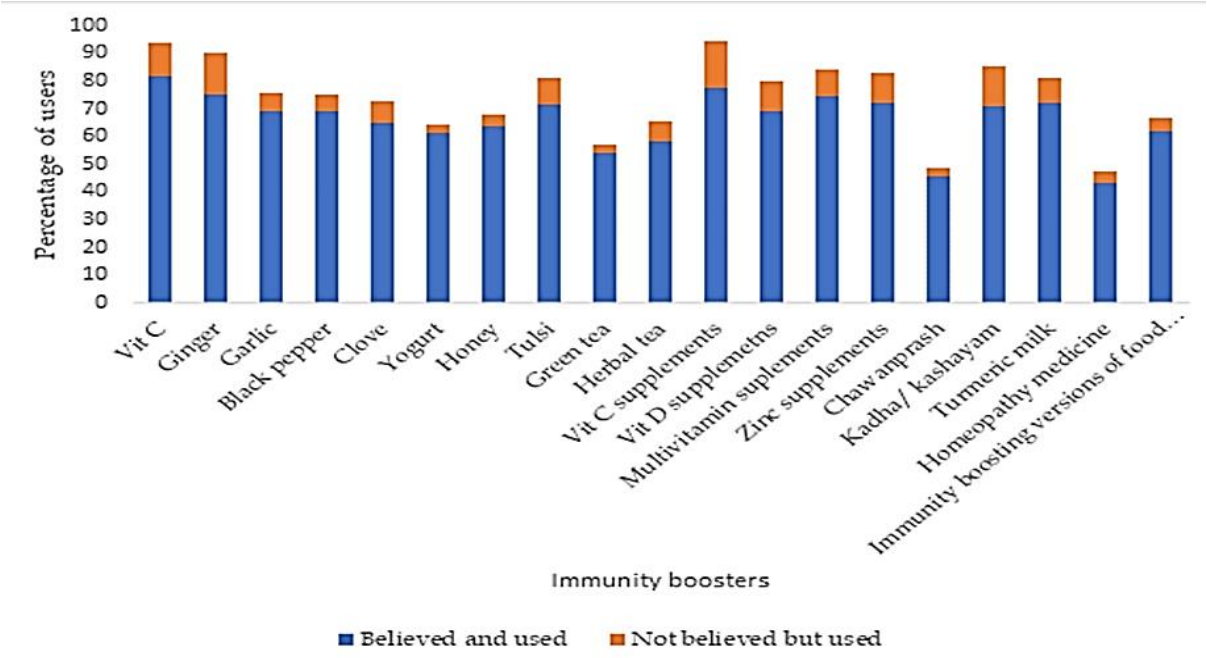


Figure 2. The perception of the respondents who reported to use each of these 'immunity boosters' about their ability to prevent COVID-19 infection.



INFERENCE AND CONCLUSION

This study highlights that there is need for promoting media literacy and health literacy among the people so as to advocate for use of information cautiously, verifying the authenticity and accuracy of health information before putting it to practice or sharing it with others. The responsibility of fighting the infodemic not only pivots on the health organizations and governments but also on the information consumers. Nutrition literacy, media literacy as a part of national programs can help in developing skills among common people to rationalize circulating information without worry of another future infodemic. These should be a part of the country's preparedness for facing the challenge of future 'infodemic in pandemic'

IX. NIN Animal Facility

PRE-CLINICAL SAFETY (REGULATORY TOXICOLOGY) OF *DURVA (CYNODON DACTYLON LINN) - DURVA SWARAS* (DS)

Hormonal replacement therapy (HRT), the first-line management of chronic menopausal syndrome (CMS) affecting women has adverse effects limiting its application. We have innovated durva swarasa-DS (*Cynodon dactylon Linn*) based on dravyaguna (ayurvedic pharmacological properties) *tikt rasa* (~improve liver metabolism) *sheet veerya* (~anti-inflammatory), Jeevaneeya (~antioxidant / immunomodulation) for treatment of MS. The pilot clinical investigations have demonstrated potential therapeutic benefits women with MS.

