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In-vivo toxicological screening of Siddha preparation Indirathi Thravagam by acute and sub-acute repeated oral toxicity studies in accordance with standard regulatory guidelines

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Abstract

Medicinal herb based preparations have evolved over millions of years, it has unique chemical diversity, which results in diversity in their biological activities and drug-like properties. Those products have become one of the most important resources for developing new lead compounds and scaffolds. Siddha system of medicine proposed by ancient siddhars are unique in their composition and its mechanism as well. The major reason behind the art of curing is siddha drugs are often made of potential bioactive herbs. Natural products will undergo continual use toward meeting the urgent need to develop effective drugs, and they will play a leading role in the discovery of drugs for treating human diseases, especially critical diseases. Hence present study aimed at investigating the toxicity profile of siddha drug indirathi thravagam (IDT) and its safety level by acute and sub-acute repeated oral toxicity studies in accordance with standard regulatory guidelines. 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 (500 and 1000 mg/kg/day) of the test drug IDT were administered for the period of 28 days and biochemical, hematological and histopathological parameters were evaluated. Acute toxicity study results reflect that the study drug IDT at the dose of 2000mg/kg haven't revealed any toxicity responses and doesn't elicit any adverse responses as observed in the clinical parameter's. Results of sub-acute toxicity study further propagates the safety level of the drug IDT with respect to the data's obtained from serology, hematology and with histopathology. Outcome of the present study substantiates the wide safety margin of the drug IDT and also advocates the long term clinical application of the drug towards several diseases.

Keywords: Medicinal herb, Siddha drug, Toxicity profile, Indirathi thravagam, Acute, Sub-acute toxicity, Safety margin

1. Introduction

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 [1]. Siddha formulation are of diversified nature most exist in the form of solid, liquid, semisolid and some are even with nano sized particles. Liquid preparations like thravagam holds greater importance since majority of the cases these are distillates derived from some potential herbs. Thravagam type of formulations have good compliance in dosing and also with respect to the bioavailability is concern. According to the tradition eighteen Siddhars were supposed to have contributed to the development of Siddha medicine, yoga and philosophy. However, literature generated by them is not available in entirety. In accordance with the well-known self-effacing nature of ancient Indian preceptor's authorship of many literary work of great merit remains to be determined [2]. Herbs used in herbal medicine herbs have been scientifically validated for the claimed medicinal effects, hence slowing down the pace of drug discovery from such plants. Among the factors responsible for this is the myth surrounding herbal medicine especially in developing nations as well as dosages administered [3].

The recognition of herbal treatment or phytomedicine as the most common form of alternative medicine has been around since time immemorial [4]. 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 [5]. 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 [6]. 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 [7,8] such as hepatotoxicity [9] and nephrotoxicity [10,11] and only a few of them have been evaluated through various phases of clinical trials [12].Hence present study aimed at investigating the toxicity profile of siddha drug indirathi thravagam (IDT) and its safety level by acute and sub-acute repeated oral toxicity studies in accordance with standard regulatory guidelines.

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/092/2018

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 Indirathi thravagam (IDT) 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 [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 IDT (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 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 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 IDT on Acute toxicity study

The dose of IDT 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 IDT in Acute toxicity study

No significant change was observed in body weight of female rats treated with IDT 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)
IDT 2000 mg/kg	183.5 ± 2.665	184.8 ± 2.317

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

3.3. Fecal Pellet consistency analysis of rats treated with IDT 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	**IDT**
Consistency	Soft
Shape	Round Headed
Colour	Pale Greenish
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	Round Headed	Round Headed
Colour	Greenish	Pale Greenish	Pale 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 rats treated with IDT on Sub-Acute toxicity study

The dose of IDT 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	IDT 500 mg/kg	IDT 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	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	7	7
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 IDT 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 IDT 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	188 ± 3.033	197 ± 3.847
IDT 500 mg/kg	190.7 ± 7.174	195.5 ± 7.609
IDT 1000 mg/kg	185.2 ± 5.115	191 ± 5.621

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 IDT for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with IDT 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	15.17 ± 2.137	25.33 ± 1.211
IDT 500 mg/kg	15 ± 2.1	24.83 ± 3.656
IDT 1000 mg/kg	17.33 ± 1.966	30.5 ± 2.588

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

No statistically significant differences were recorded in hematological parameters of rats treated with IDT 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	6.717 ± 1.03	7.2 ± 1.903	755.2 ± 182.9	11.52 ± 1.214	19.87 ± 2.179	60.92 ± 6.568
IDT 500 mg/kg	5.7 ± 0.5967	6.333 ± 2.031	594.2 ± 204.1	11.5 ± 1.468	17.7 ± 2.168	57.7 ± 4.073
IDT 1000 mg/kg	6.85 ± 0.6285	8.583 ± 0.9908	719.5 ± 192.3	10.02 ± 2.989	20.4 ± 2.133	56.57 ± 4.244

