वाधिक प्रतिवेदन Annual Report

2023 - 24





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

# वार्षिक प्रतिवेदन Annual Report

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#### **ICMR - NATIONAL INSTITUTE OF NUTRITION**

Indian Council of Medical Research Hyderabad, Telangana, INDIA Website: www.nin.res.in

### **CONTENTS**

S.No	Title of the project	Page No.
	Staff List	i
1	Modification of extracellular matrix proteins augment by non- enzymatic glycation augments fibroblast activation	1
2	Elevated WT1 expression in podocytes is linked to obesity-related glomerulopathy	5
3	Functional food mixture extricates D-galactose-induced skeletal muscle impairment in rats	9
4	Determinants of oral iron supplementation response among adolescent girls with iron deficiency anaemia	12
5	Development of new bio-marker for quantification of acetylcholinesterase (ache) enzyme activity, organophosphate, carbamates, and nerve agent in biological and ambient matrices	14
6	Information education and communication (IEC) based intervention study for chronic kidney disease (CKD) and its associated factors in tribal population—TS	
7	Study on physico-chemical and bioactive properties of bamboo rice (bambosa arundinacea willd) consumed in Southern-Western Ghats	
8	Study on the nutritional value of different processed and cooked millet	
9	Impact of the millet year 2023 campaign in shaping knowledge attitude, and practice of millets consumption among college going adult women in urban areas of Telangana	
	Meetings/Conferences/Training Programmes held at NIN	
	Scientific Publications	
	SAC Experts	44

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## 1. MODIFICATION OF EXTRACELLULAR MATRIX PROTEINS AUGMENT BY NON-ENZYMATIC GLYCATION AUGMENTS FIBROBLAST ACTIVATION

#### Introduction

Fibrosis is an abnormal tissue repair response that results in the accumulation of excess extracellular matrix (ECM) components in tissue. It is the primary manifestation of chronic inflammation, injury and repair processes of many human diseases including pulmonary fibrosis, renal fibrosis, cardiac fibrosis. The cells and the ECM share a dynamic and reciprocal relationship wherein cells are involved in the production and remodeling of the ECM, which in turn mediates the composition and topography of the ECM. Conversely, the ECM exerts an influence on cell behaviours and functions. Also, aging and any age-related disorders (such as diabetes) alter the composition and structure of the ECM due to post-translational modifications (PTMs). Glycation, nitrosylation, oxidation, and the cross-linking of ECM proteins by PTM have been shown to result in impaired tissue elasticity and a more rigid ECM, thereby serving as drivers of pathology in various age-related diseases, including renal and cardiac fibrosis. Studies have shown that people with diabetes may experience AGE accumulation in the lungs and kidneys leading to decreased function parameters. The mechanisms whereby injury, aging, and hyperglycemia-driven modification alter homeostasis between the ECM and fibroblast interactions that exacerbate the severity of fibrotic lung disease remain to be defined. Although excessive glycation and the formation of AGEs on longlived proteins such as the ECM can alter their structure and function, their accumulation and contribution to fibroblast activation and fibrosis remained unexplored. Thus, the study aims to investigate the effect of the CML-modified ECM (CML-ECM) on fibroblast activation.

#### Methodology

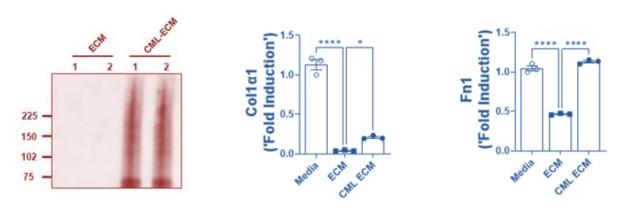
Extraction of extracellular matrix (ECM) proteins: The mouse lungs were perfused or rinsed thoroughly with decellularization buffer and incubated at room temperature with rotation for 6–10 h. Then, tissue was washed with PBS to remove the detergents and sterilized with 10% penicillin/streptomycin for 10-20 min. Tissue was further incubated with benzonase to digest all types of DNA and RNA in a buffer for 30 min at 37°C. To remove remnant DNA, the tissue was rinsed with PBS containing 10% FBS followed by homogenizing the lung extract in lysis buffer containing proteinase inhibitors. Then, it was centrifuged at 10,000× g for 10 min, after which we collected the soluble ECM and dialyzed it extensively against 20 mM phosphate buffer. The presence of ECM proteins (e.g., collagen and fibronectin) was confirmed by immunoblotting. Nε-carboxymethyllysine modification of ECM (CML-ECM): Briefly, ECM proteins were incubated in 0.2 M phosphate buffer, pH

7.8 containing glyoxylic acid (0.15 M) and NaBH3CN (0.45 M) for 24 h at 37 °C. After incubation, contents were subjected to extensive dialysis against the 20 mM phosphate buffer to remove the unbound glycating agents. Protein estimation was completed using the BCA method. CML modification was confirmed by immunoblotting using antibodies specific to CML. Preparation of lung resident fibroblasts: Briefly, lung pieces were finely minced and digested at 37 °C for 60 min in IMDM containing collagenase. Digested tissue was passed through a 100  $\mu$ m filter, washed twice with media, plated onto 100 mm tissue-culture plates, and incubated at 37 °C, 5% CO2 to allow cells to adhere and migrate away from the larger remaining tissue pieces. To isolate lung resident fibroblasts, adherent lung cultures were harvested on days 5–8 with 0.05% trypsin, and cells were resuspended in a sterile buffer containing anti-CD45 microbeads. Cells were then washed twice and loaded onto magnetic columns. Cells were then eluted in the presence of a magnetic field to isolate unbound lung resident fibroblasts (CD45cells). Immunoblotting: Briefly, after SDS-PAGE separation, proteins were transferred to nitrocellulose membrane, membranes were blocked with 5% BSA and probed with specific primary antibodies including anti-CML, anti-collagen1, antifibronectin1, anti-SMA, anti-Bcl2 and anti-GAPDH in blocking buffer at 4 °C overnight, which was followed by detection with HRP-linked appropriate secondary antibodies. Quantitative RT-PCR analysis: The total RNA from lung tissues and primary cells was extracted using the RNeasy kit. Reverse transcription was completed using SuperScript III, and real-time PCR was performed using SYBR select master mix. Target gene transcripts from mouse samples were normalized to Hprt and human beta-actin. Myofibroblast transformation assay: Lung resident fibroblasts were treated with media, the ECM, or the CML-ECM along with 2  $\mu$ M 4hydroxy-tamoxifen for 72 h. Cells were then fixed with 4% paraformaldehyde, and nuclei were stained with DAPI. Fluorescence Images were obtained using a confocal microscope.

#### Results

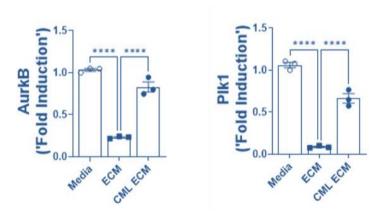
CML modification attenuates ECM-dependent inhibition of collagen and FN1 gene expression in fibroblasts: The formation of the CML-modified ECM was confirmed by immunoblotting with antibodies against CML adducts (Figure 1). To determine the effects of the ECM and CML-modified ECM, fibroblasts isolated from mouse lung cultures were treated with media, the ECM, and the CML-ECM. We observed a significant decrease in the expression of collagen 1 and FN1 gene transcripts by the ECM compared to the media control. However, CML modification of the ECM attenuated the inhibitory effects of the ECM on collagen 1 and FN1 gene expression (Figure 1).

Figure 1: CML modification attenuates ECM-dependent inhibition of collagen and FN1 gene expression in fibroblasts.



Immunoblot analysis using anti-CML antibodies shows CML-AGE formation in the ECM when incubated with glyoxylic acid and sodium cyanoborohydride (left). Quantification of gene transcripts (Col1a1 and Fn1) in lung resident fibroblasts treated with an ECM and CML-ECM (middle and right). CML Modification Attenuates ECM-Driven Inhibition of Fibroproliferation: The effects of CML modification on ECM-induced fibroproliferation, lung resident fibroblasts were treated with media, the ECM, and the CML-ECM. A significant decrease in the expression of fibroproliferative genes, Aurora kinase B (AurkB) and Polo like kinase 1 (Plk1), in fibroblasts treated with the ECM compared to those treated with media. This decrease in the expression of AurkB and Plk1 by the ECM was significantly attenuated with CML modification (Figure 2).

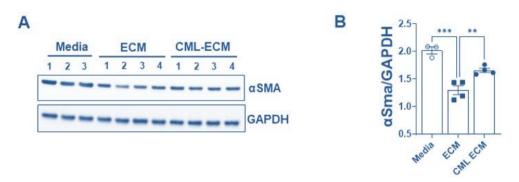
Figure 2: CML modification attenuates ECM-driven inhibition of fibroproliferation.



Quantification of AurkB and Plk1 transcripts using RT-PCR in lung resident fibroblasts treated with media, the ECM, or the CML-ECM CML modification of ECM attenuates fibroblast to myofibroblast transformation: To investigate effect of the ECM and CML-ECM on myofibroblast transformation, lung resident fibroblasts were

treated with media, the ECM, or the CML-ECM. There was a significant reduction in  $\alpha$ Sma in fibroblasts treated with the ECM compared to media, and this decrease was significantly attenuated with CML modification of the ECM (Figure 3).

Figure 3: CML modification of ECM attenuates fibroblast-to-myofibroblast transformation.



(A) Immunoblotting for  $\alpha$ Sma and GAPDH in fibroblasts treated with media, ECM, and CML-ECM. (B)  $\alpha$ Sma protein levels were normalized to GAPDH in fibroblast lysates. One-way ANOVA was used for statistical analysis.

