VOLUME - 10, ISSUE - 05, MAY- 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Rheumatology

ACUTE AND SUB ACUTE TOXICITY STUDIES OF SIDDHA HERBO-MINERAL FORMULATION IN MEGANATHI KULIGAI

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ABSTRACT Uthiravatha Suronitham (Rheumatoid arthritis) is a chronic disability disease, it affects more in the adults and occurrence of the disease is middle age groups. The Female is affected 3 times more in Male. A Herbo-mineral siddha formulation of Meganathi Kuligai can be used for pre-clinical toxicity studies in Wister albino rats, to arrive at the safe dose levels for human trials. Evaluating the toxicity in study and observation showed that Meganathi Kuligai is better effective treatment in Rheumatoid arthritits.

KEYWORDS :

INTRODUCTION

The Siddha medicine is one of the traditional medical system, widely practiced in Southern India, especially in Tamilnadu. The Uthiravatha Suronitham can be correlated with the modern aspect is Rheumatoid arthritis. It is a chronic disability disease and characterized by inflammation of Synovial membrane and hyperplasia. The RA is mainly affecting the smaller joints such as metacarpal and proximal inter phalangeal joints, finally affecting all joints and systemic illness. It affects about 0.92% of the adult population in India[5]. There are about 20-40 new cases per lakh population, each year the occurrence of the disease more frequently in females. Female to Male ratio of this disease is 3:1. About 5% of first degree relatives are more prevalence in Rheumatoid arthritis (www. Arthritis-India.com)[8].

The pre-clinical toxicity studies were essential for determining a safe dose of MK. The evaluate acute and sub acute toxicity of Herbo-mineral siddha formulation of Meganathi Kuligai (Table no. 1)[1] is very essential before the use in RA patients.

MATERIAL AND METHODS

SOURCE OF DRUG

The raw drugs were procured from Raw drug shop at Thuckalay, Kanyakumari district. After obtaining proper authentication by Botanist, Department of Medicinal Botany, at Govt. Siddha Medical College, Palayamkottai-627002.

MEGANATHI KULIGAI PREPARATION

The raw drugs were purified according to the standard operating procedure (SOP)[2,6,7].

MATERIALS :

Table No. 1 Ingredents of Meganathi kuligai

S. No.	MEDICINE	*BOTANICAL NAME*	DOSAGE
1	Purified Sivathai	*Operculina turpethum*	35 g
2	Purified Vasambu	*Acorus calamus*	35 g
3	Purified Chukku	*Zingiber officinale*	35 g
4	Purified Vaividangam	*Emblica ribes*	35 g
5	Purified Omum	*Corum copticum*	35 g
6	Purified Perungayam	*Ferula asafoetida*	35 g
7	Purified Kattamanakku	*Jatropha curcas*	35 g
8	Purified Linkam	*Cinnabar*	35 g
9	Purified Vengaram	*Borax*	35 g
10	Purified Inthuppu	*Sodium chloride*	35 g
11	Lemon juice		Qs

TOXICITY STUDY

The experimental protocol was permitted by the Institutional Ethical Committee IAEC and CPCSEA(AKCP/ IAEC/40/20-21 dated 25.11.2020)of Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Virudhunagar (Dt), Tamil Nadu.

ACUTE TOXICITY STUDY

Three female Wister rats were used for acute oral toxicity study according to OECD-423 guidelines[4]. The aqueous extract of *MK* was administered at 5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg and 2000 mg/kg body weight respectively. The results were recorded from day 0, with single oral dosing period of 14 days[3].

Animals in pain or severe signs of distress were recorded. The cage side observation includes changes in skin, fur, eyes and mucous membranes, occurrence of secretions, excretions and autonomic activity were observed. At the 14th day, sensory reactivity to stimuli of different types e.g. auditory, visual and pro proprioceptive stimuli was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/ dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups (Table no. 2 &3).

TABLE NO. 2 PHYSICAL AND BEHAVIORAL EXAMINATION

GROUP NO.	DOSE (mg/ kg)	OBSERVATION SIGN	NO. OF ANIMAL AFFECTED	MORTALITY
Group-I	5	Normal	0 of 3	0 of 3
Group- II	50	Normal	0 of 3	0 of 3
Group-III	300	Normal	0 of 3	0 of 3
Group-IV	1000	Normal	0 of 3	0 of 3
Group-V	2000	Normal	0 of 3	0 of 3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, calculated by comparing treated groups with control group . Data obtained in this study indicated $^{ns}p > 0.05$ no significance in physical and behavioral .

TABLE NO. 3 HOME CAGE ACTIVITY

Functional and Behavioural observation	Obser-vation	5mg/ kg Group (G-I)	50mg/ kg (G-II)	300mg /kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3

Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group. Data obtained in this study indicated $^{ns}p > 0.05$ 05 no significance changes in Home cage activity.

TABLE NO. 5 HAND HELD OBSERVATION

Functional and Behavioral observation	Observation	Control	5 mg/kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group. Data obtained in this study indicated $^{ns}p > 0.05$ no significance changes in hand held observation.

SUB ACUTE TOXICITY

Sub-chronic oral toxicity study was carried out according to OECD guideline 407. The study was conducted on 5 Male and 5 Female Wister rats for each group[4].

TABLE NO. 7 EFFECT OF SUB-ACUTE DOSE (28 DAYS).