In view of this a 'Reverse Pharmacology' approach has been considered and validated potential role of DS and 'ICMR–NIN–*dc'*, in preclinical data (in vitro, in vivo data), formulation is developed. ICMR–NIN–dc' (DM) is coded formulation for the Menopausal syndrome (Patent filed - PCT/IN2021/050592 &202011025626). The therapeutic potential of the DS has been evaluated in OVX rat model apart from Phytochemical profiling. This has given a scope that DS/ can be an herbal alternative (HALT).

Therefore and as per mandatory requirement, the current study is undertaken as per the regulatory guidelines described: i. NDCT-2019 - patent or proprietary medicine, ii. WHO traditional medicine guidelines 2000, iii. ASU drugs- Patent or Proprietary Drugs as defined under section 3(h) of the Drugs and Cosmetics Act, 1940/(Drug Development of Ayurvedic Formulations, Vol -I, 2018, Ministry of AYUSH,GOI).

The present study is undertaken to assess preclinical safety (regulatory toxicology) has been undertaken: i. Acute in Mice & Rats, ii. Sub-chronic in Rats for DS and ICMR-NIN-*dc*.

METHODOLOGY

All the studies have been undertaken with approval of IAEC (ICMR-NIN/IAEC/01/003/2019) in healthy Swiss albino mice, Sprague Dawley Rats obtained from NIN animal facility, ICMR-NIN (Table 1).

Acute study in Rat and Mice: For the first 48 hrs, the animals were observed at 2 hr intervals for any morbidity and mortality. After this period, observations were taken only once daily for remaining period. Live phase of animals, cage side observation, physical and neurological examinations was monitored at regular intervals. The gross necropsy changes if any of major organs viz., brain, spleen, kidneys, heart, lungs, liver, gastrointestinal tract, testis/ovaries at the end of the experimental period were monitored.

Table 1. Study design

S.no	Test Groups (G)	Test compound[@]	Species	Dose & Duration	
				Dose[$]	Duration
I	**Acute toxicity**				
	D	DS	Mice (6M+6F) Rat (6M+6F)	10X-TD*	Single exposure with 14 days observation
	DM (Coded)	ICMR-NIN-*dc*	Mice (6M+6F) Rat (6M+6F)	10X-TD	
II	**Sub-chronic DS**				
1.	Control - G1	Vehicle	Rat (6M+6F)	-	45 days (28 days –TC & 15 days recovery phase)
2.	D1 (Low)- G2	DS	Rat (6M+6F)	0.5X	
3.	D2(Medium)Therapeutic-G3		Rat (6M+6F)	1X	
4.	D3 (High)-G4		Rat (6M+6F)	5X	
III	**Sub-chronic -ICMR-NIN-*dc***				
	Control -G1	Vehicle	Rat (6M+6F)	-	45 days (28 days –TC & 15 days recovery phase)
5	DM1 (Low) -G5	ICMR-NIN-*dc*	Rat (6M+6F)	0.5X	
6	DM2(Medium)Therapeutic-G6		Rat (6M+6F)	1X	
7	DM3 (High)-G7		Rat (6M+6F)	5X	

@ D – DS (Lyophilised powder of Juice); DM- (ICMR-NIN-*dc* coded formulation).
$ Therapeutic dose-; Human dose-D-2gm/adult, DM 1.4gm/adult; -Mice :D -260mg/kg, DM-182mg/kg; Rat dose: D-180gm/kg, DM-126mg/kg.
NOTE; Control used in Sub chronic study for D and DM are common.

The animals were conditioned in experimental facility. The test material at a concentration mentioned (Acute:10X TD; Sub chronic: 0.5X, 1X, 5X) was administered to mice/Rat through oral gavages.