CML modification of ECM attenuates apoptotic clearance of fibroblasts: To assess the effects of the ECM and CML-ECM in the survival of fibroblasts, fibroblasts were treated with media, the ECM, and the CML-ECM. A significant inhibition in the expression pro-survival Bcl2 gene transcripts by the ECM compared to media, and this decrease was attenuated with CML modification of the ECM. Consistent with transcriptional changes, there was a significant decrease in Bcl2 and Bcl-XL protein levels in fibroblasts by the ECM compared to media, and this decrease was attenuated with CML modification of the ECM. Consistent with changes in survival gene expression, a significant increase in the number of apoptotic cells treated with the ECM compared to media was found, and this increase was attenuated with CML modification of the ECM.

#### **Inference & Conclusion**

The findings of our study underscore the pivotal role played by interactions between the ECM and fibroblasts in maintaining the structural integrity and functionality of lung tissue. The CML modifications were found to trigger the aberrant activation of fibroblasts, which encompasses enhanced proliferation, fibroblast-to-myofibroblast transformation (FMT), prolonged cell survival or impaired clearance of fibroblasts, and excessive production of the ECM. Our findings underscore the possibility that CML-AGEs may play a pathogenic role in fibrosis. This research not only deepens our understanding of the complex mechanisms underpinning pulmonary fibrosis but also opens up new avenues for future therapeutic interventions.

## 2. ELEVATED WT1 EXPRESSION IN PODOCYTES IS LINKED TO OBESITY-RELATED GLOMERULOPATHY

#### Introduction

Obesity is characterized by excess ectopic fat accumulation, and is prevalent across age groups, ethnicities, and geographical regions. The causes of obesity are a combination of genetic, environmental, behavioral, and metabolic factors. Obesity is a risk factor for various non-communicable diseases such as type 2 diabetes, cardiovascular disease, hypertension, stroke, certain cancers, and chronic kidney disease (CKD). Obesity and diabetes, either independently or together (known as diabesity), are the leading causes of CKD. Obesity-related glomerulopathy (ORG) is a distinct entity featuring glomerulomegaly, progressive glomerulosclerosis and gradual decline in kidney function. Besides hypertension and dyslipidemia, individuals with ORG are presented with increased kidney weight and glomerular hypertrophy. Glomerular podocytes, also known as visceral epithelial cells, constitute a barrier at the blood-urine interface and play a vital role in ensuring almost protein-free urine. Podocytes are considered to represent the filtration function of the kidney. Since podocytes are terminally differentiated and deprived of proliferating potential, the loss of podocytes elicits glomerulosclerosis. Furthermore, dyslipidemia during obesity indirectly influences systemic renal inflammation through pro-inflammatory cytokines which in turn elicits insulin resistance and endothelial dysfunction, aggravating extracellular matrix (ECM) deposition and fibrosis. Since podocytes represent glomerular permeability and ORG is presented with impaired kidney filtration and progressive proteinuria, studies on podocyte injury and response in the context of ORG merit attention. Preliminary observations indicated proteinuria in a spontaneous obese rat model, WNIN/Ob. To investigate the cellular and molecular basis for proteinuria due to obesity, we isolated glomerular lysate and analyzed the expression of podocytespecific markers.

#### Methodology

Animal experimentation: We obtained 3, 6, and 12 months old WNIN/Ob male rats and their lean littermates from the animal facility. For diet-induced obesity, 3-weeks old C57BL/6 male mice were used. Rats were fed with the AIN-93 diet, ad libitum, and mice were fed with a 40 kcal% fat. The urine collection was done at 3, 6, and 12 months of age for WNIN/Ob rats and after 16 weeks of feeding HFD diet for mice. Blood was collected from tail vein and plasma was isolated. Animals were sacrificed for harvesting the kidneys. For histology analysis, kidneys were fixed in 4% paraformaldehyde and then embedded in paraffin to obtain  $4\mu m$  thick transverse sections and mounted on silane-coated slides. Transmission electron microscopy (TEM) imaging was performed to visualize the changes in podocyte foot

processes. The animal studies were approved by the Institutional Animal Ethical Committee of ICMR-NIN. Estimation of biochemical parameters: The plasma insulin levels were estimated by radioimmunoassay and urinary albumin and creatinine, estimates were performed using a commercially available kit. Histological examination and immunostaining: Paraffin-embedded sections of kidneys were used for H&E, Masson's trichrome, PAS and Picrosirius red staining. Immunostaining and immunofluorescence for various markers including WT1 was also performed. Estimation of glomerular damage score: We used PAS staining to quantify Bowman's space and urinary space area of the glomerulus. First, we analyzed the diameter of both Bowman's space and capillary tuft area and measured the urinary space by subtracting the capillary tuft from Bowman's space. We have analyzed 20 glomeruli from each animal at x400 magnification using ImageJ. Immunoblotting: Glomerular lysate was prepared in RIPA buffer with protease inhibitor cocktail and protein was estimated. An equal amount of protein was loaded for SDS-PAGE analysis, followed by immunoblotting.

#### **Results**

WNIN/Ob rats have proteinuria: We considered WNIN/Ob rats of ages 3, 6 and 12 months for our study as incidence of obese features began at around 3–4 months of age. WNIN/Ob rats are presented with impaired kidney function as analyzed by urinary albumin creatinine ratio (UACR) and proteinuria, particularly from age of 6 months onwards (Figure 1).

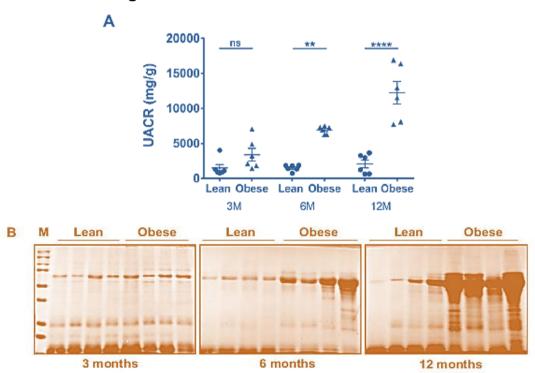


Figure 1: Proteinuria in WNIN/Ob rats.

A UACR IN WNIN/Ob rats B SDS-PAGE analysis of urinary samples of age-matched lean and obese rats

WNIN/Ob rats exhibit glomerulosclerosis: Histopathological features of obese rats using H&E, PAS and Picrosirius red staining (Figure 2), indicated that 6-months obese rats showed glomerulomegaly, mild to moderate increase in the mesangial matrix and large segmental sclerotic lesions and in 12-months, there was excess urinary space and reduced glomerular tuft region. PAS staining suggests obliteration of capillary lumina, particularly in aged obese rats. Picrosirius red staining reveals fibrotic areas and yellow non-collagen structures in WNIN/Ob rat glomeruli. Collagen deposition is evident in both glomerular and extra-glomerular regions. Masson's trichrome staining of 12-months old obese rats showed interstitial fibrosis, tubular atrophy, and chronic inflammation. Together, these observations suggest glomerulomegaly, enhanced Bowman's space, and the appearance of crescentic and sclerotic regions in the glomeruli of WNIN/Ob rats.

3 Months 6 Months 12 Months Obese Obese Obese Lean Lean Lean Α H&E В PAS C Sirius Red

Figure 2: WNIN/Ob rats exhibit glomerulosclerosis

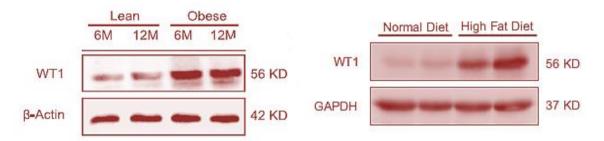
Immunohistochemical analysis were performed on 3-, 6-, and 12-month-old rat kidney sections, A H&E (Scale bar-20  $\mu$ m), B PAS, and C Picrosirius red. (Scale bar-50  $\mu$ m) staining.

Podocyte injury in WNIN/Ob rats: Since there is an array of glomerular manifestations and proteinuria in WNIN/Ob rats, next expression of podocyte-specific markers was assessed. There was a decreased expression of podocin and nephrin in obese rats of 6- and 12-months old but not in 3-months. Further, there is also increased thickness of the glomerular basement membrane. Heavy proteinuria observed in WNIN/Ob rats could be partially explained by decreased expression of slit-diaphragm proteins and compromised architecture of slit-diaphragm.

Glomerular injury and proteinuria in WNIN/Ob rats and high-fat-diet fed mice are concomitant with induction of WT1: Interestingly, we noticed an elevated expression of podocyte specific transcription factor, WT1 in WNIN/Ob rats (Figure 3). Since

WNIN/Ob rats are genetically altered rats, we next investigated whether dietinduced obesity in experimental animals could also elicit FSGS phenotype and show elevated WT1 expression. Feeding HFD to mice resulted in reduced kidney function as evidenced by altered UACR. Then, we investigated for anatomical changes in the kidney using various staining procedures and the data suggest majority of the changes were found in the glomerular regions. H&E staining of HFD fed mice glomeruli revealed increased cellularity, enhanced Bowman's space area and glomerular space. PAS staining suggests mesangial expansion in HFD fed mice glomeruli whereas Masson's trichrome staining indicates accumulation of extracellular glomerular matrix. Interestingly, we also observed elevated expression of WT1 and  $\alpha$ -SMA in the glomerular region of HFD-fed mice (Figure 3).

Figure 3: Expression of WT1 in WNIN/Ob rat (left) and in high-fat-diet fed mice (right).



#### **Inference & Conclusion**

This study revealed that WNIN/Ob rats and HFD-fed C57BL/6 mice were proteinuric and presented with anatomical deformities that resemble ORG. Both animal models showed glomerular hypertrophy, deposition of glomerular ECM, lesions, and capillary obliteration with mild tubular injury and atrophy. Interestingly, there is an elevated expression of WT1 in podocytes from both animal models, and it is concomitant with over expression of  $\alpha$ -SMA and fibronectin. In conclusion, the current study demonstrates a previously unknown role for WT1 in the pathogenesis of ORG.

