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AN ACUTE ORAL TOXICITY STUDY ALONG WITH HEAVY METAL ANALYSIS OF A SIDDHA FORMULATION MANJANAATHI KUDINEER

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ABSTRACT

Manjanaathi Kudineer (MKR) an herbal formulation of Siddha medicine, which is prescribed for *Neerkana Maantham* (Acute nasopharyngitis) in Children. To evaluate its safety, acute oral toxicity study was performed following OECD test guidelines 423. MKR was administered orally at 5, 50, 300 and 2000 mg/kg body weight. Animals were observed for toxic signs for 14 days. There were no significant behavioral changes and also in body weight, water intake, food intake. There is no treatment related death or toxic signs were observed. Heavy Metal Analysis was done by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. Which shows there is no traces of heavy metals. This study provides scientific validation for the safety of MKR.

KEYWORDS: *Siddha, Manjanaathi Kudineer*, Acute Oral Toxicity, *Neerkana Maantham.*

INTRODUCTION

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. [1] Siddha medicine, an alleviate system of healing which emerged in Tamilnadu and is considered to be one of India's oldest systems of medicine. The Siddha system of medicine is based on a combination of ancient medicinal practices and spiritual disciplines as well as alchemy and mysticism. It is through to have developed during the Hindus

civilization. Siddha medicines include a part of Tamil culture.[2]

Siddha system of medicine not only cures the disease but also plays a major role in increasing the immune system. The herbal preparation of the Siddha medicine can be given right from birth to prevent the illness. Thereby its plays a major role in pediatric age group by increasing their immune power. And moreover it is not harmful to their body and has no side effects. It also prevents them from further infections. Children become ill easier since they aren't built with a proper immune system. And moreover they are prone to several pathogens from the surrounding environment.

Balavagadam a literature in Siddha system deals with the children diseases. It is one of the books of Pediatric in Siddha system which describes from birth to late childhood. It also explains about the treatments of each pediatric disease under the herbal formulation which is safe for the child and also helps in increasing their immune powerby preventing them from further infections.

Maantham one of the disease affecting the child from the age group of 3months to 12years is explained. It is classified into 21types in the text *Balavagadam*. As per the Siddha literature *Neer Kana Maantham* (Acute Nasopharyngitis) is one of the types of Maantham which is caused due to the derangements of the three humours (Vatha, Pitha, and Kapha) in mother which affects the children also. It affects the upper respiratory tract causing fever, irritation of throat, lack ofappetite. It gives more trouble to the children under the age group of 2-12years. In this condition mostly children are prone to antibiotics which become resistant on continuous consumption and need a higher dosage for recovery which affect the children in future. The trial drug *Manjanaathi Kudineer* which is used to treat *Neer Kana Maantham* (Acute nasopharyngitis).[3]

MATERIALS AND METHODS

Ingredients of manjanaathi kudineer

Nuna ilai *(Morinda Tinctoria)* – 1 pidi (70gram)
Notchi thulir (*Vitex Negundo)* – 1 pidi (70gram)
Uthamani ilai (*Pergularia Daemia)* – 1 pidi (70gram)
Kazharchi ilia **(***Caesalpinia Bonduc)* – 1 pidi (70gram)
Omam (*Carum Copticum)* – 1 varagan (4gram)
Vasambu (*Acorus Calamus)* – 1 varagan (4gram)

Chukku (*Zingiber Officinale)* – 1 varagan (4gram)
Milagu (*Piper Nigrum)* – 1 varagan (4gram)
Poduthalai kaai (*Phyla Nodiflora)* – 1 varagan (4gram)
Thippili (*Piper Longum)* – 1 varagan (4gram)

Preparation of trial drug

The drugs are taken in the ratio mentioned above and are purified. Then they are grinded to the powder form and mixed with pure water and this mixture is boiled until the concentrated decoction of the ingredient is obtained.

Dose: 1 Sangalavu (8gram)- Twice a day daily

Preparation of kudineer: Add 8gram of chooranam to 60ml of water. Then boil the water till reaches to 8ml of kudineer.

Duration: 7 days

Acute oral toxicity study of *manjanaathi kudineer* (Oecd guideline – 423)[4]

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, 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, *Manjanaathi 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 approved Number	**:**	LV/09/CLBM/2018
Test Substance	**:**	*MANJANAATHI KUDINEER*
Animal Source	**:**	TANUVAS, Madhavaram, Chennai.
Animals	**:**	Wister Albino Rats (Female-3+3)
Age	**:**	6-8 weeks
Body Weight on Day 0	**:**	150-200gm.
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

Manjanaathi Kudineer was suspended in coconut water and 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 5, 50, 300 and 2000 mg/kg body weight was administered stepwise. 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 substance 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 monitored 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 somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, 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 humanly killed. When animals are killed for human reasons or found dead, the time of death was recorded.

Table 1: Dose finding Experiment and Its behavioural signs of acute oral toxicity.

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, diarrhoea, 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-1-4) 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.

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.	2000mg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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 (g) of Wistar albino rats group exposed to *manjanaathi kudineer.*

Dose	Days		
	1	7	14
Control	200.1±65.70	201.3 ± 41.11	201.6 ±02.12
High DOSE	202.3± 6.64	202.7 ±7.42	203.2 ± 2.70
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 4: Water intake (ml/day) of Wistar albino rats group exposed to *manjanaathi kudineer.*

Dose	Days		
	1	7	14
Control	64 ± 3.20	64±6.10	58.3±5.44
High dose	63.2±1.30	65.8±6.70	66.2±5.64
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 *manjanaathi kudineer.*

Dose	Days		
	1	7	14
Control	86.03±2.42	87.2±2.46	89.7±8.16

High dose	96.6±1.44	98.4±4.20	99.8±2.27
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)*

DISCUSSION

In the acute toxicity study, the rats were treated with different concentration of *Manjanaathi kudineer* from the range of 5mg/kg to 2000mg/kg. The test groups compared to the controls when observed during14 days of the acute toxicity experimental period. This dose level of *MKR* did not produce signs of toxicity, behavioural changes, Body weight and mortality. No significant alterations were observed in food and water intake. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract. In acute toxicity test, based on OECD 423 the trial drug *Manjanaathi kudineer* was found to be nontoxic at the dose level of 2000mg/kg body weight.

HEAVY METAL ANALYSIS BY AAS

Standard: Hg, As, Pb and Cd-Sigma.

Methodology

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample digestion

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard reparation

As & Hg-100 ppm sample in 1mol/L HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO3

Test report

Name of the heavy metal	Absorption max Δ max	Result analysis	Maximum limit
Mercury	253.nm	BDL	1 ppm
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

BDL-Below detection limit

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metals such as Mercury, Arsenic, Cadmium and Lead.

CONCLUSION

From the results of present study observed that there are no significant adverse effects on oral administration of *Manjanaathi Kudineer* in rats and also there is no traces of heavy metals. This stands as an assurance of safe usage at its desirable therapeutic dosage.

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