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Safety Assessment of Siddha drug Ratha Azhutha Nivarani Chooranam by Short term and Long term toxicity studies in Accordance with Regulatory Guidelines

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Abstract

Traditional siddha formulation has a wide range of diversity of multi-dimensional chemical structures; in the meantime, the utility of natural products as biological function modifiers has also won considerable attention. Subsequently, they have been successfully employed in the treating several dreadful disorders were modern medicine fails to achieve the substantial cure. Ratha azhutha nivarani chooranam (RANC) indicated in the siddha literature is one such herbal preparation which has multiple phytotherapeutics that can act synergistically in treating specific disorders. In the acute study, a single dose of 5000 mg/kg was orally administered and experimental animals were monitored for 14 days. In the sub-acute study, repeated doses (250 and 500 mg/kg/day) of the test drug RANC were administered for 28 days and biochemical, hematological and histopathological parameters were evaluated. It was observed from the results of the present study that there were no significant changes was observed in acute and in subacute toxicity study rats with respect to behavior, gross pathology, body weight, and hematological and biochemical parameters. Further there were no significant differences in the gross and histopathology of the vital organs like brain, heart, lung, stomach, liver, kidneys, spleen and other reproductive organs of experimental animals in test drug RANC exposed groups and their counterpart control. It was concluded from the data of the present study that the LD50 value of RANC may be higher than 5000mg/kg and the drug was considered to be absolutely safe and has no hindrance with CNS, CVS and ANS of the experimental rats when treated at the dose of 250 and 500 mg/kg.

Keywords: Siddha formulation, Ratha Azhutha Nivarani Chooranam, Gross pathology, LD50, Histopathology, Hematological, Biochemical parameters

1. Introduction

Since prehistoric times, humans have used natural products, such as plants, animals, microorganisms, and marine organisms, in medicines to alleviate and treat diseases. According to fossil records, the human use of plants as medicines may be traced back at least 60,000 years [1-3]. Traditional medicines make use of natural and herbal products and are of great importance. Such forms of medicine as traditional value all over the world for hundreds or even thousands of years, and they have blossomed into orderly-regulated systems of medicine. In their various forms, they may have certain defects, but they are still a valuable repository of human knowledge [4].Safety of most herbal products is further compromised by lack of suitable quality controls, inadequate labeling, and the absence of appropriate patient information [5]. It has become essential, therefore, to furnish the general public including healthcare professionals with adequate information to facilitate better understanding of the risks associated with the use of these products and to ensure that all medicines are safe and of suitable quality. Hence it's become mandate for the herbal formulations to prove its safety before entering in to clinical application.

The major issue with the Indian Systems of Medicine (ISM) is that still there is only very little scientific evidence to their safety and efficacy; which in part is aggravated by the fact, that it's difficult to evaluate poly herbal medicines using the conventional array of pharmacological and toxicological methods. And thus the proponents theorize on holistic use of plant parts or extracts. The fact to be borne in mind is that these materials consist of hundreds of active ingredients. Many ISM products in use today are based on the principle of single-chemical isolation from plants or large-scale synthesis. But in many instances, these single chemical entities elicit adverse effects when used alone. Therefore, practitioners feel that the active constituents in a plant are rightly balanced within the plant and any possible untoward or toxic effects of one component would be neutralized by the presence of complementary constituents [6]. The main aim of the present study is to establish the safety of the siddha formulation RathaAzhuthaNivaraniChooranam by acute study and sub-acute study, repeated dose study in accordance with OECD guideline followed by evaluation of biochemical, hematological and histopathological parameters in treated rodents.

2. Materials and Methods

2.1. Animal

Healthy adult Wistar albino rats were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air supported by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ± 2˚Cand relative humidity 50–65%. They were provided with standard pelleted feed and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of Science and technology, Chennai, Tamil Nadu, India with the IAEC approval number: SU/CLATR/IAEC/X/094/2018

2.2. Acute toxicity Study

The animals were fasted overnight (08- 12 hrs) with free access to water. Study was conducted with single oral administration of study drug Ratha Azhutha Nivarani Chooranam (RANC) the dose of 5000mg/kg (p.o) to experimental rats. The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.

Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S, C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention [7].Body weight was recorded periodically. At the end of the experiment all animals were subjected to gross necropsy and observed for pathological changes.