#### **Publication status**

Obesity-related glomerulopathy is associated with elevated WT1 expression in podocytes. Jakhotia S, Kavvuri R, Raviraj S, Baishya S, Pasupulati AK, Reddy GB. Int J Obes (Lond). 2024 Aug;48(8):1080-1091.

## 3. FUNCTIONAL FOOD MIXTURE EXTRICATES D-GALACTOSE-INDUCED SKELETAL MUSCLE IMPAIRMENT IN RATS

#### Introduction

Skeletal muscle comprises about 40% of the total human body mass in a healthy individual and plays a vital role in metabolic health, weight control, bone strength, and flexibility to stress and disease. Ageing is one of the crucial factors affecting muscle health and function. Sarcopenia is the age-related, progressive loss of muscle mass and strength/function with unfavourable effects. Hence, preventing or improving muscle wastage during ageing is paramount for upholding the quality of life. Various experimental animal models are used to investigate age-related changes in target organs, and D-galactose-induced senescence is one of the widely used models for accelerated ageing. Functional foods are evident in improving body functions and help prevent or even cure chronic ageing-related diseases. Polyphenols like curcumin, resveratrol, and 8-prenylnaringenin are also shown to be beneficial in preventing muscle atrophy in various experimental models. We have been working on the effect of functional foods in preventing age-related diseases. Further, we showed that combining amla, turmeric, cinnamon, pepper, and ginger was more efficient due to synergism in preventing diabetic complications like cataracts in rats. In the current study, we tested the effect of a functional food of amla, turmeric, black pepper, cinnamon, and ginger at a specified dose (based on our earlier experiments) on D-galactose-induced muscle abnormalities in rats.

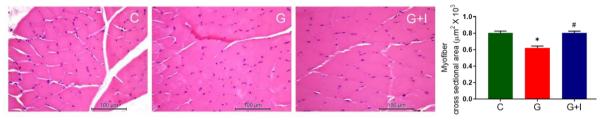
#### Methodology

Functional food mixture: Amla (fruit), turmeric (dry rhizome), black pepper (seeds), cinnamon (bark), and ginger (rhizome) were obtained from local markets in Hyderabad. Dried turmeric, cinnamon, and pepper were ground to fine powder separately. Fresh ginger and pericarp of amla were freeze-dried and powdered separately. The powders of 2 g of amla, 0.5 g of turmeric, 1 g of pepper, 2 g of cinnamon, and 3 g of ginger; a total of 8.5 g was mixed with 100 g of AIN-93 rodent diet. This specified dose is based on the earlier experiments. Animals and treatment Six months old female Wistar rats were procured and maintained at the Animal Facility of the Institute. The rats were randomly divided into 3 groups: the Control (C), the D-galactose (G), and the D-galactose + functional food intervention (G+I). Group-G and -G+I rats were injected with D-galactose (300 mg/Kg/day)intraperitoneally for days. Group-G+I 90 supplementation of functional food at 8.5 g/100g diet from the first day of Dgalactose injection. After three months of the experimental period, the rats were sacrificed to collect gastrocnemius muscle.

#### **Results**

There was no significant difference in food intake and body weights among the experimental groups. Functional food prevented the reduction in myofiber area: Group-G rats showed a decreased mean cross-sectional area of the gastrocnemius muscle fibre compared to Group-C. However, functional food intervention prevented the galactose-induced reduction in the cross-sectional area of myofibers (Figure 1).

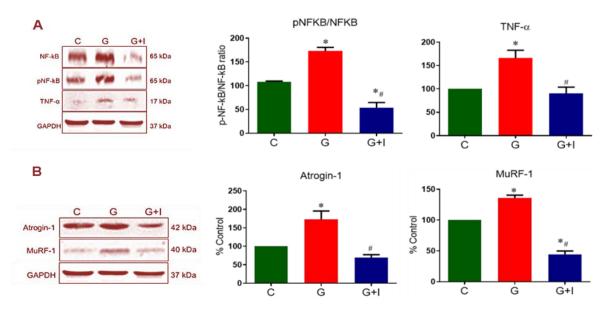
Figure 1: Representative H&E-stained muscle cross-sectional images



Scale bar = 100  $\mu$ m. Magnification = 40X. The myofiber cross-sectional area was measured on H&E-stained sections.

Anti-inflammatory potential of functional food: Ageing and chronic inflammatory responses occur along with one other. Elevated inflammatory cytokines adversely affect muscle health. Hence, we investigated the protein expression of  $TNF\alpha$  and NF-kB in the muscle of rats by immunoblotting. The results showed elevated levels of both  $TNF\alpha$  and NF-kB in group-G compared to group-C (Fig. 2A). However, intervention with the functional food mix (group-G+I) prevented an upsurge of inflammatory cytokines induced by galactose feeding.

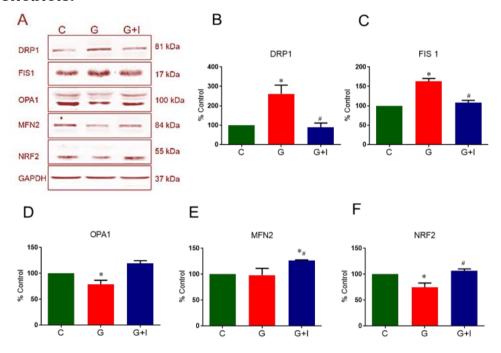
Figure 2: A: Representative immunoblot images of NF-kB, pNF-kB, and  $\mathsf{TNF}\alpha$  in the skeletal muscle of rats and their Quantification data. B: Representative immunoblot images of muscle-specific E3 ligases and Quantitative data.



Functional food prevented excessive activation of the ubiquitin-proteasome system (UPS): Atrogin-1 and MuRF-1 are two muscle-specific E3 ubiquitin ligases that target structural proteins for proteasomal degradation. They were found to be higher in group-G than group-C (Figure 2B). Interestingly, functional food prevented the elevation of these proteolytic UPS components.

Functional food role in mitochondrial dynamics: Mitochondrial dysfunction during aging is a key factor that contributes to sarcopenia. The mitochondrial fission markers DRP1, FIS1 and fusion markers OPA1 and MFN2 were evaluated by immunoblotting (Figure 3). The D-Galactose treatment showed enhanced expression of DRP1 and FIS1 whereas functional food prevented these changes. Further, the fusion proteins OPA1 and MFN2 were decreased in the group-G, and prevented in the group-G+I. The master regulator of the cellular redox homeostasis NRF2 was decreased in group-G but not in group-G+I.

Figure 3: A: Representative immunoblot images of DRP1, FIS1, OPA1, MFN2 and NRF2 proteins in the rat skeletal muscle. B: Quantification data of immunoblots.



#### **Inference & Conclusion**

Galactose feeding in rats displayed muscle aging and sarcopenic characteristics. Increased inflammation and atrogenes, and mitochondrial abnormalities could be the reason for the decreased muscle fiber cross-sectional area. However, the intervention of the functional food mixture of amla, turmeric, black pepper, cinnamon, and ginger significantly prevented the galactose-induced muscle degeneration. The anti-inflammatory and mitochondria-protective effects of these individual components might have synergistic effects in displaying positive effects

against muscle atrophy. In conclusion, the results showed that this functional food mixture prevented D-galactose-induced skeletal muscle degeneration and may help avert age-related muscle disorders in the geriatric population.

## 4. DETERMINANTS OF ORAL IRON SUPPLEMENTATION RESPONSE AMONG ADOLESCENT GIRLS WITH IRON DEFICIENCY ANEMIA

#### Introduction

India has been reported to have a high prevalence of anemia among adolescent girls, which is apparently higher when compared with the other developing nations. Iron deficiency is the primary cause of anemia, accounting for more than 50 percent of anemia cases. Oral iron supplementation is considered to be standard front-line therapy for prevention of iron-deficiency anemia. Despite routine iron supplementation and preventive measures, iron deficiency anemia (IDA) continues to be a major public health concern due to unpredictability of the response to oral iron interventions. There are very few studies available on the characterization of the response and probable factors associated with poor response to oral iron therapy. In this observational study, we aim to determine unexplored micronutrients, inflammatory and genetic factors associated with the oral iron response among adolescent girls with IDA, who are under supervised IFA administration. Our understanding on the ways to improve oral response is very limited. Considering the current scenario of an increasing number of nonresponders to oral iron, this study is expected to deepen our understanding of the response towards IFA.

#### Methodology

This is an exploratory study which was conducted in adolescent girls who were studying in Telangana Residential Educational Institutes. The estimated sample size was 310 adolescent girls. A total of 650 girls were screened from three colleges, of which about 334 girls were recruited based on inclusion and exclusion criteria. Information on socio-demographic characteristics, dietary habits, clinical history, Physical activity, stress and depression were obtained using validated questionnaires. Anthropometric measurements of height, weight and MUAC were recorded. From each participant, 6 ml of blood sample was collected and Hb levels were estimated. plasma/serum were extracted for biochemical measurements. Prior to the start of IFA 60 mg of Iron + 500 mcg of folic acid) supplementation, the study participants were given a deworming tablet (Albendazole 400 mg). IFA supplementation was administered and followed for up to 3 months duration as per I-NIPI guidelines. For participants who were found to have normal Hb,

prophylactic IFA (one tablet) was administered weekly once for 12 weeks. Participants under the mild and moderate category were administered two IFA tablets/day accounting to 120 mg of iron and 1mg of folic acid for therapeutic treatment during the 90 day period. End point blood samples (6 ml) were collected from participants after 90 days of supplementation and Hb was estimated. In order to define Iron deficiency anemia biochemically, serum ferritin and CRP levels were assessed using ELISA. The response to oral iron in this study is defined as change in Hb levels from baseline after three months of oral iron supplementation. Subjects were categorized based on increase in Hb levels, 1. Good (≥1.0 g/dL), 2. Poor (<1.0) responders. Serum vitamin A, D, B12 and folic acid has been estimated using HPLC and ELISA. Serum IL-6, IL-1beta and TNF-alpha, TfR and hepcidin estimated using ELISA. We employed whole exome sequencing using the Illumina Novaseq 6000 platform, on severe IDA and poor response subjects. Statistical analysis was performed using using the IBM SPSS version 26 statistical software and R language, p-value <0.05 was considered significant.

