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Toxicological Screening of Siddha polyherbal formulation Shanmuga chooranam by acute and sub-acute repeated oral toxicity studies in rats

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Abstract

Traditional system of medicine includes siddha, ayurveda and unani are the oldest form of health care in Asia and even worldwide used in the prevention, and treatment of physical and mental illnesses. Different societies historically developed various useful healing methods to combat a variety of health- and life-threatening diseases. Siddha system of medicine is believed as a brilliant achievement and symbol of Tamil culture which originated from southern parts of India. Siddha medicine invented from dravidian culture and is grown in the time of Indus valley civilization. Still now there are several novel preparations which are restricted for clinical application due to lack of preclinical toxicity data's. Hence the main aim of the present research work is to establish the safety of the formulation shanmuga chooranam (SC) at preclinical level through systematic regulatory toxicity studies. 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 (500 and 1000 mg/kg/day) of the test drug SC were administered for the period of 28 days and biochemical, hematological and histopathological parameters were evaluated.In the acute toxicity study, the experimental rats did not show any form of morbidity or mortality. For the sub-acute toxicity study there is no significant difference were observed in the plasma levels of SGOT, SGPT, BUN, creatinine, lipid profile parameters of rats treated with SC at the dose of 500 and 1000 mg/kg when compared with control rats. The histopathological study also advocates the normal cytoarchitecture on the tissue of all vital organs in both male and female rats subjected to the study. It was concluded that present study provided an evidence based data on the safety nature of the siddha drug SC and also justifies the safe clinical application on humans.

Keywords: Siddha formulation, Polyherbal, Shanmuga chooranam, Safety, Preclinical, drug, SGOT, SGPT, Histopathology

1. Introduction

Plant medicine is the oldest form of health care known to mankind. Herbal medicine flourishes today as the primary form of medicine for perhaps as much as 80% of the world's population [1]. Usually, a specific part of the plant (root, leaves, fruit, flowers, and seeds) is used in traditional preparations or as pure active principles formulated into a suitable preparation. Many medicines commonly used today are of herbal origin. Indeed, about 25% of prescription drugs contain at least one active ingredient derived from plant material [2,3]. Plant derived medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide. In most developing countries, the indigenous modes of herbal treatment are parts of the culture and the dominant method of healing therapy. These remedies, with a considerable extent of effectiveness, are socially accepted and economically viable and, mostly, are the only available source [4,5].

Since natural herbal remedies are being used on large scale, it is now the major focus of the researchers to conduct studies on efficacy and safety of medicinal plants [6]. 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 [7].

The plants having medicinal activity should have low toxicity because of their long-term use in humans. However, various medicinal plants used in folklore medicines have been reported to exhibit toxic effects [8,9]. Paracelsus, known as father of toxicology, has given a statement which is often quoted: “All substances are poisons; there is none which is not a poison. It is the right dose which differentiates remedy from poison” [10]. A large number of modern medicines are produced from the natural sources. Out of them many preparations rely on the use of agents in traditional medicines [11].Another implication in the toxicity of certain herbs is the presence of toxic minerals and heavy metals like mercury, arsenic, lead and cadmium [12]. Lead and mercury can cause serious neurological impaiment when an herbal medicinal product contaminated with these metals is ingested. Shanmuga chooranam is a potential siddha formulation with consist of the following biologically active therapeutics Rock salt, Piper longum, Cuminum cymium, Piper nigrum, Ferula asafetida, Murraya koenigii. The main objective of the present work is to evaluate the toxicological profiling of the siddha formulation Shanmuga chooranam in rodents by acute and sub-acute (repeated oral) toxicity study in accordance with OECD guideline.

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/095/2018

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 Shanmuga chooranam (SC) at 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 [13].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 SC (500 and 1000 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 [14].

