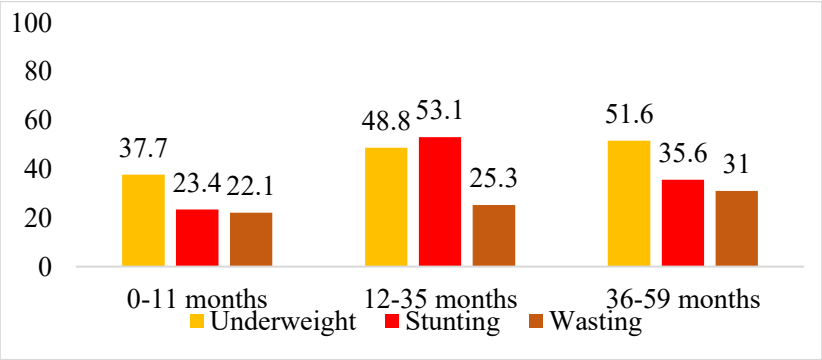
ICMR-NATIONAL INSTITUTE OF NUTRITION
RESEARCH HIGHLIGHTS (2022-23)

Major projects undertaken during the year 2022-23 and their achievements.

Epidemiological Studies

1. **Comprehensive Nutrition Assessment of Tribal Population in *Attappadi* Taluk, Kerala – A Rapid Exploratory Study: The objective of the study was** to assess the comprehensive nutritional status of various age and physiological groups of people in Attapadi Taluk of Kerala. The survey covered 480 households, 523 children, 150 adolescent girls, 40 pregnant women, and 110 lactating mothers from 20 randomly selected tribal villages under ITDA of Agali, Palakkad district.The prevalence of underweight (48.3%), stunting (41%), and wasting (27.4%) among children (0-59 months) was a serious public health problem. Anaemia was prevalent among young children (91.2%), adolescent girls (96.6%), and pregnant women (86.8%), with iron deficiency anaemia being prevalent among 65% of children aged 12-24 months. The prevalence of various micronutrients like vitamin B12, D, folate, and vitamin A was reported in 35%, 20%, 16%, and 12% of children, respectively. The mean daily intakes (g/day) of all the foods except for roots & tubers among children (1-3 years) residing in *Attappadi* were lower than the recommended daily intakes (RDIs). The consumption of green leafy vegetables, milk, milk products, fruits, and animal-sourced food was poor, leading to a substantial deficiency in various micronutrients. Inadequate dietary intake of a nutritious diet was a significant contributor to undernutrition and hidden hunger. Although there has been a marginal reduction in the prevalence of malnutrition from 2013 to 2022, underweight children prevalence has remained a serious public health concern.

Fig-1: Prevalence (%) of under-nutrition among children by age (in months)



2. **Efficacy of cooked millet diet and their effect on hemoglobin and micronutrient status among anemic women of reproductive age between 17-22years – A cluster randomized control trial:** Over 2764 subjects in the age group of 17-22 years were screened of which, 1147 subjects were included for cooked millet intervention and rest were in regular diet group. A total of 409 mild and moderately anaemic subjects from cooked millet intervention group and 414 subjects from regular diet group have been included for Hemoglobin and other blood biomarker status analyses (Sr.Ferritin, Sr. Iron, Sr.Ca, Sr.Zinc,Vit-B12, Folate, vita A and D,Hs-CRP and STFR) to assess the impact of Cooked Millet meal replacement. Regular rice and wheat-based meals are being replaced

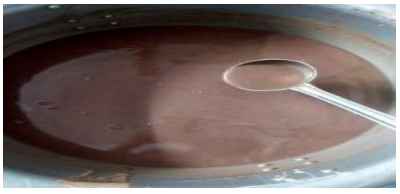
with three recipes from Pearl Millet and 2 from Foxtail and 3 from finger millet per week. Supplementation has been completed for 90 days without any adverse events and haemoglobin and other blood biomarker status will be assessed after 6 months of supplementation.

