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Systematic Toxicity Profiling of Siddha Formulation Thoothula Pazha Chooranam by Acute and 28-Day Subacute Toxicity Studies in Swiss albino mice

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Abstract

Siddha system of medicine plays a pivotal role in dealing with human life since several centuries. In general siddha formulation are preferentially safe and efficacious whereas growing clinical consideration provokes the need of exploring the safety profile of some novel siddha preparation prior to clinical application. Thoothula pazha chooranam (TTPC) is one of its kind comprises of herbal bioactive components prescribed as ailment for several diseases as per the siddha literature. Still now there is no proper documentary evidence claiming its safety at preclinical level, hence the present investigation aimed at evaluating the toxicity profile of siddha drug TTPC by acute and sub- acute toxicity studies in selective rodent model. In the acute study, a single dose of 5000 mg/kg was orally administered and mice were monitored for 14 days. In the sub-acute study, repeated doses (250 and 500 mg/kg/day) of the test drug TTPC were administered for 28 days and biochemical, hematological and histopathological parameters were evaluated. 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 TTPC at varying doses in tested mice. 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 mice. Further no changes in the gross observation of all the vital organs in both male and female mice. Single and repeated oral administration of the siddha drug TTPC may be safe and considered as relatively non-toxic at the varying doses of 250 and 500 mg/kg dose level. It was concluded from the results of the study that formulations like TTPC has wide margin of safety and may be advised for treating chronic ailments.

Keywords: Siddha system ,Thoothula Pazha Chooranam, Acute , Sub- acute, Toxicity studies, Preclinical

1. Introduction

The use of natural products as medicines must, of course, have presented a tremendous challenge to early humans. It is highly probable that when seeking food, early humans often consumed poisonous plants, which led to vomiting, diarrhea, coma, or other toxic reactions—perhaps even death. However, in this way, early humans were able to develop knowledge about edible materials and natural medicines [1]. Subsequently, humans invented fire, learned how to make alcohol, developed religions, and made technological breakthroughs, and they learned how to develop new drugs.

The recognition of herbal treatment or phytomedicine as the most common form of alternative medicine has been around since time immemorial [2,3]. This is because a larger percentage of the world's population (about 80% according to World Health Organization's estimation) depends on these plant-based remedies as a viable option to diseased conditions most especially in developing and/or developed countries where conventional or modern drugs are majorly used [4]. Similarly, it is worth mentioning that the popularity, as well as the usage of these traditional medicines, has continued to increase all over the world [5]. Despite this popularity and wide usage, the safety of these herbal therapies has, in recent times, raised a lot of questions as a result of revelations due to illnesses and fatalities [6,7] such as hepatotoxicity [8] and nephrotoxicity [9,10] and only a few of them have been evaluated through various phases of clinical trials [11].

Many countries including India, many a times the nutritional/beneficial claims made for a herbal based supplement or drug are not backed by sufficient preclinical and animal toxicity data, which is otherwise a prerequisite for any allopathic formulation. A scientifically carried out screening is therefore important in order to ascertain safety and efficacy of traditional and herbal products and also to establish the active components in them [12]. Toxicity, safety, and efficacy data for any herbal preparation in suitable animal models as per regulatory norms can greatly help in predicting toxicity and providing guidelines for selecting a safe dose in humans. Thoothula pazha chooranam is a siddha formulation indicated for treating several diseases as per siddha literature but still now there is no proper documentary evidence claiming its safety at preclinical level. Hence the present investigation aimed at evaluating the toxicity profile of siddha drug TTPC by acute and sub-acute toxicity studies in selective rodent model.

2. Materials and Methods

2.1. Animal

Healthy swiss albino mice 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/087/2018

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 Thoothula Pazha Chooranam (TTPC) the dose of 5000mg/kg (p.o) to experimental mice. 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 mice used for the study were

nulliparous and non-pregnant. The animals were randomly divided into control group and drug treated groups of 18 swiss albino mice (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 mice 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 28th day, the animals were fasted for overnight with free access to water. On 29th 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 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 [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 mice treated with TTPCon Acute toxicity study

The dose of TTPC 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 mice on Acute toxicity study

Clinical Signs Parameters	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 mice treated with TTPC in Acute toxicity study

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

Table 2: Body weight of mice in Acute toxicity study

Dose	Body weight in gms	
	Initial Body Weight (Before Treatment)	**Final Body Weight (After Treatment)**
TTPC 5000 mg/kg	20.5 ± 1.049	22.5 ± 1.049

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

3.3. Fecal Pellet consistency analysis of mice treated with TTPCin acute and sub-Acute toxicity study

Mice 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 mice in acute and sub-Acute toxicity study

