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Toxicological Profiling of Novel Siddha Formulation Kalladaippu Chooranam by Acute and Sub-Acute Toxicity Studies

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Abstract

Siddha medicine attracts a great deal of attention due to its nontoxic nature and traditional use. Toxicity studies on siddha formulation commonly used to evaluate the possible health risk of the intrinsic chemical compounds in the preparation which could result in adverse effects. As per the regulatory needs traditional medicines including siddha drugs has to establish the safety in preclinical studies before clinical recommendation in humans.Kalladaippu Chooranam (KC) is one such novel siddha preparation clinically used for the condition of renal calculi and urolithiasis. The main aim of the present investigation is to establish the safety profile of the test drug KC by acute and sub-acute oral toxicities in both male and female wistar rats.In the acute study, a single dose of 5000 mg/kg was orally administered and animals were monitored for 14 days. In the sub-acute study, repeated doses (50, 250 and 500 mg/kg/day) of the test drug KC were administered for 28 days and biochemical, hematological and histopathological parameters were evaluated.Results of the present investigation showed that there was no sign of toxicity and no mortality after single and repeated administration of *the test drug KC in experimental animals.* The mean body weight and most of the biochemical and hematological parameters showed normal levels in both male and female rats. There were mild changes was observed in ALP level of treated rats. No significant toxicity was observed in the histological examination of kidney, liver and spleen tissues at the highest dose of KC.Single and repeated oral administration of the siddha drug KC may be safe and considered as relatively non-toxic at the varying doses of 50,250 and 500mg/kg dose level.This safety studies renders useful information to the clinicians to utilize the KC as a drugs of choice of clinical management of kalladaippu noi.

Keywords: Siddha medicine, Kalladaippu Chooranam, Safety profile, Acute , Sub-acute

1. Introduction

Siddha system of medicine. During the passage of time it interacted with the other streams of medicines complementing and enriching them and in turn getting enriched. The materia medica of Siddha system of medicine depends to large extent on drugs of metal and mineral origin in contrast to Ayurveda of earlier period, which was mainly dependent upon drugs of vegetable origin. Siddha system also follows ashtanga concept with regards to treatment procedures. However the main emphasis is on the three branches - *Bala vahatam* (pediatrics), *Nanjunool* (toxicology) and *Nayana vidhi* (ophthalmology) [1].

Safety pharmacology is a subdivision of pharmacology which focuses on identification and characterization of pharmacological activities that affect the clinical safety of a drug. The guideline recommends assessing effects on functions of cardiovascular, central nervous and respiratory systems, which are referred as the core test battery of safety pharmacology [2,3].

The target organ of toxicity most frequently involved in systemic toxicity is the CNS (brain and spinal cord). Even with many compounds having a prominent effect elsewhere, damage to theCNS can be demonstrated by the use of appropriate and sensitive methods. Next in order of frequency of involvement in systemic toxicity are the circulatory system; the blood and hematopoietic system; visceral organs such as the liver, kidney, and lung; and the skin. Muscle and bone are least often the target tissues for systemic effects. With substances that have a predominantly local effect, the frequency with which tissues react depends largely on the portal of entry (skin, gastrointestinal tract, or respiratory tract).The safety of using most of siddha preparations are not well established due to its complexity in composition, although most of this information comes from case reports rather than systematic investigations.Hence the present research work aimed at evaluating the short and long term safety of the siddha formulation Kalladaippu Chooranam by acute and sub-acute toxicity studies in suitable rodent model.

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. A 12 light / dark cycle were maintained .Room temperature was maintained between 22 $\pm$ 2^0 Cand relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) 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 C.L.Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India. XLVIII/01/CLBMCP/2016

2.2.Acute toxicity Study

The animals were randomly divided into control group and treatment groups of 6 female wistar albino rats of 3 in each group.The animals were fasted overnight (12- 16 hrs) with free access to water. Group I served as control and the study was conductedwith single oral administration of study drugKalladaippu Chooranam (KC) 5000mg/kg (p.o). 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 [4].Body weight was recorded periodically. At the end of the experiment all animals were subjected for gross necropsy and observed for pathological changes.

2.3. Sub-Acute toxicity Study

The animals were randomly divided into control group and drug treated groups 48 wistar albino rats (24 male and 24 female) were selected and divided into 4 groups. Each group consist of 12 animals (6 males and 6 females). First group served as a control and other three group were treated with test drug (50, 250 and 500 mg/kg/day) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. The female rats were nulliparous and non-pregnant.

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 anesthesia. 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 [5].

2.4.Hematological analysis

Blood samples were analyzed using established procedures and automated Bayer Hematology analyzer. Parameters evaluated include Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Packed Cell Volume (PCV), 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 using hematological analyser.

