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ACUTE TOXICITY STUDY OF A SIDDHA HERBAL FORMULATION VALIKANAKUDINEER

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ABSTRACT

The present study investigated acute toxicity of Valikana kudineer, a Siddha herbal formulation indicated for the valikanam (Acute pharyngitis) in experimental animal model. Valikana kudineer contains *Vilva ilai (Agle marmoles), Vendhayam (foenum gracum), Narseeragam (cuminum cyminum), Eera vengayam (Allium cepa).* The aim of the study is to evaluate the safety of the Valikana kudineer through acute toxicity study. In an acute toxicity study the drug was administrated orally at a dose 15ml twice a day and the animals were observed for any toxic symptoms upto 72 hours.

KEYWORDS: Valikana kudineer, Acute toxicity, Siddha medicine.

INTRODUCTION

Siddha system is a well known traditional systems of medicines always played important role in meeting the global health care needs. The term **"Siddha"** means **"Achievements"** and "**Siddhars"** were "**saintly persons"** who achieved results in medicine. Eighteen siddhars were said to have contributed towards the development of this medical system.

Lord Shiva who unfolded the knowledge of siddha system of medicine to his concert Parvati who handed it down to Nandhi deva and from Himto the Siddhars. Siddhars adapted principles of Saiva Siddhantham.

Siddhars classified diseases in different categories which accounts for 4448 diseases in human body.

Agathiyar was considerd the foremost Siddhar with his later Lord Subramaniyar.

According to the ancient Siddha text, the human body is made up of several elements. It is amicroscopic componant of the universe. The elements that form the human body are the Earth (Mann), Fire(thee), Water(neer), Air(vayu) and Space (akasam).

Additionally, There are three humors or the DOSHAS called,

- Vata
- Pitta
- Kapha

Siddha medicine believes that diseases occur when there is a disequilibrium or imbalance in these humors or if their individual hormony is disturbed.

The balance can be restored by correcting the underlying dosha by the application of the Siddha system of medicine.

The three doshas are considered the three pillars of health and support the structures and functions of the body. These tridoshas are involved in regulating all the function of the body and maintain the balance in physical, emotional and mental spheres.

As per Siddha aspect,paediatric diseases are carried from gene. It defines that the paediatric diseases occur at the time of fertilization to gestational period those paediatric diseases where classified in to **Agakkarana noigal** and **Pura karana noigal.**

Pharyngitis is being 1/3 of the primary system of the upper respiratory tract infection in children. Acute pharyngitis is the inflammation of the pharynx arise from a variety of irritants and infections.Upper respiratory tract infections(URTI) are extremely common in the children on anaverage of 6-8 times in a year.Pharyngitis is the primary symptom in 1/3 of URTI's caused by

Infective and non-infective Infections like viruses,bacteriae and fungi.

Clinical features of acute pharyngitis correlates with the symptoms of valikanam fever, cold, cough, loss of appetite, sore throat, urinary infection described in the Siddha text. In siddha Literature valikanam is one of the 24 types of "Kanam" that occurs in children. The medicine was chooses for treatment and management of the Valikanam was Valikana kudineer 15-30 ml internally, twice a day after food described in Pillaipini maruthuvam. Valikana kudineer considerably high antimicrobial activity against the tested pathogenic microorganisms as well as Anti inflammatory activity. The aim of the study is to evaluate the safety of the Valikana kudineer through acute toxicity study.

MATERIALS AND METHODS

Drug Authentication and preparation

Valikana kudineer is a herbal formulation comprising of 4 type of herbs that is Vilva ilai (Agle marmoles), Vendhayam (foenum gracum), Narseeragam (cuminum cyminum), Eera vengayam (Allium cepa). The drugs were authenticated by Medicinal botany department in Government Siddha Medical College, Arumbakkam, Chennai. The purified raw drugs are made into coarse powder, then the coarse powder is taken in mod pot, 60ml of water is added and heated, till it is reduced into 30ml.

Animals

Animals Albino rats (Wister stain) of either sex weighing 150-180gm were used in the study. The animals were kept in plypropylene cages and maintained by providing balanced food and water added libitum. Experiments were perfomed 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, Thuraipakkam, Chennai 97. The IAEC Approvel number XLVIII/09/CLBMCP/2017

Acute Toxicity Study

Acute Oral Toxicity-Oecd Guide Guidelines – 423

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co-operation and Development), Guideline -423 the project was completed on **13-04.2018.**

Animal: Sexually mature Female Wistar albino rats weighing 150-200gm.

Studied carried out at three female rat under fasting condition, signs of toxicity was 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.

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.

Priciple

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.

Methodoloy

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 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

Preparation for Acute Toxicity Studies
Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, **VALIKANA**

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: LI/20/CLBMCP/2017

Test Substance	**: VALIKANA 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
Valikana 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 study of Valikana Kudineer

Table 1: Dose finding experiment and its behavioral Signs of acute oral Toxicity Observation done.

SL	Group Control	Observation	SL	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

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	232.12± 1.13	232.2± 1.52	234.2 ± 1.64
2000 mg/kg	242.1± 1.38	244.2± 1.66	246.4 ± 1.60
P value (p)*	NS	NS	NS

Table 4 (Water intake (ml/day) of Wistar albino rats group exposed to (VALIKANA KUDINEER *):*

DOSE	DAYS		
	1	6	14
CONTROL	32.2 ± 1.14	32.2± 4.23	34.4± 1.43
2000 mg/kg	35.5±1.15	35.4±2.21	35. 6± 2.19
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) of Wistar albino rats group exposed to VALIKANA KUDINEER.

DOSE	DAYS		
	1	7	14
CONTROL	33.64±2.10	33.78±2.14	35.22±2.16
2000 mg/kg	36.12±2.14	36.22±1.12	38.42±1.04
P value (p)*	NS	NS	NS

Statistical Analysis

Data was expressed as mean ± standard error of mean. Significance was evaluated by one-way ANOVA followed by dunnet's test. P value less than 0.05

RESULT AND DISCUSSION

No weight loss, abnormal animal behaviors, metabolic functions (urination, lacrymination, defecation etc,) and mortality were noted on oral administration of Valikana kudineer formulation and all the animals were found to be normal during and at the end of the observation period (14days).

CONCLUSION

The study revealed that Valikana kudineer formulation at different doses did not provoke toxic effects in the animal's tissues and it was safe when administrated to Acute pharyngitis patients.

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