#### **Results**

Nearly 52.4% (175) adolescent girls were anemic with hemoglobin levels < 12g/dL. And 25 (twenty-five) subjects were found to have <8 g/dL Hb (severe anemia). Our analysis revealed 131 (74.8%) girls suffering from anemia due to iron deficiency, the majority of them (nearly 60%) have Hb values in the moderate category and 40% were in the mild category. The Compliance to the Iron Folic Acid supplementation is found to be 81% with regular supervision. Baseline (0 time point) and Endline (90 days' time point) hemoglobin and ferritin levels among subjects with mild and moderate IDA were found to have significant increases. About 52% were found to be in good response and 48% in poor response categories. All blood and serum biomarkers changed significantly from baseline to endline after IFA supplementation. Nutrient biomarkers vitamin A, vitamin B12 and folic acid showed significant improvement in the endline. The overall mean DIL-6 and  $\Delta TNF-\alpha$ , TfR and Hepcidin significantly decreased. Further, we assessed the changes in the blood and serum biomarkers based on the response criteria. In the good response all the measured serum parameters followed the same pattern of significant change as observed in the overall except serum TNF-alpha levels, no significant difference obtained at day-0 to day-90 of IFA supplementation. The Poor response group showed a different pattern of biomarker change during IFA supplementation. Here vitamin A change was found to be nonsignificant, whereas vitamin D change was significant. The levels of IL-6 at day 0 were significantly different from the good response subjects. To understand the relationship between changes in cytokine levels and Hb levels, we performed a correlation analysis. In the poor response group, IL-6 and Hb levels were significantly associated at day-0 (r= -.38, p<0.05). A significant observation is that vitamin A levels increased from baseline to follow-up in the good response group (4.73 $\mu$ g/dL, p<0.01), but there was no significant change in vitamin A levels from baseline to follow-up in the poor response group. The mean baseline vitamin A levels were significantly different between good (30.82μg/dL) and inadequate (27.48μg/dL) response groups (p<0.05). Logistic regression showed an increase in serum retinol levels of 1 g/dL reduced the chance of being in the inadequate Hb response. The effect was more evident in the individuals with moderate anemia at day-0, which showed for every 1g/dL decrease results in 28% (OR=1.28) more likely to show inadequate Hb response. In addition, this study reported 6 coding sequence variants related to membranopathies in severe IDA subjects. Of these two variants, ANK1.c.5633G>A, SPTA1.c.1422G>T, are first time reported. No variants were detected in the poor response category.

#### **Inference & Conclusion**

The study highlights the impact of low vitamin A status on adolescent girls, suggesting it can limit hemoglobin (Hb) increment during iron and folic acid (IFA) supplementation. This finding is significant, especially in regions where vitamin A deficiency (VAD) is a public health issue. Furthermore, this research paves the way for future studies to explore the efficacy of integrated interventions designed to enhance both iron and vitamin A status. Such interventions could offer a comprehensive approach to addressing iron deficiency anemia (IDA) in regions where VAD is still a public health challenge.

# 5. DEVELOPMENT OF NEW BIO-MARKER FOR QUANTIFICATION OF ACETYLCHOLINESTERASE (ACHE) ENZYME ACTIVITY, ORGANOPHOSPHATE, CARBAMATES, AND NERVE AGENT IN BIOLOGICAL AND AMBIENT MATRICES

#### Introduction

There is strong evidence that acute and chronic exposure to organophosphorus and carbamate chemicals harms the neurological system. The major enzyme that breaks down the neurotransmitter acetylcholine (ACh) in the nervous system, acetylcholinesterase (AChE), is crucial to neuromuscular and brain function [1]. The measurement of erythrocyte AChE activity is the best way to diagnose OP's exposure and intoxication [2,3] developed the first potentiometric method for determining AChE activity by measuring pH for one hour.

#### Methodology

2 ml of blood was collected into EDTA tubes from healthy people (n=10), farmers (n=78) exposed to pesticides while spraying, and suicidal patients (n=3)

hospitalized after ingesting pesticides. All the blood samples were subjected to the reaction procedure and analyzed using the developed method, and AChE levels were estimated. Following that,  $10\mu L$  of blood was diluted in  $280\mu L$  of phosphate buffer and mixed with  $10\mu L$  of  $50\mu M$  1-naphthol acetate. The reaction was carried out at room temperature and up to 20 minutes, the reaction was stopped using with  $700\mu L$  acetonitrile. The acetonitrile was used to stop the reaction to inhibit AChE enzyme activity on RBC, and thereafter the reaction mixture was filtered using  $0.2\mu$  syringe filters. The  $20\mu L$  filtrate was injected into the RP-HPLC system. The AChE in the blood catalysis the conversion of 1-naphthol acetate to 1-naphthol, and the data is integrated with the chromatogram. The enzyme activity is measured in the units which indicate the rate of the reaction catalyzed by that enzyme expressed as micromoles of substrate transformed (or product formed) per minute. The enzyme activity was expressed as U/mL.

#### **Results**

The physicochemical properties of 1-naphthol and 1-naphthol acetate, used to evaluate the ruggedness by different methods of acetylcholinesterase on different matrices were obtained from the literature (Table 1). Attempts to separate the reaction products using polar and nonpolar HPLC solvents such as methanol, ethyl acetate, water, and acetonitrile, as well as combinations of these solvents, failed. It may be noted that enzymatic hydrolysis behaved entirely different in basic and acidic medium and, therefore acidic and basic conditions were not used for reaction. The reaction was carried out in neutral pH 7 (sodium phosphate buffer). The compounds were dissolved at various concentrations in acetonitrile and the absorbance was measured. The spectra were acquired, and it was discovered that at 280 nm, both 1-naphthol and 1-naphthol acetate have a significant absorption. It was found that the separation was easily achieved using acetonitrile—water (45:55, v/v) as mobile phase on C18 reversed-phase column with a flow rate of 1.0 mL/min.

#### **Inference & Conclusion**

This reported prototype, a field-friendly method, would also help to measure exposure and effect which are crucially important to give the prognosis in real- or near real-time to save the life of the exposed person. Furthermore, the reported method is sensitive, accurate, reproducible and easy to run for the analysis of toxicity due to carbamate, metals and pesticides. In addition, only  $10\mu L$  of blood is the target sample which could provide instant and accurate result. In future, this method could be considered as a milestone and could fill up the gap between analytical toxicology, mechanistic toxicology and clinical toxicology.

# 6. INFORMATION EDUCATION AND COMMUNICATION (IEC) BASED INTERVENTION STUDY FOR CHRONIC KIDNEY DISEASE (CKD) AND ITS ASSOCIATED FACTORS IN TRIBAL POPULATION-TS

#### Introduction

In the Telangana State (TS), according to Aarogyasri reports since from 2008-09 to 2016-17 the kidney disease patients were increased six folds. From 2009 to 2017, 19564 cases were enrolled, among these 3393 were enrolled in 2016-17. The number of CKD cases may be increased if consider in ESI, RTC, Singareni, CGHS and private corporate hospital reports30. According to print media report on 27th September 2017, the tribal villages of Bethampudi, Chunchupalli, Loddiguda, Lingapur and Gaddisiguda in Telangana state have a high prevalence of CKD. This report highlighted that unusually high kidney related disorders and more than 40 deaths with CKD among the tribal population in above said villages of Bhadradri Kottagudem and KumaramBheem districts of Telangana, India. A new form of kidney disease of unknown etiology has emerged in the above villages of TS. Almost 30% of people are suffering from kidney disease in these villages.

#### Methodology

Study design: It is community based cross-sectional study using random sampling procedure.

Study area: Bhadradri Kottagudem, Adilabad, Mahabubabad, Nalgonda and Kumaram Bheem districts-TS. The study is propagated in two phases.

Phase 1: In this phase the drinking water and food samples were screened for contaminants which include toxic heavy metals (metal toxicity) and pesticides, the levels of metals in drinking water and identification of source of contamination. Blood samples were collected from selected subjects and analyzed for biochemical markers related to kidney disorders like serum creatinine, to assess albuminglobulin (A/G) ratio, blood urea and Hemoglobin and enzyme markers like LDH and GST etc and anthropometric measurements. Surveys were carried out to know the frequent intake of NSAIDs (Painkillers) and side effects associated with that intake.

**Phase II:** Based on the study findings, the health aspects, sanitation measures, preventive measures of CKD was monitored with the help of ITDA, DMHO, local health bodies (ICDS workers), NGOs and village leaders and subjects were educated through IEC. Sample size: 5 Districts x 5 Mandals x 5 Villages X 20 HH=2500 subjects were selected for the study.

#### **Results**

Report shown that the tribal people in the selected areas are suffering with severe chronic kidney diseases and malnutrition problems. BMI ranges shown that 38% were normal and remaining were either suffering with any one of the mal nutrition related problems and CKD. Drinking water samples, food and biological samples were collected from the subjects after explained the purpose of the study. Pesticides and heavy metal levels are analyzed using LCMS and ICPMS in samples. The results found that the heavy metal content in the water analyzed shown that the levels are above permeable limits. The biomarkers related to CKD, analyzed are serum creatinine, urea, uric acid, A/G ratio etc. The group meetings were conducted and educated the subjects about malnutrition, hygienic and importance of locally available foods with the help of local leaders, NGOs, ITDA and Medical professionals.