PEARL MILLET PULAV

PEARL MILLET BISIBELE BATH

RAGI IDLY

RAGI LADDU

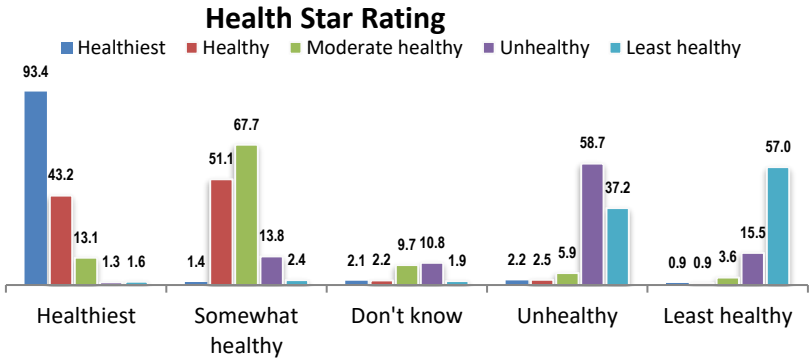


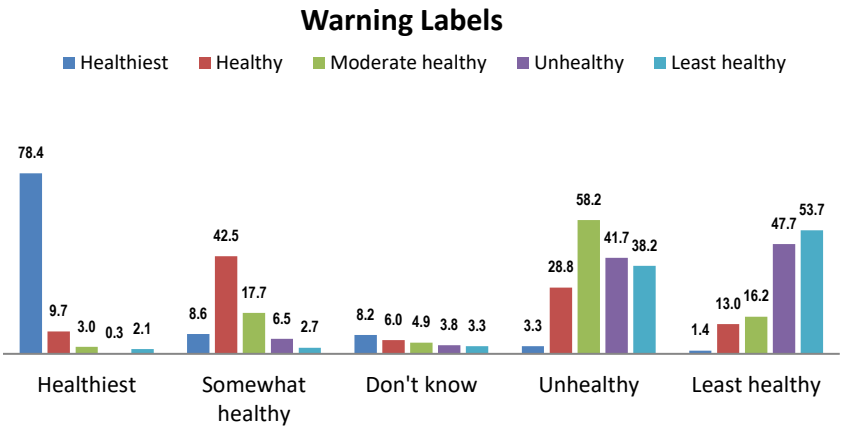
RAGI MALT

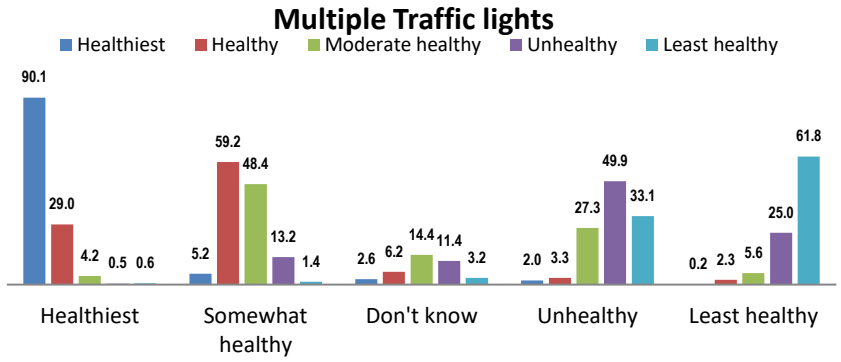
3. **Assessing Effectiveness of Front-of-Pack Nutrition Labels for Processed Pre-packaged Food Products in India – A Cross-Sectional Study on Formats, Acceptability and Potential Use:** Front of Pack Nutrition Labelling (FOPNL) system is one of the policy tools to help reduce nutrition related non-communicable diseases. FOPNL on pre-packaged foods and beverages can provide quick and easy-to-understand information for consumers at the point of purchase, therefore aiding them to make healthier food choices. Globally various formats of FOPNL have been implemented, however decision on the suitable FOPNL format to be implemented in India is under consideration with the Food Safety and Standards Authority of India (FSSAI). The

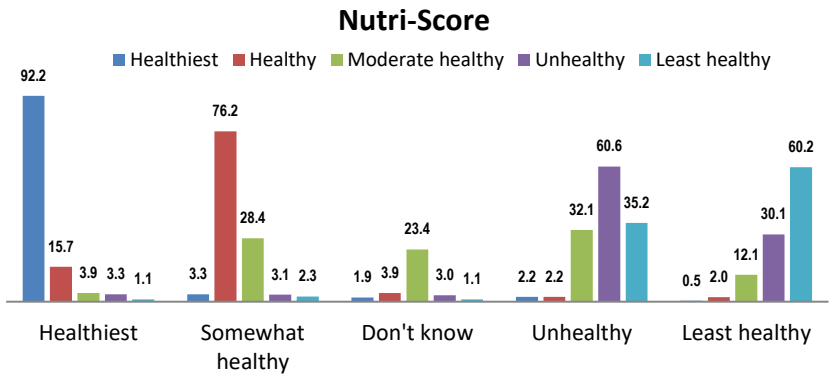
decision on which FoPNL format will work in Indian context should be based on data on consumer acceptability, and understandability of the different label formats. This was a cross-sectional study conducted among 3231 participants (Adults – 2616, and adolescents – 615) in the age group 10-60 years in five regions of India namely New Delhi, Jorhat (Assam), Kolkata, Pune and Hyderabad. The data collection was completed in a single contact with the participants using a validated questionnaire. Apart from socio-demographic details, and packaged food purchasing behaviours, the participants perception on likeability, attractiveness and perceived cognitive workload of the five different formats of the FOPNL i.e., Nutri-Score (NS), Health Star Rating (HSR), Warning label (WL), Multiple traffic lights (MTL), and Nutri-Star (NSR) was assessed. Additionally, 1/5th of the participants was randomized to one of the five FoPNL formats and asked questions about objective understanding, perceived product healthfulness, purchase intention and willingness to change purchase behaviour. The findings showed that most respondents ranked NS as the preferred first choice (41.3) of FOPNL and it scored highest in terms of likeability. Participants felt MTL (78.3) and WL (70.5) were more reliable than NS, HSR, and NSR. NS, HSR, MTL, and NSR performed better in identifying healthiest option correctly compared to WL. More participants in the WL group were not confident of healthiness healthy and moderately healthy variants, and they responded that they would not buy a product even if one warning sign was present. WL performed better in influencing purchase intention, product choice, and eating behaviour across the product variants compared to other FOPNL formats. WL and NSR had greater impact in altering the health perception of the food products, as presence of even one octagon or absence of stars prompted more cautious behaviors in choosing the foods. However, among the summary indicators, even presence of 2 stars (in HSR) or Code D (orange shades in NS) prompted higher choice of the same variants of food and lesser willingness to opt for others. In conclusion, to identify healthiest or unhealthiest variants any format of FOPNL can work. However, for promoting healthier food choices among the available variants, summary indicators (NS and HSR) seem to work better, but to deter consumption of even moderately unhealthy foods, Warning Labels (NSR or WL) are a better option.