***Sub-chronic study in Rats*:** The SD rats, aged 4-6 weeks, weighing approximately 180-200 gm were obtained after certifying the health records. They were conditioned, for 10 days. A total of 48 rats were divided into seven groups (table 1) The control group animals were administered with distilled water and all the treatment groups were administered with test substance (DS / ICMR-NIN-dc) orally daily once for twenty-eight days in three different concentrations with constant volume of 1.5 ml. All the animals were observed during the exposure period (28 days) followed by 15 days of washout period for pre-terminal morbidity and mortality. Live phase of animals, cage side observation; physical and neurological examinations were conducted at regular intervals. All the animals were subjected to biochemistry and haematology analysis followed by necropsy and histopathological examination of all vital organs viz., brain, thymus, spleen, bone marrow, kidney, skin, heart,

lung, trachea, thyroid, adrenals, sternum, liver, gastrointestinal tract, testis/ovaries, uterus at midterm i.e. 50% of the animals from all groups (D- G1-G4; DM-G5-G7) after 28 days of test compound exposure and final term i.e. remaining 50% from all groups served as recovery group on last day of experimental period (43rd day).

RESULTS

Acute study in Mice

i. DS

- There was no mortality recorded till end of the experimental period.
- No morbidity and behavioural changes in mice observed after 24 hours of single high dose (10XTD).
- The clinical signs, behavioural activity, etc., were found normal in all animals during the 14 days.
- No gross necropsy changes were observed in organs collected at the end of experiment in any group of animals.

ii. **ICMR-NIN-dc**

- There was no mortality recorded till end of the experimental period.
- No morbidity and behavioural changes in mice observed after 24 hours of single high dose (10XTD).
- The clinical signs, behavioural activity, etc., were found normal in all animals during the 14 days.
- No gross necropsy changes were observed in organs collected at the end of experiment in any group of animals.

Acute study Rats

i. DS

- There was no mortality recorded till end of the experimental period.
- No morbidity and behavioural changes in mice observed after 24 hours of single high dose (10XTD).
- The clinical signs, behavioural activity, etc., were found normal in all animals during the 14 days.
- No gross necropsy changes were observed in organs collected at the end of experiment in any group of animals.

ii. ICMR-NIN-*dc*

- There was no mortality recorded till end of the experimental period.
- No morbidity and behavioural changes in mice observed after 24 hours of single high dose (10XTD).
- The clinical signs, behavioural activity, etc., were found normal in all animals during the 14 days.
- No gross necropsy changes were observed in organs collected at the end of experiment in any group of animals.

Sub-chronic study in Rats

i. DS

- No pre-terminal deaths.
- No significant treatment related effect on food intake, body weight gain, clinical signs, behavioural activity etc.
- Haematological parameters were in normal range.
- Clinical chemistry parameters were in normal range.
- No specific test compound-induced pathological changes in organs studied.

ii. ICMR-NIN-*dc*

- No pre-terminal deaths.
- No significant treatment related effect on food intake, body weight gain, clinical signs, behavioural activity etc.
- Haematological parameters were in normal range.
- Clinical chemistry parameters were in normal range.
- No specific test compound-induced pathological changes in organs studied.

INFERENCE AND CONCLUSION

No mortality recorded till 14 days of observation period after single exposure (10X TD) of DS / ICMR-NIN-*dc* in mice and rats.

The rats exposed with DS / ICMR-NIN-*dc* daily for 28 days to three different concentrations did not show any abnormal findings in body weight gain, live phase, biochemical and haematological activity. The gross necropsy of organs was normal. No Histopathological alteration related to dose and duration of exposure to test compound was observed. No post exposure (15 day) effect was recorded.

The above study data is suggestive of human equivalent safety dose of DS (2g/day/ adult), ICMR-NIN-*dc* (1.4g/day/adult).

PhD Scholars

S.No	Name of Student	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		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	Dr. Sudip Ghosh	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

S.No	Name of Student	Name of Guide	Thesis Title
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		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	Hanuma Naik	Dr. P. Raghu	Mechanism of Iron and Zinc interactions in intestinal cells
24	Puneeta Singh Yaduvanshi		Modulation of Iron storage and regulation by Zinc in Hepatocytes
25	Konda Venu		Role of Zinc in erythropoeisis
26	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
27	Anuradha R		Effect of paternal calorie restriction of diet induced obese on metabolism of their offspring
28	V. Sai Kanth	Dr. Sanjay Basak	Maternal exposure of endocrine disrupting chemicals during reproductive development: impact on reprodructive and metabolic programing in the offspring
29	Swetha Boddula	Dr. M. S. Radhika	Etiology of severe anemia and efficacy of treatment in school going children
30	Thenarangam Sangita		Optimization and testing of nutrient dense no sugar and low sugar complementary food mixes for infants and young children - A comprehensive study
31	Shrunga Shree S		To be decided
32	Shally Vishnoi		To be decided
33	Pavithra R C		To be decided

