RESEARCH HIGHLIGHTS

1. COMMUNITY STUDIES

1.1 Childhood obesity in India: A multi-centric study on its measurement and determinants

It is a task force study of ICMR carried out at two sites in Hyderabad and New Delhi to develop reference curves of body fat (content and distribution) among Indian children aged 6 to 18 years and to identify the best possible cut-off measures of body fat that predict unacceptable cardio-metabolic risk factors for defining body fatness among Indian children. The study also aims to identify determinants of childhood body fatness that operate at different levels across five diverse socio-cultural and geographical contexts of India.

1.2 Assessment of effect of consumption of Khesari dal on human health

This epidemiological study was carried out by NIN at the request of the planning commission, with an objective to assess the effect of consumption of Khesari dal on human health in the state of Chhattisgarh, to help the government take decision on whether to lift or not to lift the ban. The mean consumption of Khesari dal was only 3.5 g/CU/day, out of 50g of total pulses consumed daily. Only nine suspected old cases of neurolathyrism have been identified in the state.

1.3 Assessment of nutritional status of children below the age of five years and performance of ICDS functionaries in the state of Haryana

The prevalence of undernutrition especially among children under the age of five years may vary geographically. Therefore, for the development of area specific intervention strategies, divisional/district level mapping of undernutrition is very essential. The prevalence of undernutrition (<Median -2SD) among <5 year children such as underweight, stunting and wasting was 28%, 34% and 11% respectively. However, the magnitude of undernutrition was higher in the divisions of Gurgaon and Hissar compared to Ambala and Rohthak.

2. MICROBIOLOGY AND IMMUNOLOGY

2.1 Vitamin D status, vitamin D receptor expression and their link with CD23/ CD21 interaction and regulatory T cell function in children with allergy

Regulatory T cells and IgE receptors (CD23 & CD21) on B cells were assessed in vitamin D deficient pregnant women. The results are as follows:

The maternal and cord blood Treg cell population and regulatory cytokines, TGF β and IL-10 were significantly lower in pregnant women with vitamin D deficiency, while CD23 and CD21 were higher. The regulatory T cell transcription factor FOXP3, vitamin D receptor (VDR) and retinoic acid receptor (RXR) expressions were down-regulated, while CD23, CD21 and VDBP expressions were up-regulated in placenta of vitamin D deficient pregnant women. Vitamin D regulating enzymes (CYP24A1, CYP2R1 and CYP27B1) expression were also altered in placenta of women with vitamin D deficiency. The current study shows that impaired maternal vitamin D during pregnancy influences the spectrum of immune cells such as regulatory T cells and B cells with IgE receptors and this in turn may be linked to allergy and asthma in neonates.

2.2 Towards the Development of edible vaccine against Helicobacter pylori with recombinant lactobacilli expressing Heparan Sulfate binding protein

Cell surface display of antigens on L. rhamnosus GG strain was developed by using signal peptide CspI from L. plantarum and anchor peptide PrtR from L. rhamnosus having MPQTG type anchor that is specific for L. rhamnosus strains.

The food-grade host, L. rhamnosus GG with pSIP503 expression vector was used with erythromycin genes as selection marker and nisRK promoter for cell surface expression of foreign proteins. This system developed by here may be very useful as antigen delivery vehicle in oral vaccine formulation.

2.3 Characterization of vaginal *lactobacilli* isolated from healthy women to formulate potential probiotic for reproductive health

L. crispatus (100%) was found to be the most predominant *lactobacilli* species present in pregnant women with normal flora followed by *L. jensenii* (72%), *L. gasseri* (66%), *L. acidophilus* (32%)

L. crispatus was found in 76% of women with abnormal flora followed by *L. jensenii* (61%), *L. gasseri* (41%) and L. acidophilus (24%). Whereas, *L. reuteri* was more (24%) commonly associated with abnormal flora than normal flora (7%). This preliminary data might be useful for the development of probiotic formulations for reproductive health of women in India.

3. BASIC STUDIES

3.1 Innovative strategies to promote early child development among low-income rural infants and pre-schoolers in India through multiple micronutrient fortification and early learning opportunities

Project Grow-Smart, a randomized controlled interventional trial was carried out among infants and pre-schoolers in 26 villages of four state administrative blocks (mandals) from Nalgonda District in the state of Andhra Pradesh (now in Telangana), India. The infant (6-12 mo) intervention was a home-based four-arm trial consisting of two interventions of 12 months duration i.e., multiple micronutrient powder, early learning, combination and a placebo. Pre-school (36-48mo.) phase was a point-of use cluster-randomized two arm trial (MNP and placebo) stratified across low and high quality Anganwadi centres for 8 mo. The MNP intervention consisted of iron and six haemopoietic micronutrients i.e., vitamins A, C, B2, B12, folic acid and zinc and placebo only vitamin B2 based on Indian RDA for the corresponding group.

