RESEARCH HIGHLIGHTS

The research activities of the institute this year covered a wide spectrum of projects in the fields of HIV/AIDS, adolescent obesity, osteoporotic hip fractures, diabetes mellitus, spices, fats-cancer interface, effect of pesticide exposure, ayurvedic bhasmas, nutrition in school curriculum etc. The studies conducted had a fine blend of hospital, community and laboratory based research work. Also, studies done in the realms of basic research, molecular biology and laboratory animal sciences revealed some interesting findings. Rich data were generated on health enhancing properties of roots, tubers and vegetables. New information pertaining to protein, iron and zinc content in several rice varieties were collected this year. The new research findings have indeed provided the much needed value addition to the existing research culture of the institute.

1. COMMUNITY STUDIES

1.1 Integrated behavioural and biological assessment among high risk groups for HIV in select districts of Andhra Pradesh revealed the following findings:

Female sex workers (FSWs)

The mean age was about 30 years, average duration of sex work was 5 to 8 years, with mean number of clients during the previous week ranging from 5 to16. Most of them (>90%) had occasional clients also. Consistent condom use ranged from 15 to 85% with regular clients and 36 to 89% with occasional clients. The prevalence of HIV ranged from 8 to 26%, and that of syphilis was 5 to 17%.

Men having sex with Men (MSM)

The mean age ranged from 25 to 30 years. Majority were literate (58% - 82%) and nearly 90% reportedly had non-commercial male/hijra partners. The extent of consistent use of condom was very low and ranged from 1 to 40%. However, condom use during the last sexual intercourse with paying male partners was more than >70% in all the districts. A considerable proportion of MSM (28 to 64%) reportedly had 'paid female partners' and consistent condom use with them ranged from 4 to 37%. The prevalence of HIV ranged from 9 to 26%, and that of syphilis was about 4 to 16%.

Clients of Female sex workers

The mean age ranged from 28 to 31 years, with a majority of them being literate (64% to 90%). Consistent condom use with regular or occasional FSWs, was in general low and ranged from 16% to 38%, while it was negligible with main/steady partner. The prevalence of HIV ranged from 2 to 8%, while that of syphilis ranged from a low 3 to10%.

1.2 Assessment of Prevalence of overweight/ obesity and its determinants among 12-17 year urban adolescents in Andhra Pradesh

Obesity is one of the fast emerging public health problems among adolescents and adults, both in the developed as well as developing countries, leading to high incidence of hypertension, type 2 diabetes, coronary artery diseases, stroke, etc. The present study, was therefore, carried out to assess the prevalence of overweight/obesity and identify its determinants among adolescents of 12 to 17 years old in the urban areas of Andhra Pradesh. A total of 8,142 adolescents of 12–17 years age group were covered in the present study.

The overall prevalence of overweight was about 6%, which was significantly ($p< 0.05$) higher among girls (7.1%) as compared to boys (4.4%) and among the adolescents of high socio-economic status (9.6%) as compared to low socio-economic group (2.3%). The average duration of 'TV watching' was significantly higher ($p< 0.05$) among overweight and obese children (1.4 hrs/day) as compared to controls (1.2 hrs/day) and participation in outdoor games and sports for 6hrs/week was significantly ($p< 0.05$) higher among the non-obese children (31.9%) compared to overweight and obese (17.5%). The proportion of children consuming soft drinks was significantly

higher ($p < 0.05$) among the overweight and obese adolescents (21%) as compared to normal children (16%). The prevalence of hypertension (JNC Criteria VII) was significantly ($p < 0.05$) higher among the overweight and obese children (8.3%) as compared to non-obese children (3.7%). The study highlights the need to educate the adolescents to promote physical activity, healthy food habits and other life style practices to prevent overweight and obesity and the consequent diet-related chronic degenerative diseases.

2. CLINICAL STUDIES

2.1 Neonatal anthropometric data and body composition in Small for Gestational Age (SGA) babies compared to Appropriate Gestational for Age (AGA) babies

Barker's hypothesis speculates that under-nutrition in intra-uterine period predisposes to increased adiposity in later life. A study was done to compare the body composition of SGA babies with full term normal babies. Contrary to the earlier studies which have shown relatively higher body fat percentage in low birth weight (LBW) babies, this study assessing over 600 newborns has shown that neonatal body fat percent is increasing with increasing birth weight, and fat percent and fat free mass (FFM) were significantly low in LBW babies. No significant differences were observed in trace element levels in either maternal or cord blood.

