Acute And Sub Acute Toxicity Study on Siddha Drug Kadhalipoo Rasayanam

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Abstract- *The polyherbal formulation kadhalipoo rasayanam has been used for the treatment of menorrhagia. Menorrhagia is one of the major bleeding disorders in clinical practice. As a mandate, steps were taken to evaluate safety profile of kadhlipoo rasayanam in rats using OECD guidelines. In acute oral toxicity study, a single dose of KR was administered and observed for 14 days. The results of acute toxicity study of revealed no mortality,abnormal signs behavioral changes in rats at the dose of 2000 mg/ kg body weight. Sub acute toxicity studies were carried in three different groups in which KR was administered orally to rats once daily for 28 days in various doses ranging from 100 and 200mg/ kg body weight respectively. Detailed haematological, biochemical, necropsy and histopathological evaluation of organs were performed for all animals. Histopathological analysis revealed that brain, heart, kidney, liver, lung tissues of treated groups did not show any signs of toxicity. No toxic effect was observed in acute and sub-acute toxicity studies of kadhalipoo rasayanam.*

Keywords- Kadhalipoo rasayanam, Menorhagia, Acute toxicity, Sub-acute toxicity

I. INTRODUCTION

Menorrhagia is the most common type of abnormal uterine bleeding characterized by heavy & prolonged menstrual bleeding which affects both the physical and mental health of women.[1] Totally 30% of world wide population having heavy menstrual blood loss. Among them around 5% seek medical care for excessive blood loss. And 50% have no organic pathological cause and diagnosed as DYSFUNCTIONAL UTERINE BLEEDING. Other common causes are hormonal imbalance, bleeding disorders or stress related disorders.

In India, about 20% of DUB cases are seen among adolescent girls and 40% of cases among women above 40 years of age.

A descriptive study was conducted in KEM Hospitals, Mumbai (India), to assess the incidence of DUB among women who underwent hysterectomy. The study revealed that total hysterectomies were for benign conditions were 719 cases out of which 357 hysterectomies were performed for DUB alone forming the incidence of 49.65%.[2]

As hysterectomy is very cost effective and there is no permanent treatment.The Siddha drug KADHALIPOO RASAYANAM mentioned in the classical literature BOGHAR 700 to give best solution for menorrhagia.[3] The pre-clinical toxicity studies were essential for determining a safe dose for human trials.Consequently an effect was made to evaluate acute and sub-acute toxicity of the herbal siddha formulation Kadhalipoo rasayanam in laboratory animals.

II. MATERIALS AND METHODS

Aim:

Aim of the study is to evaluate the acute and sub-acute toxicity of the siddha drug Kadhalipoo rasayanam.

Source of raw drugs:

The required raw drugs are procured from a well reputed indigenous raw drug shop. The raw drugs taken for study will be authenticated by the Pharmacognosist of Gunapadam Dept, Govt. Siddha Medical College, Chennai.

Ingredients

Kottam ver(*Costus speciosus*), Kandathippli(*Piper longum*), Sukku(*Zingiber officinale*), Sathikkai(*Myristica feagrans*), Lavagam(*Syzygium aromaticum*), Chiragam(*Cuminium cyminum*), milagaranai(*Toddalia asiatica*), Changan(*Azima tetracantha*), kathalipoo(*Musa paradisiaca*), Sugar, Milk, Honey.

Reference : Bhogar 700, (Page no-16-17)

Preparation:

The purified raw drugs are made in to fine powder, then adding sugar and boiled with milk till the correct constituency is obtained.Then honey added and stirred well. Finally made

into rasayanam form and the drug is stored in clean dry air tight container.

Animals

Rats of female sex weighing 150-200 g were obtained from the animal house of K.K college of pharmacy. The animals were used with the approval of the Institute animal ethics committee (KKCP/2013/009) and obtained from k.k.college of Pharmacy Chennai. They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-28° C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum. Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group. The animals were acclimatized for one week under laboratory conditions.