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 [15]

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 [16]

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[17]

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 SC on Acute toxicity study

The dose of SC 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 SC in Acute toxicity study

No significant change was observed in body weight of female rats treated with SC 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)
SC 5000 mg/kg	183.5 ± 3.017	184.7 ± 2.251

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

3.3. Fecal Pellet consistency analysis of rats treated with SC 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	**SC**
Consistency	Soft
Shape	Point ended
Colour	Dark 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	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 SC on Sub-Acute toxicity study

The dose of SC used for sub-acute toxicity study is 500 and 1000 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	SC 500 mg/kg	SC 1000 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	Nil	Nil
Respiratory Distress	Nil	Normal	Normal
Skin Color	Normal	Absence	Absence
Stereotype behavior	Absence	Absence	Absence
Piloerection	Absence	Absence	Absence
Limb Paralysis	Absence	Normal	Normal
Posture	Normal	Normal	Normal
Open field behavior	Normal	Normal	Normal
Giat Balancing	Normal	Absent	Absent
Freezing Behaviour	Absent	None Observed	None Observed
Sings of Stress and Anxiety	None Observed	Normal	Normal
Muscular coordination	Normal	Normal	Normal
Muscle grip	Normal	Absence	Absence
Sedation	Absence	Normal	Normal
Social Behavior	Normal	No Abnormality	No Abnormality
Urine Analysis	No Abnormality	Yellowish	Yellowish
Urine Colour	Yellowish	Yellowish	Yellowish
Urine pH	7	6	6
Urine -Glucose	Absence	Absence	Absence
Urine -Ketones	Absence	Absence	Absence
Urine- Bilirubin	Absence	Negative	Negative
Urine-Blood Cells	Negative	Negative	Negative
Urine - Pus cells	Negative	Negative	Negative
Mortality	Nil	Nil	Nil

3.5. Effect of SC 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 SC at low and high dose of 500 and 1000 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
SC 500 mg/kg	186 ± 4.94	210.8 ± 42.47
SC 1000 mg/kg	184.2 ± 3.869	187.8 ± 3.43

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 SC for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with SC at low and high dose of 500 and 1000 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
SC 500 mg/kg	15.33 ± 1.366	25.17 ± 2.401
SC 1000 mg/kg	14.67 ± 3.011	25.17 ± 2.401

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

No statistically significant differences were recorded in hematological parameters of rats treated with SC at low and high dose of 500 and 1000 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	6.517 ± 0.7468	10.37 ± 1.479	418.3 ± 84.88	10.73 ± 4.054	19.13 ± 1.655	63.63 ± 4.752
SC 500 mg/kg	7.583 ± 0.5115	7.983 ± 1.347	699.5 ± 176.2	12.38 ± 1.863	18.8 ± 1.367	56.75 ± 5.088
SC 1000 mg/kg	7.85 ± 1.214	8.717 ± 0.902	696.3 ± 156.8	10.58 ± 1.306	19.65 ± 3.286	55.02 ± 2.977

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

No statistically significant differences were recorded in hematological parameters of rats treated with SC at low and high dose of 500 and 1000 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
SC 500 mg/kg	2.017 ± 0.7055	1.417 ± 0.2858	0.1667 ± 0.4082	73.98 ± 5.547	1.933 ± 0.7202
SC 1000 mg/kg	2.467 ± 0.9852	1.55 ± 0.3146	0.1667 ± 0.4082	67.52 ± 11.6	2.917 ± 0.9196

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 SC 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 SC at low and high dose of 500 and 1000 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
SC 500 mg/kg	16.17 ± 4.07	0.8667 ± 0.1366	0.4 ± 0.08944	114.3 ± 19.42	23.83 ± 6.178
SC 1000 mg/kg	16.17 ± 2.483	0.65 ± 0.1871	0.3333 ± 0.08165	87.83 ± 24.55	32.5 ± 8.479

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 SC 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 SC at low and high dose of 500 and 1000 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
SC 500 mg/kg	150.7 ± 15.67	67.17 ± 4.792	66.83 ± 12.97	16.72 ± 1.538	30.33 ± 6.121
SC 1000 mg/kg	137.9 ± 7.962	59.5 ± 7.314	63 ± 10.58	15.38 ± 2.496	27.83 ± 4.491