#### **Inference & Conclusion**

- 1. Pesticides and heavy metals were detected in samples collected from subjects.
- 2. Data from the analysis of biomarkers revealed a high incidence of blood urea nitrogen, serum uric acid, serum creatinine, and the A/G ratio.
- 3. More than 50% of subjects were anaemic and 60% of subjects were suffering with CED (Chronic energy deficiency) overweight and obesity.
- 4. The subjects were using combination of multiple analysesics such as paracetamol, prophyphenazone and caffeine, naproxen, diclofenac, (Doloside Tp8) which is the combination of Aceclofenac, Paracetamol and Thiocolchicoside to provide immediate pain relief.

# 7. STUDY ON PHYSICO-CHEMICAL AND BIOACTIVE PROPERTIES OF BAMBO RICE (BAMBOSA ARUNDINACEA WILLD) CONSUMED IN SOUTHERN-WESTERN GHATS'

#### Introduction

The bamboo plant is a flowering perennial evergreen plant which is known to be one of the most useful trees as almost all parts can be used either as food or medication or for industrial purposes. Bamboo rice (Bambusa arundinacea) refers to the seeds of edible wild bamboo grass (Bambusa bambos (L.) Voss). Even though it is nutritionally superior to rice in terms of crude protein and true protein contents, their utilization is restricted mainly to the indigenous population in the country. Various inflammatory conditions are treated in Indian folk medicine using extracts of Bambusa arundinacea and it also exhibits anti-diabetic activity. It is known to have antiulcer activity and was also proved to have the most potent anti-

inflammatory activity experimentally with least toxic (no ulcerogenic) activity. There are very few studies with information on few nutrients being reported during 1950s—1960s. There is no recent study on the nutritional qualities and health benefit of Bamboo rice in India and elsewhere. Therefore, this project was proposed to conduct a detailed study on Bambusa arundinacea. The project is expected to generate information on the comprehensive nutritive profile of bamboo rice, including its bioactive efficiency. Thus, the data will help planners in considering and including Bamboo rice for planning nutritionally adequate and therapeutic diets. The data on nutrients and therapeutic significance of Bamboo rice can provide basis for research to improve on nutritional quality and help to understand to identify techniques to enhance their viability during ageing and thus improve its consumption.

#### Methodology

Sample collection and processing: The Bamboo rice samples were collected from different regions of the south western ghats. Morphological, physical and cooking properties of different landraces of bamboo rice. Cooking properties: All rice samples were cooked for the minimum cooking time, as per the method reported by Singh et al. (2003). Water absorption after cooking was measured by the increase in weight of cooked rice. Gruel solid loss was measured by drying an aliquot of water obtained after washing 2g of the cooked samples at 70°C in a hot air oven until it was dried completely. The solids obtained after drying were weighed and per cent gruel solids was estimated. Elongation ratio was estimated by dividing cumulative length of cooked kernels by raw kernel length (Singh et al., 2003).

Nutrient profiling of bamboo rice land races: Nutrients analysed included proximate composition, minerals and vitamins. The anti-nutrient phytic acid was measured in bamboo rice. Bioactive profiling of bamboo rice landraces.

Individual polyphenols: Individual polyphenols were quantified as per the method by Giusti et al. (2018).

Total polyphenols and flavanoids: Total polyphenols (free and bound) were analysed by the Folin–Ciocalteau method (Tachakittirungrod et al., 2007) and total flavonoids by the Aluminium chloride colorimetric method (Sandhu et al., 2016). Individual polyphenols were extracted from test portion by acidified polar solvent, segregated by HPLC under a gradient program of low pH buffer as the mobile phase, detected by UV-DAD/ PDA at 250, 280, 320 and 370 nm and quantified from corresponding external standard retention time and calibration curve.

Total antioxidant activity: The antioxidant activity was estimated by DPPH assay according to Jang et al. (2007) with slight modifications whereas ABTS and FRAP

assay was according to a method by Tachakittirungrod et al. (2007) with slight modifications.

Amino acid composition: Amino acid analysis was carried out by hydrolyzing the defatted samples with 6 N HCl in sealed ampoules in an oven at 110°C for 22 hours (Moore, 1963). Excess acid was removed by continuous flash evaporation under reduced pressure. The sample was then dissolved in citrate buffer (pH 2.2) and aliquot of the sample were loaded into an automatic amino acid analyzer (Biochrom-30, Cambridge, UK). Methionine and cysteine were determined separately after performic acid oxidation. Each amino acid was identified and quantified using authentic standards (National Institute of Standards and Technology, SRM 2389).

Fatty acid composition: The fatty acid methyl esters of the samples were prepared according to the method of O'Fallon et al. (2007). Fatty acid analysis was carried out in a Shimadzu 2010 GC equipped with Flame Ionization Detector (FID) and SP2560 column (100 m x 0.25 mm x 0.2 um). Nitrogen was used as carrier gas and the temperature program was from 140°C to 250°C with a ramp rate of 4°C/min. Individual peaks were identified and quantified using authentic standards (Nu-Chek).

#### **Results**

The investigation of bamboo rice revealed the presence of prominent protein molecules and its nutrient analysis confirmed a better profile of nutrients such as protein, dietary fibre, vitamin B2, B5, zinc, calcium, copper and iron when compared to brown and polished rice with lesser phytic acid content. The study revealed that in comparison to polished and brown rice studied, bamboo rice landraces had greater concentrations of total polyunsaturated fatty acids. Predominant fatty acids found were palmitic acid, oleic and linoleic acids which ranged between 23.18-38.90, 24.90-29.96 and 23.86-39.98 % respectively. The phytosterol, β-sitosterol was found to be highest in all the bamboo rice landraces. Amino acids present in higher concentrations were glutamic acid, aspartic acid and arginine which ranged from 15.78-16.94, 8.89-9.98 and 4.31-8.96 g/100g respectively. The polyphenols detected and quantified in bamboo rice landraces included glutamic acid, protocatechuic acid, catechinin, caffeic acid, sinapic acid, ferulic acid, 4-coumaric acid, ellagic acid, 2-coumaric acid, 4-hydroxy benzoic acid, luteolin 7-O-Glucoside and myricetin. Moreover, the total bound phenolic content was found to be at par with brown rice. The water extracts of bamboo rice landraces exhibited better antioxidant activity for ABTS and DPPH assay while methanolic extracts showed better activity for FRAP assay. From the in vivo study carried out, it was evident that bamboo rice has anti-inflammatory activity and is effective in the treatment of arthritis.

#### **Inference & Conclusion**

The data obtained from the study will help planners in considering and including Bamboo rice for planning nutritionally adequate and therapeutic diets. The data on nutrients and therapeutic significance of Bamboo rice can provide basis for research to improve on nutritional quality and help to understand to identify techniques to enhance their viability during ageing and thus improve its consumption.

## 8. STUDY ON THE NUTRITIONAL VALUE OF DIFFERENT PROCESSED AND COOKED MILLET

#### Introduction

Millets are small-course cereals and are part of staple diet in early independent India and by any nutritional parameter, millets are miles similar or ahead of rice and wheat [6]. Due to adaptation of improved technologies millet production has increased significantly in the last few years from 87.7 thousand tonnes (2009) to 1 lakh tonnes. In past few decades the global millet consumption has declined (0.9%), per capita consumption of millets fell marginally from 4.6 kg (1982) to 3.6 kg around the world while in India it was 12 kg (1982) and reduced to 8 kg (2009) [10]. Prospective and randomized clinical trials have provided evidence that replacement of refined grains with whole grains results in reduction of T2DM and cardiovascular risk factors In our recently reported systematic reviews, we found that consumption of millets on various health benefits including reducing total cholesterol, LDL, and increasing HDL [14], improving hemoglobin and reduced aneamia in adolescences [15], reducing risk of developing T2DM and lowering fasting blood glucose. In India, about 400 released varieties of millets are available and substantial differences in their nutrient density, fiber content, protein, essential amino acid profile and glycemic index (GI) is expected. Indian Food Composition Data recently released by National Institute of Nutrition, ICMR, India provides comprehensive nutritional profile of all the millets, of raw samples not from the cooked or processed samples. Therefore, in order to develop and promote healthy millet recipes, it is necessary to have a comprehensive nutrition composition and its retention

#### Methodology

Millet Samples: Millets such as finger millet (Eleusine coracana), foxtail millet (Setaria italica) and pearl millet (Pennisetum glaucum) was procured from local whole sale market in Secunderabad

Recipe preparation: The millet recipes such as millet pulav, millet kidhadi, foxtail millet bisibeli bath, ragi idly, ragi dosa, pearl millet upma will be prepared as per

the standard recipes recommended by Indian Institute of Millet Research (IIMR). All the cooked foods will be allowed to bring to room temperature and homogenised in to fine past for below given various nutrient analyses.

Macronutrients: The Association of Official Analytical Chemists (AOAC) methods will be used for the determination of moisture (2001.12), crude protein (984.13) ash (942.05), fat (2003.05) and dietary fibre (985.29). Carbohydrate content will be calculated by difference (100- moisture+fat+protein+ash+total dietary fibre). Vitamin content: Analysis of water soluble vitamins such as vitamin C, B2, B3 B6, B5 and B9 using Ultra-High Performance Liquid Chromatography (U-HPLC Technique) will be carried out. Fat soluble vitamins namely vitamin-A, and E (all tocopherols and tocotrienols) will be quantified using UPLC method.

