Research Article

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ACUTE TOXICITY STUDY OF A SIDDHA FORMULATION KANDHAGA RASAYANAM IN RATS

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ABSTRACT

Kandhaga Rasayanam, a compound Siddha herbo-mineral formulation is indicated for skin diseases, arthritis, venereal diseases, Urinary tract infections etc. So far no safety profile has been published for this formulation. Hence, in the present study acute toxicity study for Kandhaga Rasayanam was carried out according to WHO guidelines 2000. The acute toxicity study was conducted by administering ten times the therapeutic rat dose as a single dose to overnight fasted rats. The observations such as body weight changes, food and water intake as well as cage side observations were reported. There was no mortality reported till the end of 14 days observation at the highest dose. This shows that the drug is safe at the maximum dose in rats. However chronic toxicity studies has to be conducted to establish a safety profile.

Key words: Siddha, Herbo-mineral, Kandhaga Rasayanam, Acute toxicity study, WHO guidelines.

INTRODUCTION

Kandhaga Rasayanam (KR) is a sastric Siddha medicine which is in practice for more than 3 decades. So far no toxicological data are available for this formulation. It is a herbo-mineral preparation chosen from the classic Siddha text Siddha Vaidhya Thirattu. The medicine is indicated for skin diseases, arthritis, venereal diseases, and urinary tract infections etc[1]. Sulphur, the main ingredient is a potent antimicrobial agent. The GC-MS analysis of the drug Kandhaga Rasayanam was reported. It has 24 chemical constituents.[2] Preliminary phytochemistry and physico chemical analysis results were reported by Meena *et al*[3]. Till date, no safety profile is established for this formulation. Clinically no adverse drug reactions are reported so far. But to validate the safety of the drug scientifically, the present study was designed. The objective of this study was to evaluate the acute toxicity by single dose administration in rats. The toxicity study results of each individual raw drug of the compound formulation KR was compiled in a review article by the authors[4].

MATERIALS AND METHODS

Trial drug preparation

The raw drugs were purchased from reputed raw drug stores in Chennai. All the herbal ingredients were identified by Assistant professor, Department of Medicinal botany of National Institute of Siddha, Chennai. Sulphur was authenticated by Research Officer, Department of Chemistry of Siddha Central Research Institute, Minisrty of AYUSH, Government of India, Arumbakkam, Chennai. All the raw drugs were purified as per the methods mentioned in Siddha literature. The list of ingredients, their scientific names, part used and quantity are given in table 1 below:

Table 1: Ingredients of Kandhaga Rasayanam

S.No	Tamil name	Botanical name/ Chemical name	Part used	Quantity
1.	Kandhagam	Sulphur		350 grams
2.	Amukkara kizhangu	*Withania somnifera.*Dunal	Root tuber	175 grams
3.	Parangi chakkai	*Smilax china.* Linn	Root	70grams
4.	Kadukkai	*Terminalia chebula.* Retz	Fruit	35grams
5.	Nellikai	*Phyllanthus emblica.* Linn	Fruit	35grams
6.	Thandrikkai	*Terminalia bellerica.*Roxb	Fruit	35grams
7.	Chukku	*Zingiber officinale.*Roscoe	Rhizome	35grams
8.	Thippili moolam	*Piper longum.*Linn	Root	35grams
9.	Milagu	*Piper nigrum.*Linn	Fruit	35grams
10.	Vaividangam	*Embelia ribes.*Burm	Seeds	35grams
11.	Ealam	*Elataria cardamomum.*Linn	Seeds	35grams
12.	Kirambu	*Cinnamomum zeylanicum.*Breyn	Inflorescence	35grams
13.	Chandhanam	*Santalum album.*Linn	Wood	35grams
14.	Kadalai	*Cicer arietinum.*Linn	Seeds	35grams
15.	Senkottai	*Semecarpus anacardium.*Linn	Nut	35grams
16.	Chithiramoolam	*Plumbago zeylanica.*Linn	root bark	35grams
17.	Sugar			Sufficient quantity
18.	Honey			Sufficient quantity
19.	Ghee			Sufficient quantity

Method of preparation [5]:
The above mentioned ingredients were powdered separately and mixed together. Sufficient quantity of sugar, honey and ghee were then added.

Drugs and chemicals
The chemicals for the animal experiments were purchased from Sigma chemical Co, St.Louis, Mo, USA.

