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Acute and sub- acute oral toxicity evaluation of siddha herbomineral formulation Rasa Chendooram in wistar rats

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Abstract

Indian system of traditional medicines like siddha has been in practice over many centuries. Further its popularity and extensive use by a large number of people have challenged orthodox practices in several manners, although, traditional medicine still thrives to be officially to be recognized in few countries. Lack of regulation on formulation and evaluations is one of the primary concern in alternate therapies. This may be due to lack of scientific research data and adequate research methodology for evaluating herbo mineral formulation like rasa chendooram (RC), Thus, several siddha preparations have been recently tested for its safety and efficacy worldwide. The main objective of the present investigation is to evaluate the siddha formulation RC for its safety in preclinical level using rodent model.In the acute study, a single dose of 2000 mg/kg was orally administered and experimental animals were monitored for 14 days. In the sub-acute study, repeated doses (100 and 200 mg/kg/day) of the test drug RC were administered for the period of 28 days and biochemical, hematological and histopathological parameters were evaluated.Results of the acute study reflects no significant change in clinical observation after close observation and long-term 14-day observation of traded rats. Data's of urine biochemistry, food/water intake, hematology, serology, gross organ observation and histopathological analysis shown no significant difference between RC (100 and 200 mg/kg) treated and control group animal's. From the results obtained from the present preclinical investigation it was concluded the acute or sub-acute oral administration of the test drug RC is considerably very safe and doesn't induce any toxicity in the treated animals.

Keywords: Herbo mineral formulation, Siddha, Rasa chendooram, Acute, Sub-acute toxicity, Rodent model

1. Introduction

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system of resource of many developing countries. About 80% of the population in developing countries use traditional medicines because they cannot afford the high cost of western pharmaceuticals and health care, and because traditional medicines are more acceptable from a cultural and spiritual perspective [1].

Indian system of traditional medicine has the most diverse range of materia – medica. But despite this pharmacotherphy forms as an alternative therapy for chronic illnesses which often cause untoward side effects due to long time exposure of allopathic medication [2]. As interest is being renewed in these traditional systems the benefits of this is hampered by the fear of toxicity some of the categories of these products might elicit especially metal and mineral based preparations. Accumulated toxicity data on the hazardous effects of heavy metals as propounded by the modern medicine has made the world vary of heavy metals. Siddha system of medicine majorly relies on ancient traditional preparations for treating several infectious and non-communicable diseases. As per the vedic literature it has been provoked that this method of treatment has emerged from southern region of India and progressed though out the world [3].

Beneficial effects produced by heavy metal and other herbo – metallic compounds of ISM are often viewed with suspicion and rightly so. But ISM of medicine has a documented history of safe usage of these medications for the past 2500 years[4,5]. The metals that are extensively described in Indian and other ancient systems of medicine include gold, silver, arsenic, copper, iron, lead, mercury, and zinc. Knowledge regarding the therapeutic, toxicological effect of plants, minerals and other substances go back to the prehistoric times when people have migrated to into the Indian subcontinent. Several evidences indicated that in Indian subcontinent medical intervention like dentistry and trepanation were exercised as early as 7000 BCE [6].

General opinion among the public regarding usage of metal based preparations due safety issues whereas most of the metal based siddha formulations are processed and purified to nullify the level of toxicity and also administered with suitable vehicle to counteract the action. Rasa chendooram is one such potential formulation advocated for treating various disorders. The main objective of the present investigation is to evaluate the siddha formulation RC for its safety in preclinical level using rodent model.

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/091/2018

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 Rasa chendooram (RC) at the dose of 2000mg/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 two groups were treated with test drug RC (100 and 200 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 Cholesterol, 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 RC on Acute toxicity study

The dose of RC used for acute toxicity study is 2000mg/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 2000mg/ 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 RC in Acute toxicity study

No significant change was observed in body weight of female rats treated with RC at the dose of 2000mg/ 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)
RC 2000 mg/kg	183.3 ± 2.066	184.8 ± 2.858

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

3.3. Fecal Pellet consistency analysis of rats treated with RC 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	**RC**
Consistency	Soft
Shape	Oblong
Colour	Reddish green
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	Oblong	Oblong
Colour	Greenish	Reddish Green	Reddish Green
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 RC on Sub-Acute toxicity study