2.2 Bone parameters of men and women with osteoporotic hip fractures

A study was carried out to measure the bone parameters of men and women with confirmed osteoporotic fractures. The results of the study show that osteoporotic fractures in the study population (Hyderabad, hospital-based study) occurred at least 10-15 years earlier as compared to Western population.

3. BASIC STUDIES

3.1 MICRONUTRIENTS

Micronutrient research group has been involved in developing novel strategies to combat micronutrient malnutrition, especially of iron, zinc and vitamin A and understanding their mechanism(s) of absorption. Towards this, a rapid and sensitive common immuno assay (ELISA) for phytoferritins (plant iron-storage protein) has been developed as a screening tool for identifying high iron in biofortified crops. This was used to quantify phytoferritin levels in pulses which correlated well with their iron density. Zinc bioavailability using Caco-2 cells was developed, standardized and validated by demonstrating modulation of bioavailability in the presence of known absorption enhancers and inhibitors. Caco-2 cells were used to understand the mechanism of negative interactions between iron and zinc during uptake at the enterocyte. Kinetic studies show that zinc inhibition of iron uptake does not occur at DMT-1 (apical iron transporter) and cellular zinc status profoundly affects quantity and pattern of iron uptake.

4. DEGENERATIVE DISEASES

4.1.1 Metabolic programming of insulin resistance: Role of maternal and peri / postnatal chromium status in the offspring. Muscle development and function

Earlier studies demonstrated that chronic maternal micronutrient restriction altered the body composition [body fat %, lean body mass [LBM] and fat free mass [FFM]] in rat offspring and may predispose them to adult onset diseases. Since chromium regulates glucose and insulin metabolism, the effect of maternal Cr restriction (CrR) on muscle development and function in the offspring was studied. In the offspring of WNIN female rats fed a control or CrR diet throughout their phases of growth, pregnancy and lactation or CrR mothers rehabilitated from conception, parturition or weaning indicate that maternal CrR significantly decreased LBM % and FFM % in the male and female offspring suggesting a decreased muscle and / or bone mass. Expression of the myogenic genes : Pax3, MyoD, Myf5 and MyoG, was significantly decreased in their muscle indicating that impaired muscle development could be a contributor to the decreased LBM % and FFM %. Although basal glucose uptake by muscle was higher in CrR than CrC offspring, the fold increase with insulin was comparable suggesting no change in its insulin sensitivity. Interestingly, body composition changes were seen in male offspring only at 18 months of age, whereas, in females they were observed from 12 months onwards.

Rehabilitation from conception but not parturition or weaning partly corrected the changes in expression of myogenic genes but not those in LBM % or FFM % or glucose uptake by the muscle *in vitro*. Thus, maternal Cr restriction in WNIN rats appears to irreversibly impair muscle development and function in the offspring.

4.1.2 Hypoglycemic / insulin like activity in camel milk: Quantification of the effect in animal models of diabetes / insulin resistance

Both the camel milk as well as cow milk (raw, pasteurized or boiled) has no hypoglycemic effect in the streptozotocin induced model of hyperglycemia / type 1 diabetes in WNIN rats at the dosage level tested. However, they have a comparable hypoglycemic effect in high sucrose diet induced model of hyperglycemia / type 2 diabetes in WNIN rats and the effect is heat stable in general in both the milks. Further, the hypoglycemic effect of both these milk samples, appears not to be due to their ability to modulate basal levels of plasma insulin or the secretion to a challenge of oral glucose load. From the results it appears that at the dosage level employed in these studies, the hypoglycemic effect of camel milk does not seem to be greatly different from that of cow milk in both the models of hyperglycemia (i.e., drug or diet induced).