Experimental animals
Healthy adult male and female wistar albino rats 8-10 weeks old were obtained from National Institute of Nutrition, (NCLAS), Hyderabad on 12.2.2013. Animals were acclimatized for 7 days in the Animal house of National Institute of Siddha prior to the experimentation. The animals were housed in polypropylene cages (55x 32.7x 19 cms). Paddy husk was the bedding material used. The animals were maintained in a controlled environment with the temperature of 23±2°c and 12 hours dark light cycle. The humidity was set to be maintained between 50-70%. The animals were fed with standard laboratory animal feed (Nutrilab rodents) supplied by Provimi India limited and RO water *ad libitum*. Randomization procedure was followed for grouping before starting the experiment. The animal weight ranged between 100-180 grams.

Ethical aspects
The animal experimental protocol was approved by the Institution Animal Ethics Committee of National Institute of Siddha. The IAEC NO assigned was NIS/IAEC/I/2011/6(A). Date of approval: 12.12.2012. The animals were handled humanely as per CPCSEA guidelines.

Acute toxicity study
Acute toxicity study was performed as per WHO guidelines 2000 [6]. The toxicity study was carried out in the animal house of National Institute of Siddha, Chennai. The trial drug was dissolved in 10% Carboxy Methyl Cellulose. A normal control group and a vehicle control group were maintained. Vehicle control group received 10% CMC alone. For acute toxicity three groups were maintained. The experimental group received 10 times the clinical therapeutic dose of Kandhaga Rasayanam in 10% CMC. A single dose was given orally with the aid of gavage needle.

Groups
Each group was constituted with 5 animals per sex. A normal control group and a vehicle control group were maintained. The animals in normal control group received 5ml/kg b.wt. of normal saline. Vehicle control group received 10% CMC alone. The experimental group received 10 times the clinical therapeutic dose of Kandhaga Rasayanam in 10% CMC.

Dose calculation
Clinical dose of Kandhaga Rasayanam was 2gm b.d which means 4gm/ day. The clinical dose was converted to rat dose according to the body surface area. For a rat weighing 200 gm, the dose of Kandhaga Rasayanam was calculated as 4gm x 0.018= 0.072gram (therapeutic dose). The dose for 10 times the therapeutic dose was: 0.720grams.

Experiment details
A single dose was given orally with the aid of gavage needle. Based on the body weight of the animal, the dosage of drug administration was calculated. Food was withheld for 2 hours after drug administration. The bedding material was changed on alternate days.

OBSERVATION

The animals were observed continuously for the first half an hour without disturbing the animal attention and then they were observed periodically for first 4 hours after dosing. Daily observation includes changes in skin color (blanching, cyanosis, vasodilatation) fur, mucous membrane, abdominal distension, condition of teeth, salivation, respiration (depression, stimulation, failure), behavioral pattern such as motor activity- increased (tremors, chronic convulsions, tonic extension, pilo-erection, muscle spasm, spasticity, opisthotonos, hyperesthesia, loss of rigidity reflex), motor activity-decreased (ataxia, sedation, muscle relaxation, hypnosis, analgesia, anesthesia, arching and rolling, ptosis, lacrimation, exophthalmos, diarrhea, writhing, moribund status/death), feed and water intake, signs of toxicity and mortality. Body weight was recorded once in a week. All the animals were observed for 14 days and on 15th day, the rats were sacrificed for necropsy examination. They were kept fasting for 16-18hours the before day. The internal organs and body orifices were carefully observed for morphological and pathological changes.

Statistical analysis
Results were given as Mean ± Standard Error of Mean (S.E.M). Statistical analysis was determined by one way analysis of variance. Statistical analysis: The data was analyzed by using SPSS software (version 12.0, SPSS, Chicago, IL, USA). The

RESULTS

The observations in the study were:
- Recording body weight once in a week,
- Daily recording of feed and water intake,
- Gross observation during necropsy.

There was no death or signs of toxicity developed in all groups throughout the study period. There was no significant difference in the weight of animals in group 2 and 3 when compared to group 1. There was significant increase in body weight within the groups. The observations were tabulated in Table 1. In gross necropsy, there were no changes noted in vital organs of treated group when compared to control group.

Effect of Kandhaga Rasayanam on the body weight of animals:
The body weight of rats at the end of each week was recorded in grams. There was no significant difference in body weight of KR treated group when compared to control group. This is shown in Table 2.

Effect of Kandhaga Rasayanam on feed intake of experimental animals:
The effect of Kandhaga Rasayanam on feed intake of male albino rats were expressed as a bar diagram in Figure 1. There was significant difference in the feed intake of drug treated group when compared to control in male albino rats. The feed intake was improved in the treated group.

The effect of Kandhaga Rasayanam on feed intake of female albino rats was expressed as a bar diagram (Figure 2). There was no impact of the drug on feed intake of female rats.