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 SC at low and high dose of 500 and 1000 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
SC 1000 mg/kg - Male	1.74 ± 0.2663	0.6633 ± 0.2656	1.543 ± 0.616	1.31 ± 0.3051	5.35 ± 0.7713	0.57 ± 0.197	1.163 ± 0.2616	1.353 ± 0.2887
SC 500mg/kg - Male	1.503 ± 0.171	0.4033 ± 0.03215	1.307 ± 0.5686	1.243 ± 0.1882	4.227 ± 0.6928	0.3167 ± 0.1137	0.8967 ± 0.1021	1.627 ± 0.8071

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 SC at low and high dose of 500 and 1000 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

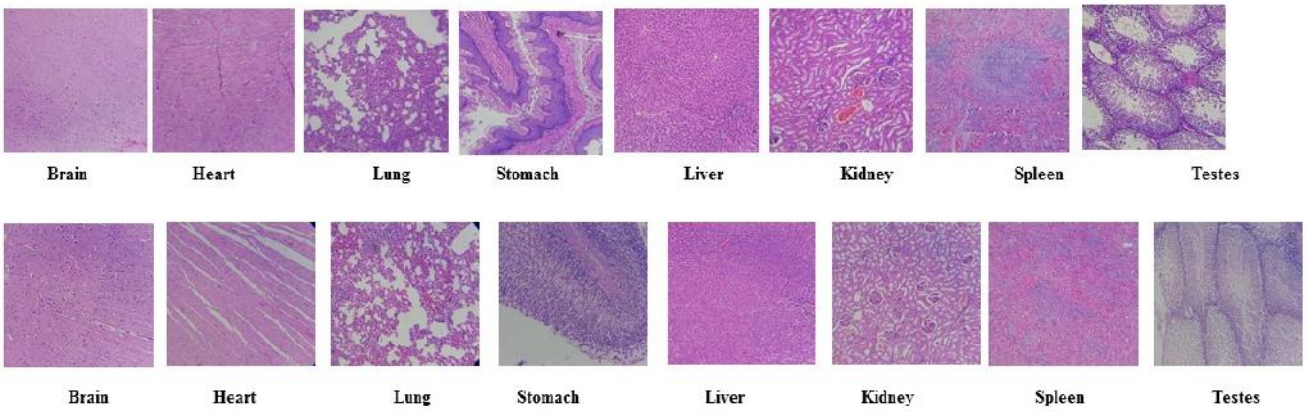
Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Uterus	Ovaries
Control - Female	1.75 ± 0.07835	0.9737 ± 0.09408	1.994 ± 0.4402	1.787 ± 0.09815	5.126 ± 0.6717	0.956 ± 0.1266	1.335 ± 0.08231	0.7957 ± 0.1555	0.4473 ± 0.04244
SC 500 mg/kg - Female	1.737 ± 0.07572	0.48 ± 0.2227	1.45 ± 0.151	1.82 ± 0.2343	4.827 ± 0.8883	0.5467 ± 0.06429	1.277 ± 0.2001	0.8667 ± 0.01528	0.26 ± 0.06083
SC 1000 mg/kg - Female	1.919 ± 0.05749	0.92 ± 0.1572	1.27 ± 0.2762	1.463 ± 0.2434	6.307 ± 2.474	0.8767 ± 0.1002	1.477 ± 0.2237	0.93 ± 0.02646	0.2833 ± 0.1756

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

3.13. Effect of SC 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.

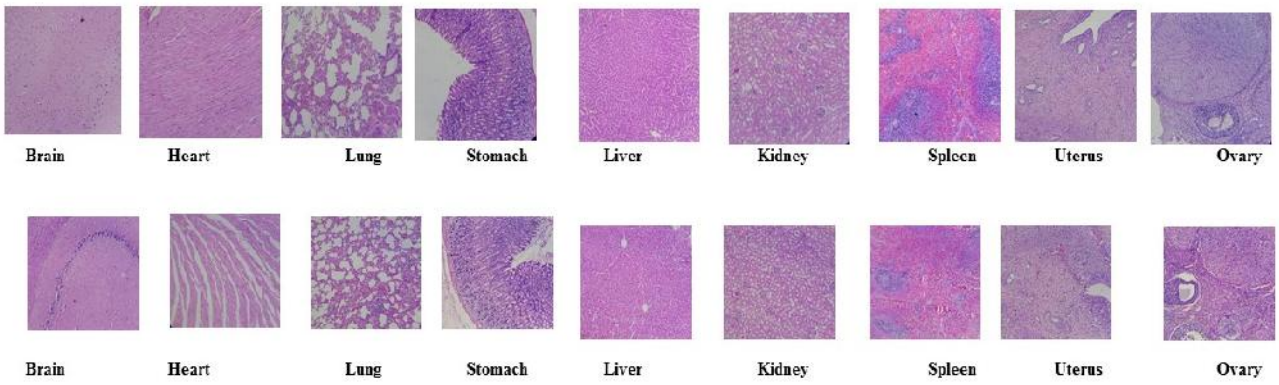
Figure 1: Histopathology of Male belongs to control and high dose treated group