2.3. Sub-Acute Toxicity Study

Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the start of treatment. The female rats used for the study were nulliparous and non-pregnant. The animals were randomly divided into control group and drug treated groups of 18 wistar albino rats (09 males and 09 females) were selected and divided into three groups. Each group consist of 06 animals (03 Males and 03 Females). First group served as a control and other three group were treated with test drug RANC (250 and 500 mg/kg/day) for 28 days.

The rats were weighed periodically and observed for signs of toxicity pertains to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28^{th} day, the animals were fasted for overnight with free access to water. On 29^{th} day the animals were sacrificed with excess dose of anesthesia as listed in the CPCSEA annexure. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra acetate) for Hematological analysis and for serum generation for biochemical analysis. The vital organs were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation [8].

2.4. Hematological analysis

Blood samples were analyzed using established procedures using automated mindray hematology analyzer 2800. Parameters evaluated includes Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

2.5. Biochemical analysis [9]

Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL), Very low density Lipoprotein (VLDL), Triglycerides (TGL), Total Cholestero , Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP) using Mind ray auto analyzer model BS 120.

2.6. Histopathological evaluation [10]

Vital organs were harvested and the histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic analysis. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

2.7. Statistical analysis[11]

The statistical analysis will be carried by one-way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error. A statistical comparison was carried out using the Dunnet's test for the control and treatment group. P-values less than 0.05 were set as the level of significance.

3. Results

3.1. Assessment of clinical signs in rats treated with RANC on Acute toxicity study

The dose of RANC used for acute toxicity study is 5000mg/kg is higher than the normal therapeutic dose. No mortality observed at this dose level, further no significant change with respect to clinical signs on acute toxicity observed for (24-48 h) and a long period (14 days). The results were tabulated in Table 1.

Table 1: Clinical signs in rats on Acute toxicity study

Clinical Signs Parameters for the duration of 14 days	Test Drug 5000mg/ Kg
Lacrimation	Absence
Salivation	Absence
Animal appearance	Normal
Tonic Movement	Absence
Clonic Movement	Absence
Laxative action	Absence
Touch Response	Normal
Response to Sound	Normal Response
Response to Light	Normal Response
Mobility	Normal Response
Respiratory Distress	Nil
Skin Color	Normal
Stereotype behavior	Absence
Piloerection	Absence
Limb Paralysis	Absence
Posture	Normal
Open field behavior	Normal
Giat Balancing	Normal
Freezing Behaviour	Absent
Sings of Stress and Anxiety	None Observed
Muscular coordination	Normal
Muscle grip	Normal
Sedation	Absence
Social Behavior	Normal
Urine Analysis	No Abnormality
Urine Colour	Yellowish
Urine pH	6
Urine -Glucose	Absence
Urine -Ketones	Absence
Urine- Bilirubin	Absence
Urine-Blood Cells	Negative
Urine - Pus cells	Negative
Mortality	Nil

3.2. Quantitative data on the body weight of rats treated with RANC in Acute toxicity study

No significant change was observed in body weight of female rats treated with RANC at the dose of 5000mg/ kg. The results were tabulated in Table 2.

Table 2: Body weight of rats in Acute toxicity study

Dose	Body weight in gms	
	Initial Body Weight (Before Treatment)	**Final Body Weight (After Treatment)**
RANC 5000 mg/kg	181.5 ± 1.643	184.2 ± 3.189

Values are mean ± S.D (n = 6 per group).

3.3. Fecal Pellet consistency analysis of rats treated with RANC in acute and sub-Acute toxicity study

Rats of control and treatment group were allowed to explore to open field on clean and sterile Stainless steel tray. The collected pellets were analyzed for consistency, color, Shape, Presence of blood cells etc. The results were tabulated in Table 3.

Table 3: Fecal Pellet consistency analysis of rats in acute and sub-Acute toxicity study

Acute Toxicity Study	
Analysis	**RANC**
Consistency	Soft
Shape	Point ended
Colour	Greenish Brown
Mucous Shedding	Absent
Blood Cells	Absent
Signs of Infection	None Observed

Sub-Acute Toxicity Study			
Analysis	**Control**	**Low Dose**	**High Dose**
Consistency	Rigid	Soft	Soft
Shape	Oblong	Point ended	Point ended
Colour	Greenish	Greenish Brown	Greenish Brown
Mucous Shedding	Absence	Absence	Absence
Blood Cells	Absent	Absent	Absent
Signs of Infection	None Observed	None Observed	None Observed

3.4. Assessment of clinical signs in rats treated with RANC on Sub-Acute toxicity study

The dose of RANC used for sub-acute toxicity study is 250 and 500 mg/kg. No mortality observed at this dose level, further no significant change with respect to clinical signs on sub-acute toxicity observed for the period of 28 days. The results were tabulated in Table 4.