Acute Toxicity Study	
Analysis	**TTPC**
Consistency	Dry
Shape	Slender
Colour	Greenish
Mucous Shedding	Absence
Blood Cells	Absent
Signs of Infection	None Observed

Sub-Acute Toxicity Study			
Analysis	**Control**	**Low Dose**	**High Dose**
Consistency	Soft	Dry	Dry
Shape	Oblong	Slender	Slender
Colour	Greenish Brown	Greenish	Greenish
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 mice treated with TTPC on Sub-Acute toxicity study

The dose of TTPC 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 mice in Sub-Acute toxicity study

Clinical Signs Parameters for the duration of 28 days	Control Normal Saline	TTPC 250 mg/kg	TTPC 500 mg/kg
Lacrimation	Absence	Absence	Absence
Salivation	Absence	Absence	Absence
Animal appearance	Normal	Normal	Normal
Tonic Movement	Absence	Absence	Absence
Clonic Movement	Absence	Absence	Absence
Laxative action	Absence	Absence	Absence
Touch Response	Normal	Normal	Normal
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	6	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 TTPC on Body weight of Mice in Sub-acute toxicity study

No significant change was observed in body weight of both male and female mice treated with TTPC 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 mice in Sub-Acute toxicity study

Dose	Body weight in gms	
	Initial Body Weight (Before Treatment)	**Final Body Weight (After Treatment)**
Control	21 ± 2.191	24.83 ± 1.722
TTPC 250 mg/kg	21.5 ± 2.881	25.83 ± 1.722
TTPC 500 mg/kg	21.17 ± 2.401	23.5 ± 1.871

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 mice treated with TTPC for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of mice treated with TTPC 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 mice in Sub-acute toxicity study

Dose	Average Food and Water Intake	
	Food Intake in gms	**Water intake in ml**
Control	2.833 ± 0.7528	5 ± 0.8944
TTPC 250 mg/kg	3.167 ± 0.7528	5.167 ± 0.9832
TTPC 500 mg/kg	3 ± 0.8944	4.833 ± 0.7528

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 TTPC on Hematological parameters of mice in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of mice treated with TTPC 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 mice 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	6.65 ± 0.6775	3.583 ± 1.342	396.8 ± 114.1	11.85 ± 1.093	14.47 ± 0.8116	47.35 ± 4.799
TTPC 250 mg/kg	5.95 ± 0.9894	3.1 ± 1.584	545.2 ± 155.7	13.13 ± 2.146	14.47 ± 2.951	51.02 ± 6.457
TTPC 500 mg/kg	5.383 ± 0.407	2.617 ± 1.003	418.8 ± 167.5	12.32 ± 1.155	14.68 ± 1.189	47.97 ± 6.194

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 TTPC on Hematological parameters of mice in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of mice treated with TTPC 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 mice in Sub-acute oral toxicity study

Group	Neutrophils 10^3 /mm ^{3}	Eosinophils (%)	Basophils (%)	Lymph (%)	Mon (%)
Control	14.93 ± 4.938	2.3 ± 1.18	0.1667 ± 0.4082	64.48 ± 8.659	4.717 ± 1.158
TTPC 250 mg/kg	18.5 ± 5.249	3.017 ± 1.332	0.1667 ± 0.4082	73.57 ± 6.814	3.2 ± 1.692
TTPC 500 mg/kg	19.68 ± 5.885	3.533 ± 0.9873	0.1667 ± 0.4082	62.83 ± 7.25	2.567 ± 1.196

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 TTPC on Serum Bio-chemistry profile of mice in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of mice treated with TTPC 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 mice in Sub-acute oral toxicity study

Group	BUN (mg/dl)	Serum Creatinine (mg/dl)	Total Bilirubin (mg/dl)
Control	23.33 ± 5.125	0.3333 ± 0.2066	0.7833 ± 0.3189
TTPC 250 mg/kg	22.17 ± 2.787	0.3167 ± 0.1941	0.9 ± 0.3521
TTPC 500 mg/kg	19.33 ± 4.633	0.4667 ± 0.1366	0.8833 ± 0.2858

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 TTPC on Serum Bio-chemistry profile of mice in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of mice treated with TTPC 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 mice in Sub-acute oral toxicity study

Group	SGOT (IU/L)	SGPT (IU/L)	Total cholesterol (mg/dl)	TG (mg/dl)
Control	91 ± 20.36	55.67 ± 20.77	77.08 ± 9.891	76 ± 22.7
TTPC 250 mg/kg	134.3 ± 42.35	61.83 ± 21.48	90.78 ± 5.117	84.17 ± 13.72
TTPC 500 mg/kg	92.33 ± 20.31	50.83 ± 19.36	99.47 ± 11.35	83.33 ± 7.528