S.No	Name of Student	Name of Guide	Thesis Title
34	Aruna Talari	Dr. Devindra S	Nutritional quality, prebiotic potential and health benefits of raffinose family oligo-saccharides of Pigeonpea (Canjanus Cajan, L)
35	Deepika T		Studies on resistant starch of some plant foods and development of low glycemic index food products
36	Soumya Ranjan Pradhan		Development of database on macronutrients, minerals, glycemic index and glycemic load of commonly consumed ready-to-eat meals
37	Shreya Elma Mathew		Nutritional quality, prebiotic potential and health benefits of raffinose family oligosaccharides of chickpea (*Cicer arietinum*))
38	Sumi MS		Nutritional quality, prebiotic potential and health benefits of raffinose family oligosaccharides of green gram (*Vigna radiata (L.) Wilczek*))
39	Dr. Alekhya G		Identification and Characterization of Anti-Glycating compounds from natural sources for the control of post-diabetic complications.
40	Pallabika Gogoi	Dr. Paras Sharma	Nutritional characterization and bioaccessibilty studies of polyphenols and nutrients from pigmented rice and maize
41	Anwesha Mahajan		Characterization Encapsulation and bioaccessibility studies of polyphenols extracted from fruits and vegetables by products
42	S. Gomathi	Dr. S. Sreenivasa Reddy	Unraveling the essence of riboflavin in retinal function and development
43	Shrabani Das	Dr. Gargi Meur	Impact pf hypoxia on placenta and neonate development

Meetings/ Conferences/ Workshops and Important Events during the year

- India Post released a Picture Post Card on ICMR-NIN which was unveiled by Dr. Hemalatha R, Director, Mr. S Rajendra Kumar, Chief Postmaster General, Telangana Circle (10th March).
- The third E-Dialogue of the monthly "Maternal Nutrition Technical E-Dialogue series" on "Women's groups for maternal nutrition" organised by ICMR- NIN, FOGSI and UNICEF was conducted (5th April).
- The fifth technical e-dialogue on "Maternal Diets and Nutrition Counselling" in the monthly maternal Nutrition technical E-dialogues series was organized by ICMR-NIN and Federation of Obstetric and Gynecologic Society of India (FOGSI) (28th May).
- Teams of NIN Scientists and Technical staff completed the Fourth Round of COVID19 sero-prevalence survey which concluded in 3 Districts of Telangana. This survey included subjects of all age groups, health workers and 6-9 yr children for the first time.
- Dr. Hemalatha R, Director and Mr. J. Sreenivasa Rao, Scientist E were invited by Dr. Tamilisai Soundararajan, Hon'ble Governor, TS to discuss and design a Nutritional Intervention for Primitive Tribal Groups in Telangana on 14th June 2021. NIN is collaborating with Raj Bhavan to develop a nutritional intervention.
- The 6th E-Dialogue of the monthly "Maternal Nutrition Technical E-Dialogue series" jointly organised by ICMR-NIN, FOGSI and UNICEF India on June 25, 2021 on "Maternal Anaemia." It was chaired by Dr. Mrudula Phadke, Senior Advisor, NRHM, GOI.
- Dr. Hemalatha R, Director and Dr. Bharati Kulkarni, Scientist-F, HoD-Clinical Division, met Dr. Tamilisai Soundararajan, Hon'ble Governor, Telangana State briefed about a study being conducted by NIN "UKRI GCRF Action against Stunting Hub", which aims to examine the multifactorial determinants of stunting in a cohort of pregnant women and the offspring recruited from Hyderabad slums. The Hon'ble Governor offered to extend her support for creating awareness on child stunting and improving nutritional status of women and children among the people (30th July).
- The 7th E-Dialogue in the monthly Maternal Nutrition Technical E-Dialogue series being organised by ICMR-NIN along with FOGSI and UNICEF India was held on the topic "The FIGO Nutrition Checklist" co - organized by International Federation of Gynaecology and Obstetrics (FIGO).
- The 8th E-Dialogue in the monthly Maternal Nutrition Technical E-Dialogue series jointly organized by ICMR-NIN with FOGSI and UNICEF India was held on 27th August on the topic "The role of social marketing in maternal health and nutrition".
- ICMR-NIN conducted as part of FREEDOM RUN 2.0 *Azadi Ka Amrit Mahotsav* to commemorate Platinum Jubilee of Independent India on 24th Sept. All the Scientists, Technical & Administrative staff, PhD scholars, PG Students & Project staff participated in this "Fit India Run".