4.1.3 Generation of database on health beneficial effects of plant foods commonly consumed in India: Roots, Tubers and other vegetables

As a part of efforts to generate the database on the phenolic content and antioxidant activity (AOA) of plant foods commonly consumed in India, data has been generated this year on roots, tubers and vegetables. In general there was a wide variation in the phenolic content and AOA of the foods analysed. Among the roots and tubers, beetroot and carrot had the highest and the lowest phenolic content and AOA as determined by the DPPH scavenging and FRAP methods. Among the vegetables, red cabbage and ridge gourd had the highest and the least phenolic content. DPPH scavenging activity was the highest in beet root followed by red cabbage in a close second place while ridge gourd had the least activity. On the other hand, FRAP was highest in red cabbage followed by a distant second in raw mangoes while pumpkin had the lowest activity. In general, there was a significant correlation between the phenolic content and AOA (both methods) in the foods studied.

4.2 RESEARCH ON CATARACT AND RETINAL DEGENERATION

4.2.1 Erythrocyte aldose reductase activity and sorbitol and diabetic retinopathy

Activation of polyol pathway due to increased aldose reductase (ALR2) activity has been implicated in the development of diabetic complications including diabetic retinopathy (DR), a leading cause of adult blindness which is also the most common complication of diabetes. However, the relationship between hyperglycemia-induced activation of polyol pathway in retina and DR is still uncertain. The relationship between ALR2 levels and human DR in a case-control study was investigated. Type-2 diabetes (T2D) patients with DR showed significantly higher specific activity of ALR2 as compared to T2D patients without DR. Elevated levels of sorbitol in T2D patients with DR substantiated the increased ALR2 activity in erythrocytes of DR patients. Thus, levels of ALR2 activity and/or sorbitol in erythrocytes may have value as a quantitative trait to be included among other markers to establish a risk profile for development of DR.

4.2.2 Effect of curcumin on hyperglycemia-induced vascular endothelial growth factor expression in streptozotocin-induced diabetic rat retina

Diabetic retinopathy is one of the most devastating microvascular complications of diabetes. Neovascularization stimulated by hyperglycemia mediated induction of vascular endothelial growth factor (VEGF) has been implicated in the pathogenesis. Various small molecules have been investigated for their ability to inhibit angiogenesis. In this study, it was demonstrated that feeding of curcumin and turmeric to diabetic rats inhibited expression of VEGF. This study highlighted the importance of biologically active compounds derived from dietary agents that could be explored further for the prevention and/or treatment of diabetic retinopathy.

4.2.3 Effect of turmeric and curcumin on oxidative stress and antioxidant enzymes in streptozotocin-induced diabetic rat tissues

There is increasing evidence that complications related to diabetes are associated with increased oxidative stress. Curcumin, an active principle of turmeric, has several biological properties including antioxidant activity. The protective effect of curcumin and turmeric on streptozotocin (STZ)-induced oxidative stress in various tissues of rats was studied. Results of this study indicate that curcumin and turmeric controlled diabetes-mediated oxidative stress by inhibiting the lipid and protein oxidation and reversing altered antioxidant enzyme activities without altering hyperglycemic state in most of the tissues. Hence, turmeric and curcumin might be beneficial in preventing the diabetes-induced oxidative stress.

4.2.4 Anticataractogenic effect of ginger against streptozotocin - induced diabetic cataract in rats.

Formation of advance glycation end products (AGE) through non-enzymatic glycation of proteins is one of the mechanisms that is implicated in the development of diabetic complications. Therefore, inhibition of AGE formation is of considerable value in ameliorating the complications of diabetes like cataract. A number of dietary sources for their antiglycating activity was evaluated and ginger, one of the agents, has shown antiglycating activity in the *in vitro* studies. In this study, it was demonstrated that ginger is effective in delaying the onset and progression of diabetic cataract in rats. These results, thus, provide a basis for the antiglycating effect of ginger that may have pharmacological implications in the treatment of diabetic complications.

