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RESEARCH ARTICLE

Acute Toxicity and 28 Days Repeated Oral Toxicity Study of a Siddha Medicine Kirubagara Shanmuga Chenduram in Rats

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ABSTRACT

Siddha system of medicine is simple practical traditional, sasthric and bearing the cultural heritage of India. Siddha medicines have significant amount of heavy metals. It was propounded that hazardous effect of heavy metals would cause toxic accumulation. Based on mode of preparation, Indian system of medicine documented safe usage of drugs since 2500 years. Kirubakara Shanmuga Chenduram (KSC), a siddha medicine contains mostly metallic ingredients. In this study we have evaluated toxicity of KSC in Wistar albino rats. The acute and 28 days repeated oral toxicity study was done according to OECD guidelines 423 and 407 respectively. In acute study, high dose 2000mg/kg/bw of drug was produced neurotoxic symptoms but there were no remarkable changes in 28 days repeated oral toxicity study. Haematological and biochemical parameters did not show any change. In quantitative analysis (ICP-OES), the heavy metals like lead, cadmium, arsenic was found in BDL and mercury was found in 0.317 ppm. In histopathology study brain, heart, lungs, kidney, liver, spleen did not show any pathology. So KSC was toxic only in high dose of 2000 mg/kg. It reveals that the therapeutic dose of KSC (202mg-thuvarai alavu) is the safe dose for clinical use for human being.

KEYWORDS

Kirubakara Shanmuga Chenduram, Toxicity, Siddha Medicine, OECD Guideline

INTRODUCTION

Siddha system of medicine is one of the oldest traditional systems of medicine followed in Tamilnadu. It starts at the time period as the tamil language have originated. The strength of siddha medicine is it's effect in balancing and strengthening the body's own healing mechanism instead of suppressing or disturbing it as many modern drugs tends to do knowing that medicinal herbs are not enough and efficient to cure the diseases completely siddhar's have made thorough study on the utility of mineral substances and formulated the medicinal preparation consisting of Metals, Organic secondary minerals (uparasam), Organic salts (karasaram), Arsenic compounds (padanams)[1,2]. The single most important factor that determines the harmfulness or safeness of a substance is the dose (i.e) the amount or "how much" chemical is taken up by the living system.[3,4].

Since Kirubakara shanmuga chenduram (KSC) is a sasthric preparation being in use still today. This preparation contains 7 purified inorganic compounds such as vaalai rasam (mercury), lingam (cinnabar), veeram (hydrargyrum per chloride), pooram (calomel), thalagam (arsenic trisulphide), gandhagam (sulphur), 2 botanicals

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and common salt (Nacl)[5]. It cures many threatful and challenging diseases, but the toxicological evaluation has not yet been studied. So using globally accepted scientific methodology OECD for toxicological profile, the author planned to observe the acute and 28 days repeated oral toxicity study for KSC in the animal model wistar albino rats[6-8]. Animal research is usually done for the benefit of human (i.e) prevention or treatment of human diseases and sufferings.

The type nature and extent of the effect obtained during toxicological study help to differentiate the medicine (kirubakara shanmuga chenduram) as non-toxic, moderately toxic or severely toxic on the selected biological system.

MATERIALS AND METHOD

Drug- Kirubakara Shanmuga Chenduram

Raw drugs were collected from raw drug store in Chennai. Identified and authenticated from the department of Pharmacognosy in Siddha Central Research Institute, Chennai. The raw drugs were purified as per gunapadam thathu vagupu text and prepared sasthrically according to the text Pathartha guna vilakkam (thathu vadupu) as mentioned with the therapeutic dose of 202 mg twice daily with the adjuvant honey[1, 5].

Heavy Metal Analysis of KSC by ICP- OES

ICP- OES study done at IIT Madras. Sample was prepared by taking weight of 0.25 g of KSC and 9 ml of sulphuric acid was slowly added and mixed thoroughly. Allow it to react for few minutes. Quantitative analysis was achieved by measuring the intensity of specific wavelength and after performing the calibration using known standards[9,10].

Experimental Animals

Male and Female adult Wister albino rats, weighing 150- 200g purchased from Sri Ragavendra Enterprizes, Bangalore. The animals were housed in polypropylene cages provided with bedding of husk and maintained laboratory conditions (Temperature 22°C±3°C, relative humidity between 30% and 70% , air changes 10- 15 per hour and dark, light cycle each of 12 hours).