The dose of RC used for sub-acute toxicity study is 100 and 200 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	RC 100 mg/kg	RC 200 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	Dark Brownish	Dark Brownish
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
Negative	Negative	Negative	Negative
Mortality	Nil	Nil	Nil

3.5. Effect of RC 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 RC at low and high dose of 100 and 200 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	184.5 ± 4.37	211.5 ± 42.02
RC 100 mg/kg	192 ± 2.098	199.2 ± 2.787
RC 200 mg/kg	193 ± 4.858	201 ± 5.762

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 RC for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with RC at low and high dose of 100 and 200 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	14.83 ± 1.722	23.67 ± 2.066
RC 100 mg/kg	17.67 ± 1.033	27.17 ± 1.472
RC 200 mg/kg	16 ± 1.265	28.17 ± 2.787

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 RC on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with RC at low and high dose of 100 and 200 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 ($\times 10^6$ µl)	WBC ($\times 10^3$ µl)	PLT ($\times 10^3$ µl)	HGB (g/dl)	MCH (pg)	MCV (fl)
Control	6.517 ± 0.7468	10.37 ± 1.479	418.3 ± 84.88	10.73 ± 4.054	19.13 ± 1.655	63.63 ± 4.752
RC 100 mg/kg	7.65 ± 1.157	8.817 ± 1.484	696.3 ± 173.1	11.22 ± 1.15	19.55 ± 3.059	60.62 ± 3.997
RC 200 mg/kg	6.45 ± 1.495	7.567 ± 1.95	715 ± 208.1	12.03 ± 1.675	18.77 ± 3.083	59.38 ± 5.254

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 RC on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with RC at low and high dose of 100 and 200 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	2.15 ± 0.6979	1.55 ± 0.251	0 ± 0	80.2 ± 8.415	2.25 ± 0.8313
RC 100 mg/kg	2.467 ± 0.7033	1.417 ± 0.3312	0 ± 0	71.43 ± 2.997	3.95 ± 1.305
RC 200 mg/kg	2.667 ± 0.7941	1.45 ± 0.2881	0.1667 ± 0.4082	70.62 ± 7.796	3.85 ± 0.8167

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 RC 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 RC at low and high dose of 100 and 200 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	16.5 ± 1.871	0.75 ± 0.1761	0.2333 ± 0.08165	102 ± 26.15	29.33 ± 11.2
RC 100 mg/kg	15.5 ± 3.728	0.75 ± 0.1049	0.4667 ± 0.1033	97 ± 19.83	16.17 ± 1.472
RC 200 mg/kg	13.83 ± 2.639	0.6667 ± 0.1633	0.3167 ± 0.07528	79.67 ± 15.2	18.83 ± 1.169

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 RC 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 RC at low and high dose of 100 and 200 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	138.1 ± 7.627	59.83 ± 3.251	63.17 ± 6.242	15.08 ± 1.388	28.17 ± 4.262
RC 100 mg/kg	125.8 ± 7.39	65 ± 5.177	46 ± 12.66	14.83 ± 2.31	27.5 ± 5.718
RC 200 mg/kg	123.2 ± 19.38	54.33 ± 5.82	53.83 ± 17.67	15.07 ± 2.352	34.83 ± 7.574

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 RC at low and high dose of 100 and 200 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	1.425 ± 0.1886	0.762 ± 0.1525	1.7 ± 0.7264	1.379 ± 0.5352	6.824 ± 1.46	0.69 ± 0.4311	1.586 ± 0.2905	1.9 ± 0.5
RC 100 mg/kg - Male	1.63 ± 0.1229	0.5967 ± 0.07024	1.237 ± 0.332	1.573 ± 0.3272	4.94 ± 0.6776	0.9467 ± 0.121	1.1 ± 0.08718	1.69 ± 0.2339
RC 200 mg/kg - Male	1.503 ± 0.3439	0.5933 ± 0.3099	1.45 ± 0.2905	1.38 ± 0.2816	5.267 ± 0.4772	0.5833 ± 0.1762	1.13 ± 0.2762	1.783 ± 0.6452