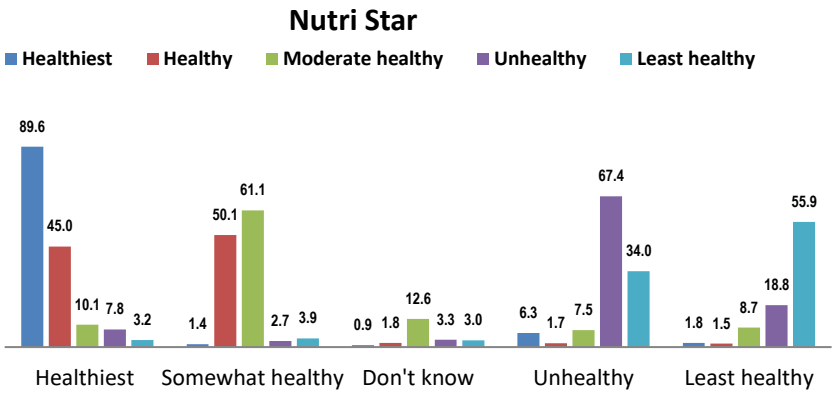
Participants' responses of perceived product healthfulness of product variants showed under different FOPNL formats (N=3231)











4. **Eggs for Improving Nutrition, cognitive development and Reducing linear growth retardation among Infants and Young Children (ENRICH):** An egg supplementation trial among 9-18 months old children in Hyderabad, India along with a unique behavioural change communication (BCC) methods are being used to improve adherence to egg consumption in this age group directed against stigma and lack of awareness of

the benefits of egg consumption in infants and young children. Our preliminary findings show better compliance rates.

A Mother Displaying Egg Compliance Calendar

A child who is consuming whole eggs

A child who is consuming whole eggs Compliance Calendar

A Mother Displaying Egg

5. **Health, Nutrition, and demographic surveillance system at Addagutta, Hyderabad and MRHRU- Chandragiri, Andhra Pradesh (HNDSS):** 10350 people from 4679 rural and urban households were enrolled in the study, of which, 1341(28.66%) households were from Rural area and 3338(71.34%) households were from urban area. Out of 10350 people, 8317 (80.36 %) live in urban area and 2033 (19.64 %) live in rural areas. The study is under progress

6. **Anakapalle outbreak investigation:** On 2nd August, 2022, over 129 women employees of an apparel industry located at Anakapalle district, Andhra Pradesh, South India, have reported acute gastrointestinal and neurological symptoms and got admitted in government and private hospitals. This has attracted media glare from national communities. A multi-disciplinary team from NIN consisting of epidemiologists, food toxicologists and nutrition

experts has conducted an invitation to identify the cause of the outbreak. An unmatched case control methodology was adopted for investigating the cause of the outbreak. Hence, a control group without symptoms were also interviewed and their biological samples were also collected. A total of 64 blood samples (52 cases and 12 controls), 38 urine samples (26 cases and 12 controls) were collected from both hospital and community visits. Clinical symptoms through a case report forms (CRF) were collected from 59 participants of which 47 were cases and 12 were controls. Mean age of Cases was 30.8 years and Controls was 34.3 years. Major Symptoms in cases were giddiness (77 %), vomiting (66 %), comatose (66 %), breathlessness (58 %) and burning sensation of Eyes (45 %), Nausea (35%). Out of 52 blood samples, four cases were found positive for Imidacloprid, an Insecticide. All blood samples of controls, urine samples of cases and controls, and water samples from the factory were found negative for Imidacloprid. The clinical picture and epidemic pattern are characteristic of a common source, single exposure outbreak suggestive of inhalation of Imidacloprid, organophosphate insecticide. It might be due to spraying of insecticides for treatment of bedbugs on the fabrics in the factory.

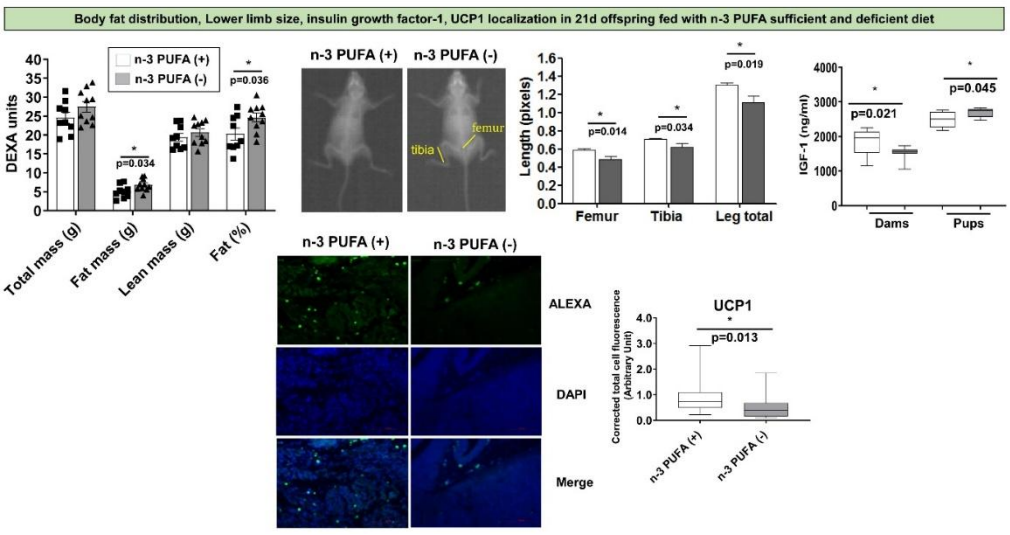
7. **"Let's Fix Our Food" – E dialogue series- supported by UNICEF:** Childhood and adolescent obesity are significant public health concerns in India. This worldwide epidemic has important consequences like an increased risk of developing non-communicable diseases (NCDs) later in life. Since food choices, food habits, and food environment along with the lifestyle inculcated during the formative years have a significant role in development and prevention of NCDs among adolescents, focusing on food and nutrition literacy and measures to restrict unhealthy food consumptions can navigate children towards a healthier food environment. With overweight and obesity becoming a public health priority, strategies to reduce the consumption of non-nutritive caloric intake are being discussed in this multi-stakeholder initiative along with youth engagement for advocating a healthy food environment. In this study 'dialogues' have been used as a tool to gain deeper insights into the issue. Communication among the consortium partners along with national and international subject experts for developing policy directions to combat the adolescent obesogenic environment. Six E-dialogues with an average duration of 90- 120 minutes have been conducted so far under the project. Each E-dialogue initiated with flagging the issues, followed by international and national experts sharing perspectives on the topic, followed by a panel discussion. Finally, few important take away points were assembled to be considered for policy recommendations. The six E-dialogues were held on (i) restricting advertising and marketing exposure of unhealthy foods and beverages for children (ii) Impact of advertising and marketing on adolescent food choices (iii) Front of Pack Nutrition