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 mice belongs to control and drug treated group in sub-acute toxicity study

No statistically significant differences were recorded in organ weight of male mice treated with TTPC 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 mice in sub-acute toxicity study

Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Testes
Control - Male	0.4 ± 0.01732	0.1267 ± 0.02082	0.24 ± 0.04583	0.3267 ± 0.03055	0.959 ± 0.1817	0.09333 ± 0.02517	0.2767 ± 0.02887	0.25 ± 0.0866
TTPC 250mg/kg - Male	0.3533 ± 0.04933	0.15 ± 0.06083	0.22 ± 0.01732	0.31 ± 0.02646	1.103 ± 0.2754	0.1 ± 0.04359	0.3033 ± 0.04509	0.2533 ± 0.01528
TTPC 500mg/kg - Male	0.3733 ± 0.01528	0.1367 ± 0.03215	0.1867 ± 0.03055	0.32 ± 0.05292	0.87 ± 0.06557	0.1 ± 0.02	0.31 ± 0.02646	0.2333 ± 0.03055

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 mice belongs to control and drug treated group in sub-acute toxicity study

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

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

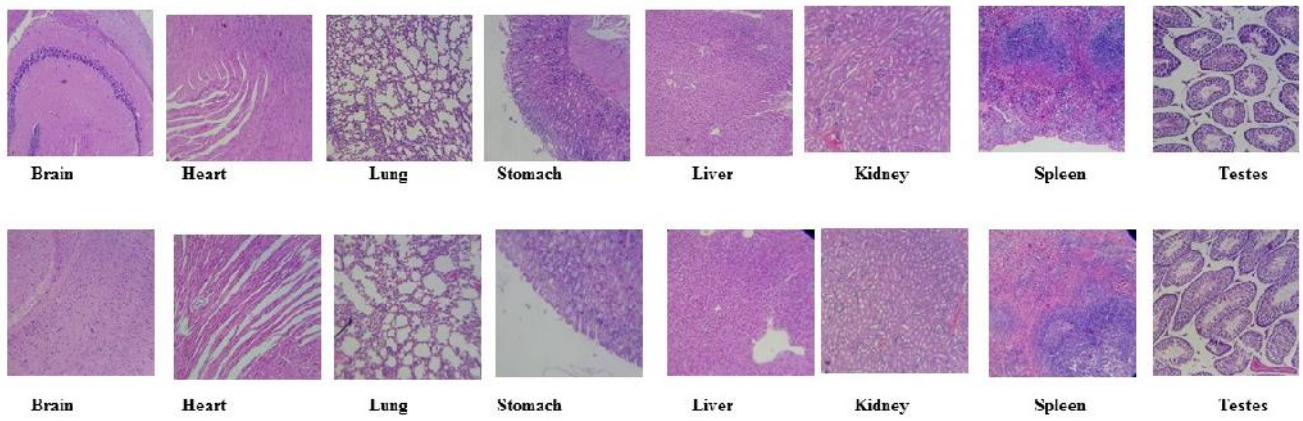
Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Uterus & ovary
Control - Female	0.4033 ± 0.0503	0.18 ± 0.04	0.23 ± 0.01	0.3167 ± 0.03055	0.94 ± 0.05568	0.08 ± 0.02646	0.21 ± 0.02	0.2267 ± 0.05686
TTPC 250mg/kg - Female	0.4033 ± 0.05033	0.18 ± 0.04	0.23 ± 0.01	0.3167 ± 0.03055	0.94 ± 0.05568	0.08 ± 0.02646	0.21 ± 0.02	0.2267 ± 0.05686
TTPC 500mg/kg - Female	0.3633 ± 0.02887	0.1067 ± 0.04933	0.23 ± 0.01732	0.34 ± 0.09539	1.013 ± 0.07572	0.05667 ± 0.03512	0.2567 ± 0.05508	0.2697 ± 0.1325