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 RC at low and high dose of 100 and 200 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

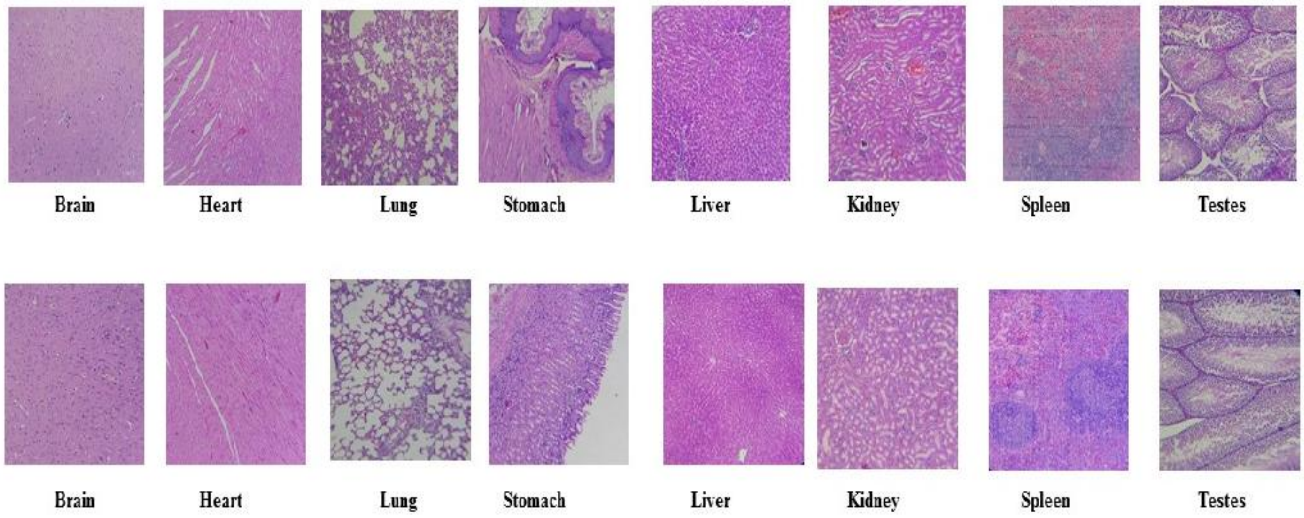
Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Uterus	Ovaries
Control - Female	1.75 ± 0.07835	0.9737 ± 0.09408	1.994 ± 0.4402	1.787 ± 0.09815	5.126 ± 0.6717	0.956 ± 0.1266	1.335 ± 0.08231	0.7957 ± 0.1555	0.4473 ± 0.04244
RC 100 mg/kg - Female	1.583 ± 0.08505	0.5833 ± 0.03055	1.2 ± 0.2066	1.393 ± 0.1102	5.13 ± 1.167	0.6 ± 0.1819	1.087 ± 0.1305	1.247 ± 0.05033	0.2567 ± 0.07506
RC 200 mg/kg - Female	1.627 ± 0.1137	0.58 ± 0.08185	1.397 ± 0.3313	1.643 ± 0.1305	5.143 ± 0.4614	0.53 ± 0.1044	1.03 ± 0.1212	0.9533 ± 0.03215	0.1333 ± 0.04933