Effect of Kandhaga Rasyanam on water intake of experimental animals:

The effect of Kandhaga Rasayanam on water intake of male albino rats were expressed as a bar diagram (Figure 3). From the figure 3, it is evident that there was no significant difference in water intake of male rats of KR treated group when compared to normal control. The water intake of vehicle control group was slightly increased at the end of the study.

The effect of Kandhaga Rasayanam on water intake of female albino rats were expressed as a bar diagram (Figure 4). The water intake of female rats with KR was increased gradually from the start of the study till end of 14th day. There were fluctuations in the water intake of rats, which might be attributed to the external environment and other factors.

Table 2: Effect of Kandhaga Rasayanam on the body weight of animals in acute toxicity study

Treatment	No. of animals (male:5, female: 5)	0th day	Week 1	Week 2
Group 1(normal control)	10	156.10 ± 5.675	167.40 ± 7.090	178.40 ± 6.877
Group 2 (vehicle control-10% CMC)	10	155.90 ± 6.456	171.80 ± 10.050	185.40 ± 10.135
Group 3 (10 times the dose of KR)	10	153.10 ± 3.016	173.00 ± 4.279	184.10 ± 5.409

Values are expressed as Mean ± Standard Error of Mean. Significance with Turkey's test followed by one way ANOVA is evaluated as * p< 0.5, **p< 0.01, ***p<0.001 versus group 1 (normal control)

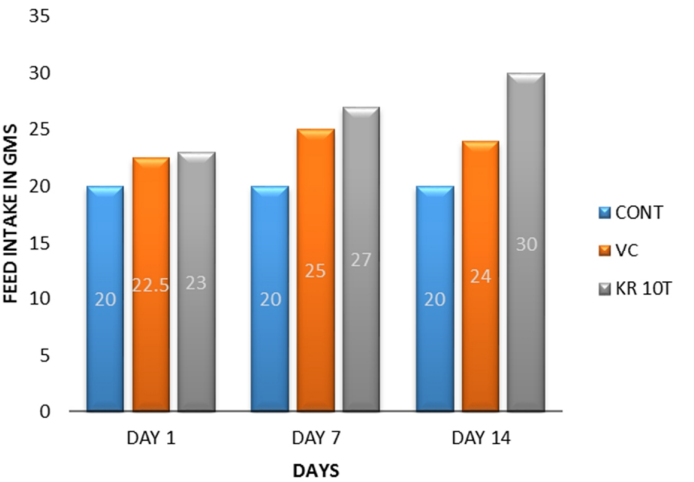

Figure 1: Effect of Kandhaga Rasayanam on feed intake of male albino rats- Acute toxicity study

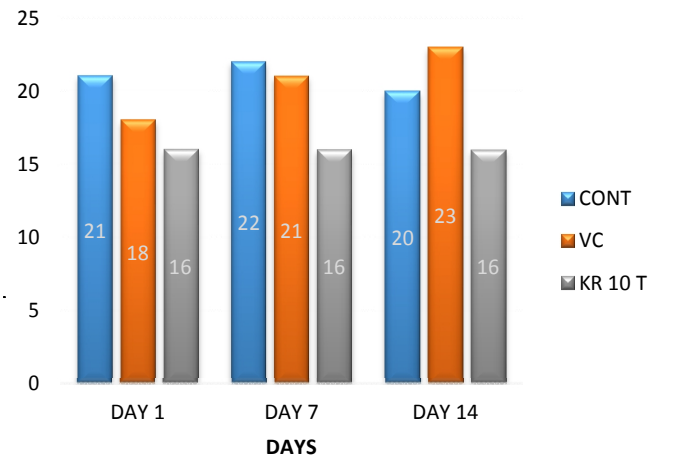

Figure 2: The effect of Kandhaga Rasayanam on feed intake of female albino rats- Acute toxicity study

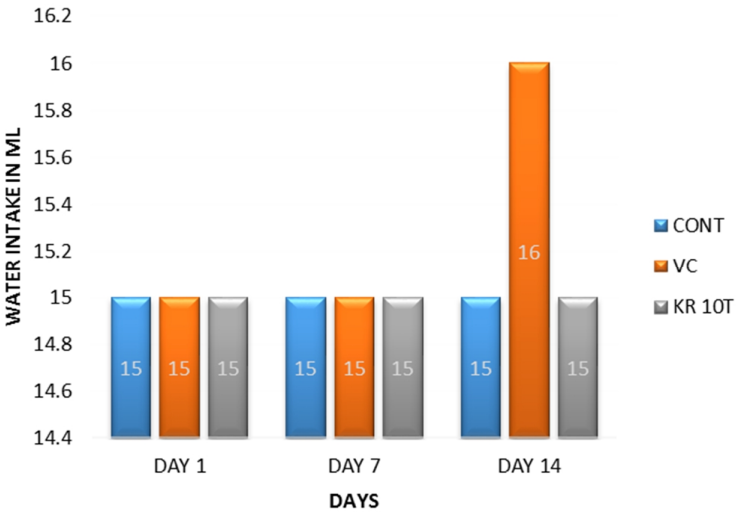

Figure 3: The effect of Kandhaga Rasayanam on water intake of male albino rats-Acute toxicity study