2.5. Biochemical analysis [6]

Serum samples were analyzed for Bilirubin, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP), High Density Lipoprotein (HDL), Low density Lipoprotein (LDL) , Very low density Lipoprotein (VLDL) , Triglycerides (TGL), Total Cholesterol ,Total protein, Urea, Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, using auto analyzer.

2.6.Histopathological evaluation [7]

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 bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

2.7.Statistical analysis[8]

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. Effect of KC on clinical signs of rats in Acute Oral Toxicity Study

The dose of KC 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:Effect of Kalladaippu Chooranam on clinical signs in Acute Oral Toxicity Study

SN	Group Control	Observation	SN	Group Test Group	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

3.2.Effect of KC on Body weight of rats in acute toxicity study

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

Table 2:Effect of Kalladaippu Chooranam on Body weight of rats in acute toxicity study

Dose	Days		
	1	7	14
Control	186.6± 2.75	189.2± 3.87	194.2 ± 7.62
KC 5000 mg/kg	182.5± 4.08	184.2± 2.16	187.4 ± 2.67
P value (p)*	NS	NS	NS

3.3.Effect of KC on Body weight rats in Sub-acute oral toxicity study.

No significant toxicity was observed in rats during the 28 consecutive days of treatment via oral route with KC at low, mid and high dose of 50, 250 and 500 mg/ kg b.w. The results were tabulated in Table 3.

Table 3:Effect of Kalladaippu Chooranam on Body weight of rats in Sub-acute toxicity study

Dose	Days				
	1	7	14	21	28
Control	165.6± 2.76	166.4 ± 3.42	167.7 ± 3.26	169.2 ± 3.73	170.7 ± 1.31
Low dose	160.2 ± 2.12	162.7 ± 3.64	164.4± 1.51	165.2 ± 1.66	166.42± 2.76
Mid dose	166.6± 1.64	167.3 ± 2.74	159.4 ± 8.12	162.1 ± 3.36	163.7 ± 3.11
High dose	167.4± 6.74	169.6 ± 3.72	162.6 ± 2.46	167 ± 6.81	161.92 ± 2.49
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.4.Effect of KC on food and water intake of rats in Sub-acute oral toxicity study.

No significant change was observed in body weight of both male and female rats treated with KC at low, mid and high dose of 50, 250 and 500 mg/ kg b.w. The results were tabulated in Table 4 and 5.

Table 4:Effect of Kalladaippu Chooranam on food intake of rats in Sub-acute toxicity study

Dose	Days				
	1	7	14	21	28
Control	37.12 ±5.37	38.5±3.22	39.5±3.37	38.5±3.37	37.12±3.12
Low dose	43.7±2.68	44.3±1.12	44.1±1.18	44.4±2.12	44.6±2.42
Mid dose	46.2±3.75	45.2±3.60	45.2±4.25	45.4±2.68	47.2±2.44
High dose	47.2±2.34	47.2±2.64	48.6±2.66	49.2±3.20	49.0±3.62
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test).

Table 5:Effect of Kalladaippu Chooranam on water intake of rats in Sub-acute toxicity study

Dose	Days				
	1	7	14	21	28
Control	31.5 ± 8.95	32.0 ±6.23	28.5±6.23	29.12±8.19	31.5±3.96
Low dose	21.5±3.28	21.4±3.22	21.7±3.02	21.2±3.29	24.9±3.11
Mid dose	26.7±4.33	26.3±2.11	27.1±2.43	28.4±2.11	32.4±2.34
High dose	20.1±1.32	20.2±2.13	22.7±2.13	25.2±1.73	28.4±2.65
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.5.Effect of KC on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with KC at low, mid and high dose of 50, 250 and 500 mg/ kg b.w. The results were tabulated in Table 6.

Table 6: Haematological parameters of rats exposed to Kalladaippu Chooranam

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	14.8±1.88	13.88±1.66	14.94±0.66	15.28±0.96	N.S
Total WBC ($\times 10^3$ l)	10.91±2.59	11.25±3.73	11.48±3.91	12.20±3.17	N.S
Neutrophils(%)	32.65±1.06	33.23±2.14	35.61±1.36	35.40±2.20	N.S
lymphocyte (%)	69.34±2.48	72.12±3.12	72.48±2.66	73.10±3.16	N.S
Monocyte (%)	0.78±0.17	0.79±0.09	0.82±0.03	0.84±0.06	N.S
Eosinohil(%)	0.64±0.09	0.68±0.02	0.70±0.06	0.72±0.04	N.S
Platelets cells10^3/µl	687.17±8.76	702.71±8.16	725.18±9.0	726.16±9.74	N.S
Total RBC 10^6/µl	7.99±0.12	7.82±0.57	8.82±0.59	8.38±0.72	N.S
PCV%	37.79±0.6	43.35±1.13	45.2±1.68	46.82±2.54	N.S
MCHC g/dL	33.6±2.23	35.09±1.29	35.98±1.22	36.03±1.24	N.S
MCV fL(µm^3)	49.17±3.64	50.20±1.22	52.28±1.24	53.24±1.44	N.S

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.6.Effect of KC on Biochemical parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in biochemical parameters of rats treated with KC at low, mid and high dose of 50, 250 and 500 mg/ kg b.w. The results were tabulated in Table 7 -9.