Acute oral toxicity-experiment procedure: [4]

Acute toxicity studies were carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Healthy female rats, weighing 150–200 g, were selected and oral administration of the single doses of *Kadhali Poo rasayanam* were done aseptically by suspending in 1% SCMC (Sodium carboxymethyl cellulose).

Administration of doses:

Kadhali Poo rasayanam in 1% SCMC was administered as a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. An oral (p.o) dose of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviors of the rat were continuously monitored for 1 h after dosing, periodically during the first 24 h with special attention given during the first 4 hours and then daily thereafter, for a total of 14 days. Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which signs of toxicity or mortality were recorded.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 12 h prior to the administration of the test substance. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels:

Three animals are used for each step. The dose level used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level was most likely to produce mortality in some of the dosed animals. The available information suggests that mortality is likely at the highest starting dose level 2000 mg/kg body weight, so the trial or limit test was conducted. The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs.

Limit test:

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out. [(2)]

OBSERVATIONS

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somato motor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The principles and criteria

summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death should was recorded

Table 1: Dose finding experiment and its behavioural signs of toxicity of kadhalipoo rasayanam

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2000 mg/kg	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-

1.Aggressiveness 2.Alertness 3.Alopecia 4.Circling 5.Diarrhoea 6.Edema 7.Eye closure at tough 8.Grip strength 9.Grooming 10.Lacrimation 11.Loss of writing reflex 12.Mortality 13.Nasal sniffing 14.Piloerection 15.Rearing 16.Righting 17.Reflex 18.Seizures 19.Straub tail 20.Urine stains

III. RESULTS AND DISCUSSION

The trail drug Kadhalipoo Rasayanam was administered to wistar albino rats at the dose of 2000mg/kg. They were observed for a period of 14 days in the 14 days observation mild alopecia was observed in all the three animals. The animals recovered from alopecia at the end of 14 days. Based on OECD 423 the drug is considered to be non toxic upto the dose of 2000mg/kg.

sub-acute oral toxicity study of *Kadhali Poo Rasayanam* on rats(OECD – 407 guidelines[5]

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). *Kadhali Poo Rasayanam* was administered to rats at the dose of 100 & 200 mg/kg/day for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection.

Justification for Dose Selection:

The results of acute toxicity studies in mice indicated that *Kadhali Poo Rasayanam* was non toxic and no behavioral changes was observed up to the dose level of 200mg/kg and 100 mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose:

Kadhali Poo Rasayanam at two doses level respectively was suspended in of 1% SCMC in distilled water. It was administered to animals at the dose levels of 100 and 200 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

IV. METHODOLOGY

Randomization, Numbering and Grouping of Animals:

Ten Rats (Five Male and Five Female) in each group randomly divided into three groups for dosing up to 28 days. Animal's acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliporous and non-pregnant.

OBSERVATIONS

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0 and at 5 days intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

V. TERMINAL STUDIES

***Laboratory Investigations*:**

Following laboratory investigations were carried out on day 29 in animals' fasted over-night. On 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for

immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 ℃ for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

***Haematological Investigations*:**

Blood samples of control and experimental rats was analyzed for haemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Mean corpuscular volume (MCV) and packed cell volume (PCV). From the estimated values of RBC count (millions/mm3) and PCV (volumes percent), mean corpuscular volume (MCV) was calculated.

***Biochemical Investigations*:**

Serum was used for the estimation of biochemical parameters. Samples of control and experimental mice were analyzed for protein, bilirubin, urea, uric acid, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, adrenals, spleen, brain, heart, uterus and testes/ovaries were recorded. The relative organ weight of each animal was then calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \times 100$$

Histopathology:

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 200 mg/kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

The organs included brain, heart, kidneys, liver and lungs of the animals were preserved they were subjected to histopathological examination.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way Anova. Followed by dunnett's test using a computer software programme. (Graph Pad Prism 5.0)

