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EVALUATION OF ACUTE TOXICITY STUDY AND PHYSICO-CHEMICAL PROPERTIES OF MURUVILI KUDINEER

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ABSTRACT

The present study was designed to investigate one such Siddha formulation *Muruvili Kudineer*. It is herbo mineral formulation of a Siddha medicine and has been used for various herbs and one mineral. To evaluate its safety, acute oral toxicity studies were performed following studies organization for economic co-operation and development (OECD) test guidelines 423. Acute oral toxicity was carried out in sexually mature female wister albino rats (150-200gm) and the animals were treated more than ten times with the therapeutic dose of *Muruvili Kudineer* and they were observed for toxic signs for 14 days. Acute oral toxicity study showed no lethality in experimental animal with the ten times of therapeutic dose. The *Muruvili Kudineer* proves to be a safe medicine. The Physico chemical evaluation of *Muruvili Kudineer* is also proves to be a safe medicine.

KEYWORDS: *Muruvili Kudineer*, Physico chemical, Acute toxicity study, wister albio rats.

INTRODUCTION

Siddha system of medicine is a great heritage of India. It is developed by the siddhars, the ancient supernatural spiritual saints of India. The Siddha system of medicine uses a fascinating combination of herbs, minerals, metals to promote good health and longevity. Siddha system is an earliest medical that stress on positive health, a harmonious blending of physical, mental, social, moral and spiritual. *Muruvili Kudineer* is a sastric Siddha medicine. So far no toxicological data are available for this formulation. It is a herbo mineral preparation chosen from the classic sidha text (*Kuzhanthai Maruthuvam) Balavagadam*. The medicine was chooses for treatment and management of the Singimantha kanam was *Muruvili Kudineer* 15-30ml internally, twice a day after food described in *Balavagadam (Kuzhanthai Maruthuvam)*. The Physico chemical analysis shows PH is 4.8, Total ash value 09.06%. Acute toxicity studies shows, it has no significant toxic effect.

MATERIALS AND METHODS

Drugs Authentication and Preparation

Muruvili Kudineer is a herbomineral formulation comprising of fifteen type of herbs and one type of mineral, that is,Muruvili (*Cuscuta reflexa*), Vengayam (*Allium cepa*), Vellai ver (Root of *Gynandropis pentaphylla*), Keezhanelli ver (Root of *Phyllanthus amarus*), Nannari ver (Root of *Hemidesmus indicus*), Vishnukaranthai (*Evolvus alsinoids*), Nelli vatral *(Phyllanthus emblica*), Kadukkai (*Terminalia chebula*), Narathai ver (Root of *Citrus medica*), Nilaposani ver (Root of *Ipomoea mauritiana*), Parsorti *(Ruellia secunda*), Vasambu (*Acorus calamus*), Kuratai ver (*Trichosanthes tricuspidata*), Ellumichai ver (Root of *Citrus lemon*), Malaithangi ver (Root of *Sida acuta*), Indhuppu (*Sodium chloridum impura*). The drugs were identified and authenticated by Medicinal botany department in Government Siddha Medical College, Arumbakkam, Chennai-106. The purified raw drugs are made into coarse powder, then the coarse powder is taken in a mud pot, 60ml of water is added and heated, till it is reduced into 30ml.

Animals

Animals Albino rats (wister stain) of either sex weighing 160-200g were used in the study. The animals were kept in polypropylene cages and maintained by providing balanced food and water added libitum. Experiments were performed complied with the rulings of the committee for the purpose of control and supervision of experiments on animals, New Delhi India. The present study was approved by the Institutional Animal Ethical Committee. C.L. Baid metha college of pharmacy, Thoraipakkam, Chennai 97.

Acute Oral Toxicity Study of Muruvili Kudineer (Oecd Guideline – 423)

Introduction

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.
- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.
- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

Principle of the Test

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

- No further testing is needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology

Selection of Animal Species

The preferred rodent species is the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions

The temperature in the experimental animal room should be 22ºC ± 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of Animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Test Animals and Test Conditions

Sexually mature Female Wistar albino rats (150-200gm) were obtained from TANUVAS, Madhavaram, and Chennai. All the animals were kept under standard environmental condition (22±3 ° C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore)

Preparation of Acute Toxicity Study

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, **Muruvili Kudineer**

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design IAEC No: XLVIII/07/CLBMCP/2017.

Test Substance	**Muruvili Kudineer**
Animal Source	TANUVAS, Chennai.
Animals	Wister Albino Rats (Female-3+3)
Age	>6 weeks
Body Weight on Day 0	150-180 gm.
Acclimatization	Seven days prior to dosing.
Veterinary examination	Prior and at the end of the acclimatization period.
Identification of animals	By cage number, animal number and individual marking by using Picric acid.
Number of animals	3 Female/group,
Route of administration	Oral
Diet	Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	Aqua guard portable water in polypropylene bottles.
Housing & Environment	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	Between 22ºC + 3ºC.
Relative humidity	Between 30% and 70%,
Air changes	10 to 15 per hour and
Dark and light cycle	12**:**12 hours.
Duration of the study	14 Days

Administration of Doses

Muruvili Kudineer was administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 2000 mg/kg body weight 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 as per the guideline after drug administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitered for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Observations

Animals are 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 will be taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanly killed. When animals are killed for humanly reasons or found dead, the time of death was recorded.