Table 7: Biochemical parameters of rats exposed to Kalladaippu Chooranam

Biochemical Parameters	Control	Low dose	Mid dose	High dose	P Value (p)*
Glucose (R) (mg/dl)	75.45±13.4	78.16±8.44	78.26±11.20	78.42±11.6	N.S
T.CholosteroL(mg/dl)	115.26±1.83	115.45±1.83	116.42±1.78	116.22±1.73	N.S
Trigly(mg/dl)	45.35±1.48	46.32±1.48	44.58±1.30	45.66±1.33*	N.S
LDL	73.81±2.13	71.24±2.14	72.8±2.14	71.64±4.32	NS
VLDL	14.2±2.44	15.42±4.64	15.44±6.64	15.64±4.36	NS
HDL	25.66±6.88	26.86±2.24	26.68±4.66	31.78±2.22	NS
Ratio 1(T.CHO/HDL)	3.42±2.44	4.16±3.14	4.34±8.44	4.46±2.22	NS
Ratio 2(LDL/HDL)	3.83±4.22	2.84±2.22	2.86±2.20	2.96±6.02	NS
Albumin(g/dl)	2.63±0.17	3.43±0.12	3.14±2.02	3.24±6.86	NS

NS- Not Significant,**(p > 0.01), * (p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 8: Renal function test of rats group exposed to Kalladaippu Chooranam

Parameters	Control	Low dose	Mid dose	High dose	P Value (p)*
Urea (mg/dl)	13.35±0.99	14.31±0.16	13.06±1.08	13.48±1.12	N.S
Creatinine(mg/dl)	0.28±0.08	0.36±0.06	0.52±0.04	0.66±0.02	N.S
BUN(mg/dL)	15.02±0.10	16.10±0.60	16.22±0.44	18.10±2.12	NS
Uric acid(mg/dl)	5.17±0.35	5.31±0.43	5.72±1.25*	5.58±0.23	S

NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 9: Liver Function Test of rats exposed to Kalladaippu Chooranam

Parameters	Control	Low dose	Mid dose	High dose	P Value (p)*
Bilirubin(mg/dl).	0.45±0.07	0.53±0.06	0.51±0.08	0.48±0.05	N.S
SGOT/AST(U/L)	78.95±1.39	78.35±0.51	76.01±1.53	81.55±1.03	N.S
SGPT/ALT(U/L)	30.23±1.28	30.91±1.59	28.34±1.48	34.32±0.68	N.S
ALP(U/L)	142.25±8.70	142±16.17	147.16±24.07*	149.33±14.65*	S
T.Protein(g/dL)	5.31±0.38	6.48±0.34	7.01±0.23	7.53±0.46	N.S

NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.7. Effect of KC on Histopathological changes of rats in Sub-acute oral toxicity study

No abnormality were detected in the histopathological analysis of organs (Kidney, Liver and Spleen) retrieved from the rats treated with KC at high dose of 500 mg/ kg b.w. The results were illustrated in figure 1 and 2.

Figure 1: Histopathological representation organs of control group rats in Sub-acute oral toxicity study