3.14. Effect of SC 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 measures relative toxicological response of an experimental organism to single or brief exposure to a test substance. In accordance with regulatory guidelines rats are the most widely used species for ascertaining the toxicity. Acute toxicity test is also used to calculate median lethal dose (LD50) of a substance, using various standardized methods including Lorke's and acute toxic class methods. Following administration of a test drug, animals are observed individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days in the case of delayed toxicities [18].

In the present acute toxicity study, there was no mortality up to a maximum dose of 5000 mg/kg body weight of SC 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 SC is nontoxic at the administered dose of 5000mg/kg.

Repeated dose toxicity testing is carried out for a minimum of 28 days. The test substance is administered daily for a certain period through the oral route. If this route is not convenient, the test substance may be administered parenterally. The test substance is administered regularly at a specific time. Usually, a rodent of any gender and age 5–8 weeks is used for repeated dose toxicity testing. In sub-acute toxicity study treatment with SC at 500 and 1000 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 SC in humans.

Serum liver function tests provide information about the status of the liver. The liver enzymes (aminotransferases; ALT and AST) describe its cellular integrity, while albumin and total protein levels describe its functionality [19]AST and ALT are principally produced by the liver cells and any assault to the liver may lead to an increase in the serum level of these enzymes . High levels of liver enzymes are signs of hepatocellular toxicity [20], whereas a decrease may indicate enzyme inhibition [21]. However, ALT is the most sensitive marker of liver damage or toxicity since AST is also found in abundance in kidneys, testes, cardiac and skeletal muscles [22].

Serology study reveals that 28-day daily dose treatment with the SC has no significant election in any of the hematological and serological parameters in drug treated rats in comparison with control group animals, further no clinical signs of toxicity, morbidity, or mortality across all the treatment groups hence it may have concluded that the formulation SC is safe at the tested doses over the observation period. Results of the present investigation showed that there was no sign of toxicity and no mortality after single and repeated administration of the test drug SC at varying doses in tested rats. There was no significant difference in mean body weight, food/water intake in male and female rats.

Histopathological examination of brain reveals normal cerebral region shows the neuronal populations within the brain with scattered combination of medium- to large-sized neurons with prominent nucleus, normal appearance of heart fibres without any histological alterations. Pulmonary alveoli and blood lumen appears normal in lungs and no signs of ulcer and glandular degeneration were observed in stomach. Regular radiated hepatic cords from the central vein to the peripheral of lobule, central vein observed in liver. Normal renal structure with rounded renal corpuscles formed of the Glomerulus (G) and the Bowman's capsule (B) seen in kidney. Regular histology of marginal zone along with marginal and germinal centre were observed in spleen. Testicular tissue shows

well differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed. Appearance of endometrium, myometrium and uterine glands was normal in uterus and appearance of antral follicle, primary oocyte and secondary follicles are normal in ovary.

5. Conclusion

Herb derived medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide. In most developing countries, the indigenous modes of herbal treatment are parts of the culture and the dominant method of healing therapy. Results of the study concluded that the formulation has highly safety index and doesn't affect any of the vital organs, hence based on the safety nature of the siddha drug Shanmuga chooranam it was well justifying the exploration of the drug for clinical application in humans may be safe and efficacious due to presence of phytotherapeutics in the formulation.

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