Mineral content: Elemental analysis will be carried out after wet digestion according to AOAC (968.08) method. Potassium, sodium, iron, calcium, copper, manganese, magnesium, zinc, selenium and chromium will be determined in atomic absorption spectrometer (flame / graphite furnace). Other trace elements will be quantified Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Phosphorus will be estimated by the Fiske and Subbarow method as described in AOAC method (931.01).

Amino acid composition: Amino acid analysis would be carried out by hydrolyzing the defatted samples with 6 N HCl in sealed ampoules in an oven at 110 °C for 22 hours. Excess acid will be removed by continuous flash evaporation under reduced pressure. The sample was then dissolved in citrate buffer (pH 2.2) and aliquot of the sample will be loaded into an automatic amino acid analyzer (Biochrom-30, Cambridge, UK). Methionine and cysteine will be determined separately after performic acid oxidation. Tryptophan will be determined after barytic hydrolysis. Each amino acid would be identified and quantified using authentic standards (National Institute of Standards and Technology, SRM 2389). Anti-nutrients: Tannin and phytic acid content: Condensed tannins or its monomeric components will be extracted from 200 mg of dry powdered sample in duplicate using 1% HCl in methanol by placing them in a shaking water bath for 30 min at room temperature. The clear extract would be mixed with vanillin reagent or 4% HCl in methanol (blank) and the resulting soluble complex is measured calorimetrically after 15 minutes at an absorbance of 510 nm. Amount of tannin in the original sample will be calculated as D-Catechin equivalents/g.

#### **Results**

The nutritional analysis of macro nutrients and micro nutrients along with antinutrients were analysed using standardized methods

#### **Inference & Conclusion**

The outcome of the project would be the detailed nutritional composition of different cooked and processed millet recipes. In addition, nutritional retention of various macro and micro nutrients were determined. The study also would address the reduction of antinutritional factors which affects micronutrition absorption, after different processing of millets. Overall, this data is extrapolated in various millet food intervention studies.

9. IMPACT OF THE MILLET YEAR 2023 CAMPAIGN IN SHAPING KNOWLEDGE ATTITUDE, AND PRACTICE OF MILLETS CONSUMPTION AMONG COLLEGE GOING ADULT WOMEN IN URBAN AREAS OF TELANGANA

#### Introduction

The year 2023 has been designated as the International Year of Millets (IYoM-2023) for promoting a global celebration and recognition of the importance of millets in our lives. Millets have been a staple diet in some parts of India. In Telangana, where millets have been a staple for generations, there has been a renewed emphasis on promoting millet consumption through government initiatives and awareness campaigns.

College-going adult women, as an important demographic group, play a crucial role in shaping their own dietary choices and those of their families. Due to lifestyle and dietary modifications early adolescents onwards, they are suffering with nutritional deficiencies particularly micronutrients this impact is on growth and development of these young adolescents (https://pib.gov.in; Sireesha and Kusuma, 2015).

A recent study reported that, 80.6% of women in the study (Bengaluru) are aware of millet but only 62.7% of women are consuming millet (Chakraborty, 2019). However, there is also a growing interest in millets among urban consumers, especially those who are health-conscious and environmentally aware. Given this background, the current study has been taken up with the following objectives

#### **Objectives:**

- 1. To assess the existing knowledge on nutrition values of millets and culinary uses among the college-going adult women in urban areas of Telangana.
- 2. To identify the perceived authentic information sources from where they seek information on importance of millets and their nutritional and health benefits.

- 3. To assess the impact of the millet year 2023 campaigns on the consumption of millets among the study participants in urban areas of Telangana.
- 4. To assess the food frequency and factors influence the consumption of millets in the daily diets

#### Methodology

This was a cross-sectional study which employed mixed methods approach (combining qualitative and quantitative methods) to collect data.

**Area of research:** The study was conducted in urban setting of two districts of Telangana state namely, Hyderabad and Karimnagar.

**Universe of the research:** The universe of the study is undergraduate college going adult women from the study districts of Telangana pursuing undergraduate programme both in Government and Private Degree Colleges. The exclusion criteria for the study are those pursuing B.Sc. nutrition/Home science/ Life Sciences.

**Sampling:** A multistage random sampling technique was used to enrol the study participants. The first stage involved random selection of two districts. In the second stage, colleges within a district both private and government degree colleges were randomly chosen. In the final stage, courses were stratified as B.A, B.Sc, B.com, and BBA and strata wise college-going women were selected using systematic random sampling approach.

**Sample Size:** Assuming the prevalence of millet consumption by 25%, a 95% confidence interval, a 5% absolute precision, a non-response rate of 10%, and a design effect of 1.5, the sample size determined for the study is 480. However, rounded to 500 participants.

Tools for data collection: The study administered a structured self-administered, pre-tested questionnaire for quantitative data collection. The questionnaire was developed, based on review of literature and iterations within the research team before it was pre-tested among college going adult women and validated for its content by subject experts. A moderator guide is prepared to conduct in-depth interview (IDI) to explore the cultural practice and attitude towards the millet consumption. Content validity had been done by subject experts such as medical practitioners, nutritionists, and social scientists.

**Data analysis:** Quantitative data was analysed using SPSS 28 version statistical software. Descriptive statistics were employed to summarize the demographic characteristics, knowledge levels, and awareness of millets and their practices among participants. Inferential statistics, specifically chi-square tests, were used to

identify associations between dependent variables (family type, college type, study year, residence, religion, marital status, ration card ownership, and caste) and millet consumption. Additionally, associations between information sources and millet consumption were calculated.

The qualitative data from the interviews was transcribed, coded, and analysed manually, employing thematic analysis techniques. Themes and patterns related to attitudes and traditional food habits consumption were identified. After coding, the findings were reviewed by two investigators.

#### **Results**

The mean age of participants was 18.85 years (range: 18y to 25y). Most of the respondents 360(72%) belonged to nuclear family; Hindu religion 420(84%) and 292(58%) belonged to Backward class (BC) and almost all of them 483(97%) were unmarried. More than half of the participants reported that they stayed in hostels. Over a half of the participants 272 (54%), although studying in urban colleges hailed from rural areas. Majority 483 (97%) of hem belonged to high Standard of living index (SLI) (Table.1).

#### Awareness on millets (Types of Millets, Benefits, knowledge):

Majority of the participants knew about millets 493(99%). Of them, 66% perceived that they had little knowledge millets,8% on had adequate knowledge and 24% had only heard of it, but have no knowledge about millets. While, 1% never heard about millets (Figure 1).

Regarding awareness about government initiatives, only 27% were aware of government initiatives on millet promotions and majority of the participants (73%) were not aware of any initiatives on millet promotion taken up either by union or state governments (Figure 2).

Figure 1: Frequency of awareness level on millets

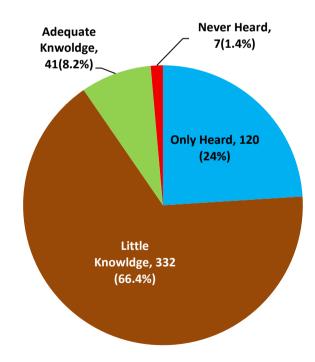


Figure 2: Participants' awareness on government campaigns on millets promotion (number of participants on x-axis)

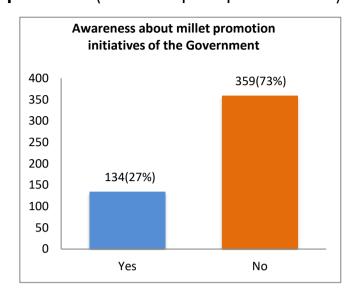


Table 1: Socio-demographic characteristics of the participants

Variable	Frequency (N=500)	Percentage (%)		
Family type				
Nuclear	360	72.0		
Extended	106	21.2		
Joint	34	6.8		
Religion				
Hindu	420	84.0		
Muslim	47	9.4		
Christian	28	5.6		
Others	5	1.0		
Caste				
General	79	15.8		
BC	292	58.4		
SC	101	20.2		
ST	28	5.6		
Domicile				
Rural	272	54.4		
Urban	228	45.6		
Marital Status				
Single	483	96.6		
Married	6	1.2		
Separated	1	0.2		
Do not want to disclose	10	2.0		

College Type				
Government	255	51.0		
Private	245	49.0		
Year of study				
First	279	55.8		
Second	122	24.4		
Third (Final)	99	19.8		
Stay				
Day Scholar	229	45.8		
College Hostel	160	32.0		
Hostel Outside College	111	22.2		
Income status				
<3 Lakh	189	37.8		
>3 lakh to <5Lakh	37	7.4		
>5 lakh to <10 lakh	13	2.6		
>10 Lakh	16	3.2		
Don't Know	255	51.0		
Standard of living index (SLI)				
Low	0	0		
Middle	17	3.4		
High	483	96.6		

#### Source of information about millets:

The major source of information on millets for participants are (i) family members 404 (92%) (ii) Social media165 (38%) and (iii) government campaigns on millet promotion 39 (9%) (Figure 3).

social media God. Campaigns BOOKS Friends Ushons MENS Papers colleges

Figure 3: Source of information about Millets to participants (N)

#### **Association between Millet Consumption and Dependent variables**

Cross tabulation between millet consumption cross-tabulation and dependent variables such as type of type of family, type of college, study year, stay, religion, marital status, having ration card, and caste etc., has been done. It was found that highly significant association between millet consumption and type of family and college (chi-square value=22.397, p=0.001, chi-square value=17.521, p=0.001 respectively), age (chi-square value=9.582, p=0.022). Similarly study year, course, stay and annual income were also was also significantly associated with millet consumption. Whereas as religion, caste, marital status, type of ration card, and having ration card, were not associated with millet consumption.

