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Toxicological Investigation of Siddha Preparation Agasthiyar Elathy Chooranamin accordance with OECD guideline

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Abstract

Siddha system of medicine is practiced in some parts of India especially in the state of Tamil Nadu. As the global use of herbal medicinal products continues to grow and many newer products are introduced into the market, public health issues, and concerns surrounding their safety are also increasingly recognized. Although some siddha medicines have promising potential and are widely used, many of them remain untested and their use also not monitored. This makes knowledge of their potential adverse effects very limited and identification of the safest and most effective therapies as well as the promotion of their rational use more difficult. The main aim of the present study is to evaluate the safety profile of the siddha drug agasthiyar elathy chooranam (AEC) through short term (acute) and long term (sub-acute) toxicity studies in accordance with OECD (Organization for economic co-operation and development) guideline. 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 AEC were administered for the period of 28 days and biochemical, hematological and histopathological parameters were evaluated. Results of acute and sub-acute oral toxicity studies of the drug AEC revealed no toxic signs and or evidence of mortality including behavioral, sensory or organ related toxicities. There were no significant changes in the body weights, food intake, water intake, organ weights and haemato-biochemical parameters in both the dose level of 500 and 1000 mg/kg. Microscopic observation of organ justifies the safety level of the drug by projecting normal morphology in drug treated group when compare to that of the control group rats.These results further suggest that acute or sub-acute oral administration of the test drug AEC is safe and doesn't causes any potential toxic effect in rats.

Keywords: Siddha, Agasthiyar elathy chooranam, Acute, Sub-acute toxicity, OECD, Biochemical, Hematology, Histopathology

1. Introduction

Traditional herbal medicines are naturally occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. Traditional herbal medicines are getting significant attention in global health debates [1]. In India, about 80% of the rural population uses medicinal herbs or indigenous systems of medicine [2]. It is estimated that nearly 960 plant species are used by the Indian herbal industry, and the turnover of the industry is more than Rs 80 billion. Herbal exports include medicines of AYUSH (Ayurveda, Unani, Siddha, and homoeopathy) products, which occupy a share of 3% of total Indian pharmaceutical export. Although the AYUSH industry represents one of the oldest traditional forms of medicine in India, it has not been able to exploit the opportunities of the emerging market [3,4].

Traditional medicine particularly herbal formulation playing important role in maintain of health in rural and remote areas. Inclusion of traditional herbal medicine in clinical practice will help to achieve the target 'health for all'. Indian traditional medicine has sound scientific background of effectiveness and also acknowledged by the recent researches. Although efforts are needed to overcome barriers like irrational use, quality control and standardization issues, high pharmacovogilance etc. Stick implementation of rules, monitoring and periodic revision of regulations are absolute necessary to promote Indian traditional medicine [5].

In screening traditional herbal preparations for pharmacological activity, the evaluation of the toxic characteristics of medicinal products is usually a preliminary step. During such evaluation, the determination of LD_{50} is usually an initial step to be conducted. The acute toxicity study may provide initial information on the mode of toxic action of an agent, acts as the basis for classification and labelling, and helps in deciding the dose of novel compounds in animal studies. Moreover, if a high dose (e.g., 5000 mg/kg) is found to be survivable, no further acute testing will be conducted [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]. Agasthiyar Elathy Chooranam is a poly herbal siddha formulation that majorly comprises of the following ingredients *Eletteria cardamomum, Syzygium aromaticum*, Rock salt, *Myristica fragrans, Costus speciosus, Rhus succedanea, Piper nigrum, Piper longum,* Dried Rizhome of *Zingibero fficinalis*, skin of *Terminalia chebula,* skin of *Terminalia bellirica*, Dried fruit of *Emblica officinalis, Nardostachys grandiflora, Cleodendrum serratum,* Flower of *Michelia champaca.* But still now there is no proper documentary evidence available on its safety and efficacy of this novel drug. Hence present study aimed at investigating the toxicity profile of siddha drug AEC 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/089/2018

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 Agasthiyar elathy chooranam (AEC) 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 [8].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 AEC (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 [9].