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

3.13. Effect of TTPC on Histopathological changes of Male mice in Sub-acute oral toxicity study

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

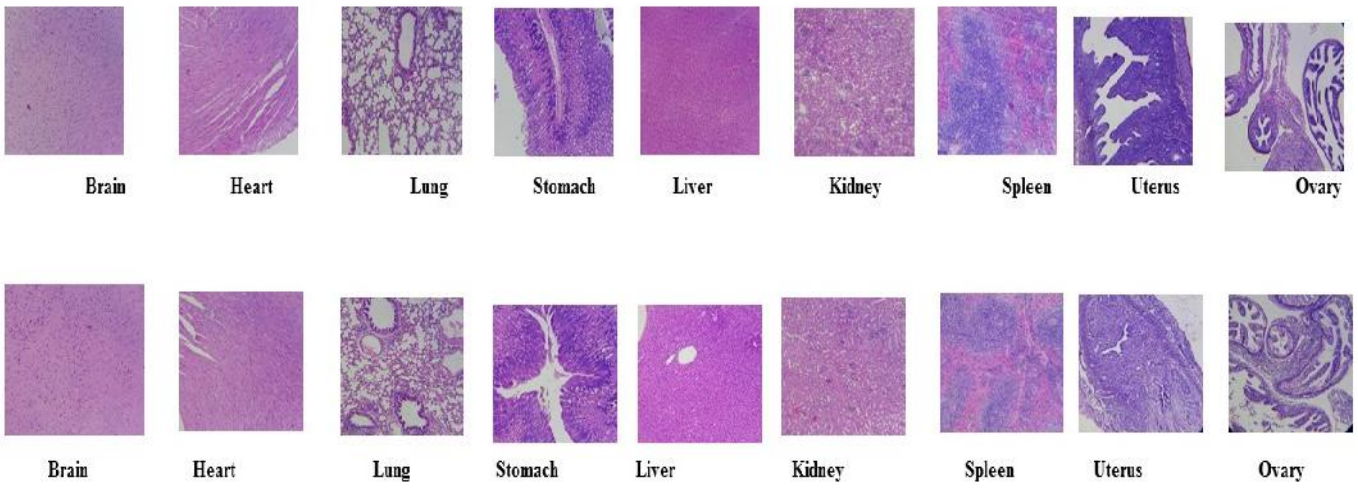
Figure 1: Histopathology of Male mice belongs to control and high dose treated group



3.14. Effect of TTPC on Histopathological changes of Female mice in Sub-acute oral toxicity study

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

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



4. Discussion

The principal aim of evaluating the safety of any formulation is to identify the nature and significance of adverse effect and to establish the exposure level at which this effect is observed [18]. Toxicological evaluation of siddha formulation TTPChas provided an evidence based data with respect to C.N.S, A.N.S and C.V.S system on the tested animals. In acute toxicity study siddha formulation TTPC administered at the dose of 5000 mg/kg had no adverse effect on the treated mice in up to 14 days of observation. No significant change in the body weight, behavioral and sensory parameters were observed in acute toxicity study.

In acute toxicity study, there was no mortality up to a maximum dose of 2000 mg/kg body weight of TTPC 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 mice as compared to control group, it can be inferred that siddha formulation TTPC is nontoxic at the administered dose of 5000mg/kg.

The haematological parameters can be used to determine the blood relating functions of plant extract. The haemopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological status in both humans and animals [19]. Hematology results of sub – acute toxicity study indicated a non-significant difference in all the blood cell count parameters indices which suggested that the TTPC does not affect erythropoiesis, morphology, or osmotic fragility of red blood cells.

The role of liver and kidney functions are important for survival of animals. Their functionality can be measured by serum biochemical analysis, which are crucial in the toxicological evaluation of xenobiotics. Sub-acute serology profiling reports clearly suggested that the drug TTPC was absolutely safe and there is no elevation in SGOT, SGPT and creatinine levels. istopathological analysis of the vital organ of control and TTPC treated group mice reveals the morphology of neurons in CA1, CA2 and CA3 zones are normal, The CA zones of brain are filled with densely packed Pyramidal cells, No evidence on accumulation of adipose tissue on interstitium of the heart, Pulmonary alveoli and blood lumen appears normal, Regular arrangement of muscularis externa and outer longitudinal muscle were observed in stomach and liver hepatic cords appears normal with radiating morphology were observed.

Microscopic observation of kidney showing normal, intact renal tubules as well as renal glomeruli. Histology of spleen reveals regular appearance of red pulp is composed of a three dimensional meshwork of splenic cords and venous sinuses were observed. 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. Section of ovary showing well follicular development, Pre-ovulatory follicle surrounded by granulosa cells with normal zona pellucida and theca interna and externa of the ovary.

5. Conclusion

Societal need of alternate therapy grabs the attention of researcher in exploring the efficacy and potential of numerous siddha formulation. But at the same time the preclinical documentary evidence of the same were seriously limited. The results of the present study have strongly suggested that the siddha drug Thoothula Pazha Chooranam is safe and well tolerated at the tested oral doses in both acute and sub- acute toxicity studies.

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