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Investigation on safety profiling of siddha formulation Kaadikkara Chenduram in accordance with OECD guideline

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Abstract

Siddha formulation made of metals and minerals have absolutes potency as described in the vedic traditional literatures. Despite of its potency the public awareness on utilization of such formulations seems limited because of the growing concern on emergence of systemic and other organ related toxicities. Since prehistoric times, humans have used natural products, such as plants, animals, minerals, metals and marine organisms, in medicines to alleviate and treat diseases. Above all according to siddha system of traditional medicines mineral and metal based preparation's are equipotent in managing several disorders and are still plays a key role in clinical management in many countries today. Kaadikkara Chenduram (KKC) majorly comprises of cinnabar, red sulphide of mercury and nitrate of silver indicates for treating gastro intestinal disabilities. Present study aimed at establishing the safety of the drug KKC using acute (OECD 423) and sub-acute (OECD 407) repeated oral toxicity studies in both male and female wistar rats in accordance with regulatory guidelines.In the acute study, a single dose of 1000 mg/kg was orally administered and experimental animals were monitored for 14 days. In the sub-acute study, repeated doses (50 and 100 mg/kg/day) of the test drug KKC were administered for the period of 28 days and biochemical, hematological and histopathological parameters were evaluated.In acute study, single oral administration of the drug KKC shown no dose-dependent general behavior adverse effects and mortality. The LD50 values of the drug may be higher than 1000mg/kg. Results of repeated oral administration of KKC at the dose of 50 and 100 mg/kg doesn't provokes any change in serological and hematological parameter's.No significant pathological difference was observed in the histological examination of all the vital organs includes brain, heart, lungs, liver, kidney, spleen and other reproductive organs of rats treated with KKC at both the dose level. It was concluded from the results of the acute or sub-acute oral administration of the test drug KKC that this drug may be considerably safe and may render clinical benefits in patients upon short and long term usage.

Keywords: Metalo-mineral, Siddha, Kaadikkara Chenduram , OECD, Acute, Sub-acute toxicity , Serological and Hematology.

1. Introduction

Toxicology is a branch of science that deals with toxins and poisons and their effects and treatment. Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. The US Food and Drug Administration (FDA) states that it is essential to screen new molecules for pharmacological activity and toxicity potential in animals (21CFR Part 314). The toxic effects of chemicals, food substances, pharmaceuticals, etc., have attained great significance in the 21st century. Toxicity tests are mostly used to examine specific adverse events or specific end points such as cancer, cardiotoxicity, and skin/eye irritation. Toxicity testing also helps calculate the No Observed Adverse Effect Level (NOAEL) dose and is helpful for clinical studies [1].

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 [2]. It has close affinity to Ayurveda yet it maintains a distinctive identity of its own. This system has come to be closely identified with Tamil civilization. The term '*Siddha*' has come from '*Siddhi*'- which means achievement. *Siddhars* were the men who achieved supreme knowledge in the field of medicine, yoga or *tapa* (meditation) [3].Warnings have been issued by the Food and Drug Administration as regards the potential toxic effects of many commonly consumed medicinal plants and/or herbal preparations [4]. Toxicological risks of medicinal plants used in different parts of the world have been documented [5]. Safety testing is needed in order to popularize acceptance, standardize and/or regulate the market of herbal medicines currently being offered [6]. However, in recent times, scientists have inquire into scrutinizing the quantity and quality of the safety and efficacy potential acclaimed in folklore medicine in order to provide data to meet the criteria needed to support its use worldwide [7-10].

