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Research Article

TOXICOLOGICAL STUDIES OF *'VITHU RASA MEZHUGU'* IN ACUTE AND CHRONIC INFLAMMATION MODELS IN EXPERIMENTAL RATS

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Abstract: The paper focusses on evaluating anti - inflammatory effect of a Siddha formulation *'Vithu Rasa Mezhugu'* to prove its claim. The trial drug is subjected for its toxicity studies on rat models. Carrageenan induced hind paw edema model and Cotton pellet granuloma method was followed for studying anti inflammatory effect. The formulation has proved to be non toxic upto 2000 mg /kg and possessed positive anti inflammatory effect on both methods with compared to Diclofenac sodium used as standard drug.

Key words: Vithu Rasa Mezhugu, *Semicarpus ancardium*, mercury, vatha disease, anti- inflammatory.

INTRODUCTION

'Vithu Rasa Mezhugu' (VRM) is a Siddha formulation, tamil lyrics containing *Rasam* (mercury) and *Vithu* of Seraankottai (seed of ***Semicarpus anacardium***) after proper purification prepared in *Mezhugu* form (semisolid dosage form) which are very effective in vatha diseases (arthritic pains) at dose of 65mg twice a day with palm jaggery after meals as described in Siddha texts[1,2].

Method of preparation[1]

Ingredients:

1. Purified Rasam- 22.5g
2. Seed of ***Semicarpus anacardium*** – 35g

All the two ingredients mentioned above are ground to *Mezhugu* (after proper purification).

Dosage: 32-65mg, twice a day.

Adjuvant: Palm jaggery.

The formulation is subjected for toxicity studies and anti – inflammatory effect was evaluated to prove its efficacy compared with a standard drug.

MATERIALS AND METHODS

Test drug

The Siddha formulation used in the study was processed by the methods prescribed in standard text books of Siddha medicines.

Preparation of drug for dosing

Vatha Rasa Mezhugu is shortly mentioned as **'VRM'** in this paper. **'VRM'** was not soluble in water and made into a suspension in sodium carboxy methyl cellulose before administration. The drug suspension was administered at the dose of 2000 mg/kg/p.o. for acute toxicity study and at the dose of 12 mg/kg/p.o. for 14 days repeated oral toxicity and other pharmacological studies.

Drugs and chemicals

Carrageenan, Histamine and fine chemicals used in these experiments were obtained from Sigma Chemicals Company, U.S.A. Other analytical grade chemicals were obtained from S.d. Fine Chemicals Ltd., Mumbai.

Experimental animals

Colony inbred animals strains of wistar albino rats of either sex weighing 200-250 g were used for the pharmacological and toxicological studies. The animals were kept under standard conditions 12:12 (day/night cycles) at 22^0C room temperature, in polypropylene cages. The animals were fed on standard pelleted diet (Hindustan Lever Ltd., Bangalore) and tap water *ad libitum.* The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Acute oral toxicity study

Acute oral toxicity was conducted as per the OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). The acute toxic class method is a stepwise procedure with 3 animals of a single sex

per step. Depending on the mortality and /or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

Wistar albino rats of either sex weighing 200-250 g were fasted overnight, but allowed water *ad libitum.* Since the formulation is relatively non toxic in clinical practice the highest dose of 2000mg/kg/p.o. (as per OECD guidelines "Unclassified") was used in the acute toxicity study. The animals were observed closely for behavioral toxicity, if any by using FOB (Functional observation battery).

Repeated oral toxicity study

Repeated oral toxicity studies can be used to get additional information regarding the toxicity profile of a chemical. Repeated oral toxicity studies are defined as those studies where the chemical is administered to the animal for a period covering approximately 10% of the expected life of the animal. Usually, the dose levels are lower than for acute studies and allow chemicals to accumulate in the body before lethality occurs, if the chemical possess this ability.

Experimental procedure:

The following experimental procedure was followed to evaluate the repeated oral toxicity study of **'VRM'.**

Group I: Control animals received 10 ml/1000 g b.w. of 1% carboxy methylcellulose for 14 days

Group II: Suspension of VRM at the dose of 12mg/kg/p.o. for 14 days.

Dose calculation:

The human dose 130mg (65mg b.d.) is converted into rat dose by multiplying the human dose with a factor 0.018 (corresponding to body surface area) to get the dose for a rat weighing 200g. Multiply the dose for 200 grams rat x5 to get the dose for kg weight of the rat 130 x 0.018=2.34mg for rat weighing 200 grams Multiply by 5 to get the dose for kg body weight of rat.2.34 x 5=11.70mg/kg Actual dose taken is 12mg/kg.

Body weight, food intake and water intake was recorded at two intervals with simultaneous observation for toxic manifestation and mortality, if any. At the end of 14 days treatment all the animals were sacrificed by over dosage of ether anaesthesia. Blood was collected and used for haematological studies. Section of liver, brain, kidney, pancreas, heart, lung and testis were dissected out and kept in 10% formalin for histopathological studies.

Biochemical studies

The procedures were followed as per standard text[3].

Estimation of glucose

Glucose was estimated using commercial Glucose estimation kit (Span Diagnostics) by standard methods[4].

Aspartate aminotransferase (AST)

Aspartate aminotransferase was estimated[5] using commercial AST kit (Span Diagnostics).

Alanine aminotransferase (ALT)

Alanine aminotransferase was estimated using commercial AST kit (Span Diagnostics)[5].