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 [10]

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 [11]

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[12]

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 AEC on Acute toxicity study

The dose of AEC 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 AEC in Acute toxicity study

No significant change was observed in body weight of female rats treated with AEC 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)
AEC 5000 mg/kg	184.7 ± 2.733	188 ± 3.633

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

3.3. Fecal Pellet consistency analysis of rats treated with AEC 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		Sub-Acute Toxicity Study			
Analysis	**AEC**	**Analysis**	**Control**	**Low Dose**	**High Dose**
Consistency	Soft	**Consistency**	Rigid	Soft	Soft
Shape	Oblong	**Shape**	Oblong	Oblong	Oblong
Colour	Pale green	**Colour**	Greenish	Pale green	Pale green
Mucous Shedding	Absence	**Mucous Shedding**	Absence	Absence	Absence
Blood Cells	Absent	**Blood Cells**	Absent	Absent	Absent
Signs of Infection	None Observed	**Signs of Infection**	None Observed	None Observed	None Observed

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

The dose of AEC 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 in rats on Sub-Acute toxicity study

Clinical Signs Parameters for the duration of 28 days	Control	AEC 500 mg/kg	AEC 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	7	7
Urine pH	7	Absence	Absence
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 AEC 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 AEC 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
AEC 500 mg/kg	184.5 ± 3.728	190 ± 3.742
AEC 1000 mg/kg	182.5 ± 1.871	189.7 ± 3.327

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 AEC for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with AEC 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
AEC 500 mg/kg	14.33 ± 2.805	28.33 ± 1.862
AEC 1000 mg/kg	16.67 ± 1.211	28.83 ± 2.927

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

No statistically significant differences were recorded in hematological parameters of rats treated with AEC 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 ($\times 10^6$ µl)	WBC ($\times 10^3$ µl)	PLT ($\times 10^3$ 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
AEC 500 mg/kg	5.883 ± 0.4491	8.267 ± 1.412	727.7 ± 177.8	11.72 ± 1.657	19.47 ± 1.669	61.68 ± 4.838
AEC 1000 mg/kg	6.133 ± 1.319	7.833 ± 2.062	630.5 ± 223.6	11.53 ± 1.757	20.43 ± 1.303	58.45 ± 7.762

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.

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

No statistically significant differences were recorded in hematological parameters of rats treated with AEC 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
AEC 500 mg/kg	2.45 ± 0.6535	1.433 ± 0.2582	0.1667 ± 0.4082	76.43 ± 7.748	2.367 ± 1.071
AEC 1000 mg/kg	2.05 ± 0.8666	1.5 ± 0.2608	0.1667 ± 0.4082	77.63 ± 8.131	3.483 ± 1.505

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 AEC 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 AEC 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
AEC 500 mg/kg	14.83 ± 3.43	0.7 ± 0.2191	0.4 ± 0.08944	86.5 ± 20.04	25.83 ± 5.707
AEC 1000 mg/kg	12.67 ± 1.633	0.4 ± 0.1095	0.4167 ± 0.1169	92 ± 22	27.33 ± 8.165

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 AEC 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 AEC 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
AEC 500 mg/kg	130.9 ± 22.31	61.83 ± 7.055	54.83 ± 17.07	14.18 ± 2.547	25 ± 2.828
AEC 1000 mg/kg	125.5 ± 10.5	54.83 ± 4.119	53.33 ± 11.81	17.3 ± 2.375	33.5 ± 4.764

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 AEC 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
AEC 500 mg/kg - Male	1.583 ± 0.08021	0.4633 ± 0.02517	1.117 ± 0.1721	1.1 ± 0.1044	4.013 ± 1.037	0.5533 ± 0.05508	0.9833 ± 0.2203	1.727 ± 0.3837
AEC 1000 mg/kg - Male	1.64 ± 0.1473	0.5233 ± 0.04041	1.387 ± 0.4486	1.123 ± 0.1358	4.21 ± 0.6684	0.59 ± 0.1249	1 ± 0.1652	1.37 ± 0.2193