4.2.5 Inhibition of aldose reductase by rutin

Inhibition of ALR2 represents one of the means for the treatment of diabetic complications. In the course of investigations on the evaluation of aldose reductase inhibitory activity from natural sources, a significant inhibition with some dietary sources was found. Based on the data base search it was found that rutin is a common flavonoid in these sources. Further, rutin is one of the flavonols, which is abundantly present in many fruits and vegetables. Studies conducted at NIN indicate that rutin inhibited ALR2 with an IC_{50} value 16 µM. It is specific towards ALR2 (over ALR1) and prevented the accumulation of sorbitol in RBC. These results suggest the significance of rutin as a specific and potent ALR2 inhibitor, which could be explored further for preventing or delaying of diabetic complications.

4.3 MOLECULAR BIOLOGY

4.3.1 Polymorphisms in adiponectin and TNF alpha and its association with insulin resistance, obesity and hypertension

The etiology of T2DM has a strong genetic component and variations in several candidate genes have been widely implicated. Earlier, in spite of a very high incidence of type 2 diabetes, efforts to detect any association of a common variant Pro12Ala (rs1801282) of PPAR with type 2 diabetes failed, indicating that the Indian population is unique in its genomic architecture and possibly some yet uncharacterized genetic variants may be responsible for predisposing them to type 2 diabetes. Therefore, whether other polymorphisms in candidate genes such as TNF , adiponectin and resistin are associated with type 2 diabetes in the Indian population was investigated.

Hence, four polymorphisms in the adiponectin gene and one in TNF. in a cohort (n=699) from Hyderabad were screened. It was found that G308A variant in TNF was significantly associated with waist circumference (p=0.0125) but not with T2DM, hypertension and BMI. On the other hand, the T+45G variant of the adiponectin gene was not found to be associated with any of the mentioned disease conditions. Interestingly, in contrast to other populations, any of the G+276T, G-11377A and C-11391G variants of adiponectin gene in this cohort could not be detected. These data suggest a certain level of genetic uniqueness in Indian population and varied effects of different factors on different populations. It will be interesting to screen the Indian population for novel variants, which might be responsible for making it vulnerable to metabolic disorders as a result of changing environmental factors including demographic and life style changes.

5. FOOD COMPOSITION AND NUTRIENT AVAILABILITY

5.1 Evaluation of the nutritional potential of Eri Silkworm Pupae

Studies on the nutritional potential of eri silk worm pupae, a byproduct of the silk industry has shown that it offers tremendous scope for the utilization of eri protein and fat as food source. Nutritional and toxicological evaluation of eri silk worm oil has shown that it is safe for consumption and the high α-LNA in its oil could be used to nutritional and commercial advantage.

6. PATHOLOGY

6.1 Role of type of dietary fat in the etiopathogenesis of carcinogen – induced breast neoplasm in Fischer female rats

The study was undertaken to investigate the possible etiological roles played by different types of dietary fats in female Fisher 344 rats using a carcinogen and also to study the role of insulin resistance and hyperinsulinemia in mammary carcinogenesis in these animals.

A total of 80 animals were divided into 5 groups and given either Transfats / saturated / n3 rich / n6 rich and n6 + n3 fats in their diets at 10% level for 4 months followed by administration of carcinogen DiMethylBenzAntherecene (DMBA) in 50% of animals in each group. DMBA was given (40mg/kg body weight per dose) at weekly intervals for 4 weeks by oral route and all animals were continued on their respective diets for a further period of 8 months (32 weeks).

Based on the results obtained, it appears that the **body weights** of animals not given carcinogen (C-) were, as expected, significantly higher than in carcinogen administered (C+) animals. When both the above groups were analysed separately, it was seen that n3 + n6 diet fed animals showed better body weights than others and that n3+n6 diet was much better acceptable than other diets. As regards **tumor load** and **tumor typing**, adenocarcinomas, squamous cell carcinomas and benign tumors were more in n6 fed groups as compared to others. Both the above parameters were found to be lower in n3 group as compared to n3+n6 group, suggesting that n3 may probably have a protective effect. **Immunohistochemistry** study of adenocarcinomas, showed that n3+n6 diet followed by n3 diet alone had better results than other groups studied.

Biochemical estimates indicated that carcinogen administration did not have much effect on glucose and lipid metabolism. **Haematological parameters** were unremarkable in all groups studied.