Kaadikkara Chenduram (KKC) majorly comprises of cinnabar, red sulphide of mercury and nitrate of silver indicates for treating gastro intestinal disabilities. Only few of the formulation of mineral origin have been scientifically validated in the siddha system of medicine. Among the factors responsible for this is the myth surrounding metalo-mineral medicine especially in developing nations as well as dosages administered. To this end, fewer side effects and high therapeutic values are two key criteria used to scientifically evaluate siddha mineral formulations as adjunct or sole pharmaceuticals. Hence present investigation aimed at establishing the safety of the drug KKC using acute (OECD 423) and sub-acute (OECD 407) repeated oral toxicity studies in both male and female wistar rats in accordance with 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/096/2018

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 Kaadikkara Chenduram (KKC) at the dose of 1000mg/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 [11].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 KKC (50 and 100 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 [12].

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

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 [13]

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[14]

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 KKC on Acute toxicity study

The dose of KKC used for acute toxicity study is 1000mg/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 1000mg/ 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 KKC in Acute toxicity study

No significant change was observed in body weight of female rats treated with KKC at the dose of 1000mg/ 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)**
KKC 1000 mg/kg	183 ± 2.098	185.2 ± 2.563

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

3.3. Fecal Pellet consistency analysis of rats treated with KKC 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	**KKC**
Consistency	Soft
Shape	Oblong
Colour	Greenish Brown
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 KKC on Sub-Acute toxicity study

The dose of KKC used for sub-acute toxicity study is 50 and 100 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	KKC 50 mg/kg	KKC 100 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	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 KKC 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 KKC at low and high dose of 50 and 100 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
KKC 50 mg/kg	182.5 ± 2.168	185.2 ± 1.941
KKC 100 mg/kg	185.5 ± 4.506	192 ± 4.195

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 KKC for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with KKC at low and high dose of 50 and 100 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
KKC 50 mg/kg	19.17 ± 1.941	27.17 ± 1.602
KKC 100 mg/kg	18.17 ± 1.722	28.5 ± 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.

3.7.Effect of KKC on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with KKC at low and high dose of 50 and 100 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
KKC 50 mg/kg	5.35 ± 0.3017	8 ± 1.195	854.2 ± 106.3	13.12 ± 2.207	18.35 ± 1.363	62.08 ± 4.826
KKC 100 mg/kg	5.867 ± 0.8981	6.883 ± 2.084	829 ± 80.09	11.95 ± 1.962	18.8 ± 2.154	59.82 ± 5.187

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

No statistically significant differences were recorded in hematological parameters of rats treated with KKC at low and high dose of 50 and 100 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
KKC 50 mg/kg	2.75 ± 0.493	1.333 ± 0.1966	0.5 ± 0.5477	71.7 ± 11.66	4.083 ± 1.292
KKC 100 mg/kg	3.1 ± 0.5404	1.467 ± 0.3502	0.3333 ± 0.5164	77.23 ± 16.22	2.117 ± 0.8931

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 KKC 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 KKC at low and high dose of 50 and 100 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
KKC 50 mg/kg	12.2 ± 6.138	0.5667 ± 0.2582	0.2833 ± 0.1602	103.8 ± 23.53	25.5 ± 6.745
KKC 100 mg/kg	15.5 ± 3.391	0.75 ± 0.2074	0.4 ± 0.08944	110.3 ± 20.27	21.33 ± 7.815

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 KKC 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 KKC at low and high dose of 50 and 100 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
KKC 50 mg/kg	127.2 ± 15.23	63.83 ± 4.491	48.5 ± 16.83	14.8 ± 1.963	26.83 ± 3.189
KKC 100 mg/kg	124.3 ± 12.94	63 ± 9.187	47.33 ± 10.21	13.93 ± 2.582	26.33 ± 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.