Table-2: Body weight changes of rats exposed to kadhalipoo rasayanam

Treatment	0th day	5th day	10th day	15st day	20th day	25th day	28th day	% increase
Control	175.83± 6.84	179.50± 6.28	181.83± 6.46	184.83±6.31	187.16± 6.01	190.66± 6.46	193.66± 5.70	9.79
100 mg/kg	163.16± 6.38	163.33± 6.60	165.33± 6.39	167.83±6.610	169.66± 6.99	171.16± 7.32	174.83± 7.37	6.67
200 mg/kg	150.83± 9.58	150.66± 9.09	153.50± 9.58	155.01±9.56	157.33± 9.54	159.53± 9.31	163.16± 9.67	7.56

Table-3:Effect of kadhalipoo rasayanam on Organ weight in rats

	Relative Organ Weight of rats					
Dose	**Liver**	**Kidney**	**Brain**	**Lungs**	**Heart**	**Spleen**
Control	2.8± 0.1	0.66±0.02	0.38±0.22	0.29±0.01	0.29±0.01	0.15±0.01
100mg/kg	2.88 ±0.1	0.645± 0.02	0.422± 0.01	0.320± 0.02	0.311± 0.01	0.167± 0.01
200mg/kg	3.01 ±0.1	0.661± 0.03	0.422± 0.01	0.351± 0.01	0.321± 0.01	0.156± 0.01

Table-4: Effects of kadhalipoo rasayanam onHaematological Parameters in rats

Haematological parameter	Control	*Trial drug*	
		100 mg	200mg
Total R.B.C. count ($\times 10^6$ mm−3).	9.09±0.15	9.13±0.12	9.13±0.18
Total W.B.C. Count ($\times 10^3$ mm−3).	12.67±0.22	12.42±0.688	12.23±0.812
Haemoglobin (Hb) (g/dl)	15.61±0.36	15.877±0.176	15.68±0.78
Hematocrit (%).	44.21±1.01	42.42± 0.952	44.7±1.68
Platelets (×103 mm−3).	834.91±24.01	845.21±16.55	833.58±16.68
Lymphocytes (%).	84.7±1.32	79.28±2.67	82.8±5.49
Neutrophils (%).	20.6±0.65	19.6±1.277	20.952±0.66

Data are expressed as mean ± SEM

Table-5:Effect of kadhalipoo rasayanam on biochemical Parameters in rats

Biochemical parameter	Control	*Trial drug*	
		100 mg	200mg
Creatinine (mg/dl)	0.5890±0.079	0.56±0.04	0.54±0.11
Urea (mg/dl)	15.30 ± 0.47	15.01±0.49	15.17±1.078
Triglycerides (mg/dl)	52.20±1.13	51.73±1.98	49.89±2.86
Total Cholesterol (mg/dl)	46.60±1.21	50.460±0.98	50.66±1.05
Total protein (mg/dl)	4.40±0.26	4.72±0.35	4.76±0.765
Albumin (g/dl)	3.20±0.41	3.29±0.75	3.19±0.85
AST (IU/L)	121.41±2.68	119.3±1.67	119.76±3.065
ALT (IU/L)	69.40±1.57	69.712±2.32	69.72±1.558
ALP (IU/L)	112.6±4.67	115.01±1.021	113.41±4.108
T. Bilirubin (mg/dl)	0.2569±0.32	0.254±1.012	0.254±01.82

Data are expressed as mean ± SEM

VI. RESULTS

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for sub acute toxicity study. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days.

The results of haematological investigations such as Erythrocytes, Total leucocytes and Platelets count conducted on day 29, revealed no significant changes in the values when compared with those of respective controls. This gave clear justification that bone marrow and spleen were not influenced by *Kadhali Poo Rasayanam*.

Results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT,SGPT, Bilirubin were within the limits.

The other cardio vascular risk markers were also within normal ensured that *Kadhali Poo Rasayanam* did not influence the Cardio vascular system. Urine analysis data of control group and treated group of animals determined in week 4 did not reveal no abnormalities.

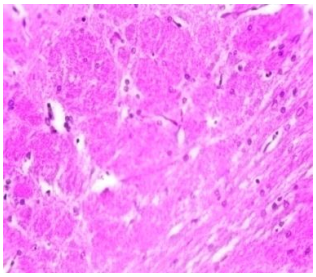
Group Mean Relative Organ Weights (% of body weight) are recorded Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable with respective control group. Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities.

