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ACUTE AND 28 DAYS REPEATED ORAL TOXICITY STUDY OF SIDDHA DRUG KARAPPAN MARUNDHU ON WISTAR ALBINO RATS

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ABSTRACT

The herbal formulation Karappan Marundhu is used for skin disease particularly for eczema. Siddha medicines are very effective in the treatment of dermatological conditions. Hence to document the safety of the drug, toxicity study has been conducted in rats following OECD guidelines. Acute toxicity studies, were carried out on female wistar albino rats as per OECD guidelines 423 and the repeated oral toxicity study was carried out on wistar albino rats of both sex as per OECD guidelines 407. Acute oral toxicity study of KM revealed no mortality at the dosage of 2000 mg/kg body weight and the median lethal dosage of KM is estimated to be above 2000mg/kg body weight. Repeated oral toxicity study of KM does not exhibit any mortality even at the high dosage of 90 mg/kg body weight during the 28 days of drug administration period in the rats. At the end of 28 days no specific changes are observed in hematological, hepatic, renal and other biochemical parameters. No gross morphological changes were observed in the organs. The KM was found to be safe in animals. No toxic effect was observed of Karappan Marundhu in acute and sub-acute toxicity studies. The above studies recommend that KM is a safest drug under intended clinical dosage in human (500mg) as illustrated in the literature Agasthiyar 2000.

KEYWORDS

Karappan, Skin disease, Siddha system and Toxicity studies.

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INTRODUCTION

Varal Karappan has been described in the Siddha literature as the disease in children. According to the Siddha texts it is characterized by severe itching, papules, pustules, oozing and foul smelling. There is no complete cure for Karappan, but it can be managed with regular medical treatment. Effective Karappan management requires a

combination of prevention and treatment. In addition to preventing eczema flare-ups by avoiding any known triggers factors, treatment is also an important part of eczema management. It is not a contagious disease. Eczema is not generally serious condition, but there is a potential to develop complications such as a secondary bacterial or fungal infection due to eczema rash. In addition it also induces psychological stress among them with social avoidance.

Many herbal formulations have been indicated for skin diseases. Most of them have been proved effective clinically. As it is one of the chronic diseases which is difficult to cure the author selected a herbal formulation Karappan Marundhu for the treatment of ezcema. The main herbs in this trial drugs are,

PerumaraPattai (Sterculia foeitida),
Kadugurohini (Picorrhiza kurroh),
Sengathaari (Capparis seipiara),
Karunseeragam (Nigella sativa),
Chitrarathai (Alpinia galanga)
SanganVaer (Azima tetracantha),
Kandangathiri (Solanum surratense).

All these drugs are individually indicated for karappan in siddha texts. Moreover it has mostly anti-inflammatory effect, antibacterial activity, antimicrobial activity, antioxidant, and antihistamine activity. But a clear picture of toxicokinetics of KM has not been studied earlier. So this article ventured to evaluate the acute and sub-acute toxicity of herbal formulation Karappan Marundhu in laboratory animals.

MATERIAL AND METHODS

Standard operating procedure

The trial drug was prepared by the Author in the Gunapadam practical laboratory of National Institute of Siddha, after getting proper authentication of raw drugs from the Medicinal botany department at NIS, Chennai 47. The trial drug was prepared by the standard operating procedure as mentioned in the protocol. The drug is stored in clean and dry air tight container. PerumaraPattai (Sterculia foetida) Chukku (Zingiber officinale) Seeragam (Cuminum cyminum) Karunseeragam (Nigella sativa) Kadugurohini (Picorrhiza kurroh) Chitrarathai (Alpinia galanga) Sengathaari (Capparis sepiaria) SanganVaer (Azimate tracantha) Kandangathiri (Solanum surratense)

All the above drugs are collected in equal quantity and dried in sun shade. Then it is powdered.

Animals for acute oral toxicity study

Wistar Albino female rats, weighing 150g to 175g were purchased from TANUVAS, Madhavaram Vertinary College, Chennai and stocked at National institute of siddha, Chennai. The animals were fed on standard rodent pellet and RO water was provided ad libitum. All the animals were kept under standard environmental condition (27+ or – 2 degree c). The animals were kept on fasting overnight before starting the experimentation.