3.11. 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 KKC at low and high dose of 50 and 100 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
KKC 50 mg/kg - Male	1.63 ± 1.597	0.52 ± 0.5133	1.26 ± 1.367	0.95 ± 1.06	3.35 ± 3.643	0.43 ± 0.48	0.93 ± 1.3	0.86 ± 1.143
KKC 100 mg/kg - Male	1.637 ± 0.106	0.5467 ± 0.05033	1.48 ± 0.26	1.1 ± 0.1646	4.083 ± 0.157	0.4867 ± 0.06506	1.207 ± 0.1858	1.417 ± 0.2354

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 KKC at low and high dose of 50 and 100 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

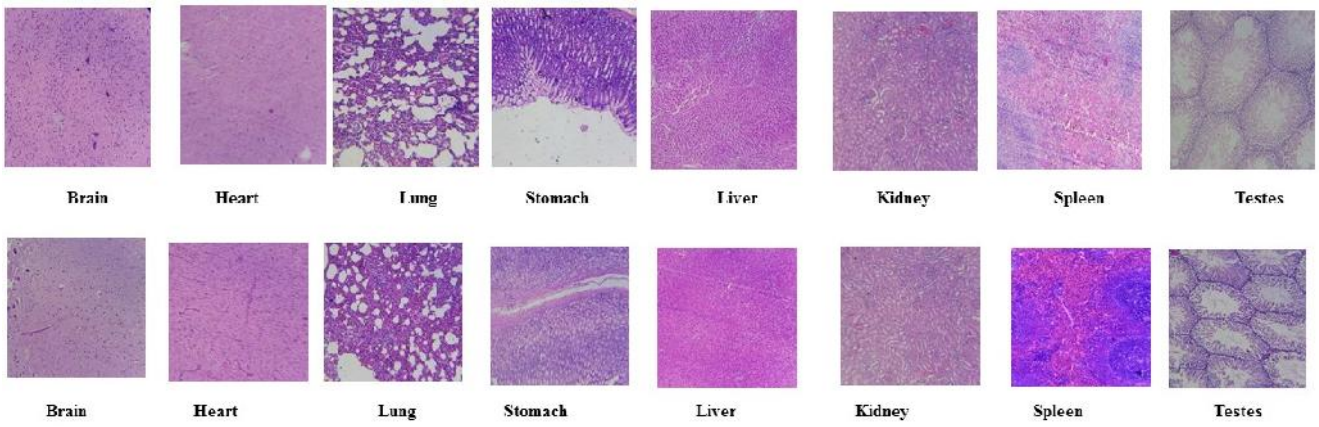
Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Uterus	Ovaries
Control -Female	1.72 ± 0.13	0.5833 ± 0.07767	1.32 ± 0.4493	1.253 ± 0.3253	4.62 ± 1.218	0.5233 ± 0.04041	1.093 ± 0.2397	0.9533 ± 0.03512	0.1633 ± 0.04041
KKC 50 mg/kg - Female	1.46 ± 1.59	1.18 ± 0.7467	1.11 ± 1.193	1.43 ± 1.43	3.93 ± 4.11	0.32 ± 0.4033	0.87 ± 0.9833	1.02 ± 1.093	0.15 ± 0.1267
KKC 100 mg/kg - Female	1.58 ± 0.02	0.5067 ± 0.04509	1.1 ± 0.2506	1.353 ± 0.1779	3.777 ± 0.465	0.4633 ± 0.06658	0.97 ± 0.02646	0.9433 ± 0.005773	0.1233 ± 0.09238

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

3.13. Effect of KKC 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 KKC 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.