The animals were identified by cage number, animal number and individual marking on fur by using picric acid and were given Pelleted feed supplied by Sai meera foods Pvt Ltd. and aqua guard portable water in polypropylene bottles. Rats were acclimatize to these condition for 7 days prior to dosing. All animal experiments were conducted after getting approval from Institutional Animal Ethics Committee (IAEC Protocol No. 1248/ac/09/CPCSEA/4-37/2011). The study was done at National Institute of Siddha, Chennai.

Acute (OECD- 423) and 28 days Repeated Oral Toxicity Study (OECD- 407) of KSC in Rats

In acute oral toxicity study three female rats in each group randomly divided into 5 groups. KSC was suspended in 10% aqueous tween 80 solution with vigourous mixing and was administered to the groups in a single oral dose by gavage using a feeding needle. I group kept as control. II, III, IV, V group at a dose level 5, 50, 300 and 2000 mg/kg body weight. After administration, food was withheld for 3-4 hours. Control group received equal volume of vehicle[12,13].

In 28 days repeated oral toxicity study, six rats (3 males and 3 females) were in each group randomly divided into four groups for dosing up to 28 days. Three different dose levels low, mid and high were selected. Selected doses were calculated according to the body surface area (0.018) and the therapeutic dose level (404 mg) i.e 404 x 0.018 and it was fixed as x dose as low dose, 5x as mid dose and 10x as high dose. KSC was suspended in 10% aqueous tween 80 solution. It was administered to group I (low dose) x dose (7.272 mg/animal), group II (mid dose) at 5x dose (36.36 mg / animal), group III (high dose) at 10x dose (72.72 mg/animal). The control animals were administered vehicle only. Administration was given orally using an oral gavage once in daily for 28 consecutive days.

In acute study animals were observed for first 24 hours, special attention given for first 4 hours and daily thereafter, for a total of 14 days. The times at which signs of toxicity appear and disappear are important to be noted and behavioral changes were seen. Body weights were recorded at 1, 2, 7 and 14th day. At the end of 14 th day were sacrificed and gross necropsy of the external surface of the body, all orifices, and organs like brain, thymus, lungs, heart, spleen, liver, kidneys, adrenals and sex organs of all animals were noted.

In 28 days repeated oral toxicity study, body weight of animals evaluated weekly. Water, food consumption and mortality events evaluate daily. At the end of 28 day, the animals were sacrificed. Blood samples were collected by Heart puncture method and kept in vaccum container tube which was coated with sodium heparin for blood chemistry and potassium EDTA for haematology as anticoagulant. Hematological parameters included red blood cell count, leucocyte count, hemoglobin, platelet count, erythrocyte sedimentation rate (ESR) and bio chemical parameters included Liver Function Test, Renal Function Test, lipid profile, blood glucose were seen[14,15].

Gross necropsy was seen. Control and highest dose group animals were initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group was also be examined. Organs like brain, thymus, lungs, heart, spleen, liver, kidneys, adrenals and sex organs of all animals were collected and preserved in 10% buffered neutral formalin, sliced 5 or 6µm sections and it was stained with hematoxylin and eosin, examined for histopathological changes were examined by pathologist.

Statistical Analysis

Data were expressed in mean ± standard error mean(SEM). The findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and biochemistry parameters were subjected to One-way ANOVA followed by dunnet't' test using a computer software programme -INSTAT-V3 version[16].

RESULTS

Quantitative Analysis of KSC

The quantitative analysis of KSC by ICP- OES contains the presence of mercury in ppm and arsenic, cadmium, lead were present in below detectable limit showed in Table 1.

Table 1: Heavy Metals Analysis of KSC by ICP- OES

S.No.	Elements (Inorganic compounds)	Wave Length (nm)	Observation (KSC)
1	Arsenic (As)	193.696	BDL
2	Calcium (Ca)	317.933	8.99 mg/L
3	Cadmium (Cd)	226.502	BDL
4	Mercury (Hg)	253.652	0.317 ppm
5	Phosphorus (P)	214.914	7.44 mg/L
6	Lead (Pb)	230.204	BDL

Acute Toxicity

Data were summarized in tabular form (Table-2). In the dose level of 2000mg/kg/body weight after 4 hrs of administration produces the following characteristic signs like alertness, grooming, touch response listlessness, unching, porphyrin, motor incordination, muscular spasm in all the three animals. After 24 hrs 2 animals were found dead and after 48 hrs third animal was also found dead. 5, 50, 300 mg/kg/body weight displayed no signs of toxicity. Alertness, grooming, touch response were present. From these findings, as per the OECD guidelines the LD50 cut off value was concluded as 500 mg/kg/body weight.