Labelling for promoting healthy food choices among adolescents (iv) Nutrition literacy among adolescents (v) Taxation on HFSS foods to discourage consumption (vi) Priorities in nutrition education for school age children. A host of knowledge products and policy briefs were co-developed with PHFI and other consortium partners to be shared with NITI Ayaog

BASIC STUDIES

8. **Impact of maternal omega-3 (n-3) fatty acid deficiency on placental development & functions and offspring's body and brain development in mice:** Maternal omega-3 (n-3) polyunsaturated fatty acids (PUFAs) deficiency can affect offspring's adiposity and metabolism by modulating lipid and glucose metabolism. However, the impact of n-3 PUFA deficiency on the development of fetal thermogenesis and its consequences is not reported. Using an n-3 PUFA deficient mice, fetal interscapular brown adipose tissue (iBAT), body fat composition, insulin growth factor-1 (IGF-1), glucose transporters (GLUTs), and expression of lipid storage & metabolic proteins in the offspring were assessed. The n-3 PUFA deficiency did not change the pups' calorie intake, organ weight, and body weight. However, the offspring's skeletal growth was altered due to excess fat to lean mass, reduced tibia & femur elongation, dysregulated IGF-1 in the mother and pups. Localization of uncoupling protein 1 (UCP1) in iBAT exhibited a reduced expression in the deficient fetus. An altered musculoskeletal growth in the offspring is associated with impaired browning of the fetal adipose, dysregulated thermogenesis, growth hormone, and expression of glucose and fatty acid metabolic mediators due to maternal n-3 PUFA deficiency (**Fig.3**). BAT had higher metabolic sensitivity compared to WAT in n-3 PUFA deficiency. Maternal n-3 PUFA intake may prevent excess adiposity and growth failure by modulating fetal development of thermogenesis and skeletal growth dynamics in the mice offspring.

Fig.3



9. **Impact on the maternal exposure of Bisphenols: implication to developmental programming of glucose homeostasis and insulin resistance in offspring**: Plastic-derived endocrine disrupters like bisphenols are very predominant in terms of exposure to our daily life. Early life development is the most susceptible window for genome programming. Current efforts have been initiated to assess the impacts of maternal exposure to Bisphenols on the developmental programming of metabolic homeostasis

and its in-utero effects on risks of metabolic and reproductive diseases. We investigated the effects of orally administered BPS and BPA (0.4, 4.0, 40.0 µg/kg bw/d) during gestation (gD4-21) on metabolic and reproductive development in the offspring. Initial data showed obesogenic effects in pups (**Fig.1**) when their mothers were administered BPA and BPS at concentrations reported in the exposure population. The bisphenol-exposed offspring were overweight, with altered body fat composition, higher cortisol levels, and potential disruption in testicular development and sperm maturity (**Fig.2**).