Baseline data showed iron deficiency and maternal anaemia as the major factors associated with anaemia among young children. Additionally, inflammation was associated with anaemia among pre-schoolers.

MNP intervention resulted in significant anaemia reduction in both infants (67 to 50 % in MNP against 66 to 72% in placebo) and pre-schoolers (46 to 10 % in MNP against 48 to 35 % in placebo). There was a significant improvement in biomarkers of iron in both groups. MNP and early learning improved cognition in infants. Among pre-schoolers, MNP reduced disparities between children in low and high quality anganwadis by improving development in children in low quality preschools. Findings from this home and pre-school based integrated trial can be used to guide larger-scale policy and programs designed to promote the developmental, education and economic potential of young children in rural India.

3.2 Efficacy of polyphenol-rich dietary ingredients as proteasome inhibitors and their role as anticancer agents

Dietary ingredients are rich sources of polyphenols/ flavonoids and these plant metabolites are known to act as proteasome inhibitors. Inhibition of the proteasome leads to cancer cell death and is thought to be a promising approach for cancer therapy. Murraya *koenigii* leaf (curry leaf) is a rich source of polyphenols. M. *koenigii* leaf extract (MLE) inhibited enzyme activities of the 26S proteasome and led to cell death and growth arrest in two human breast cancer cell lines. Further, the leaf extract resulted in growth suppression of breast tumor xenografts. Reduction in tumor growth was associated with decrease in proteasomal enzyme activities in the treated groups. Increased caspase-3 activity and TUNEL-positive cells indicated enhanced apoptosis with MLE treatment. Decreased expression of angiogenic and anti-apoptotic gene markers is indicative of inhibition of angiogenesis and promotion of apoptosis in the leaf extract treated tumors. In addition, increased phosphorylation of p38 and decrease in phosphorylation of Akt may contribute to the anti-proliferative and pro-apoptotic effect of M. *koenigii* leaf extract.

3.3 Reduced longevity in WNIN/Ob rats: Possible role of changes in neuronal metabolism and neurochemical profile

Volume changes in the brain determined in the WNIN/Ob obese and normal WNIN rats of comparable age

using Magnetic Resonance Imaging (MRI) did not show any significant differences between the age matched obese and control rats in various brain regions analyzed. This falls in line with our previous observations of wet brain weights, which although were lower in WNIN/Ob obese rats than WNIN normal control rats, the differences were not statistically significant. Further, our findings on neurochemical profile indicate that at a young age itself the neurochemical profile of these animals is getting altered and resembles the changes seen in the control rat brains at later age (*e.g.* 15-18 months, further corroborating the accelerated ageing of the WNIN/Ob obese rats. Also, it was observed that neuronal glucose metabolism was somewhat lower in WNIN/Ob rats compared to controls of comparable age further corroborating accelerated ageing of the WNIN/Ob obese rats.

3.4 Possible utility of human umbilical cord blood or placenta derived mesenchymal stem cells for Toxicity studies

◆ The in vitro micronucleus test is a well-known test for the screening of genotoxic compounds. However, until now, most studies have been performed on either human peripheral lymphocytes or established cancer cell lines. It is perhaps demonstrated for the first time that the use of a normal diploid umbilical cord-mesenchymal stem cells can be used as an alternative to the conventional micronucleus test.

◆ Umbilical cord mesenchymal stem cells (UC-MSCs) were grown on the coverslips eliminating the cumbersome technique involving hypotonic treatment, fixation and preparing smears required for suspension culture (lymphocytes). A platform was created for simultaneous testing of cytotoxicity and genotoxicity of pharmaceuticals.

◆ The cytotoxic and genotoxic effects of two known mutagens, mitomycin-C and hydrogen peroxide (H2O2), on UC-MSCs, lymphocytes and A549 cells were inspected. Treatment with mitomycin-C and H2O2 demonstrated drastic differences in the degree of cytotoxicity and genotoxicity suggesting a constitutional difference between normal and cancer cells.