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

No statistically significant differences were recorded in hematological parameters of rats treated with IDT 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	2.467 ± 0.9352	1.4 ± 0.3225	0.1667 ± 0.4082	82.77 ± 6.514	5.083 ± 1.324
IDT 500 mg/kg	2.583 ± 0.8773	1.333 ± 0.2944	0.1667 ± 0.4082	71.63 ± 7.63	2.417 ± 1.415
IDT 1000 mg/kg	3.167 ± 0.6976	1.4 ± 0.3033	0.1667 ± 0.4082	68.3 ± 25.02	3.85 ± 1.311

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 IDT 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 IDT 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	13.67 ± 3.141	0.6833 ± 0.2639	0.4167 ± 0.07528	123.3 ± 16.67	18.83 ± 1.169
IDT 500 mg/kg	14.17 ± 3.189	0.6 ± 0.3098	0.4333 ± 0.1862	95.17 ± 11.86	32 ± 7.127
IDT 1000 mg/kg	16.83 ± 3.312	0.6333 ± 0.216	0.3167 ± 0.1169	111.7 ± 18.86	28.67 ± 5.82

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 IDT 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 IDT 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	131.6 ± 15.34	58.67 ± 2.582	59.17 ± 12.25	13.78 ± 2.234	35 ± 21
IDT 500 mg/kg	125.4 ± 16.6	58.33 ± 7.866	52.33 ± 14.42	14.72 ± 2.053	44.17 ± 11.58
IDT 1000 mg/kg	128.4 ± 11.71	60.67 ± 8.454	54.17 ± 17.63	13.55 ± 3.393	26.33 ± 5.046

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 IDT 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	1.593 ± 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
IDT 500 mg/kg - Male	1.567 ± 0.1115	0.5 ± 0.09644	1.28 ± 0.09644	1.397 ± 0.1168	5.317 ± 1.142	0.4333 ± 0.04509	1.077 ± 0.1185	1.487 ± 0.4267
IDT 1000 mg/kg - Male	1.547 ± 0.1079	0.4633 ± 0.06658	1.223 ± 0.05508	1.143 ± 0.343	3.893 ± 0.6837	0.5633 ± 0.09452	0.9233 ± 0.04163	1.19 ± 0.347

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 IDT 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	1.72 ± 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
IDT 500 mg/kg - Female	1.423 ± 0.2663	0.4533 ± 0.138	1.147 ± 0.2403	1.033 ± 0.6117	3.797 ± 1.234	0.39 ± 0.148	0.94 ± 0.2066	1.027 ± 0.06351	0.1167 ± 0.07024
IDT 1000 mg/kg - Female	1.62 ± 0.1418	0.4967 ± 0.1193	1.187 ± 0.1801	1.3 ± 0.09644	3.8 ± 0.3279	0.3967 ± 0.04726	0.8833 ± 0.2205	1.073 ± 0.1401	0.28 ± 0.1127