Fig.1

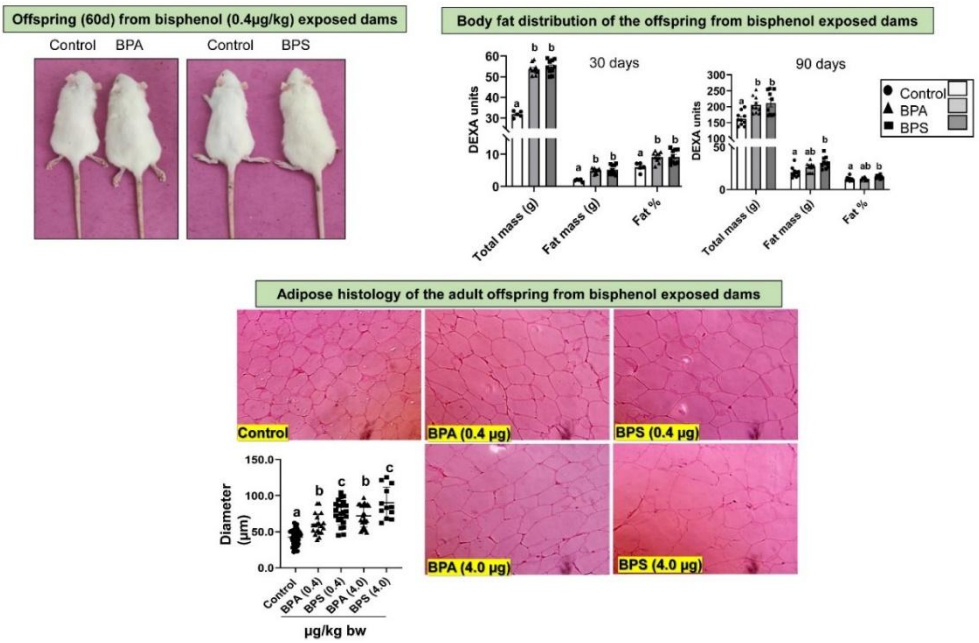
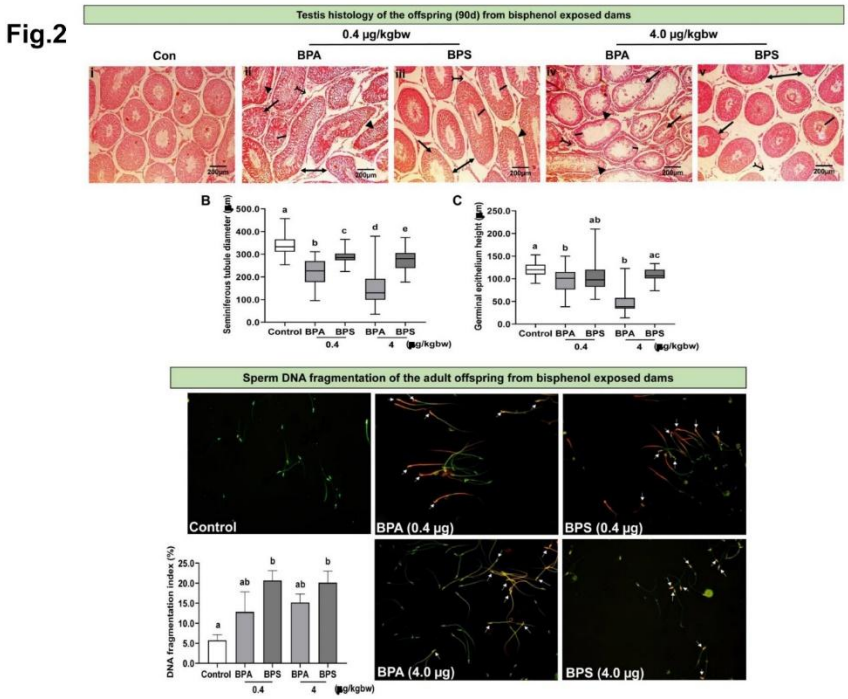


Fig.2



10. **Exploring therapeutic potentials of nano-entrapped curcumin on inflammation associated disease:** Despite its potential, the beneficial effects of curcumin are restricted due to its limited systemic availability, low bioavailability, rapid metabolism and clearance. Encapsulation of curcumin can improve its delivery and absorption into

metabolic machinery. An encapsulated curcumin was developed after entrapping it in the lipid-based nano-system and subsequently investigated the curcumin nano-emulsion's therapeutic and protective function in vitro. Nanocurcumin was prepared by producing an emulsion using combinations of oils and a surfactant detergent (methods patent awaiting). Different combinations of micelles were prepared, and their biophysical properties were evaluated using Zetasizer (DelsaTM Nano, Beckman Coulter). The micellar solution containing nanocurcumin was characterized based on its dynamic light scattering (nm) for size measurements, negative zeta potential for optical stability in solution, and polydispersity index as heterogeneity index of the particle size. DLS data indicates that the nanoparticles' size ranged from ~150-200nm. The negative zeta potential of the combinations was measured around -30mV, indicating stable particles in a liquid medium. The polydispersity index (PDI) was around ~30%, indicating a monodisperse population of particles needs to be lowered. DLS data suggests that spherical substances (micelle) are likely present in the solution. However, further confirmation of the particle size of the nanocurcumin using a transmission electron microscope is required. The zeta potential and DLS data satisfy the optical stability and nanoparticle size of the micelle we created. Treatment with nanocurcumin indicates that much of the curcumin in nano-formulation penetrated the cells, as it shows higher fluorescence when compared to native curcumin. In native states, curcumin covers fewer cellular areas than nano form. The anti-inflammatory potential of nano curcumin and native curcumin was assessed by LPS-induced HeLa cells. Pro-inflammatory mediators such as IL-1β and NF-κB showed a significant decrease in their expression in nanocurcumin-treated cells compared to cells treated with native curcumin. These data indicate that nano curcumin suppresses the expression of pro-inflammatory cytokines more efficiently than native curcumin and thus could have a greater anti-inflammatory effect than native molecules.

11. **Evaluation of poly-spice (functional food) formulation for diabetes and its complications:**A functional food mix (FFM) of amla, turmeric, cinnamon, black pepper, ginger and fenugreek in a specific proportion found to be very effective in delaying cataract progression in rats. As the diabetic cataract is multifactorial caused by glycation of crystallins, polyol pathway activation, oxidative stress, and inflammation, likewise the FFM with its multiple activities showed promising effect in preventing cataract progression. An IPR has been files for this functional food mix.