◆ In addition, two solvents, dimethyl sulfoxide (DMSO) and ethanol, and two drugs, metformin and rapamycin were tested. DMSO above 1% was found to be cytotoxic and genotoxic, whereas ethanol at same concentration was neither cytotoxic nor genotoxic, indicating the minimal non-toxic level of the solvents. This study thus offers UC-MSCs as a better substitute to peripheral lymphocytes and cancer cell lines for high throughput screening of compounds and reducing the animal studies.

3.5 Status of vitamin-D in type 2 diabetes patients with and without retinopathy

In this cross-sectional case–control study, the status of vitamin D (VD) in type 2 diabetic (T2D) patients with and without diabetic retinopathy (DR) has been reported. While mean plasma VD levels were significantly lower in T2D patients without (NDR) and with DR groups compared with the control group, there were no significant differences between the diabetes groups. The prevalence of VD deficiency (VDD) was higher in NDR and DR groups (66% and 63%) compared to age-matched controls (45%), suggesting that prevalence of VDD was higher in diabetic groups, irrespective of presence or absence of retinopathy.

3.6 Ellagic acid ameliorates diabetic retinopathy by inhibition of AGE formation in rats

Hyperglycemia in diabetes leads to accumulation of AGE which activates VEGF through the receptor for advanced glycation end products (RAGE) to trigger angiogenesis in retina. AGE also induces apoptosis of pericytes through interaction with RAGE and induction of VEGF. Using a range of *in vitro* protein glycation models, we reported that ellagic acid (EA), present in many dietary sources, prevents AGE formation. In this study we demonstrated that EA inhibit AGE formation and activation of RAGE in retina under hyperglycemic conditions. Further, AGE-RAGE mediated cellular events and subsequent functional abnormalities are modulated by EA which may throw light on the potential of therapeutic avenues for treatment of diabetic retinopathy.

3.7 Response of small heat shock proteins in diabetic rat retina

Small heat shock proteins (sHsp) play a critical role under stress conditions to maintain cellular homeostasis. The hyperglycemia in diabetes may impose cellular stress on retina. In this study, we reported that increased

expression of sHsp: αA-crystallin (αAC), αB-crystallin (αBC) and Hsp22 in diabetic retina; There was increased phosphorylation of αBC under diabetic conditions. Moreover, diabetes activated the p38MAPK signaling cascade by increasing the p-p38 MAPK in retina. These results suggest that specific sHsp are crucial for neuronal protection in diabetic retinopathy (DR) and may aid in developing therapeutic strategies for DR.

3.8 Altered ubiquitin-proteasome system leads to neuronal cell death in a spontaneous obese rat model

Obesity is associated with various progressive age-related diseases, including neurological disorders. However, underlying molecular basis for increased risk of neurodegeneration in obesity is unknown. Using a spontaneous obese rat (WNIN/Ob) model we have described neurodegeneration due to obesity. Altered ubiquitin-proteasome system (UPS), existence of ER stress, up-regulation of apoptotic markers and apoptosis was found in the cerebral cortex of obese rats. This study highlights the role of altered UPS in neurodegeneration due to obesity.

3.9 Role of growth hormone in podocyte injury and depletion: Implications in proteinuria

Loss of glomerular podocytes is a critical event in the pathogenesis of proteinuria during diabetic nephropathy (DN). Increased level of growth hormone (GH) is implicated as a causative factor in the development of nephropathy in type 1 diabetes. However, the molecular basis for the effects of GH on podocyte depletion is not understood. Our studies reveal that GH increases expression of transforming growth factor-beta-induced protein (TGFBIp) and also increased secretion of extracellular TGFBIp. Both GH and TGFBIp induced apoptosis and epithelial mesenchymal transition (EMT) of podocytes. Administration of GH to rats induced EMT and apoptosis in the kidney. Therefore, GH-dependent increase in TGFBIp in the podocyte could be one of the mechanisms responsible for podocyte depletion in DN.

3.10 Effect of reactive oxygen species on macrophage signalosome: Impact on antigen presentation function and T cell priming responses

Although reactive oxygen species (ROS) are important for induction of cytotoxic effects against invading pathogens, it is not very clear whether excess ROS production can actually underweigh the beneficial outcome of the innate immunity. Oxidative stress has been implicated in manifesting detrimental effects of various disorders like cancer, aging, diabetes, atherosclerosis and infection. Interestingly, most of these pathophysiological disorders are found to be associated with severe immune suppression, indicating that excessive production of ROS could be one of the factors responsible for development of immune suppression in these situations. Therefore, it was aimed to understand whether oxidative stress has a direct link with the observed immunosuppression in pathophysiologcal conditions, in other words whether it has any actual direct effect on the T cell priming responses and the macrophage signaling cascades required for development of subsequent adaptive immune response.