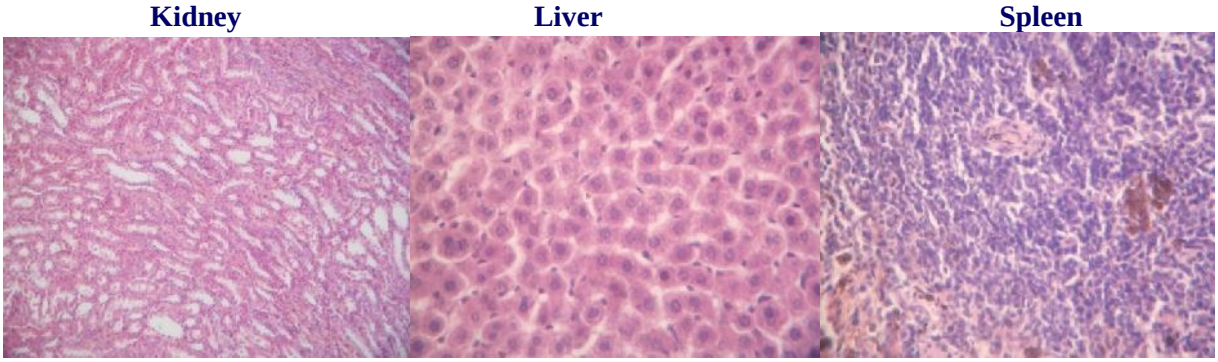
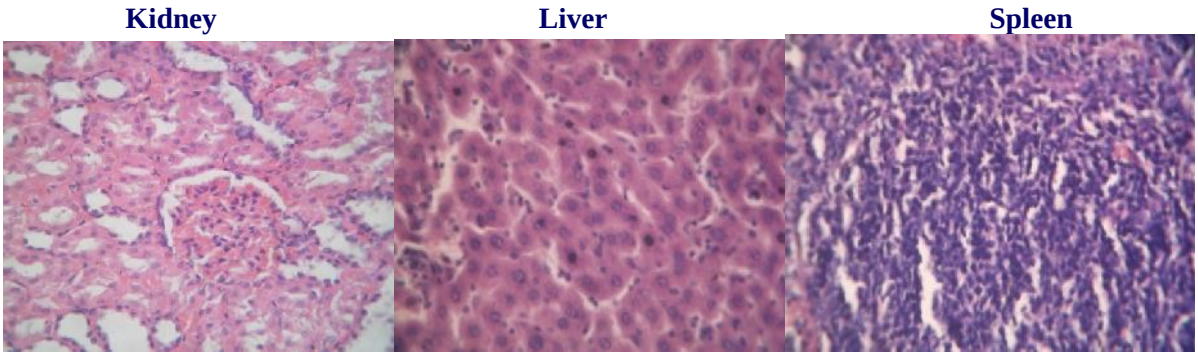


Figure 2: Histopathological representation organs of high dose treated rats in Sub-acute oral toxicity study



4. Discussion

Siddha medicines have been used for thousands of years, and their preparations are commercially available for the folk remedies or for the promotion of health. In contrast to conventional drugs, siddha medicines are regarded to be non-toxic and safe, because of their natural origin. But accumulating clinical data claim the toxicity of herbal medicine. Toxicological evaluation of siddha formulation Kalladaippu Chooranam has provided an evidence based data with respect to C.N.S, A.N.S and C.V.S system on the tested animals.KC at a dose of 5000 mg/kg had no adverse effect on the treated rats in up to 14 days of observation.

There were no significant changes in the weight and the organs of the rats. Sub-acute toxicity study were carried out in accordance with OECD 407 in which the KC were administered at three dose viz 50, 250 and 500 mg/kg/day level which was fixed based on the human therapeutic dose.

Blood a fluid connective tissue involved in supply of nutrient's and also drug to the target organs often exposed to the ill effect of certain cytotoxic drugs. Most of the siddha preparation seems to be safe with hematopoietic system but certain drugs and chemicals which particularly have tendency to disturb the bone marrow and causes hemolysis disturbs the cardiovascular physiology. The haemopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological status in both humans and animals [9]. The hemo toxic nature of the drug exerted by the fluctuation in blood cell count in particular to RBC and WBC cells. Low hemoglobin content reflects the low level of RBC which in turn affects the oxygen carrying capacity of the blood. At the end of the most of the toxicity studies the blood collected from the animal before sacrifice will be subjected to whole blood analysis and also to serological analysis.

The hematological parameters such as Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Packed Cell Volume (PCV), 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 between control and treated groups showed that treatment with KC was non-toxic to the haemopoietic system.

Additionally, most of the biochemical parameters were not altered. No relevant changes were found in levels of SGOT, SGPT, urea, BUN and creatinine which are good indicators of liver and kidney functions. The lack of significant alterations in the levels of ALT, AST, ALP, creatinine, and uric acid, which are good indicators of liver and kidney functions [10].The body weight changes serve as a sensitive indication of general health status of animals [11]. After 28 days of treatment of the test drug KC, all the animals exhibited a normal increment in body weight. It can be stated that treatment with KC did not interfere with the normal metabolism of animals. The significant increment in food and water intake is considered as being responsible for augmentation in body weight gain. No gross lesions were found in histopathology examinations of kidney, liver and spleen samples of the drug treated (KC 500mg/kg) rats when compare to that of the control group animals.

5. Conclusion

The basic rationale of using rodents as ideal model for prediction of toxicity is all because of their genomic resemblance, drug metabolism and response towards toxic chemicals and drugs are almost similar to that of the humans. It was observed from the results of the acute toxicity study that treatment with KC at the dose of 5000mg/kg did not alters any of the physiology and behavioral pattern of the rats. Further there was no mortality observed for the period of 14 days. Similarly in sub-acute toxicity study there is no significant changes were observed in the body weight, food intake, water intake, hematological and serological profiling of the treated rats. From the results of the present investigation it was concluded that the siddha formulation Kalladaippu Chooranam was safe for both short and long term usage.

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