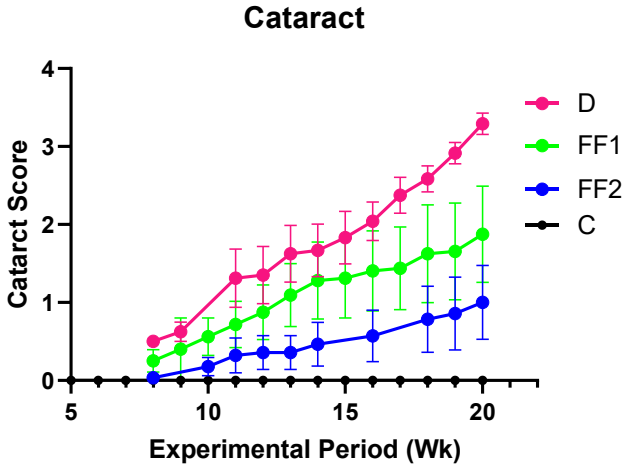


Figure: A functional food mix delayed the progression of cataract in diabetic rats. Quantitative representation of cataract progression in the experimental groups with time. Data is mean ± SEM. C-control, D-diabetes, FF1-diabetic rats treated with functional food dose-1, FF2-diabetic rats treated with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

A functional food formulation containing amla, turmeric, cinnamon, ginger, and black pepper powder in tackling the polyol pathway, inflammatory, and AGEs pathways in diabetic renal manifestations has been developed. This FFF has also shown an antifibrotic effect. The synergistic effect of these five compounds targeting various molecular pathways is well evident in this study in preventing diabetic nephropathy in rats.

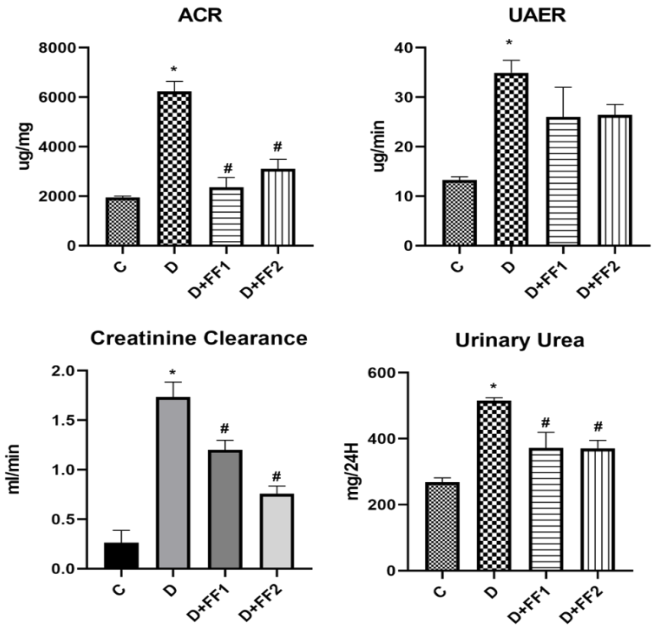


Figure: A functional food formulation (FFF) attenuated proteinuria and renal pathological changes in diabetic rats. Effect of FFF on renal parameters. Data is mean ± SEM. C-control, D-diabetes, FF1-diabetic rats treated with functional food dose-1, FF2-diabetic rats treated with functional food dose-2.

12. **Effect of maternal protein restriction (quantity and quality of protein) on body composition and protein quality control processes in the muscle of the offspring:** Investigated the effect of combined prenatal to postnatal protein restriction and prenatal to perinatal protein restriction with postnatal rehabilitation on protein quality control processes and proteolysis in the offspring. Results suggest that maternal protein restriction induced muscle atrophy and accelerated muscle proteolysis by augmenting the protein quality control processes, including unfolded protein response, ubiquitin proteasome system, autophagy, and muscle-specific E3 ligases in the off- spring. However, prenatal to perinatal protein restriction with postweaning rehabilitation showed little effect or no effect on SM proteolysis.

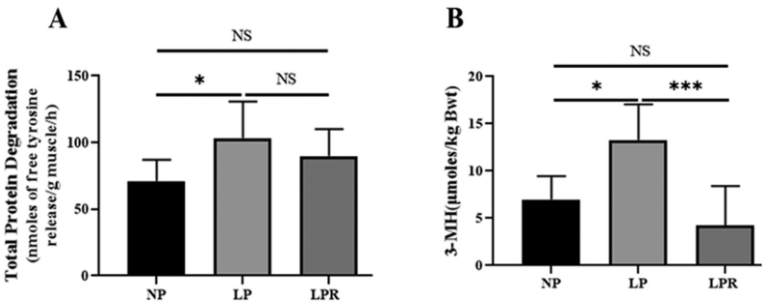


Figure: Effect of chronic protein restriction on skeletal muscle proteolysis of the offspring: Total protein degradation (TPD) was measured in the gastrocnemius muscle. ***(Panel A)*** the TPD was measured by the amount of free tyrosine released into the medium ***(Panel B)*** estimation of 3-methylhistidine excreted in the urine. Values are the mean ±SD; $*P < .05$, and $***P < .001$. NP, normal protein; LP, low-protein; LPR, low-protein group rehabilitated with NP diet.

13. **Identification and characterization of suitable biomarkers for zinc deficiency using Next Generation Sequencing (NGS) based platforms:** Transcriptome analyses in the small intestine of rats fed with zinc deficient diet (0.4 mg/kg) showed that the expression o of total 531 genes were significantly modulated, out of which 214 genes were significantly down regulated compared to the control animals with standard diet containing 29.6 mg/kg zinc. On the other hand, supplementation of 100% of zinc present in the control diets of these cells caused significant modulation in the expression 93 genes of which 44 were found to be upregulated and 49 were down regulated. Gene ontology analyses revealed that, in zinc deficient animal genes related to the immunological functions are mostly affected which include chemokine signalling pathways, B cell receptor signalling pathways, leukocyte functions etc. This finding is in line with previous reports where role of zinc is highlighted in regulating functions of immune cells and explains why zinc deficiency causes susceptibility to various infections. In addition, several noncoding RNAs like lncRNAs and snoRNAs were found to be differentially expressed indicating their possible roles in zinc homeostasis which would be an interesting area to investigate further. Since expression of many of these regulatory RNAs appear to be condition specific, they can be explored further for developing diagnostic markers to assess zinc deficiency