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 AEC 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

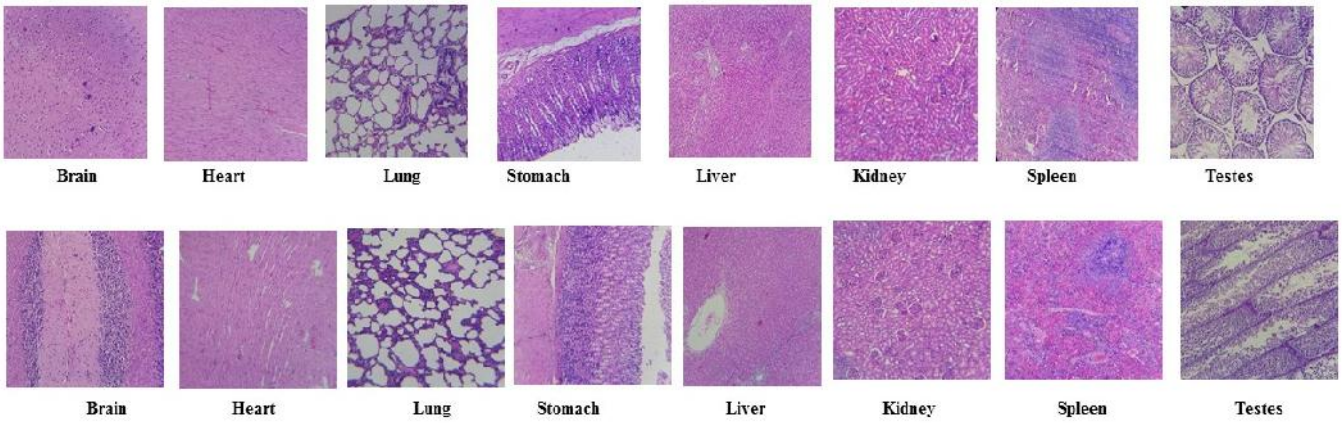
Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Uterus	Ovaries
Control -Female	1.72 ± 0.13	0.5833 ± 0.07767	1.32 ± 0.4493	1.253 ± 0.3253	4.62 ± 1.218	0.5233 ± 0.04041	1.093 ± 0.2397	0.9533 ± 0.03512	0.1633 ± 0.04041
AEC 500 mg/kg - Female	1.613 ± 0.01155	0.5267 ± 0.09452	1.053 ± 0.1358	1.237 ± 0.2654	3.63 ± 1.001	0.4633 ± 0.1026	0.95 ± 0.1852	0.8233 ± 0.02309	0.05667 ± 0.02082
AEC 1000 mg/kg - Female	1.393 ± 0.2601	0.53 ± 0.1758	1.277 ± 0.03055	0.9667 ± 0.08386	3.82 ± 0.8779	0.4333 ± 0.1531	0.9267 ± 0.1069	0.8633 ± 0.03786	0.06667 ± 0.04726