Fatty acid analysis revealed that TFA which is known to induce/promote carcinogenesis did not show increased tumor incidence, thereby suggesting that at the level of consumption (10%) it may not be promoting tumor formation. As anticipated, SFA levels were more in PO fed animals as compared to others. With respect to n3 fatty acid levels, the lower tumor incidence may be because of its anti-inflammatory role which was also evident in n3+n6 fed animals as compared to n6 only fed animals. This could also be because of lower n6 : n3 ratio in SFO + FO group. N6 rich diet consumption showed highest tumor incidence and density which could be attributed to increased inflammation and this in turn could be due to increased oxidative stress/arachidonic acid. Mammary adipose tissue in C+ animals was surprisingly very minimal / not present and hence no analysis could be undertaken which could have otherwise added valuable information to data generated from this study.

Finally, it appears that n6 rich diet has deleterious effects in relation to tumorogenesis while n3 alone diet has a better outlook with respect to the same. Apart from n3 diet, n3 + n6 diet was also observed to be beneficial. SFA and TFA diets that are associated with increased incidence of CVD and other chronic diseases do not seem to show a similar trend with respect to carcinogen induced mammary neoplasms.

7. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

7.1 Genotoxicological effects of pesticides in agricultural farmers in Guntur district of Andhra Pradesh

The widespread use of pesticides and exposure is a health hazard. Although a million

cases of pesticide toxicity are documented every year around the World, there is only limited data available on its cytogentetic effects. In addition, acute exposure to pesticides leads to generation of free radicals which include oxidative stress, lipid peroxidation and alterations in antioxidant status in animals and humans. Hence, a study was conducted in cotton growing farmers in Guntur district of A.P. These cotton growing farmers use more complex mixture of pesticides when compared to the farmers of other districts of the State. Therefore, the study was taken up to assess the extent of toxicity by analyzing the different test parameters which are the best toxicity indicators of exposure assessment.

Aims & Objectives of this study were to assess the toxicity of the commonly used pesticides viz., organochlorines, organophosphates etc in the agricultural farmers of Guntur district by AchE inhibition and to assess the cytogenetic changes and also the DNA damage in the blood of agricultural farmers by chromosomal aberrations (CAs), lymophocyte micronucleus test, and sister chromatid exchange.

The results of the cytogenetic analysis indicated that out of the 4,547 metaphase plates scored in the exposed subjects (312), 213 (4.7%) found to have CAs. Similarly, in the un-exposed group (312) out of the 3,267 metaphase plates analysed 55 (1.7%) were found to be positive for CA. A significant increase in CAs in the agricultural farmers exposed to pesticides indicates that chronic/sub chronic occupational exposure to complex mixture of pesticides is genotoxic. In the present investigation, 102 of the subjects from exposed group were found to be positive for micronuclei (0.15%) whereas in the un exposed group 89 (0.13%) subjects were found to be positive for micronuclei.

The results of the AchE activity in RBC indicated that there was a significant ($p<0.0.5$) decrease in the RBC AchE activity of the experimental subjects when compared to controls. Lipid peroxidation in terms of thiobarbituric acid reactive substance (2.68 ± 0.056) was significantly increased when compared to control subjects ($p<0.01$), while the antioxidants such as reduced glutathione (40.52 ± 1.50), and -tocopherol in experimental group (7.58 ±0.22) were significantly reduced ($p<0.01$) when compared to control subjects. There was a significant reduction in the level of GSH in the experimental subjects, while the activity of catalase in the experimental subjects increased when compared to control subjects. The liver and kidney function tests such as SGPT and urea were normal in both groups.

The studies suggest that there is a need to curtail the indiscriminate use of pesticides and that farmers should be enlightened to follow good agricultural practices.