14. **Identification and functional characterization of miRNAs in genetic models of obesity and diabetes:** Hepatic expression of PPARα during obesity and the plausible role for miRNAs in mediating hepatic PPARα dysregulated expression was explored. A bioinformatics approach using TargetScan miRNA database allowed us to identify eight miRNAs belonging to the miR-19 and the miR-17 families that could potentially target the PPARα mRNA in rats of which the miR-17-5p and miR-93-5p were chosen to explore their yet-to-be-identified roles in regulating hepatic PPARα expression. Gene expression studies revealed a significant increase in the hepatic expression of miR-17 and miR-93 in the WNIN/Ob obese rats temporally at 60, 120 and 180 days compared to the lean animals. Dual luciferase reporter assays confirmed that both the miR-17 and miR-93 targeted PPARα mRNA with the reduction in the luciferase expression being significantly greater for the wild type PPARα -3′UTR which was co-transfected with miR-17 compared to miR-93 (Fig. 1). Further, the use of miRNA sponge technique using miR-17 and miR-93 sponges in H4IIEC3 rat hepatocytes revealed that there was an enhanced rescue of the miRNA targeting site in the 3'-UTR of PPARα mRNA due to sequestration of miR-17 and miR-93 by the respective miRNA sponges with a resultant increase in PPARα expression. A significantly up-regulated expression of several genes participating in peroxisomal and mitochondrial β-oxidation, lipid transport and Acetyl-CoA synthesis and a significantly down-regulated expression of lipogenesis-related genes were observed in the miR-17 sponge treated rat hepatocytes. However, the outcome of the miR-93 sponge treatment was not similar to that of the miR-13 sponge where only a few of the PPARα target genes were upregulated. Hence, miR-17 presents a novel target for addressing the hepatic PPARα mediated regulation of lipid homeostasis which could have far-reaching benefits in addressing obesity and its associated complications.

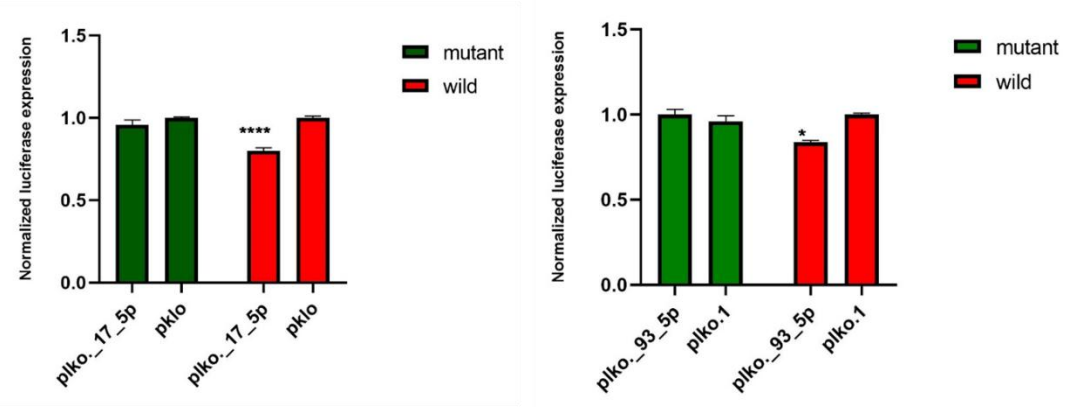


Figure 1: Validation of miR17 and miR93 targeting PPARα using dual luciferase assay.

15. **Evaluating the therapeutic potency of poly-spice formulation in neutralizing cytokine storm: This study** investigated the prophylactic effect of a functional food mix comprising three anti-inflammatory plant products: turmeric, amla, and black pepper (TAB) against lipopolysaccharide (LPS)-induced acute ling injury in rats. Results showed that TAB ameliorated LPS-induced increase in circulating inflammatory cytokines and significantly prevented lung histopathological changes. TAB also suppressed LPS-activated ER stress markers and apoptotic markers in the lung. The anti-inflammatory effects of the TAB support its potential use as an adjuvant to mitigate acute ling injury.

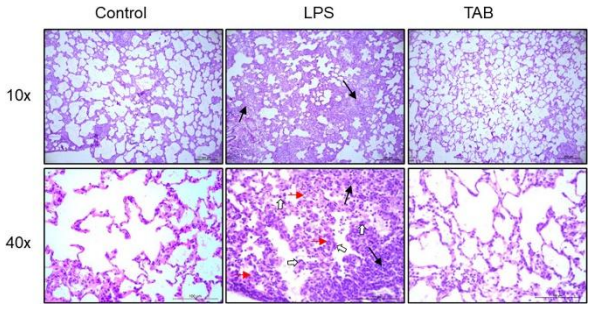


Figure: Histology of LPS-induced lung injury in rats. LPS = lipopolysaccharide group; TAB = functional food mixture (turmeric + amla + black pepper) pre-treated group. Black filled arrows are indicating decreased alveolar spaces, red arrows are indicating thickened alveolar membrane, black open arrows are indicating macrophage infiltration near alveolar space

16. **Efficacy of Vitamin D2 vs Vitamin D3 on the classical and non-classical functions in rat models:** Serum vitamin D-dependent parameters such as calcium, phosphorus, alkaline phosphatase were not different between groups given either Vitamin D2 or D3. Intestinal calcium absorption appeared to be better in the groups given Vitamin D3 compared to D2. Bone mineral content and density was similar in groups administered either D2 or D3.