All experimental procedures described were reviewed and approved by the Institutional Animal Ethical Committee at National institute of siddha, Chennai and the IAEC approval No. is 1248/AC/09/CPCSEA-9/DEC/2013/26.

ACUTE ORAL TOXICITY OECD 423 GUIDELINES

Acute toxicity studies were carried out according to the OECD (Organization of Economic Cooperation and Development) guidelines 423. Healthy female rats, weighing 150-175g, were selected and oral administration of the single doses of Karappan Marundhu were done.

Principle

Acute toxicity was carried out in Wistar albino rats with a single exposure of 10 times of the recommended therapeutic dose of test compound the study duration was 14 days.

Route of Administration

Oral route was selected, because it is the normal route of clinical administration.

Test Substance and Vehicle

Karappan Marundhu (KM) was brown in colour. The test substance is soluble in water, in order to obtain and ensure the uniformity in drug distribution the drug is dissolved by distilled water.

Administration of Doses

Karappan Marundhu (KM) was suspended in distilled water with uniform mixing and it was administered to the groups in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. After the fasting period, the animals were weighed and then the test substance (KM) was administered to the animals. After the substance has been administered, food was withheld for a further 3-4 hours. All the principles prescribed for the laboratory animal care were followed and then observations were made on the animals and they are recorded systematically and promptly as per the guidelines.

The control groups received equal volume of the vehicle. The animals were weighed before giving the drug. The dose level was calculated according to body weight and surface area. Since the clinical dose was 500 mgs/day it was converted to animal dose and then administered.

An oral (p.o) dose of 50mg/kg, 300 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviors of the rats were monitored for 1 h after dosing and then periodically during the first 24 h (with special attention given during the first 4 hours) and then daily thereafter, for a total period of 14 days. Animals were observed for any changes in the normal psychomotor activity, external morphology, and their body weights were monitored periodically before and after dosing the test substance. 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. After 24h, the number of survivors was noted and then they are maintained for a further period of 14 days and observations made daily. The toxicological effect of the drug was assessed on the basis of mortality of animals.

REPEATED DOSE 28-DAY SUB-ACUTE ORAL TOXICITY STUDY (OECD – 407 GUIDELINES)

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into 4 groups of 10 animals (5 male and 5 female). Karappan Marundhu was administered to rats at the dose of 9, 45 and 90 mg/kg/day for 28 days.

Any signs of toxic symptoms such as signs of mortality, toxicity, and body weight changes were monitored. All the rats were anesthetized with ether at the end of the treatment period of 28 days, and they were sacrificed after the blood collection.

Animal Source

Test animals were obtained from The King Institute, Chennai and kept at animal house, National Institute of Siddha, Chennai. All the animals were kept under standard environmental condition (22 ± 3ºc). The animals had free access to water and standard pellet diet (Sai Meera foods pvt. Ltd, Bangalore). The principles of laboratory animal care were followed.

Identification of Animal

Animals were identified by cage number and individual marking on the fur of each animal with picric acid. The females were nulliparous and non pregnant.

Housing and Environment

The animals were allowed for an acclimatization period of 7 days to laboratory conditions prior to the initiation of study. The animals were housed in polypropylene cages provided with bedding of husk. Dark and light cycle each of 12 hours was maintained.

Study Period

28 days

Justification for Dose Selection:

The result of acute toxicity studies in Rats indicated that Karappan Marundhu was nontoxic and no behavioral changes were observed up to the dose level of 2000mg. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose:

Karappan Marundhu was suspended in distilled water. The animal dose was fixed with the formula of total clinical dose* conversion factor (0.018) = animal dose / 200g. Hence the low dose was calculated as 9mg according to the formula. It was administered to groups II, III, IV at dose levels

of1X (9mg), 5X (45mg), 10X (90mg). The control animals were administered water only which is the vehicle for the drug KM. Administration was given orally by using an oral gavage once in daily for 28 days.

Laboratory Investigations

Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

RESULTS

Statistical Analysis

Laboratory findings such as hematology and biochemical parameters were done according to one -way ANOVA and then followed by Dunnet "t" test using a computer software -Graphpad INSTAT-V3.1.

DISCUSSION

The trail drug Karappan Marundhu was administered to wistar albino rats at the dose of 50 mg and 300 mg/kg. Even with treatment with high dose of 2000mg/kg has produced no mortality in the treated animals with the drug, Karappan Marundhu (KM). Hence based on the OECD 423 guidelines, the drug is considered to be non-toxic in the experimental stage.