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

3.13. Effect of AEC 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.

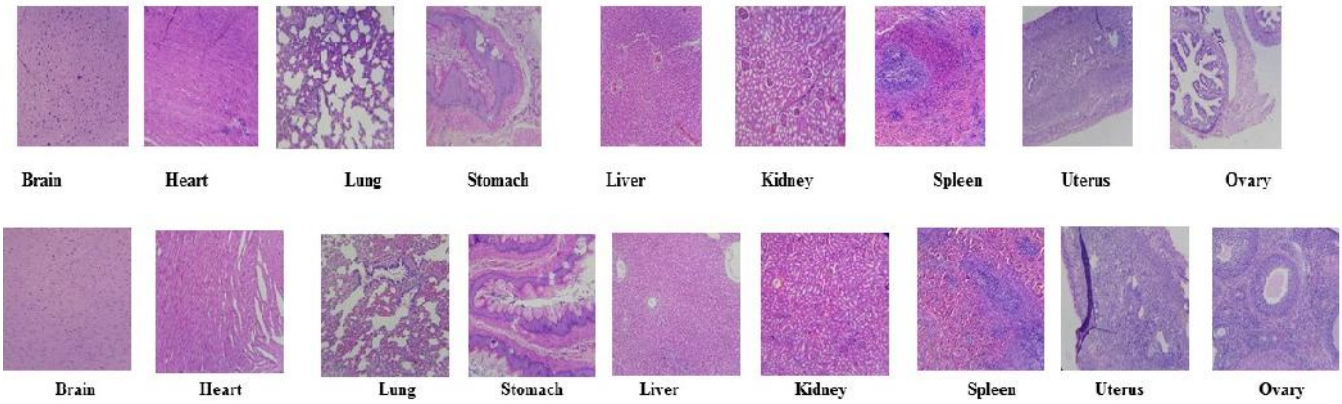
Figure 1: Histopathology of Male belongs to control and high dose treated group



3.14. Effect of AEC 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

Toxicological evaluation of siddha formulation *Agasthiyar Elathy chooranam* (AEC)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 AEC administered at the dose of 5000 mg/kg had no adverse effect on the treated rats in up to 14 days of observation.In sub-acute toxicity study treatment with AEC 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 AEC in humans. Results of the study reveals that 28-day daily dose treatment with the AEC elicited no clinical signs of toxicity, morbidity, or mortality across all the treatment groups. Analysis of haematological parameters are used to study the extent of toxicity of drug substances including plant extracts [13]. Haematopoiesis is the process of blood cell formation. Changes in the haematopoietic system have a higher predictive value for human toxicity when data are translated from animal studies [14]. All blood cells are believed to be derived from the pluripotential stem cell, an immature cell with the capability of becoming an erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet) [15].

WBC's are the first line of cellular defines that respond to infectious agents, tissue injury, or any inflammation. Furthermore, no significant changes were observed in neutrophils, lymphocytes, and monocytes in AEC treated rats suggesting that the formulation might not have exerted challenge on the immune system of the animals. Most of hematological and serological biochemical parameters including hepatic and renal biomarkers showed normal levels proposing no significant adverse effect for AEC.

The protocol of weighing relative organs in toxicity studies includes their sensitivity to predict toxicity and it correlates well with histopathological changes [16]. The results of this study revealed no significant changes in the relative organ weight of control and treated groups which showed that none of the organs were adversely affected, nor showed any signs of toxicity throughout the study.Histopathological analysis served as an important tool in predicting the organ related toxicity of the study drug, in the present study vital organs of male and female rats of control and drug treated group were subjected to histopathological examination to extrapolate the safety level of the drug AEC.

Histopathological analysis of brain reveals normal granular and purkinje cells appears of cerebellum projected with distinct cytoplasm, Normal histology of myocardial tissue with prominent inter fiber distance observed in heart, Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis.

Light microscopic observation of stomach shown normal gastric mucosa containing intact gastric gland cells, parietal cells which are spherical cell with deeply stained dark nucleus, Centrilobular zone appears normal with stable network of hepatocytes in liver. Renal tubule appeared normal and lined by flattened epithelium in kidney and marginal sinus (MS) of the spleen and its sinus lining cells appears normal. Primary spermatocytes with large centered nucleus and dense chromatin were observed in testes. Regular histology of uterine epithelium and endometrial glands observed in uterus along with normal appearance of corpora lutea (CL), atretic follicles (AF) and interstitial tissue (IT) appeared in ovary.

5. Conclusion

Indian system of medicine has a standard operational procedure in drug purification and processing even though it has been believed that siddha preparation is free of drug induced toxicity, validation is essentially required to provoke the safety among general public. These results further suggest that acute or sub-acute oral administration of the test drug agasthiyar elathy chooranam is safe and doesn't causes any potential toxic effect in rats.

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