#### Association between information source and millet consumption

Similarly, cross-tabulations between millet consumption and various information sources such as, family, friends, social media, Books/ Articles, Universities/ Colleges, TV shows, Newspapers and government campaigns. Surprisingly, except family (chi-square value = 9.416, p = 0.009) as information source, there was no significant association found between consumption and other information sources.

Findings of qualitative data: A total of 20 in-depth interviews were conducted with college going students of B.A, B.Sc, B.Com and BBA streams, in Hyderabad and Karimnagar districts. Participants involved in quantitative survey were not included in these interviews. Thematic analysis was carried out by two independent research staff. After coding the interviews, discussions were conducted to validate the codes and resolve the differences, wherever applicable. A total of 8 themes with 27 sub-themes were derived from the in-depth interviews (Table 2).

Table 2: List of themes and sub-themes

SL.No	Theme	Sub-theme
1	Views about millets as	<ol> <li>Healthy food</li> </ol>
	food	2. Nutritious
		3. Balanced diet
2	Awareness and	<ol> <li>Various types of millets</li> </ol>
	knowledge	2. Importance
		3. Nutritive values
		4. Health benefits
		5. Sources of information
3	Consumption	Pattern of consumption
		2. Frequency of consumption
		3. Different recipes
		4. Family food habits

4	Cultural/ Traditional	1. Festivals
	practices	2. Special foods
5	Millets availability	1. Affordability
		2. Own production
6	IYoM 2023 campaign	1. Awareness
		2. Exposure to campaigns
7	Barriers for consumption	<ol> <li>Costly/Financial problems</li> </ol>
	of millet	2. Lack of subsidies
		3. Lack of awareness
		4. Non availability
		5. Lack of time/busy
8	Suggestions	<ol> <li>Need for effective campaign</li> </ol>
		2. Awareness pregame at
		schools & colleges
		3. Promote in social media
		platform
		<ol> <li>Need for diversification food choices</li> </ol>

#### Conclusion

Many of the respondents did not know about IYoM, 2023, but have witnessed heightened media campaign on millets. Very few types of millet are known and used. Family tradition and cultural habits determined millet consumption. Social media is the most preferred source of information on millets.

## MEETINGS/CONFERENCES/ TRAINING PROGRAMMES HELD AT NIN

ICMR-NIN conducted a 2-days orientation programme for the newly joined permanent employees on 3<sup>rd</sup> & 7<sup>th</sup> April. The programme began with speech of the Director Dr. Hemalatha. Scientists, HoDs, Senior Technical Officers and Administration officers addressed the newly joined staff and gave orientation on topics like Good Laboratory Practices, Waste Disposal, Animal Ethics, Fire Safety, Vigilance Awareness, Cyber Awareness, Work Ethics, Code of Conduct, Leaves, CGHS, PoSH Act, etc.



ICMR-NIN celebrated 133<sup>rd</sup> Birth Anniversary of Bharat Ratna Dr. B. R. Ambedkar & 117<sup>th</sup> Birth Anniversary of Dr. Babu Jagjivan Ram were on 19th April. Guest of Honour Prof. C. Kaseem, Professor, University College of Arts and Social Sciences, Osmania University, Hyderabad and the Director Dr. Hemalatha R, Dr. G. Bhanuprakash Reddy, Scientist G & Head, Division of Biochemistry spoke on the occasion. Dr. N. Arlappa, Scientist G & SC/ST Liaison Officer, Convener Shri. S. Laxman, Co-Conveners Shri. M. Asai Thurai & Mr. G. Satyapaul; General Secretary Shri. K. Harinarayana, SC/ST Association, NIN were also present.



Scientific Advisory Committee (SAC) Meetings were held on 28<sup>th</sup> & 29<sup>th</sup> May, 2024 under the chairmanship of Prof. Anura Kurpad, St. John's Medical College, Bengaluru. SAC reviewed new research proposals presented by the scientists of the Institute.



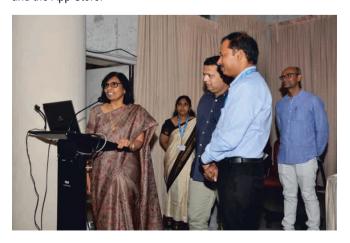
ICMR-NIN celebrated National Technology Day-2024 in association with Telangana Academy of Sciences at the institute. Dr. Chaitanyamoy Ganguly, Former Chairman & Chief Executive, Nuclear Fuel Complex, Department of Atomic Energy, India delivered the Technology Day talk. Dr. G. Bhanuprakash Reddy, Scientist G & HoD, Biochemistry Division coordinated the event.



Meeting of PhD scholars and the Academic Council (AC) was held on May 9, 2024. The AC Convener and Members interacted with the scholars to discuss the issues/problems faced by them.



Dr. Hemalatha R, Director launched "NUTRIFY INDIA NOW (NIN) 2.0" MobileApp and 'Nutrition Atlas' web application that allows users to geo-visualize & compare nutrition indicators from multiple surveys by age, gender & physiological group. This app helps users track nutritional intake & physical activity & offers suggestions for health. Both the NUTRIFY INDIA NOW 2.0 mobile app and the Nutrition Atlas web application are set to be available soon on the Google Play Store and the App Store.



An Exposure visit from International Centre for Integrated Mountain Development (ICIMOD), from Bhutan & Nepal to NIN was held on 25<sup>th</sup> June. The visit aimed to provide the visitors with an overview of NIN's research activities.



An MoU was signed between Sri Konda Laxman Telangana State Horticultural University and ICMR-NIN, for an intramural project to develop guava leather. The VC, Dr. B. Neeraja Prabhakar, Dr. A.Kiran Kumar, Research Director, Dr.V.Suchitra, Sr. Scientist & Dr. P. Harikanth, Scientist, SKLTS, Horticultural University alongside Dr. Hemalatha, Director, Dr. M. Maheshwar, Scientist-F and Dr. K.Venkatesh, Scientist-E were present during the event.



N-CET 2024 for admission into M.Sc (Applied Nutrition) & M.Sc (Sports Nutrition) was conducted successfully on June 23 in multi-centres including Hyderabad, New Delhi & Kolkata. Dr. Hemalatha R, Director released the question paper from the control room at the Academic Block of NIN. Dr. M. Maheshwar, Scientist-F, HoD, ET Division and Dr. C. Suresh, Scientist-F, Convener, M.Sc. Curriculum Committee were also present during the event.



International Yoga Day was celebrated with active participation from staff and students (21st June).



ICMR-NIN team officially launched the Diet and Biomarker Survey-India (DABS-I) in a meeting held in the office of the Chief Medical Officer, Leh, Ladakh. Dr. Motup Dorje, Director of Medical Services graced the occasion as chief guest (17th June).



The annual inspection of Institutional Animal Ethics Committee (IAEC) Meeting held at NIN Animal Facility (26th June).



NIN Animal Facility conducted an Ad-hoc training program on Animal Handling and Breeding Techniques to Medical PG students of Govt Medical College Siddipet, AIIMS, Bibinagar and Staff of GITAM University from 25th to 27th June.



ICMR-NIN Extension & Training Division conducted graduation day on 12<sup>th</sup> July. Certificates were awarded to 88 alumni from the past three batches of the M.Sc. courses in Applied Nutrition and Sports Nutrition. Dr.Biplab K. Nandi, former Senior Nutrition Officer at the FAO of the United Nations-Philippines, participated as the Guest of Honour. Dr. P. Uday Kumar, Scientist G & HoD, Pathology Division, was the Chief Guest. Dr. M. Maheshwar, Scientist F & Head, Extension & Training Division, along with Dr. C.Suresh, Scientist F & Head Cell Biology Division and Convenor of the Course Curriculum Committee, presented the Dr.Biplab K. Nandi Merit Scholarship Awards of ₹25,000/- each to the top students of each batch.



One month LATTC - Laboratory Animal Technician Training Course conducted successfully from 1<sup>st</sup> to 31<sup>st</sup> July. Nineteen candidates participated in the training programme.



Extension & Training (ET) Division, ICMR-NIN & UKRI GCRF Action against Stunting Hub, Project Staff along with Department of Women and Child Development (DWCD), Telangana conducted an awareness programme in BN Timmapur & Haumapur villages in Yadadri Bhongiri district and slums of Golconda as a part of the World Breastfeeding Week 2024.



Nationwide every year, National Sports Day (NSD) is celebrated on 29<sup>th</sup> August, to commemorate the birth anniversary of Hockey Legend Major Dhyan Chand, to instill a strong sports and fitness culture in the country. In this connection, ET Division staff conducted various sports & games and administered Fit India Pledge on 29<sup>th</sup> & 30<sup>th</sup> August.



ICMR-NIN collaborated with the Central Bureau of Communication (CBC), Hyderabad, to organize a five-day exhibition as part of Poshan Maah 2024. The exhibition was inaugurated by Hon'ble Governor of Telangana, Sri Jishnu Dev Varma. He also visited the ICMR-NIN stall 'Nutritious Diet For All' at Women's University Campus, Koti, Hyderabad (2nd to 6th Sept.).



Awareness sessions on "Balanced Diet" and "Importance of Nutrition during pregnancy" were organized in various slums and several schools in Hyderabad as part of Poshan Maah 2024 celebrations.



Extension & Training Division conducted the open/spot counselling for Admission into the new batch (2024-26) of M.Sc. (Applied Nutrition) & (Sports Nutrition). A total of 42 seats are available in both the courses for which around 150 candidates from different states of India attended the counselling. All 42 seats were filled based on the candidates' merit in the all-India entrance exam (10th Sept).



As part of Poshan Maah 2024, ET Division, ICMR-NIN organized a nutrition awareness programme for state-level junior athletes and coaches at the 10th Telangana State Junior Athletics Championships-2024, hosted by Osmania University. First-year M.Sc. (Applied Nutrition) and M.Sc. (Sports Nutrition) students and staff of the division participated in the programme (20th Sept).