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

3.13. Effect of RC 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.

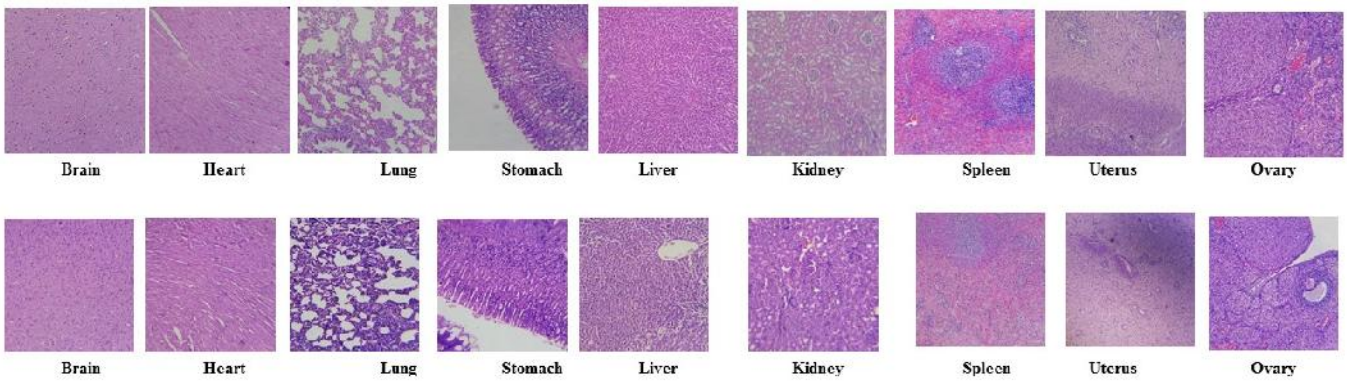
Figure 1: Histopathology of Male belongs to control and high dose treated group



3.14. Effect of RC 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

Acute toxicity study report in rodents seems highly essential in dose fixation and close monitoring of adverse event after increased single dose exposure of the study drug. Results of acute toxicity study, reveals no mortality up to a maximum dose of 2000 mg/kg body weight of RC 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 RC is nontoxic at the administered dose of 2000mg/kg.

Repeated oral toxicity study provides valued based information on long term adverse effect of the study drug with respect to the change in body weight, behavioral and other biochemical parameters including histological assessment. Investigation on the haematological parameters can be used to determine the extent of the deleterious effect of foreign compounds in herbal formulation on the blood constituents of an animal [12]. In the present study, the no significant difference in hematological parameters including RBC and HGB level following repeated daily dose treatment with siddha drug RC could be an indication that it may not be toxic to the blood. This implies that the morphology and osmotic fragility of the RBC, as well as HB incorporation into the RBC, were not affected. This may also suggest that the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following treatment with the drug intact [13]. Evaluation of indices (HCT, MCV, MCH and MCHC) relating to the status of RBC is imperative to the diagnosis of anaemia in animals. The no significant effect on these indices for the test drug RC treated animals relative to control group rats.

Liver and kidney function tests are crucial in toxicological evaluation of test drug due to the utmost involvement of these organs in xenobiotic biotransformation [14]. Significantly increased serum activities of ALP, ALT, AST are closely associated with hepatic injury [15]. The no significant differences in the serum activities of these marker enzymes in the RC treated rats relative to normal control are informative either of the fact that the drug does not impede hepatocytes function in the rats or of the fact that the integrity of the liver cells was not compromised. Additionally, concentrations of total bilirubin, BUN and creatinine levels in the serum may indicate the safety of kidney on treatment with RC.

Besides complementing biochemical investigations, histological examination of organs following exposure to pharmacological agents is an important consideration in assessing the safety of such agents on organ injury. Hence, the apparently preserved histoarchitectural features as evident from microscopic examinations of the vital organs. Granular layers of neurons in cerebrum appeared clear and distinct without any

changes in their cells in brain, appearance of fibrils and cross striations are equidistant in heart. Respiratory and terminal bronchioles appear normal in lung, normal gastric glands and gastric pits observed in stomach. Increased numbers of kupffer cells were observed in liver, Kidney basement membrane of the capillaries are thickened with narrow lumen and sme renal tubules are hypertrophic, others are dilated. Histology of spleen projects normal cytoarchitecture with no signs of immunological activities. Normal sertoli cell aligned properly on the basement membrane with oval dome shaped nucleus shows the normal morphology of the seminiferous tubule were observed in testes. Uterus exhibits normal histological aspect of endometrium and myometrium. Appearance of graafian and antral follicle was normal in ovary of female rats.

5. Conclusion

The development of regulatory toxicology during the twentieth century up through the present has continued to shadow the ability to detect both chemicals and effects. Preclinical toxicity studies occupies greater proportion of time in the entire process of new drug invention. From the results obtained from the present preclinical investigation it was concluded the acute or sub-acute oral administration of the test drug RC is considerably very safe and doesn't induce any toxicity in the treated animals.

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