8. EXTENSION AND TRAINING

8.1 Content analysis of nutrition component in School Science textbooks

Development of innovative nutrition education curricula is a continuous and demanding process. Before developing the nutrition content that can be effectively blended into the school science curricula, the first step would be to evaluate the nutrition component in the existing school science textbooks. A study was carried out with an aim to assess the over all nutrition component in the school science curricula. NCERT and AP State Education Board text books were considered for the study. The study which systematically applied quantitative and qualitative methods of Content Analysis concluded that the space allocated for biology in relation to physical sciences was lesser in higher classes (VI and above). Nutrition component is systematically organized at primary school level (till V Class), but not so much at high school level. Nutrition, in whichever class it is covered after class III in both NCERT and AP syllabi, deals only with – food groups or nutrient deficiency disorders. The study found that many important topics such as nutrition and growth, link between childhood malnutrition and non-communicable diseases in adulthood, adolescent nutrition, nutrition for girl child, hidden hunger, lifestyle factors and obesity, nutrition during pregnancy and lactation, importance of breast feeding, unhealthy foods, fortification etc. are not covered in the curricula. Considering that many earlier studies indicated that school based nutrition education is preferred mode of learning and

effective way of education, the results of this study will be useful during future revisions of the textbooks for strengthening the nutrition component.

9. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

9.1 Effect of long-term exercise in WNIN obese rats

From the original WNIN parental stock, two obese mutant strains were developed and designated as WNIN/Ob and WNIN/GR-Ob. These strains showed hyperphagia, higher body fat, hypertriglyceridemia, hyperinsulinemia, hypercholesterolemia and hyperleptinemia. Additionally, impaired glucose tolerance was observed in WNIN/GR-Ob rats. The present study is aimed to check whether long-term exercise will have any marked effect on the body composition, insulin resistance and lipid profile of these mutant rats.

So, a total of 72 animals from these two mutants along with parental strain WNIN were taken at 35 days of age and divided into 3 groups. Two groups of animals were taken for exercise on Rota-rod treadmill and the remaining served as controls. Exercise was carried out at two speeds for two different durations. Daily food intake and weekly body weights were measured and body compositions of these animals were analyzed by using total body electrical conductivity (TOBEC). Insulin resistance, lipid profile and serum lactate levels were measured by standard procedures. The study showed that exercise improves glucose tolerance and reduced insulin resistance in all three strains of rats tested.

There were only marginal changes by doubling the intensity of exercise. Simultaneous decrease in LBM, total body sodium and water suggests that there is extra cellular fluid loss in exercised animals when compared to controls. This is also reflected in terms of increased total body potassium. Exercise also had positive effect on reducing plasma triglyceride levels. The alterations in plasma lactate levels suggest that the glucose utilization is higher with low intensity exercise when compared to high intensity exercise.

10. PRE-CLINICAL TOXICOLOGY

10.1 Pre-clinical toxicity evaluation of Tetravalant Vaccine (DPT+Hep B)

Tetravalent Vaccine (Diphtheria, Tetanus, Pertussis + Recombinant Hepatitis B antigen) is a prophylactic agent against Diphtheria, Tetanus, Pertussis and Hepatitis B by eliciting immunity in sufficient doses. Tetravalent vaccines are very important for preventing diseases like Diphtheria, Tetanus, Pertussis and Hepatitis B. Indian Immunologicals has prepared this Tetravalent vaccine as per DCGI guidelines, Schedule Y, Drugs and Cosmetic (Second Amendment) Rules, 2005, Government of India following GMP. The objectives of the study were to assess the safety profile of Tetravalent vaccine which elicits immunity against the four diseases (Diphtheria, Pertussis, Tetanus, Hepatitis B) and to Test the allergenic potential of tetravalent vaccine.

The test material tetravalent vaccine (TV), DPT and Hep-B was tested for acute toxicity test (14 days) in swiss albino mice & Sprauge Dawley rats. Sub chronic toxicity test was carried out in Swiss albino mice and Guinea pigs. In acute toxicity test, mice and rats were exposed once to highest dose (10 times of intended therapeutic dose) by sub cutaneous route and observed for lethality. In sub chronic toxicity test Therapeutic Dose (TD) of DPT, TD of Hep- B, TD of TV and five times of TD of TV were tested. The results showed no abnormalities in physical, physiological, clinical chemistry, hematological, pathological, immunotoxicological and genotoxicological parameters.