Table 4: Clinical signs of rats in Sub-Acute toxicity study

Clinical Signs Parameters for the duration of 28 days	Control	RANC 250 mg/kg	RANC 500 mg/kg
Lacrimation	Absence	Absence	Absence
Salivation	Absence	Normal	Normal
Animal appearance	Normal	Absence	Absence
Tonic Movement	Absence	Absence	Absence
Clonic Movement	Absence	Absence	Absence
Laxative action	Absence	Normal	Normal
Touch Response	Normal	Normal Response	Normal Response
Response to Sound	Normal Response	Normal Response	Normal Response
Response to Light	Normal Response	Normal Response	Normal Response
Mobility	Normal Response	Normal Response	Normal Response
Respiratory Distress	Nil	Nil	Nil
Skin Color	Normal	Normal	Normal
Stereotype behavior	Absence	Absence	Absence
Piloerection	Absence	Absence	Absence
Limb Paralysis	Absence	Absence	Absence

Posture	Normal	Normal	Normal
Open field behavior	Normal	Normal	Normal
Giat Balancing	Normal	Normal	Normal
Freezing Behaviour	Absent	Absent	Absent
Sings of Stress and Anxiety	None Observed	None Observed	None Observed
Muscular coordination	Normal	Normal	Normal
Muscle grip	Normal	Normal	Normal
Sedation	Absence	Absence	Absence
Social Behavior	Normal	Normal	Normal
Urine Analysis	No Abnormality	No Abnormality	No Abnormality
Urine Colour	Yellowish	Yellowish	Yellowish
Urine pH	7	6	6
Urine -Glucose	Absence	Absence	Absence
Urine -Ketones	Absence	Absence	Absence
Urine- Bilirubin	Absence	Absence	Absence
Urine-Blood Cells	Negative	Negative	Negative
Urine - Pus cells	Negative	Negative	Negative
Mortality	Nil	Nil	Nil

3.5. Effect of RANC on Body weight of Rats in Sub-acute toxicity study

No significant change was observed in body weight of both male and female rats treated with RANC at low and high dose of 250 and 500 mg/ kg b.w.The results were tabulated in Table 5.

Table 5: Body weight of rats in Sub-Acute toxicity study

Dose	Body weight in gms	
	Initial Body Weight (Before Treatment)	Final Body Weight (After Treatment)
Control	188 ± 3.033	197 ± 3.847
RANC 250 mg/kg	188.3 ± 6.088	194.2 ± 6.113
RANC 500 mg/kg	186 ± 5.06	192.2 ± 4.792

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.6. Quantitative data on the food and water intake of rats treated with RANC for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with RANC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 6.

Table 6: Food and water intake of rats in Sub-acute toxicity study

Dose	Average Food and Water Intake	
	Food Intake in gms	Water intake in ml
Control	15.17 ± 2.137	25.33 ± 1.211
RANC 250 mg/kg	13.33 ± 1.862	22.5 ± 2.51
RANC 500 mg/kg	16.83 ± 3.312	22.83 ± 4.215

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.7.Effect of RANC on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with RANC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 7.

Table 7: Hematological parameters of rats in Sub-acute oral toxicity study

Group	RBC (×10 ^{6} µl)	WBC (×10 ^{3} µl)	PLT (×10 ^{3} µl)	HGB (g/dl)	MCH (pg)	MCV (fl)
Control	6.717 ± 1.03	7.2 ± 1.903	755.2 ± 182.9	11.52 ± 1.214	19.87 ± 2.179	60.92 ± 6.568
RANC 250 mg/kg	6.833 ± 0.689	7.117 ± 1.074	588 ± 67.63	13.08 ± 1.367	19.32 ± 2.384	58.7 ± 6.848
RANC 500 mg/kg	6.683 ± 0.2483	8.167 ± 1.384	571.3 ± 171.8	13.02 ± 2.146	17.5 ± 2.475	63.75 ± 4.333

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.8.Effect of RANC on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with RANC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 8.