It was found that excess ROS can inhibit MHC Class I and Class II presentation of exogenous ovalmumin and inhibit processing of antigens by professional APCs like macrophages. Even presence of physiological concentrations of ROS could significantly inhibited MHC Class II-mediated antigen processing and presentation. ROS was found to target c-rel transcription factors in macrophages as over-expression of c-rel was found to ameliorate antigen processing and presentation inhibited by ROS. Interestingly, excess exogenous ROS was not found to significantly affect surface expression of co-stimulatory molecules like MHC-I, MHC-II and CD80, considered to be important for antigen presentation. ROS was also found to inhibit calmodulin expression. Our results indicate that inhibition of antigen presentation by ROS might is probably due to modulation of calmodulin-c-rel signaling pathway, rather than weakened co-stimulatory signaling.

4. PUBLICATION, EXTENSION AND TRAINING DIVISION

4.1 Promoting the use of food label information among school-going adolescents

A nutrition education intervention study evaluated the impact of a label information reading kit on usage of food labels among adolescents for promoting healthy food choices. The kit titled 'Read-B4-U-Eat' was developed and theories of social-cognition and shared-learning were used to develop the module's five components – interactive sessions for guided learning; booklet for self-learning; 9 posters for shared learning;

animation film (4 min.) for edutainment; and notes for teachers for reiteration. The kit was efficacious nutrition education module to inculcate label reading skills among adolescent consumers.

5. PATHOLOGY

5.1 Knowledge, Attitude and Practices [KAP] of medical practitioners in Hyderabad regarding food allergy (FA) - a pilot study

- A total of 300 medical professionals including gastroenterologists, pediatricians, dermatologists, pulmonologists, general practitioners and homeo-physicians (50 from each category) were contacted and administered a questionnaire.

- More than 50% knew that FA is a serious problem but did not responded correctly to questions on difference between food allergy and intolerance.

- 53.7% had very little knowledge about prevalence and published literature on food allergy.

- 37.6% responded that they rarely refer such patients for further evaluation.

- The medical practitioners were not sufficiently knowledgeable regarding the diagnosis, treatment and risk factors for food allergies and related anaphylaxis.

6. FOOD CHEMISTRY

6.1 Bioactive phytochemicals in Indian foods

There is a considerable evidence for the role of antioxidant constituents of fruits and vegetables in the maintenance of health and disease prevention. Studies also showed that phytochemicals some of them not necessarily antioxidants but are also bioactive and help in preventing many chronic degenerative diseases. This research project made us to standardize the separation of many polyphenolic constituents of foods. To appreciate and to understand important minimal constituents of plant foods to our health, the composition of bioactive phytochemicals in cereals, pulses, fruits, vegetables, and their processing effects were studied. This project generated database on polyphenolic compounds in commonly consumed Indian foods.

6.2 Studies on gastro-protective effects of 'King chilli'

Studies on gastric protection and mineral absorption using Naga King Chilli was carried out in ethanol induced ulcer model for a period of 24 days (sub-chronic) and 90 days (chronic). Experiments were conducted in male SD rats using ethanol induced ulcer model. Findings of this study demonstrated the gastroprotective effect of Naga King chili against gastric mucosal damage induced by ethanol. The observed gastroprotection is possibly mediated to a major extent by the local inflammatory mechanism followed by antioxidant mechanism. Results clearly shows that increased mucosal content and CGRP were higher in the groups treated with capsaicin or Naga king chili followed by ethanol. Elevated levels of antioxidant enzymes in the capsaicin+ethanol and Naga king chili+ethanol groups clearly demonstrates the ability of restoration of epithelial cells by capsaicin and Naga king chili. Therefore, the combined effect of antioxidant and other complimentary mechanism such as CGRP and PGE are accompanied to protect gastric ulcer induced by ethanol. The present findings of gastro-protection by Naga king chili not only supports the traditional consumption of chili but also stress the need for the development of potential therapeutic drugs.

6.3 Prebiotic effect of legume raffinose family Oligosaccharides

The analysis of oligosaccharide content of commonly consumed legumes showed the presence of raffinose family of sugars with varied concentration. These sugars cannot be hydrolyzed and absorbed in the intestine, due to the lack of -galactosidase activity in the small intestine but undergo anaerobic fermentation by bacteria in the large intestine, which may result in the production of flatus gases (H_2, CO_2 and small amount of CH_4). The effect of different house hold processing methods led to decrease in the levels of raffinose family sugars.