17. **Investigating the therapeutic potential and to study the molecular mechanism of the combo of cowpea isoflavones and Alpha lipoic acid (ALA) as a natural source for the treatment of osteoporosis in terms of MC3T3-E1 osteoblast differentiation and bone remodeling**: Cellular signaling mechanisms in osteoblast differentiation and bone remodeling: various proteins marker like (Collagen-1, OPG, RUNX2, ALP, OSX,β-Catenin and Smad3) showed a significant increase when compared with their respective controls. Osteoblastic activity and osteoblastogensis after treatment indicating that CP isoflavones and ALA stimulates bone formation. Thus, the study may provide the information on the beneficial role of natural Isoflavones in protecting the bone mass.

18. **Microbiological Safety and Quality of commonly consumed herbal drugs: Over** 32% of 90 herbal drugs collected from the market were exceeding the microbiological limits of US Pharmacopoeia. About 20%, 8.9 % and 3.3% of solid, semi-solids and liquid herbal drug samples are exceeding the limits. The data developed in this research helps to establish microbiological criteria for herbal drugs regulations in India.

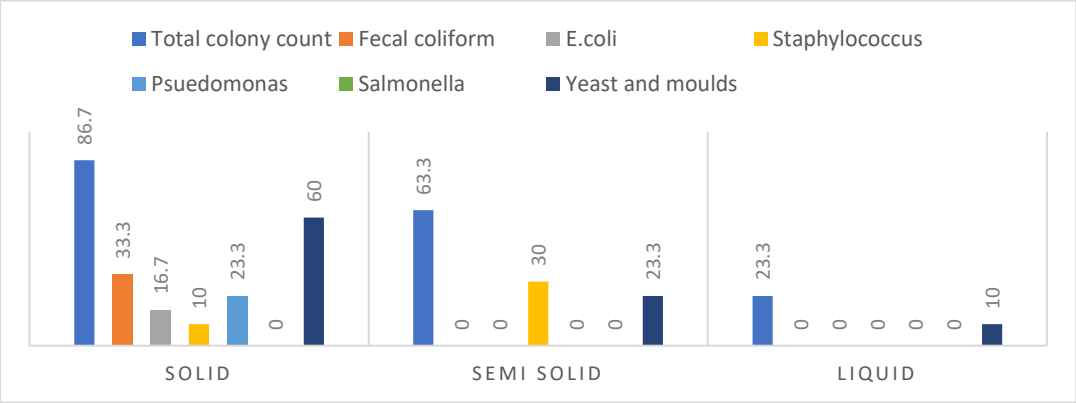


Fig. Microbial contamination of herbal drugs among solids, semi-solids and liquids

DIETETICS

19. **Development of low and moderate glycemic index, and glycemic load food products for the management of diabetes, pre-diabetes and other non-communicable diseases**: The study is unique and to our knowledge the first of its kind to estimate glycemic carbohydrate and Glycemic index of ready-to-eat, ready-to-prepare foods and beverages in India. Recently, much discussion has centered on the validity of measured GI values and how useful the concept of GI is for the general public and for food labelling. Till date not many Indian foods have been tested for GI in a scientific manner using an internationally approved scientific protocol. The developed database on glycemic carbohydrate and GI may thus be of particular benefits to Indians due to high prevalence of impaired glucose tolerance and diabetes. This Scientific evaluation of GI can be helpful for food labelling purposes and health practitioners.

20. **A comprehensive study to establish the health benefits (in vivo) of nutri-cereals A way forward for main streaming millets:** This study aims to develop accurate database on the macro and micronutrients of commonly consumed millets. Eight different varieties of millets were tested among the millets moisture content ranged from 9.55% (foxtail millet) to 11.44% (finger millet), protein from 5.41% (kodo millet) to 14.98% (foxtail millet), fat from 1.91% (finger millet) to 5.42% (pearl millet), ash content from 0.80%

(Foxtail millet) to 2.64% (little millet), available carbohydrate from 62.37% (little millet) to 66.93% (pearl millet) and dietary fiber from 6.55% (foxtail millet) to 15.94% (Kodo millet). From the results, it is evident that most of the millets contain low amount of available carbohydrates and fairly good protein and high levels of dietary fiber. Study is in progress to develop the glycemic index and loads of the millets and millet products

21. **Makhana -Nutritional Composition and Acceptability Study:** Makhana (Euryale ferox Salisb) is also known as gorgon nut and fox nut, prickly waterlily, Lotus seeds is an important aquatic crop belongs to family Nympheaceae. It is considered as nutrient rich products although scattered and limited reports are available on nutritional composition of makhana. A **study e**valuated the comprehensive nutritional profile of makhana and its value-added products, and changes in same upon storage. The Glycemic Index of Makhana Pops (Mean ± SE) is 68.7 ±2.16. The Glycemic Load of Makhana Pops was found to be 17. The roasted and seasoned Makana Pops is a recipe with a medium Glycemic index and a medium Glycemic Load

CLINICAL STUDIES

22. **Evaluation of maternal micronutrient status, inflammation and effect of COVID -19 on placentas of anaemic pregnant mothers, including fetal outcome- a pilot study:** 212 term pregnant mothers were recruited, 58% of whom were positive for COVID-19 IgG antibodies and 63.2% were anaemic, although all were asymptomatic during their entire course of pregnancy and also were RT-PCR negative for SARS-CoV-2 antigen at the time of their admission for delivery. Asymptomatic COVID-19 during pregnancyseemed to be associated with various abnormal placental histopathologic changes related to placental hypoxia independent of maternal anemia status. Our data supports an independent role of SARS-CoV-2 in causing placental hypoxia in pregnant women.