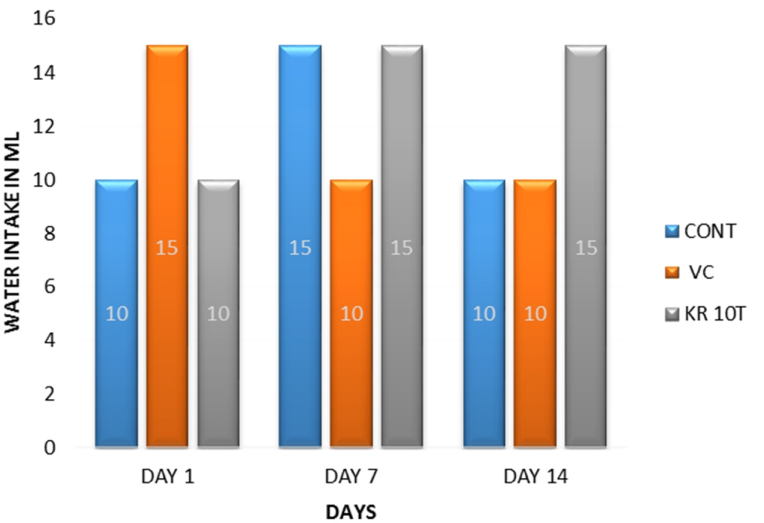

Figure 4: The effect of Kandhaga Rasayanam on water intake of female albino rats-Acute toxicity study

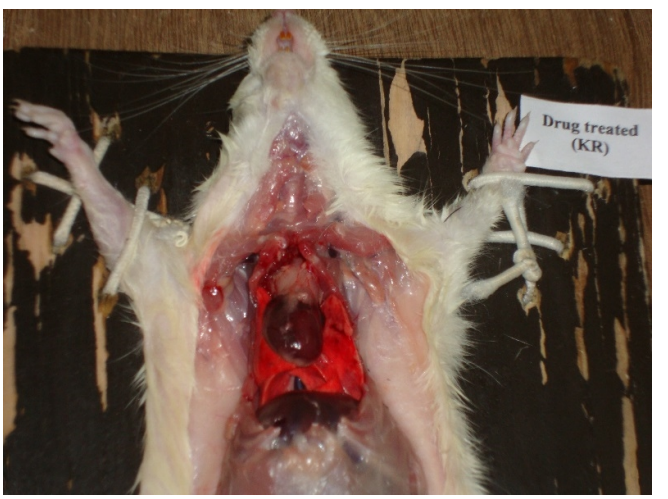

Figure 5: Necropsy of rat in treatment group

DISCUSSION

A drug which is intended to be used therapeutically in humans should be subjected to toxicity studies for safety concern. The study drug Kandhaga Rasayanam has been in use for many decades and so far no adverse reactions are reported. But, to have documentary evidence the acute toxicity study was conducted. In acute toxicity a single oral dose (10 times the therapeutic dose-0.720grams) was administered. The observations were made for 14 days. Reduction in body weight is the first indicator of toxicity. Here, in this study there was no significant difference was noted in body weight of experimental group when compared to normal control group. The feed and water intake showed no significant differences. The minor changes and fluctuation in feed and water intake variation is considered physiological. Since there was no treatment associated death, changes in home cage behavioral activity and other observations, we can assume that KR is a safe drug for single dose administration at higher dose. The gross necropsy also showed no gross changes in the organs of any study group. Though there are reports of toxicity on ingredients of KR, the compound formulation as a whole is safe in rats. It has been reported that *Semecarpus anacardium* nut oil extracts exhibit nephro- toxicity (50% W/V) in groundnut oil [7,8]. *Semecarpium anacardium* toxins lead to acute renal failure due to hemodynamic effects [9]. Jaila El Malti et al has reported in a study that *Elettaria cardamomum* produces toxicity at 0.3 mg/ g b.w.of mouse. It also affects the energy metabolism and oxidative stress [10]. No toxicity was reported in previous studies for other ingredients.

CONCLUSION

From the above observations, it is clear that the drug Kandhaga Rasayanam is non-toxic at the dose of 0.720 grams in rats. However, long term toxicity studies have to be carried out to establish a safety profile. Further trials in humans will add significance to the toxicity profile.

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