Oligosaccharide fermentation in the caeco-colon by the bacteria can give many positive health benefits as prebiotics. The prebiotic potential of legume oligosaccharides in animal model were shown decrease in blood glucose level, improved lipid profile, increased mineral absorption, improved body mass composition and inflammatory markers. The ceacum sample analysis showed that increase in the gut bacterial colonies of

lactobacillus, bifidobacteria, enterobacillus and bacteroides by decrease in the pathogenic putrificative bacterial counts. There was reduction in inflammatory cytokines like IL-1ß, IL-6, TNF-α and INF-γ and CRP in experimental animals supplemented with legume prebiotics. No such changes were seen in control group. Further research is required to understand the role of legume prebiotics to prevent or control diabetes, obesity, cardiovascular diseases, irritable bowel syndrome and other health benefits.

7. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

7.1 Serotyping RNA virus to study molecular epidemiology of dengue supplementing emergency preparedness and capacity building in metro cities of Karnataka, India

- We have tried to use both geographic data to correlate genetic analysis to disclose the spread of dengue virus lineages in urban and metro areas of city. It suggests that a pattern of dispersion is consistent with dispersion rate mostly human accidental transport.

- The collection of our samples in endemic areas averages within a diameter of 3-4 kilometers. The data stressed the importance of mosquito and human circulation; do play an important role in dispersal of viruses.

- As the egg of the vector mosquito *Aedes aegypti* are resistant to desiccation it may have important implications for cryptic maintenance of viral strain. Therefore, viral strain not detected in any season cans re-imerge in next rainy season due to trans-ovarian transmission.

- So, availability of samples serotype in any particular urban/metro areas of Bangalore and Mangalore could not be ascertained where they are autochthonous or are imported distinct strain lineages from outside.

- Alternatively, its establishment may have been hampered by decreasing availability of susceptible host in later stage outbreak. In our study, because of the time constrain, the follow-up of post viral infestation could not be done.

7.2 Empowerment of farm women in mitigating the pesticide residues at the farm and household levels

300 vegetables/fruits were analysed for various pesticide residues at different stages of household processing (1. raw; 2. after soaking in distilled water; 3. after rubbing and again soaking in distilled water and 4. after soaking in 2% sodium chloride solution).

A significant reduction in the residue levels was observed after subjecting to the rubbing and again soaking in distilled water. No significant difference was found in the mineral contents viz., Ca, Mg, K, P, Na, Cu, Mn and Zn in the vegetable/fruit samples after subjecting to any processing stage.

Further, extensive educational activities were undertaken for both the farmers and farm women. Under the translational researchwork, novel cost effective protective devices were developed for the farmers and farm women to be used while working in the agricultural farms.

7.3 Quantitative detection of heavy metals and phthalates in toys

This study was formulated with the following objectives:

- To determine the levels of heavy metals namely Lead (Pb), Cadmium (Cd), Chromium (Cr), Arsenic (As) and Mercury (Hg) in toy samples obtained from four different geographical regions (North, South, East and West) of India.

- To estimate the phthalates Di butyl phthalate (DBP), Benzyl butyl phthalate (BBP), Di ethyl hexyl phthalate (DEHP), Di–n– octyl phthalate (DNOP), and Di-isononyl phthalate (DINP) content in the sampled toys.

- To compare the level of heavy metals and phthalates in toy samples collected from rural as well as urban areas and between the three different categories of toys – local, unbranded and branded.

- To provide recommendations based on the study findings.

Important Findings

a) 95% of toy samples were found to have lead level within permissible limits (90 mg/kg).

b) 98% of toy samples were found to have Cadmium level within the permissible limits (75 mg/kg).

c) Chromium level was detected below 60 mg/kg (permissible limit) in 96.5% of the samples.

d) Arsenic level was within 25 mg/kg (permissible limit) in 97.8% of the samples. Most of the samples exceeding this limit were those collected from south zone.

e) Mercury was within the permissible limit (60 mg/kg) in all the samples.

f) The detected phthalates viz., DBP, BBP, DEHP, DNOP, DINP were above the prescribed limit in 4.6%, 0.2%, 15.8%, 0.6% and 21% of toys respectively.

g) DEHP and DINP were found to be more than 10 times above prescribed limit (0.1%) in 3.4% and 12% of the total toy samples respectively.