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

3.13. Effect of IDT 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.

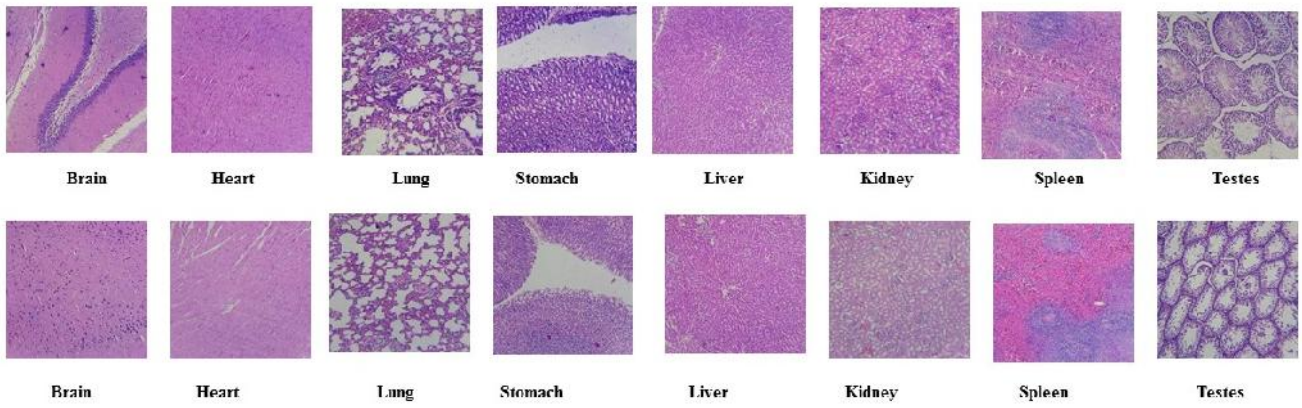

Figure 1: Histopathology of Male belongs to control and high dose treated group

3.14. Effect of IDT 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.

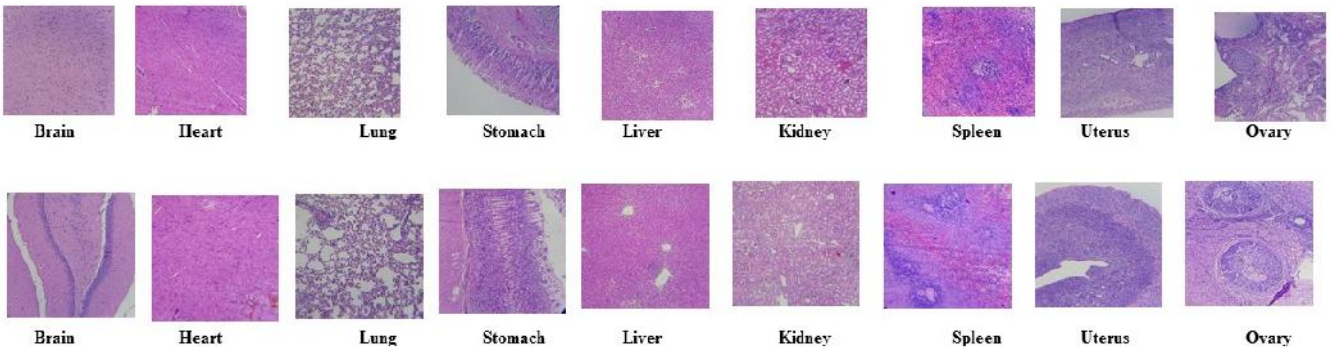

Figure 2: Histopathology of Female belongs to control and high dose treated group

4. Discussion

A more contemporary design for acute toxicity testing attempts to derive a maximum amount of information from a minimum number of animals. Study objectives include determination of the most important clinical signs attributable to high doses of the test substance, time of onset and remission of those signs, possible determination of a minimum lethal dosage, and in the event of lethality, the sequence and timing of effects leading to death or recovery. Toxicological evaluation of siddha formulation *Indirathi thravagam* (IDT)has 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 IDT administered at the dose of 2000 mg/kg had no adverse effect on the treated rats 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 IDT 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 IDT is nontoxic at the administered dose of 2000mg/kg.

The subacute toxicity study was conducted for 28 days to examine the toxicity of the extract on some blood parameters and histopathology of the liver and kidneys [18].In sub-acute toxicity study treatment with IDT 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 IDT in humans. Results of the study reveals that 28-day daily dose treatment with the IDT elicited no clinical signs of toxicity, morbidity, or mortality across all the treatment groups hence it may have concluded that the formulation IDT is safe at the tested doses over the observation period.

The degree of liver damage induced by a chemical substance can be evaluated by determining the level of biochemical markers of the liver function such as Aspertate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP). ALP is located in the cytoplasm and is release into circulation after cellular hepatic damage. ALT and AST are also enzymes released as a result of liver injury, especially damage to mitochondria of liver cells [19]. Elevation of level of these enzymes can be an indication of cellular damage, leakage and loss of functional integrity of hepatic cell membrane. Results of hematological and serological investigation clearly provoked that repeated oral treatment of the test drug IDT at both the dose level did not deviate any of the cellular and enzyme level in the treated rats when compare to that of the control group.

Histopathological observation of brain showed normal architecture in both cortex and medulla where three layers of cerebellar cortex. Appearance of fibrils and cross striations are equidistant I heart and histology of lung showing normal alveoli and collagen fibres. Microscopic observation of stomach reveals the presence of normal Sub-mucosa and gastric glands, marginal changes near portal vein was observed with migration of inflammatory cells. Epithelial lining on proximal convoluted tubule appears normal with very mild contusion observed in kidney.

Normal cyto architecture with No signs of immunological activities were observed in spleen. 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. Normal cyto architecture of uterine layers and glands were observed in uterus. Follicular cells, cytoplasm and nucleus appears normal in ovary.

5. Conclusion

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. Results of sub-acute toxicity study further propagates the safety level of the drug Indirathi Thravagam with respect to the data's obtained from serology, hematology and with histopathology. Outcome of the present study substantiates the wide safety margin of the drug Indirathi Thravagam and also advocates the long term clinical application of the drug towards several diseases.

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