Table 2: Dose Finding Experiment and its Behavioral Signs of Toxicity

No.	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	5	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	50	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	300	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	2000	+	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catanonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19. Body weight 20. Mortality +Positive Response, - Negative Response

Table 3: Body Weight (g) of Albino Rats Exposed to *KSC* for 28 Days

Dose (mg/kg/day)	Body weight (g) ± SEM				
	Days				
	1	7	14	21	28
Control	152.21±6.72	156.50±7.35	159.23±5.71	161.30±9.39	165.41±10.82
Low (x)	154.34±6.56	159.78±8.14	162.25±11.98	166.47±10.58	173.54±10.71
Mid (5x)	163.96±10.64	167.46±8.38	171.59±8.19	176.79±11.95*	182.31±12.87**
High (10x)	159.04±7.22	165.49±10.11**	168.18±10.55**	174.13±10.02**	179.22±8.41**

Values are mean of 6 animals ± SEM. (Dunnett's test). *P<0.05; **P<0.01. N=6

28 Days Repeated Oral Toxicity Study

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days. The KSC did not cause any mortality.

Effect of KSC on Body Weight, Food and Water Consumption

Results of body weight (g) determination of animals (Table 3) from control and different dose groups exhibited comparable body weight

gain significantly throughout the dosing period of 28 days. The quantity of food (g/day) and water (ml/day) consumed by animals from different dose groups was found to be comparable and normal with that of control animals.

Effect of KSC on Hematological and Biochemical Parameters

The results of haematological and biochemical investigations (Table- 4) conducted at the end of 28th day, revealed no significant changes in the values of different parameters investigated when compared with those of respective controls. The levels of RBC and hemoglobin in experimental animals were elevated when compared with that of the control. It is statistically significant ($p<0.05$). In liver function test, there is significant increase ($p<0.01$) in ALP at 5x and 10x dose when compared to the control groups. In other biochemical parameters like renal function test and lipid profile values obtained were within normal biological and laboratory limits. There was no significant change.

Table 4: Hematological and Biochemical Parameters after 28 Days Administration of KSC in Rats

Parameters	Control	Low Dose (x)	Mid Dose (5x)	High Dose (10x)
Red blood cell (mm^3)	7.14±0.18	7.92±0.16*	8.40±0.20*	8.91±0.24*
Hb (%)	12.60±0.53	13.53±1.41*	14.98±0.93*	15.97±1.00*
Leukocyte ($x10^6$ /ml)	10025±126.20	9700±389.53	9900±328.10	10025±421.21
Platelets ($x\ 10^3/mm^3$)	1163.92±59.24	1252.10±81.31	1127.03±105.37	1192.00±98.68
ESR (mm)	2.40±00	2.00±0.80	2.30±0.40	2.10±0.50
Total Bilirubin (mg/dL)	0.6±0.02	0.63±0.03	0.7±0.02	0.69±0.05
Bilirubin direct (mg/dL)	0.2±0.02	0.19±0.05	0.21±0.03	0.20±0.01
Bilirubin indirect (mg/dL)	0.2±0.05	0.19±0.01	0.21±0.00	0.2±0.04
ALP (U/L)	180.34±5.16	182.55±20.80	201.30±23.02**	189.12±27.71**
SGOT (U/L)	25.24±3.21	25.00±8.60	29.81±6.33	30.24±9.54
SGPT (U/L)	30.40±2.34	30.26±6.31	32.37±8.41	33.42±9.02
Total Protein (g/dl)	6.5±1.23	6.9±0.22	6.5±0.21	6.9±0.27
Albumin (g/dl)	3.2±0.19	3.5±0.24	3.6±0.1	3.6±0.56
Globulin (g/dl)	3.90±0.18	3.96±0.14	3.82±0.18	3.74±0.15
Urea (mg/dL)	25.19±2.79	24.20±2.82	25.48±3.10	27.30±2.69
Creatinine (mg/dL)	0.82±0.06	0.83±0.03	0.85±0.02	0.84±0.06
Uric acid (mg/dL)	4.20±0.10	4.14±0.32	4.30±0.28	4.51±0.62
Na m.mol	138.16±7.32	139.58±10.54	140.35±11.98	140.73±13.48
K m.mol	21.15±2.64	21.47±1.88	20.59±1.70	21.19±2.66
Cl m.mol	98.23±3.81	101.27±3.60	96.14±2.18	101.52±3.05
Total cholesterol (mg/dL)	42.89±2.87	40.35±2.68	39.18±3.91	41.98±3.78
Blood glucose (mg/dL)	108.16±8.26	101.29±5.65	100.29±4.21	104.40±5.62

Values are mean of 6 animals ± SEM. (Dunnett's test). *P<0.05; **P<0.01. N=6

Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities (Figs. 1, 2).