h) In total toy samples, 32.6% had any one phthalate above 0.1% and more than half of these toys were found to be teethers (Those toys which are kept in mouth).

i) The country wise distribution of any phthalate above 0.1% showed that 16.9% of the samples were made in China followed by 13% in other countries, the identity of which could not be ascertained and 1.9% were made in India.

j) Higher levels of heavy metals as well as phthalates were detected in the local category of samples.

k) Leaching (Migration and Wiping) studies did not show any significant migration of Lead and Cadmium.

l) The randomly selected sample in this study revealed that 51.3 % of the sample toys were labeled as 'Made in China' (however, it was not possible to authenticate the origin) as compared to 8.8 % labeled as 'Made in India' and 1.9 % from other countries.

7.4 Evaluation of the impact of genetic polymorphism on pharmacodynamic activity of commonly prescribed antihypertensive drugs (thiazide diuretics, ace inhibitors, CCBS and β-blockers)

Sub Title: Prescription profile anti-hypertension drugs

The prescription profile demonstrates that monotherapy is chosen over the combination therapy. The pattern of prescription adhers to JNC VII except for diuretics. The antihypertensive potential of drugs were reflected only in 30-40% of subjects, therefore, calling for an investigation to assess role of genetic variations along with nutrient interactions.

8. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

8.1 Effect of environment and microorganisms in the development of obesity in WNIN Obese rats

The species of Firmicutes detected by FAME (Fatty Acid Methyl Ester) analysis in obese rats (cecal samples) were Staphylococcus-cohnii and Staphylococcus epidermidis. Of these two species identified, Staphylo-coccus-cohnii was detected in more number of samples when compared to Staphylococcus-epidermidi. In Lean rats Bacillus-subtilis, Bacillus-GC group 22 and Paenibacillus-validus were identified. Of these three species, Bacillus-subtilis was found in more number of samples followed by Paenibacillus-validus and Bacillus-GC group 22.

The species of Bacteroidetes detected by FAME analysis in obese rats cecal samples were Prevotella-melaninogenica, and Eikenella-corrodens. Among these Prevotella-melaninogenica was found more in obese rats cecal samples when compared to Eikenella-corrodens. However, in Lean rats cecal samples among the two species identified Prevotella-melaninogenica and Eikenella-corrodens, Eikenella-corrodens was found more than Prevotella-melaninogenica.

The data supported the key idea that the gut microbiota can contribute to the patho-physiology of obesity. This could be considered when developing strategies to control obesity and its associated diseases by modifying the gut microbiota. This is a preliminary data of pilot experiment to get the lead for taking up a full fledged study. With additional studies this work could lead to identification of microbial markers that make up a kind of obesity or leanness profile in animals a vital-stats sheet of the gut world that would help understanding how people are likely to respond to microbes. Beyond that, the knowledge gained from this study can be applied to agriculture science and we could grow foods that are specifically designed to provide the optimal balance of nutrients and energy for various life stages.

9. PRE-CLINICAL TOXICOLOGY

9.1 Pre clinical safety evaluation of vegetables cultivated using deoiled Karanja seed cake

Objective: To assess the safety of vegetables (Amaranthus, Onion, Tomato) cultivated using Karanja seed cake (Expelled and deoiled cake) in rats.

Conclusion: There was no mortality in any group of animals which received test material (Amaranthus- T_1, T_5 & T_{10} , Onion - T_1, T_5 & T_{10} , Tomato - T_1, T_5 & T_{10}) cultivated using karanja seed cake in SD Rats for 90 consecutive days it did not show any adverse effects on feed and test material intake, body weights, cage side activities, clinical chemistry and heamotology profile. Histopathological study showed organ changes in various groups but it may not be attributed to test material given at various doses and for whole study period.

9.2 Pre clinical evaluation of iron filings in CTC-Tea and its brew

Objective: To assess the safety profile of CTC tea in Rats.

Conclusion: There was no mortality in groups of animals fed IF directly and CTC with Iron filling (10mints) which is 10 times more than permissible limits daily for 28 days. The early pre-terminal mortality was recorded in orthodox, CTC (5mints) and CTC+IF (5mints) tea brew perhaps due to technical errors in feeding process. The post exposure study results of live phase activity, clinical observations, chemistry and hematology and various histopathology observations did not appear to be due to exposure to test material, serum and liver ferritin, gross necropsy investigation of vital organs didn't suggest any abnormal finding in spite of exposure to CTC with iron filings more than 10times of permissible limit (250ppm) in experimental conditions.