A training programme on "Nutrition survey methods and community based management of acute malnutrition (CMAM)", was organized by Division of Public Health Nutrition, ICMR-NIN (23rd to 27th Sept).



As part of UKRI action against stunting hub an engagement and dissemination workshop was led by NIN team (Dr. Sylvia F Rao, Scientist E and Dr. B. Santosh Kumar, Scientist D) and central hub team Dr. Julie, Lynn, and Bernie alongside anganwadi teachers, supervisors, and child development officers. The workshop aimed to present project outcomes and collaboratively refine the toolkit with the input of stakeholders. Discussions focused on early years' education, cognition and ways to promote play-based learning and positive child interactions (25th Sept).



A TDS/TCS Awareness Seminar conducted by Commissioner of Income Tax (TDS), Hyderabad (3<sup>rd</sup> Sept).



आई सी एम् आर - राष्ट्रीय पोषण संस्थान, हैदराबाद में 13 से 25 सितंबर तक हिंदी सप्ताह मनाया गया। उद्घाटन समारोह 13 सितंबर को आयोजित किया गया था जिसमें कई वैज्ञानिक, तकनिकी, प्रशासनिक और अन्य कर्मचारी शामिल हुए। प्रभारी निदेशक डॉ. शांतसाबुज दास ने कार्यक्रम सभा को ऑनलाइन संबोधित किया। उन्होंने केंद्र सरकार के कार्यालयों में हिंदी दिवस मनाने के उद्देश्य पर प्रकाश डाला। केंद्रीय हिंदी निदेशालय के सहायक निदेशक श्री संतोष कुमार ने कार्यक्रमों के आयोजन के प्रयासों की सराहना की। विशेष अतिथि डॉ. भानुप्रकाश रेड्डी और हिंदी कार्यान्वयन समिति के संयोजक डॉ. सुब्बाराव एम. गवरबारपु ने भी इस अवसर पर सभा को सम्बोधित किया और 25 सितंबर तक आयोजित होने वाले कार्यक्रमों पर प्रकाश डाला। हिंदी के प्रचार-प्रसार को ध्यान में रखते हुए स्टाफ सदस्यों के लिए कई प्रतियोगिताएं आयोजित की गई और कर्मचारियों ने उत्साह के साथ भाग लिया। समापन समारोह 25 अक्टूबर को आयोजित किया गया और एक भव्य सांस्कृतिक कार्यक्रम के बाद डॉ. उदय कुमार, डॉ. भानुप्रकाश रेड्डी द्वारा प्रस्कार विजेताओं के लिए प्रस्कार वितरण की व्यवस्था की गई।



Medical camp at NIN Welfare Clinic conducted by Omega Hospitals, Hyderabad (24th Sept).



A Model Rural Health Research Unit (MRHRU) supported by Department of Health Research and Indian Council of Medical Research was inaugurated by Smt. D. K. Aruna, Hon'ble Member of Parliament at Janampet, Mahbubnagar District. Nodal Officer Dr. Putcha Uday Kumar, Scientist-G, In-charge, MRHRU-TG graced the occasion. This MRHRU will closely work in coordination with our Institute to develop and strengthen health research infrastructure to address local health challenges (30th Oct).



Vigilance Awareness Week 2024 pledge administered by NIN staff ( $25^{th}$  Oct).



The staff of Extension & Training Division conducted an awareness programme on "Nutrition & Well-being" for sanitation workers in collaboration with GHMC at Kukatpally Zone, Hyderabad. Dr. K. Rajender Rao, Scientist F & HoD, NIN Animal Facility, Dr. Sylvia F Rao, Scientist E, Ms. Sridevi, Nutritionist from ET Division delivered awareness lectures (5<sup>th</sup> Oct).



Dissertation presentations were conducted for the 2022-24 batch of M.Sc. Applied Nutrition and Sports Nutrition students on 24<sup>th</sup> & 28<sup>th</sup> October. External examiner for the M.Sc. (AN) student's dissertation presentations was Dr. Vijay Lakshmi Bodiga, I/c Director, Institute of Genetics, Osmania University. 23 students from M.Sc. (AN) presented their dissertation works. External examiner for the M.Sc. (SN) students dissertation presentations was Dr. Vivekanand Upadhyay, Sports Medicine Specialist, SMC, Artillery Centre, Hyderabad. 17 students from M.Sc. (SN) presented their dissertation works which were carried out under the guidance and supervision of NIN scientists.



A training programme on "Nutrition survey methods and community based management of acute malnutrition (CMAM)", was organized by the Division of Public Health Nutrition, ICMR-NIN. Dr. N. Arlappa, Scientist-G & HoD, Dr. I.I. Meshram, Scientist F, Public Health Nutrition presided the event (21st to 25th Oct).



Media delegation from Jammu & Kashmir visited NIN and interacted with senior scientists & visited labs. Dr. Santasabuj Das, Director In charge addressed them. The visit was coordinated by Press Information Bureau - PIB, Government of India (22<sup>nd</sup> Oct).



As part of SwachhtaHiSewa2024 the housekeeping staff were felicitated and a health camp was organized for them (1st Oct).



BATHUKAMMA- A popular conventional flower festival of Telangana was celebrated at NIN by the staff and students. On this special occasion the women staff and the students dressed in traditional attire danced around the beautiful flower stacks made with seasonal flowers with medicinal values. They danced to the traditional Bathukamma songs modified with important nutrition messages.



Vigilance Awareness Week-2024 Walkathon



NIN Animal Facility conducted the 41stLaboratory Animal Supervisors Training Course-2024 (LASTC) from 3rd Oct to 29th Nov and 3 days Ad-hoc training programme from 26th to 28th Nov.



Staff of NICHE Division conducted nutrition awareness programme in Nagaram Municipality area, TG Municipal commissioner, Municipal Chairperson & Vice-Chairman, Medical Officer & over 300 SHG women, 50 daily wage Workers attended (4th Dec 24).



The role of stakeholders in implementing nutritional guidelines was moderated by Dr. SubbaRao M Gavaravarapu, Scientist F & HoD, NICHE Division. Experts Prof. V. K. Tiwari, Dr. Meenakshi Bajaj, Dr. Aman Basheer, and Dr. Premalatha discussed the role of health programmes, media, FMCGs, government, and academia in promoting nutrition info (16th Dec 24).



Team NIN forged ties with the Indian Medical Association (IMA) India Org Food & Nutrition Initiative to popularize Dietary Guidelines for Indians. Glimpses of the National Nutrition Seminar were seen in the ongoing IMA National Conference in Hyderabad (28th Dec 24).



ICMR-NIN and Gandhi Medical college signed an MoU to collaborate and work on National Health Research projects, academic research exchange and support MDRU, at Gandhi Medical college. The team of NIN Scientists and Technical Officers was seen with Dr. Bharati Kulkarni Director, NIN (08th January, 2025).



Dr. Yellapragada SubbaRow's 130<sup>th</sup> birthday celebrations were held at Dr. YSR Academic Block by lighting the lamp and garlanding the statue by Dr. Bharati Kulkarni, Director. Earlier, Dr. M. Maheshwar, Scientist F & HOD, Extension & Training Division-MYAS, welcomed staff of the ET Division, MYAS and M.Sc. Students. Dr. C. Suresh, Scientist F & HOD, Cell Biology Division & Convener of the M.Sc. Curriculum Committee also spoke on the occasion (10<sup>th</sup> January 2025).



On the occasion of 76<sup>th</sup> Republic Day, Dr. Bharati Kulkarni, Director, unfurled the tricolour flag & addressed the staff. She also released 2025 Calendar based on Dietary Guidelines, booklets in 3 languages & distributed prizes to winners of sports events. Scientists, technical staff & PhD students also participated in the event.



An AcSIR PhD Orientation programme was conducted on 23<sup>rd</sup> January virtually for the PhD Students of AcSIR, ICMR- NIN for the batches of August 2024 and January 2025.



ICMR-NIN Animal Facility conducted a 3-day ad-hoc training programme on "Animal Sciences, Care, Handling and Experimental Lab Animal Welfare Guidelines". Dr. Rajender Rao, Scientist F & HoD, NIN Animal Facility was seen presented certificates to the participants (29th to 31st January, 2025.



ICMR-NIN and FAO are jointly working on the Education for Effective Nutrition in Action (ENACT) programme. To this effect, officials from the FAO met with the Director, and the Head of the Extension and Training Division to finalize and discuss the Letter of Agreement. The deliberations were productive, and the modalities of cooperation were discussed to facilitate the smooth running of the programme.



Under the guidance of Dr. Bharati Kulkarni, Director, ICMR-NIN, nutrition kits were provided to 22 TB patients at Uppal TB Centre on February 21, 2025, under the Nikshay Mitra programme. The kits included basic foodstuffs like rice, dals, oils, and other nutritional provisions with a view to enhancing the nutritional status of the patients by supplementing them with sufficient proteins, vitamins, and essential micronutrients. The programme was joined by Dr. Sai Ram Challa, Scientist-E, MCH Nutrition; Dr. Santosh Kumar B, Scientist-D, MCH Nutrition; Dr. Sapavat Shankar, Scientist-B, Public Health Nutrition; and S. Laxman, Technical Officer-C.



Extension & Training Division successfully conducted 60<sup>th</sup> batch of annual certificate programme PG Certificate Course in Nutrition (PGCCN)-2025 and the valedictory function was held on 18<sup>th</sup> March. Yet another group of nutrition ambassadors trained in this annual programme. Dr. M. Maheshwar, Scientist F & HoD, ET Division & Dr. C. Suresh, Scientist F & HoD, Cell Biology Division and Convener, Curriculum Committee for PG courses distributed certificates to the participants.



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