Table 8: Hematological parameters of rats in Sub-acute oral toxicity study

Group	Neutrophils 10^{3} /mm ^{3}	Eosinophils (%)	Basophils (%)	Lymph (%)	Mon (%)
Control	2.467 ± 0.9352	1.4 ± 0.3225	0.1667 ± 0.4082	82.77 ± 6.514	5.083 ± 1.324
RANC 250 mg/kg	1.917 ± 0.475	1.383 ± 0.2714	0.3333 ± 0.5164	73.4 ± 6.169	2.967 ± 1.097
RANC 500 mg/kg	1.917 ± 0.7859	1.333 ± 0.3445	0.3333 ± 0.5164	75.55 ± 13.53	2.55 ± 0.8961

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.9.Effect of RANC on Serum Bio-chemistry profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of rats treated with RANC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 9.

Table 9:Serum Bio-chemistry profile of rats in Sub-acute oral toxicity study

Group	BUN (mg/dl)	Serum Creatinine (mg/dl)	Total Bilirubin (mg/dl)	SGOT (IU/L)	SGPT (IU/L)
Control	13.67 ± 3.141	0.6833 ± 0.2639	0.4167 ± 0.07528	123.3 ± 16.67	18.83 ± 1.169
RANC 250 mg/kg	13.5 ± 1.871	0.6667 ± 0.216	0.3333 ± 0.1366	100.7 ± 21.26	38.5 ± 5.01
RANC 500 mg/kg	16 ± 3.406	0.6167 ± 0.3061	0.3333 ± 0.1211	94.17 ± 18.47	26.83 ± 10.94

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.10.Effect of RANC on Serum Bio-chemistry profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of rats treated with RANC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 10.

Table 10: Serum Bio-chemistry profile of rats in Sub-acute oral toxicity study

Group	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TG (mg/dl)
Control	131.6 ± 15.34	58.67 ± 2.582	59.17 ± 12.25	13.78 ± 2.234	35 ± 21
RANC 250 mg/kg	117.6 ± 5.011	61.67 ± 6.408	40.5 ± 10.25	15.38 ± 2.818	36.5 ± 11.57
RANC 500 mg/kg	133.4 ± 18.4	63.17 ± 7.195	56.5 ± 12.21	13.68 ± 1.169	35.5 ± 5.822

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

3.11. Quantitative data on absolute Organ weight of male rats belongs to control and drug treated group in sub-acute toxicity study

No statistically significant differences were recorded in organ weight of male rats treated with RANC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 11.

Table 11: Quantitative data on absolute Organ weight of male rats in sub-acute toxicity study

Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Testes
Control - Male	1.593 ± 0.1069	0.5367 ± 0.01155	1.49 ± 0.2265	1.317 ± 0.1069	4.303 ± 0.5074	0.4933 ± 0.1976	0.99 ± 0.07	2.267 ± 1.062
RANC 250mg/kg - Male	1.583 ± 0.01528	0.47 ± 0.1114	1.357 ± 0.1762	1.167 ± 0.06028	3.96 ± 0.4058	0.4367 ± 0.02517	0.9833 ± 0.07572	1.52 ± 0.588
RANC 500mg/kg - Male	1.657 ± 0.1501	0.5567 ± 0.05132	1.357 ± 0.2053	0.89 ± 0.6065	3.73 ± 1.181	0.5767 ± 0.1266	0.97 ± 0.1808	1.983 ± 0.2065

Values are mean ± S.D (n = 3 per group). Control and treatment groups were compared statistically

3.12. Quantitative data on absolute Organ weight of female rats belongs to control and drug treated group in sub-acute toxicity study

No statistically significant differences were recorded in organ weight of female rats treated with RANC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 12.