10.2 Safety / toxicity studies of Ayurvedic bhasmas (Vn & Wn)

Ayurvedic formulations are classified into various groups viz., Kadla (decoction), churnas (Powder), Bhasmas (Mineral + herbal preparations) etc. Bhasmas are calcined powder of metals, minerals, gems etc. Traditional literature has provided standard guidelines to prepare such formulations in non-toxic, therapeutically potential formulation. As per the traditional system, these formulations are recommended to treat chronic neurological diseases viz., liver disorder, arthritis, diabetes, neurological disorders. These are sold as rejuvenator substances. However, in the recent

past, presence of metals in such formulations sold at grocery shops in international market are reported and so these can be potential toxicants. In view of this observation, CCRAS has proposed a multicentric pre-clinical safety evaluation of various herbomineral formulations as per inter-national guidelines. The present investigation was carried out to assess the safety of products as per the international guidelines. The Objective was to assess the Pre-clinical Toxicity of coded Vn & Wn Ayurvedic Bhasmas as per the International Guidelines.

Two test formulations coded as Vn & Wn recommended in a clinical dose of 30mg and 60mg respectively for 3-4 weeks were provided by the sponsor. The present investigation involved acute toxicity test (14 days) in swiss albino mice, sub acute toxicity test (30 days) and long-term term toxicity test (120 days) in Swiss Albino mice & WNIN Rats. In acute toxicity test, mice were exposed once to highest dose (10 times of intended therapeutic dose) by oral gavage and observed for lethality. The test compound was administered daily with 33% honey water (v/v) for 15 days and 30 days in Sub acute toxicity, long term toxicity test respectively in various dose levels viz. therapeutic dose (TD), average dose (TDx5) and high dose (TDx10). The results of acute toxicity test showed no lethality in mice and rats after a single exposure to 50 times of therapeutic dose till 14th day.

In subacute toxicity test, 10% pre-terminal deaths were recorded in mice, exposed to TD & 5 times of TD in both Vn and Wn Bhasmas. No pre-terminal deaths were recorded in rats. No significant treatment related effect were seen on food intake, body weight gain, clinical signs, behavioral activity etc in the survived animals. No significant changes in hematological parameters and clinical chemistry parameters were observed. In long term toxicity test, pre-terminal deaths were recorded in VC (10%), TD (15%), AD (10%), HD (10%) in mice which received Vn and Wn Bhasmas for 90 days. Mortality was 5% in rats exposed to Wn. No significant treatment related effects were seen on food intake, body weight gain, clinical signs, behavioral activity etc. No significant changes in hematological and clinical chemistry parameters were found. Genotoxicity effect was observed in those which were given ten times of recorded therapeutic dose.

10.3 Pre-clinical toxicity evaluation of Skimmed Milk Fermentate (SMF)

The Skimmed Milk Fermentate (SMF) having bacteriocin type activity has been developed by indigenous technology, with an intention to promote it as a bio-preservative for Indian dairy products. The SMF has been produced by fermenting skim milk with a bacteriocinogenic (bacteriocin producing) strain of food grade lactic acid bacterium, *Pediococcus pentosaceus*, isolated from Cheddar cheese. National Dairy Development Board is keen to exploit this product for its use as a preservative in commonly consumed dairy products in India.

The Objective was to carry out preclinical toxicology of SMF to ensure its safety. The intended daily dietary intake (DDI) of SMF was calculated (1.2gm/day i.e.0.001%w/w). The investigation involved acute toxicity test (14 days) in swiss albino mice and WNIN rats and Sub chronic toxicity test in WNIN Rats. In acute toxicity test, mice and rats were exposed once to highest dose of test material (10 times of intended therapeutic dose) by oral gavage and observed for lethality. Sub chronic test has been conducted in Rats (Wistar NIN) receiving the diet containing 0.2%, 1%, 2% SMF. In addition, a group of animals received the diet with low protein (30%), and less fat (15%) which is considered equivalent to poor man's diet.

The results of this study showed no lethality in mice/rats after a single exposure to maximum quantity of SMF in acute toxicity test. In sub-chronic toxicity test, there were no pre-terminal deaths except one animal which died on 62nd day of drug exposure receiving 2% SMF and no significant treatment related effect on food intake, body weight gain, clinical signs, behavioral activity etc. was noticed. There were no abnormalities in hematological, clinical chemistry, histopathological parameters. At higher dose, some genotoxic effect was observed which was not significant.