Acute Oral Toxicity of Muruvili Kudineer

Table 1: Dose finding experiment and its behavioral.

S. No.	Group Control	Observation	S. No.	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	Normal	3	Signs of Convulsion,	Absence of sign (-)
	Limb paralysis			Limb paralysis	
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	7	Change in skin color	No significant color
		color change			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

Signs of acute oral Toxicity Observation done

Behaviour

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convolusion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhea, leathery, sleep and coma.

Body Weight

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

Food and Water Consumption

Food and water consumed per animal was calculated for control and the treated dose groups.

Mortality

Animals were observed for mortality throughout the entire period.

RESULTS

All data were summarized in tabular form, (Table-1-5) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight changes, food and water intake.

No of animals in each group: 3.

Table 2: (Observational study Results).

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.	**Control**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	**2000 mg/kg**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19. Respiration 20. Mortality.

(+ Present, - Absent)

Table 3: (Body weight Observation).

Dose		Days	
	1	**7**	**14**
Control	176.21± 3.22	177.2± 4.27	179.2 ± 4.82
2000 mg/kg	172.5± 3.18	174.2± 3.26	175.4 ± 3.27
P value (p)*	NS	NS	NS

Table 4: Water intake (ml/day).

Dose		Days	
	1	6	14
Control	36.7 ± 2.74	30.9± 4.33	31.4± 4.13
2000 mg/kg	30.4±1.34	34.5±1.11	36. 9± 4.19
P value (p)*	NS	NS	NS

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

Table 5: Food intake (gm/day).

Dose	Days		
	1	7	14
Control	32.56±2.16	32.92±3.26	30.92±3.26
2000 mg/kg	34.12±8.64	34.31±1.22	35.22±2.24
P value (p)*	NS	NS	NS

Physico chemical Evaluation

Percentage Loss on Drying

10gm of test drug was accurately weighed in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100.

Determination of Total Ash

3 g of test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/Wt of the Crude drug taken X 100

Determination of PH

Sample being oily in nature the direct litmus evaluation method was adopted to check the pH of the sample.

Determination of Specific Gravity

Fill the dry specific gravity bottle with prepared samples in such a manner to prevent entrapment of air bubbles after removing the cap of side arm. Insert the stopper, immerse in water bath at 50^{O}C and hold for 30 min. Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side and quickly weigh. Calculate the weight difference between the sample and reference standard.

Determination of Iodine Value

About 20 gm of oil was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 minutes. Followed by this about 25 ml of Wiji's solution was added in the same flask and shaken well. The flask was allowed to stand for 30 mins and refrigerated for anhour. About 10 ml of KI solution was added to this and titrated against 0.1 N Sodium thiosulphate solutions until the appearance of yellow colour. 1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue colour indicates end point. Repeat the above procedure without taking sample and note the corresponding reading for blank titration.

Determination of Saponification Value

About 2 gm of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added to the round bottomed flask. Repeatthe same procedure with out taking the sample for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.

Table: 6 Physico chemical evaluation report of Muruvili Kudineer.

S. No.	Parameter	Result
1.	Specific gravity	1.021g/cm3
2.	Viscosity at 50° C	0.6533 mPa.s (millipascal-second)
3.	Refractive index	2.32
4.	Weight per ml (gm/ml)	1.52±0.33
5.	Iodine value	
6.	Saponification value	
	(mg of KOH to saponify 1gm of fat)	
7.	Total iron content (mg/ml)	0.235mg/dl
8.	Loss on drying at 105° c	11.23 % by mass
9.	Total ash	09.06%
10.	PH	4.8

RESULTS AND DISCUSSION

Physi-chemical properties of the *Muruvili Kudineer* are shown in table:6. The loss on drying value obtained is indicative amount of moisture content present in drug. The loss on drying at 105ºc in *Muruvili kudineer* found to be 11.23% by mass. The total ash value was 09.06% and its an indicative total amount of inorganic material after complete incineration. The acid insoluble ash indicates improper washing of drugs. Physico chemical analysis shows ph is 4.8 and total ash value is 09.06 is a safety profile of the *Muruvili Kudineer*.

DISCUSSION

The study drug *Muruvili Kudineer* has been in use for many decades and so far no adverse reactions are reported. In Acute toxicity study a single oral dose (ten times the therapeutic dose) was administered. The observation were made for 14 days. Reduction in body weight is the first indicator is 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 difference. Since there was no treatment associated death, changes in home cage behavioural activity and other observations, we can assume that *Muruvili Kudineer* is a safe drug for single dose administration at higher dose.

CONCLUSION

From the above observations, it is clear that the drug *Muruvili Kudineer* is Non toxic in rats. Further trials in humans will add significance to the toxicity profile.

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