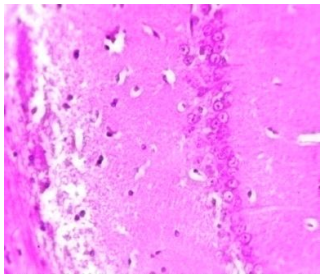
Histopathology: The vital organs such as liver, heart, kidneys, lungs and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Cross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.

VII. HISTOPATHOLOGY OF VITAL ORGANS

Low dose 100mg/kg
High dose 200mg/kg

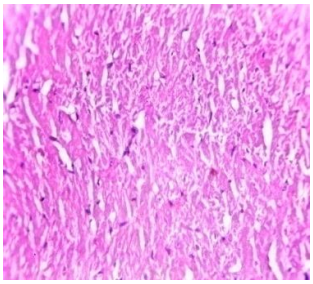
BRAIN

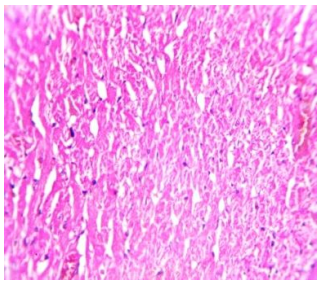




Cerebellum – Normal
Cerebellum – Mild gliosis

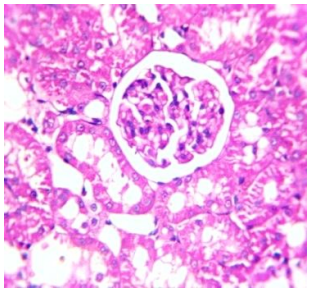
HEART

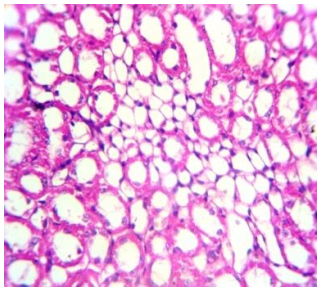




Normal Cardiomyocytes
Normal Cardiomyocytes

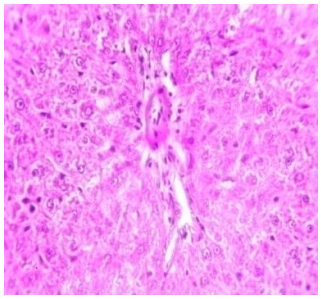
KIDNEY

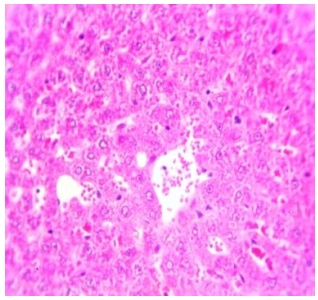




Cortex – Normocellular glomeruli
Cortex – Normocellular glomeruli

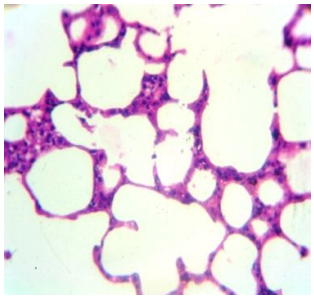
LIVER

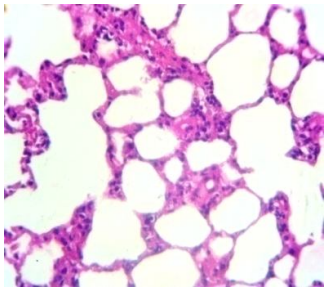




Normal
Periportal zone – Normal

LUNG





Normal
Normal

VIII. CONCLUSION

From these studies the authors conclude that the acute and sub-acute toxicity studies of Kadhalipoo Rasayanam

revealed no toxicity by oral route over a period of 28 days.So the siddha medicine can be prescribed therapeutically up to a human dose of 5gm, in menorrhagia women as per the dosage recommended in literature.

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