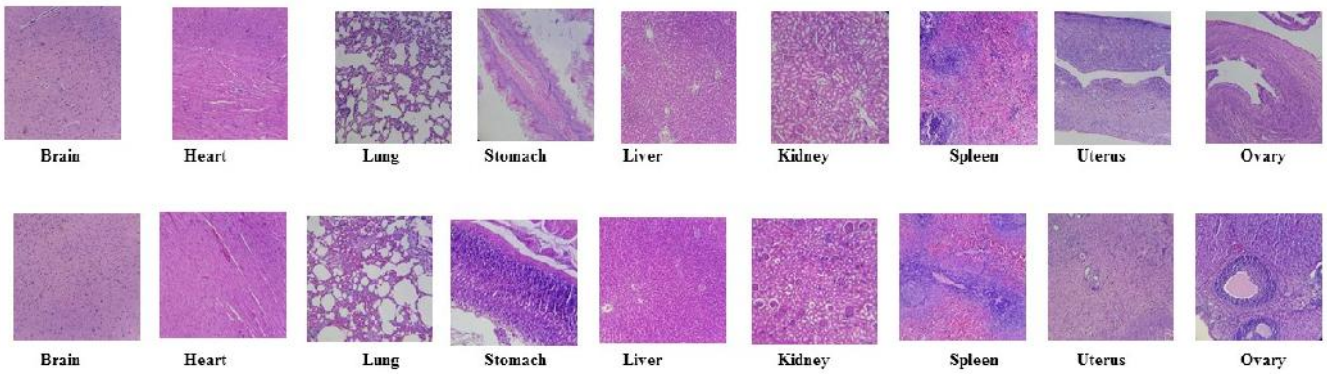


Figure 2: Histopathology of Female belongs to control and high dose treated group

4. Discussion

Acute toxicity studies are designed to determine the dose that will produce either mortality or serious toxicological effects when given once or as multiple administrations. They also serve to determine the doses that should be used in subsequent studies. These studies give another opportunity to determine what effects the test drug has in morphology, clinical chemistry, or other parameters, and also can give an early indication of possible target organ(s). In acute toxicity study, there was no mortality up to a maximum dose of 1000 mg/kg body weight of KKC after 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 KKC is nontoxic at the administered dose of 1000mg/kg.

In sub-acute toxicity study treatment with KKC at 50 and 100 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 KKC in humans. A drastic change in body weight is a critical evaluator of toxicity and may serve as a sensitive indication of the general wellbeing of animals [15]. The mean body weight gained by the animals in all the treatment groups may be an indication that the study drug KKC did not interfere with their normal metabolism as closely supported by the non-significant difference in this parameter when compared with the control group.

Results of the study reveals that 28-day daily dose treatment with the KKC elicited no clinical signs of toxicity, morbidity, or mortality across all the treatment groups hence it may have concluded that the formulation KKC is safe at the tested doses over the observation period. Liver and kidney damage may be ascertained by measurements of SGOT, SGPT, urea, uric acid, creatinine, and electrolytes, and deviations from normal in their serum concentrations are a tentative pointer to nephrotic injury [16].Increase in liver function enzyme may be consider as endpoint for hepatotoxic potential of the drug. In the present study serological investigation reports reveals that there is no significant difference observed in the liver and kidney function indices in the KKC (50 and 100 mg/lg) treated rats in comparison with control group suggestive of normal hepatic and renal function.

Histopathological examination provides reliable data on organ related adverse event in rodents. In sub-acute toxicity the histological analysis of the vital organs reveals almost normal morphology. Neuronal populations within the brain are heterogeneous with scattered combination of medium- to large-sized neurons. Appearance of myofibrillar and cytoplasmic zone was normal in heart, normal alveoli with

equidistant arrangement and prominent histology observed in lung. Histology of stomach reveals regular histology of Inner circular muscle (ICM), gastric pit (GP), and muscularis mucosae (MM). Hepatocytes appear variably pale with mild congestion on central vein in liver, The lining epithelial cells of the renal tubules appeared normal with projected signs of very mild contusion and shrunken glomeruli observed in kidney. Appearance of LF – lymphoid follicle; PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement were seen in spleen. Primary spermatocytes with large centered nucleus and dense chromatin were observed in testes. Normal endometrial gland and epithelium appears in uterus along with normal appearance of graafian and antral follicle were observed in ovary.

5. Conclusion

Safety assessment becomes a mandate regulatory needs to promise the long term tolerance of the drug which likely to get prescribed for humans. In the process of toxicological profiling several information's pertains to the dosage, compatibility, tolerance have been predicted with suitable rodent's models. It was concluded from the results of the acute or sub-acute oral administration of the test drug kaadikkara chenduram that this drug may be considerably safe and may render clinical benefits in patients upon short and long term usage.

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