- Extension & Training Division organized two National webinars on “Nutrition, Immunity and COVID-19" (1st Sept) & “Nutrient requirements - Implications of timing and policy” (21st Sept).
- Two National webinars on “Food Fortification + Precision Nutrition in Adolescents and Women” & “COVID-19 Perspectives” organized by ICMR-National Institute of Nutrition with the support of National Commission for Women (NCW) on the occasion of National Nutrition Month Celebrations (28th & 29th Sept).
- The Scientific Advisory Committee (SAC) meeting of ICMR-NIN was held on 30th Nov 2021. Scientists presented their plan of work to the SAC members.
- Orientation program held on Laboratory Animal Experimentation and Welfare for PhD Research Scholars and Ad-hoc Training Program on Laboratory Animal Experimentation and Welfare (16th -18th Nov).
- Curtain Raiser Event of the India International Science Festival (IISF) -2021 on "History of Science and Science in History of Telangana" organized in association with Vigyan Bharati Telangana on 6th Dec.
- Local Programme Advisory Committee (LPAC) of DST-SEED Division and Skill Development Council (SDC) meeting held on 17th Dec under the chairmanship of Prof. Dr. Avil Kumar, Director of Research, Water Technology Center, Prof. Jayashankar Telangana State Agricultural University (PJTSAU).
- State-wide SARS Cov-2 Sero-prevalence Survey commenced on 5th Jan. Twenty teams consisting 4 coordinators and one lead scientist, along with state officials on the field collected blood samples.
- ICMR-NIN is one of the 75 prestigious centres chosen from across the country to host the National Science Week Festival, 22- 28 Feb 2022 as part of *Azadi Ka Amrit Mahotsav* being led by Ministry of Culture, Government of India and Principal Scientific Adviser to GoI through Vigyan Prasar. Inauguration of National Science Week was by Dr. BHVS. Narayana Murthy, Distinguished Scientist and Director General - Missiles and Strategic Systems (MSS), DRDO, Dr. Hemalatha R, Director delivered the welcome address and Dr.G.Bhanuprakash Reddy, Scientist G & Organising Secretary of the festival provided outline of the event and over 600 visitors participated in the event.
- Induction orientation programme conducted for newly recruited Scientists and Technical Officers of ICMR-NIN (11th March).
- On the occasion of World Obesity Day an E-dialogue series on “LetsFixOurFood” was launched with an aim to Advance India's Young People's Right to Healthy Foods and Healthy Food Environments on 4th March. The first E-dialogue in the series was on 'Restricting Advertising and Marketing of Unhealthy Foods and Beverages to Children'. ICMR-NIN in collaboration with Indian Council of Medical Research, UNICEF India, NITI Aayog, Public Health Foundation of India (PHFI), Deakin University YuWaah - Generation Unlimited India, Ministry of Health and Family Welfare, GoI, Ministry of Women & Child Development, GoI, World Health Organization (WHO) and World Food Program USA are organizing this E-dialogue series.
- 12th Maternal Nutrition E-dialogue on 'Nutrient Requirements 2020 and its Implications for Public So is stress Health Interventions' was held on 24th March.