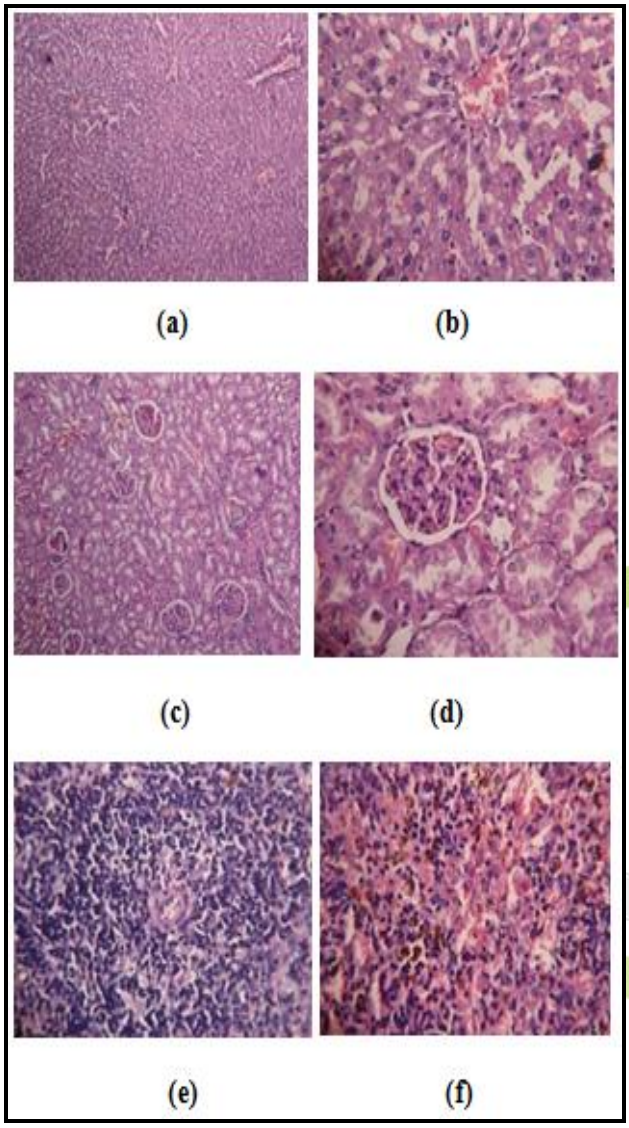

Figure 1: Histopathology of Liver, Kidney and Spleen After 28 Days Repeated Oral Administration of KSC in Rats

a) Control group liver, b) Test group liver (KSC 72.72mg/animal) c) Control group kidney, d) Test group kidney (KSC 72.72mg/animal) e) Control group spleen, f) Test group spleen (KSC 72.72mg/animal)

Liver tissues showed normal Portal area, hepatocytes, kupffer cells and sinusoidal spaces. Kidney showed normal glomeruli and renal tubes were normal. Section from the spleen showed normal appearing white and red pulp with central artery.

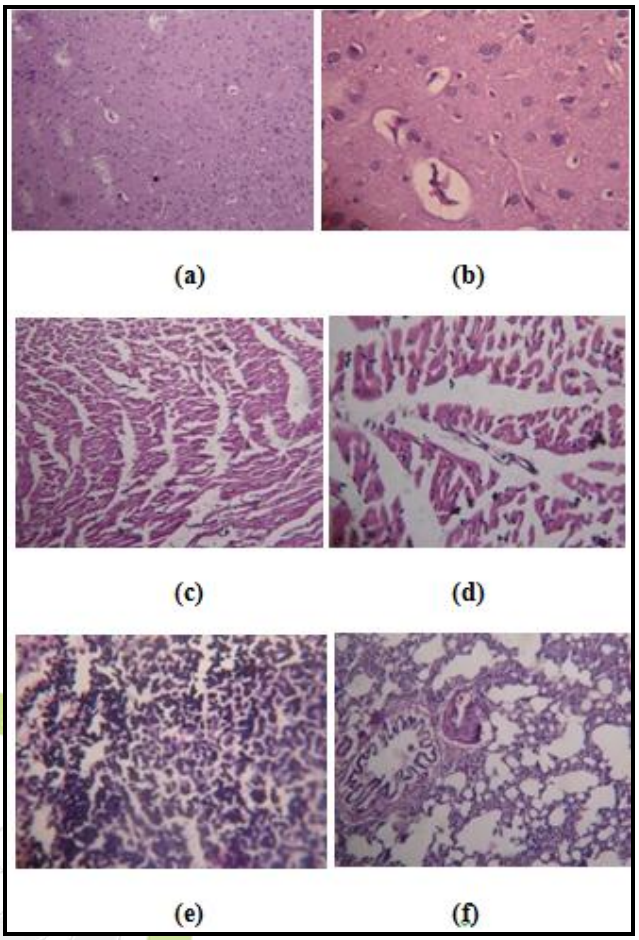

Figure 2: Histopathology of Brain, Heart and Lungs After 28 Days Repeated Oral Administration of KSC in Rats