Table 12: Quantitative data on absolute Organ weight of female rats in sub-acute toxicity study

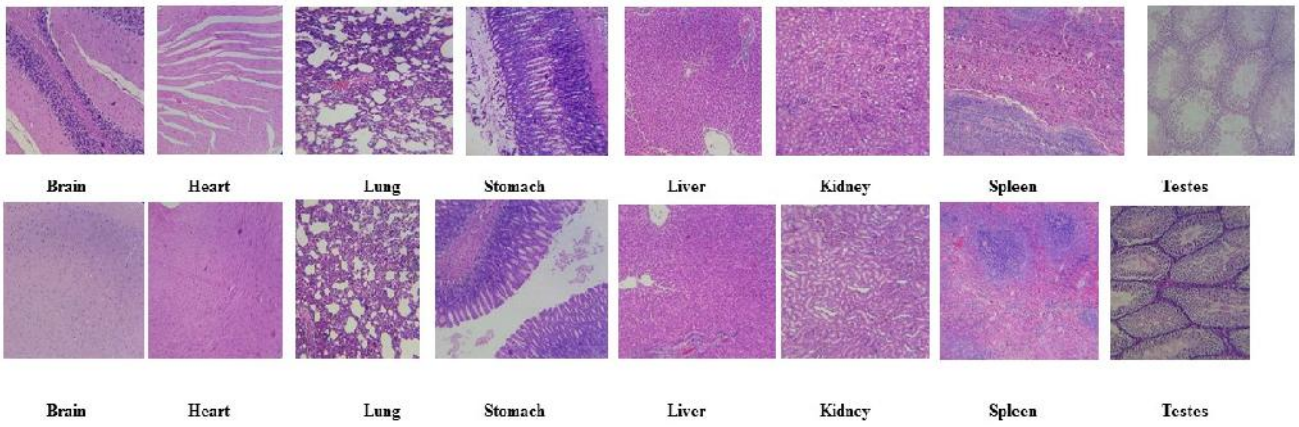
Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Uterus	Ovaries
Control -Female	1.72 ± 0.13	0.5833 ± 0.07767	1.32 ± 0.4493	1.253 ± 0.3253	4.62 ± 1.218	0.5233 ± 0.04041	1.093 ± 0.2397	0.9533 ± 0.03512	0.1633 ± 0.04041
RANC 250mg/kg - Female	1.677 ± 0.135	0.4967 ± 0.03215	1.1 ± 0.1179	1.113 ± 0.1286	3.457 ± 0.2122	0.5667 ± 0.01155	0.9033 ± 0.08622	1.157 ± 0.09452	0.1967 ± 0.07234
RANC 500mg/kg - Female	1.727 ± 0.1102	0.8167 ± 0.2285	1.243 ± 0.1955	1.403 ± 0.2434	3.64 ± 0.4838	0.66 ± 0.1552	1.093 ± 0.1343	0.9667 ± 0.05508	0.2267 ± 0.05508

Values are mean ± S.D (n = 3 per group). Control and treatment groups were compared statistically

3.13. Effect of RANC on Histopathological changes of Male rat in Sub-acute oral toxicity study

Microscopic observation of vital organs belongs to male rats presenting the following architecture as shown in figure 1.

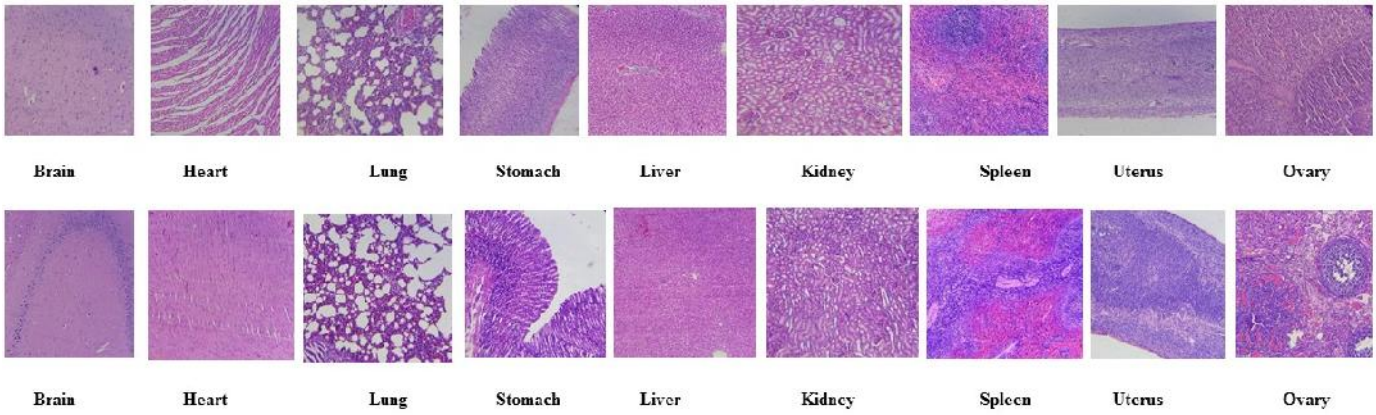
Figure 1: Histopathology of Male belongs to control and high dose treated group



3.14. Effect of RANC on Histopathological changes of Female rat in Sub-acute oral toxicity study

Microscopic observation of vital organs belongs to female rats presenting the following architecture as shown in figure 2.