Scientific Publications

PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Adivitiya MSK, Soura Chakraborty, Shobi V, Suneel K: Mucociliary respiratory epithelium integrity in molecular defense and susceptibility to pulmonary viral infections. Biology. 10, Jan'2021. https://doi.org/10.3390/biology10020095. **(CI 5) (IF 3.796)**

2. Alekhya S, Naveen Kumar R, SubbaRao GM, Balakrishna N, Sudershan Rao V: Safety of cooked foods in home and street vended conditions beyond two hours: relevance of 'safe storage temperature' message from WHO's five keys for safe food in India. Int J Food Safety Nutr Public Health. 6:104-119, Apr-Jun'2021. **(CI - -) (IF - -)**

3. Amruth Rao P, Sreevennala Rao P, Kavanashri NP, Devi CHVR: Effect of Probiotic curd (Indian Dahi) supplementation in delaying the progression of pre-diabetes to diabetes mellitus – a pilot study. Int J Diabetes Metab Disord. 5:121124, Jan'2021. **(CI - -) (IF 1.421)**

4. Anitha S, Ian Givens D, Rosemary B, Kane-Potaka J, Sulaiman NLB, Tsusaka TW, Subramaniam K, Ananthan R, Devraj JP, Raj Kumar B: Calcium from finger millet-a systematic review and meta-analysis on calcium retention, bone resorption, and in vitro bioavailability. Sustainability. 13:8677, Aug'2021. https://doi.org/10.3390/ su13168677. 102294. **(CI 2) (IF 3.251)**

5. Anitha S, Kane-Potaka J, Tsusaka TW, Rosemary B, Ananthan R, Ian Givens D, Devraj JP, Subramaniam K, Prasad KDV, Vetriventhan M, Raj Kumar B: A Systematic review and meta-analysis of the potential of millets for managing and reducing the risk of developing diabetes mellitus. Frontiers Nutr. 8:687428, Jul'2021. doi:10.3389/fnut.2021.687428.**(CI 5) (IF6.576)**

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7. Anurag B, Madhavi B, Banurekha V, Kannan T, Basilea Watson, Bharati Kulkarni, Manjula Singh, Rakesh Dayal, Rajeev RP, Anindya Mitra, Kiran R, Sachdeva KS: The RATIONS (Reducing Activation of Tuberculosis by Improvement of Nutritional Status) study: a cluster randomised trial of nutritional support (food rations) to reduce TB incidence in household contacts of patients with microbiologically confirmed pulmonary tuberculosis in communities with a high prevalence of undernutrition, Jharkhand, India. BMJ Open. 11:e047210. doi:10.1136/bmjopen-2020-047210, May 2021. **(CI - -) (IF 2.692)**

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9. Bhanuprakash Reddy G, Shalini T, Santu Ghosh, Raghu P, Naveen Kumar B, Bharati Kulkarni, Laxmaiah A, Umesh Kapil, Sila Deb, Hemalatha R, Anura VK, Sachdev HS: Prevalence of vitamin A deficiency and dietary inadequacy in Indian school-age children and adolescents. Eur J Nutr. Jul'2021. doi: 10.1007/s00394-021-02636-7. **(CI - -) (IF 4.449)**

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23. Khandare AL, Vakdevi V, Ravi Prakash J, Srinivas D, Srinivasu K, Anitha Chauhan, Naveen Kumar B, Longvah T: Potential health risks associated with the heavy metal content in commonly consumed food from Prakasam district of Andhra Pradesh, India. Biol Trace Element Res. Nov'2021. https://doi.org/10.1007/s12011-021-02918-5. **(CI - -) (IF 3.738)**

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27. Maheshwar M, Sylvia Fernandez Rao, Jagdish Buwade, Narender K, Balakrishna N: Media usage profile of adolescents in varied economic background. J Media Commun Stud. 13: 38-49, Apr-Jun'2021. **(CI - -) (IF - -)**

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29. Mallinson PAC, Bharati Kulkarni, Santhi B, Sanjay Kinra: Association between parents' socioeconomic conditions and nutritional status during childhood and the risk of cardiovascular disease in their adult offspring: an intergenerational study in south India. J Epidemiol Community Health. 75: 1091-1097, Nov' 2021. **(CI - -) (IF 3.342)**

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