a) Control group brain, b) Test group brain (KSC 72.72mg/animal)

c) Control group heart, d) Test group heart (KSC 72.72mg/animal)

e) Control group lungs, f) Test group lungs (KSC 72.72mg/animal)

Brain showed normal glial cells and astrocytes appeared normal. Cerebellum was also normal. Heart and lungs also not showed any abnormality.

DISCUSSION

Studies on siddha system will reveal many of the forgotten fundamentals. If a drug is to combat some severe illness, it must be reasonably powerful. KSC is being widely used in siddha medicine prepared according to the process found in pathartha guna

vilakkam(thaathu vagupu). It contains metalic ingredients like vaalai rasam, gandhagam, lingam, veeram, pooram, manosilai, thalagam, which cures diseases like eight types of kuttam, thadippu, megaranam, gunmam, kaasam, sanni, suram,utkuthu, purakuthu, soolai, moorchai and vatha diseases[5].

The quantitative analysis of KSC by ICP- OES contains the presence of heavy metal like mercury in 0.317ppm. It is in permissible limit[17,18]. Arsenic, cadmium, lead were present in below detectable limit. KSC was subjected to acute and repeated oral toxicity studies in wistar albino rats. During acute toxicity study the animals were fasted overnight given single oral dose of 2000 mg/kg/body weight of KSC suspended in 10% aqueous solution of tween 18 and the animals were observed for 14 days. After first 4 hrs of drug administration all the three animals showed the following characteristic activities like alertness, grooming, touch response listlessness, unching, porphyrin, motor incordination, muscular spasm in all the three animals. After 24 hrs 2 animals were found dead and 48 hrs third animal was also found dead. 5, 50, 300 mg/kg/body weight displayed no signs of toxicity. Alertness, grooming, touch response were present. The acute toxicity study of KSC reported the LD 50 value of 500 mg/kg/body weight as per OECD guidelines.

In repeated oral toxicity study, the doses selected for the study were x, 5x, 10x mg/kg body weight. During 28 days study there was no signs of toxic effect in all the animals and the body weight was gained significantly as that of control. The average water and food intake in the control group was not significantly ($p>0.05$) different from that of the test groups. the feed conversion efficiency followed the same pattern, thus indicating the normal metabolism of the animals. At the end of 28 th day all animals were sacrificed and the gross necropsy does not show any abnormality in the organs.

In hematological parameter the levels of RBC and hemoglobin in experimental animals were elevated when compared with that of the control. It is statistically significant ($p<0.05$). On the other hand, there was a significant increase ($p<0.05$) in the ALP, at mid dose (5x) and high dose (10x) dose treated groups when compared to the control groups. Other hematological and biochemical investigations the alterations in values was obtained within normal biological and laboratory limits or the effect was not dose dependent.

In the histopathology study of KSC administered to low dose (x), mid dose (5x), high dose (10x), the sections of brain, heart, lungs, kidney, liver, spleen were found normal as comparable with that of the control.

Though the drug KSC contains mostly mecurial and arsenical compounds as ingredients. In the final product these were converted into oxide form due to 12 hours heating process as mentioned in its preparation[19,5]. It reveals the foremost preparation of the medicine KSC was done as per literature.

CONCLUSION

In ICP- OES study heavy elements like arsenic, cadmium, lead were found only in below detectable limit and mercury was in acceptable limit. In acute toxicity study KSC produced neuro toxic symptoms and death in 2000 mg/kg/bw, so the LD50 cut off value was calculated as 500 mg/kg/bw as per OECD guidelines. In 28 days repeated oral toxicity study KSC at the dose of low dose (x), mid dose (5x), high dose (10x), does not produce any toxic signs or death. In histopathological study, the sections of brain, heart, lungs, kidney, liver, spleen showed normal in all the three doses. It reveals that the therapeutic dose of KSC (i.e) 202 mg (thuvarai alavu) for human being which is mentioned in Pathaartha Guna Vilakkam (thaathu vaguppu) is the safe dosage for clinical use.

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