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

GROUP	CONTROL	LOW	MID	HIGH
1st DAY	9.49±0.07	10.52±0.12	9.18±0.25	10.65±0.25
7th DAY	10.66±0.07	9.90±0.07	10.78±0.07	11.20±0.08
14th DAY	11.78±0.06*	10.89±0.08*	11.90±0.08*	11.77±0.08*
21st DAY	12.90±0.07**	19.80±0.07**	20.89±0.071**	21.21±0.06**
28th DAY	13.06±0.08***	12.41±0.06***	11.05±0.05***	12.05±0.06***

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group(Table no.7)

TABLE NO. 8 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF MK ON WATER INTAKE IN ML

GROUP	CONTROL	LOW	MID	HIGH
1st day	11.40±0.07	10.20±0.08	11.18±0.09	11.58±0.15
7th day	12.59±0.08	11.86±0.08	12.71±0.07	12.21±0.08
14th day	13.40±0.07*	13.90±0.08*	13.91±0.08*	14.91±0.07*
21st day	15.69±0.05**	15.46±0.27**	16.41±0.07*	18.50±0.09**
28th day	18.15±0.06***	18.41±0.07***	17.77±0.06**	19.59±0.08***

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group.

TABLE NO. 9 EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF MK ON BODY WEIGHT IN GRAM

The body weight of each rat was recorded on an one week before the start of treatment, and once during the course of the treatment.

GROUP	CONTROL	LOW	MID	HIGH
1st day	180.06±0.70	181.15±0.72	182.80±0.75	184.75±0.78
7th day	183.13±0.72	185.21±0.73	187.29±0.75	188.75±0.77
14th day	185.11±0.69	188.17±0.80	190.98±0.84	192.81±0.76
21st day	192.03±0.76	193.09±0.07	194.42±0.83	195.95±0.71
28th day	195.15±0.77*	197.14±0.70*	198.05±0.74*	199.86±0.75

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group.

TABLE NO. 10 MK ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, Pancreas and Stomach.

GROUP		CONTROL	LOW	MID	HIGH
HEART		0.41±0.01	0.42±0.05	0.44±0.06	0.51±0.08
LIVER		3.21±0.14	3.40±0.07	3.45±0.14	3.85±0.08
LUNGS		0.65±0.08	0.84±0.09	0.89±0.07	0.98±0.13
KIDNEY	L	0.90±0.08	0.94±0.09	1.18±0.09	1.60±0.07
	R	0.98±0.07	1.11±0.08	1.48±0.09	1.58±0.09

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group.

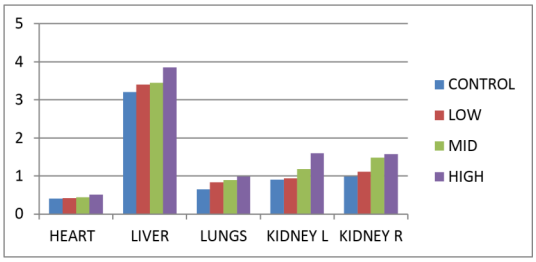

FIGURE 1 MK ON ORGAN WEIGHT

TABLE NO. 11 EFFECT OF SUB-ACUTE DOSE (28 DAYS) OF MK ON HAEMATOLOGICAL PARAMETERS

The following hematological parameters were analyzed using Autoanalyser.

Drug treatment	RBC million cells/cumm (10^{12}) lit	WBC cells/cu mm (10^{9})lit	Haemoglobin gm/ml	Differential count %			
				Neutrophils	Eosinophils	Monocyte	Lymphocyte

Control	5.88 ±0.07	6.18±. 040	11.59± 0.07	54.31 ±0.07	1.68± 0.08	7.17± 0.07	38.20± 0.0.08
LOW	4.93± 0.06	7.50± 0.23	11.65± 0.08	54.72 ±0.16	1.76± 0.07	6.42± 0.02	39.41± 0.7
MID	5.37± 0.07	6.65± 0.15	12..95 ±0.07	55.47 ±0.14	1.96± 0.07	7.79± 0.08	37.88± 0.08
HIGH	5.35± 0.07	6.93± 0.45	13.00± 0.07	56.88 ±0.18	2.15± 0.06	5.80± 0.07	37.04± 0.06

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

The effects of **MK** were observed for its effect on hematological parameters in experimental rat. Final study, not significant ($p<0.05$) in the values of treated groups. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV (Table no 11).

TABLE NO. 12 EFFECT OF SUB-ACUTE DOSE(28 DAYS)OF MK ON BIOCHEMICAL PARAMETERS

The following clinical Bio parameters were analyzed using auto analyser.

Drug Treatment	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	29.20± 0.08	57.19± 0.08	122.29 ±0.045	26.59± 0.08	0.82±0.077
LOW	30.51± 0.25	58.15± 0.06	123.77 ±0.65	28.62± 0.07	0.87±0.06
MID	31.62± 0.55	60.84± 0.67	126.16 ±0.75	29.44± 0.49	0.94±0.08
HIGH	31.88± 0.70	61.17± 0.75	127.89 ±0.65	30.86± 0.60	1.12±0.08

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

DISCUSSION

The acute and repeated 28 days oral toxicity studies of MK did not produce any toxicity signs in Wister albino rats. Daily administration of Mk at different doses of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg body weight of animals as suspension along with water for 28 days was observed (Table no.1,2,3)

Results showed indication of drug tolerance among the rats against MK without any mortality and morbidity. Toxicity signs such as Lacrimation, Palpebral closure, Salivation, Piloerection abdominal tone, Limb tone were not observed.

Also, other Biochemical and Hematological parameters were almost similar in all experimental groups(Table no. 11 & 12). The assessment in the Liver, Heart and Lung did not reveal any vascular changes. Analysis of kidney tissue revealed macroscopic examination normal in all treatment groups.

CONCLUSION

The study concluded that MK is suitable for clinical use in human with dosage recommendation based on body weight. Mk is safe and it can be used for widespread clinical application of Uthira Vatha Suronitham (Rheumatoid arthritis).

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