Figure 2: Histopathology of Female belongs to control and high dose treated group



4. Discussion

An important objective of toxicity testing is the detection of toxic compounds derived thereof in the early (pre-clinical) and late (clinical) stages of drug discovery and development from plant sources. This will facilitate the identification of toxicants which can be discarded or modified during the process and create an opportunity for extensive evaluation of safer, promising alternatives [12]. For certain compounds, modifications such as dosage reduction, chemical group or structural adjustments may improve their tolerability.

In acute toxicity study, there was no mortality up to a maximum dose of 5000 mg/kg body weight of RANC after per oral administration. The changes in bodyweight and other Clinical signs like skin color change, fecal consistency, gait analysis, urine analysis, sensory responses, animal behavior abnormalities, neuro muscular coordination have been used as an indicator of adverse effect. Since no remarkable changes were observed in animal behavior, body weight and organ weight at dose in treated rats as compared to control group, it can be inferred that siddha formulation RANC is nontoxic at the administered dose of 5000mg/kg.

In sub-acute toxicity study treatment with RANC at 250 and 500 mg/kg reveals no significant change in the body weight of the treated rats. The low and high dose selected for toxicity covers the average therapeutic dosage of the test drug RANC in humans. Results of the study reveals that 28-day daily dose treatment with the RANC elicited no clinical signs of toxicity, morbidity, or mortality across all the treatment groups hence it may have concluded that the formulation RANC is safe at the tested doses over the observation period. The liver and kidneys have fundamental roles in the metabolism and excretion of drugs or plant products. Exogenous chemicals and their metabolites might result in toxicity or cell damage on these organs [14,15]. Kidney and liver function enzymes including BUN, creatinine, SGOT,SGPT, bilirubin and other lipid profile are within the limit in both low and high dose treated male and female rats in sub-acute toxicity study.

In this study, administration of RANC at both the dose level in female and male rats for a period of 28 days produced no significant change in all blood parameters such as Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

Results of the present investigation showed that there was no sign of toxicity and no mortality after repeated administration of the test drug RANC at varying doses in tested rats. There was no significant difference in mean body weight, food/water intake, behavioral, C.N.S, C.V.S, A.N.S vitals in control and test group rats. Further no changes in the gross observation of all the vital organs in both male and female rats. Single and repeated oral administration of the siddha drug RANC may be safe and considered as relatively non-toxic at both the doses levels of 250 and 500 mg/kg dose level.

Histopathological analysis of samples from control and RANC treated rats reveals the regular marginal alignment on the neurons with promising histology were observed in brain , Myocardial cells appears normal with well-defined mycoplasma and prominent nucleus and nucleolus in heart, Regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis in lung.Pathology of stomach shown normal pyloric and fundus zone, Numerous hepatocytes appears with shrunken nucleus in liver, Lumen of vessels and bowman's space appears normal with mild tubular dilatation in kidney of drug treated rat. Morphology of capsule, nodes, red and white pulp appears normal in spleen. Section of testis showing normal interstitial connective tissue. Histology of uterus projects regular uterine epithelium and endometrial glands and appearance of corpora lutea (CL), atretic follicles (AF) and interstitial tissue (IT) was normal in ovary of drug treated female rat.

5. Conclusion

By the WHO estimates, increasing population world-wide is depending on herbal medicine as a source of primary health care. Herbal medicine is used by about 60% of the world population both in the developing and in the developed countries where modern medicines are predominantly used. The results of the present study have strongly suggested that the siddha drug Ratha Azhutha Nivarani Chooranamis safe with wide safety margin established in both acute and sub-acute toxicity study further preclinical validation has to be carried out before clinical application of the drug for chronic ailment in humans.

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