All animals from control and the entire treated dose (9, 45 and 90 mg/kg) groups survived throughout the dosing period of 28 days for sub-acute toxicity study. The results based on body weight determination of animals from the control group and different dose level groups showed comparable increase in the body weight throughout the dosing period of 28 days.

Haematological Investigations

All the blood samples of the control group and the experimental rat groups were analyzed for the white blood corpuscles (WBC), red blood corpuscles (RBC), packed cell volume (PCV), and hemoglobin content.

Biochemical Investigations

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

Test Substance	Karappan Marundhu (KM)
Animal Source	Animal house of King Institute of Preventive Medicine
Animals	Male and Female Wistar Albino Rats
Age/Weight	150- 175gms
Acclimatization	Seven days prior to dosing
Veterinary examination	Prior to the start and at the end of the acclimatization period
Identification of animals	By animal number, cage number, and individual marking on fur
Diet	Standard pellet feed provided by Sai meera foods Pvt Ltd, Bangalore
Water	Portable water in polypropylene bottles ad libitum
Housing and	The animals were housed in Polypropylene cages provided with bedding of
Environment	husk
Relative humidity	Between 30% and 70%
Dark and light cycle	Each of 12 hours

Table No.1: Haematological Parameter

S.No	Haematological Parameters	Control	Low dose	Mid dose	High dose
1	Haemoglobin	11.29±0.077	11.22±0.182	11.55±0.146	11.47±0.33
2	Total WBC	10.67±0.438	10.96±0.525	9.71±0.351	9.22±0.065
3	Neutrophils	66.32±0.05	66.46±0.25	61.97±1.32	68.57±1.24
4	Lymphocyte	35.69±0.183	35.59±0.403	32.46±0.283	39.43±0.384
5	Monocyte	0.5±0	0.52±0.04	0.67±0.051	0.9±0
6	Eosinohil	0.47±0.014	0.47±0.048	0.4±0.052	0.49±0.046
7	Basophill	0 ± 0	0.166±0.408	0.166±0.408	0.666±0.516
8	Platelets cells	564.27±1.506	565.83±1.777	572.79±1.641	576.74±0.368
9	Total RBC	8.245±0.403	8.23±0.340	7.49±0.430	6.67±0.287
10	PCV	43±1.414	43.67±0.516	41.83±0.408	43±0.00
11	MCHC	38.31±0.063	38.49±0.261	34.56±0.212	35.47±0.215
12	MCV	52.5±0.707	53±0.00	50.33±0.516	54.33±0.516

Table No.2: Biochemical Parameters

S.No	Biochemical Parameters	Control	Low Dose	Mid Dose	High Dose
1	Glucose (F)	77.23±0.071	78.45±1.712	79.09±0.82	83.58±0.19
2	T. Cholosterol	94.19±0.014	94.36±0.207	105.28±0.11	106.57±1.343
3	Sr.Creatinine	0.8± 0.0	0.8± 0.0	0.78±0.04	0.7± 0.0
4	T.Bilirubin	0.8± 0.0	0.8± 0.0	0.78±0.048	0.7± 0.0
5	Sr.Total Protein	4.56±0.06	4.51 ± 0.30	4.52 ± 0.35	4.4 ± 0.19
6	Sr. Triglycerides	33.2±0.03	33.3±0.17	39.78±0.74	47.41±0.19
7	BUN	14.57±0.095	14.36±0.61	14.35±0.54	15.48±0.29
8	SGPT	64.42 ±0.15	64.5±0.27	63 ±0.77	60.34±0.213
9	SGOT	76.13±0.28	76.15 ±1.36	78.71±1.5	84.76±2.7
10	ALP	366.72±0.2	366.3±1.04	378.35±3.51	391.81±1.13

CONCLUSION

The acute and sub-acute toxicity study of Karappan Marundhu (KM) revealed no toxicity by oral route over a period of 28 days. So, it can be concluded that the Karappan Marundhu (KM) can be prescribed for therapeutic use in humans. Higher dose is 2000mg/kg. In this trial drug no mortality was noted. The clinical dose was fixed as 500 mg/day.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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