Alkaline phosphatase (ALP)

Alkaline phosphatase was assayed using commercial ALP kit (Span Diagnostics) by following standard method[6].

Urea

Urea was assayed[7] using the commercial kit (Span Diagnostics).

Blood Urea Nitrogen (BUN)

Blood Urea Nitrogen was estimated[8] using the Diagnostic kit.

Reduced Glutathione (GSH)

Reduced Glutathione was assayed using the kit by the method of standard text[9].

Haematological studies

Erythrocyte count

Erythocyte count was estimated by Hemocytometer method[10].

Total Leukocyte Count (WBC)

Total Leukocyte Count was estimated by standard method[11].

Haemoglobin

Haemoglobin was estimated as per standard procedure[10].

Histopathological studies

Animals were sacrificed at the end of repeated oral toxicity and tissues were processed for histopathological studies.

Anti - inflammatory activity

Anti - inflammatory activity of **'VRM'** was evaluated in both acute and chronic models of inflammation.

Acute model

a. Carrageenan induced hind paw edema

The carrageenan assay procedure[12] was carried out. Edema was induced by injecting 0.1 ml of a 1% solution of carrageenan in saline into the plantar aponeurosis of the left hind paw of the rats. The extracts, reference drug and the control vehicle (distilled water) were administered 60 minutes prior to the injection of the carrageenan. The volumes of edema of the injected and contra lateral paws were measured at +1, 3 and 5 hrs after induction of inflammation using a plethysmometer[13] and percentage of anti-inflammatory activity was calculated.

Chronic model

b. Cotton pellet granuloma

Sterile cotton pellets (weighing 10 ± 2 mg) were implanted subcutaneously along the flanks of axillae and groins of wistar albino rats[14]. The extracts, reference drug and the control vehicle (distilled water) were administered as per protocol to rats everyday for a period of 7 days. On day + 8 the rats were sacrificed by cervical decapitation and cotton pellets were removed surgically, freed from extraneous tissue and weighed immediately for wet weight. One half of the pellets were dried in an incubator at 60ºC until a constant weight was obtained.

RESULTS AND DISCUSSION

Acute oral toxicity study

Treatment of **'VRM'** at the dose of 2000mg/k,g./p.o. did not exhibit any mortality in rats. As per OECD 423 guidelines the dose is said to be "Unclassified" under the toxicity scale. Hence further study with higher doses was not instituted.

Repeated oral toxicity for 14 days

The drug '**VRM'** at the dose of 12 mg/kg/p.o. was administerd orally for 14 days in rats did not show toxicity as evidenced by Haematological parameters (Table-01) However a siginificant ($P<0.05$) alteration in the kidney function was observed with the test drug. There was no significant changes in the liver function were observed with the drug (Table-02).

Table-01: Effect of 'VRM' on Haematological parameters after 14 days repeated dosing in rats

Groups	Hb (gm/100ml)	RBC (millions/ cu.mm)	WBC (cells/ cu.mm)	Differential Leucocyte count (%)		
				Lymphocytes	Monocytes	Granulocytes
Control	12.08±0.348	5.20±0.347	5583.33±334.94	77.00±3.89	5.50±1.04	15.66±3.07
'VRM' 12mg/kg/p.o..	12.32±0.24 ns	5.27±0.53 ns	5443.±349.23 ns	78.33±4.32 ns	6.00±2.28 ns	17.5±4.27 ns

n=6; Values are expressed as mean ± S.D followed by Students Paired 'T' Test
ns - Non significant as compared with control.

Table-02: Effect of 'VRM' on Biochemical markers of liver and kidney after 14 days repeated dosing in rats

Groups	ALP (K.A.Units)	AST (IU/L)	ALT (IU/L)	Urea (mg/100ml)	BUN (mg/ 100ml)	GSH (mmol/L)
Control	2.76±0.37	72.16±1.16	26.91±1.19	11.25±0.67	4.92±0.74	1.22±0.02
'VRM' 12mg/kg/p.o.	2.58±0.39 ns	72.58±1.64 ns	27.17±1.09 ns	12.08±0.85 ns	6.16±0.21 *	1.24±0.03 ns

n=6; Values are expressed as mean ± S.D followed by Students Paired 'T' Test
ns - Non significant as compared with control
*P<0.05

Histopathological study

'VRM' did not exhibit evidence of pathological lesions in the tissue after 14 days repeated oral dosing.

Anti - inflammatory studies

Administration of **'VRM'** at the dose of 12mg/kg/p.o. exhibited significant anti-inflammatory activity in both acute (carrageenan induced hind paw) and chronic (Cotton pellet granuloma) models of inflammation in rats. A 44.6 % reduction in paw edema volume was observed in the **'VRM'** treated animals when compared to control at the end of 240 minutes (Table-03). Similarly significant reduction in dry granuloma weight (60.8%) was also observed in animals treated with **'VRM'** (12mg/kg/p.o.) for one week in chronic model of inflammation when compared to control animals (Table-04). The results were comparable to that of Diclofenac sodium (5 mg/kg/p.o.).

Table-03: Anti - inflammatory activity of 'VRM' in Carrageenan induced Hind paw edema in rats

Groups	Paw volume (ml) by Mercury Displacement at Regular interval of Time				
	0min	**30min**	**60min**	**120min**	**240min**
Control	0.773±0.027	0.916±0.040	1.246±0.090	1.766±0.103	2.105±0.080
'VRM' 12mg/kg/p.o.	0.853±0.070^{ns}	1.36±0.057**	1.16±0.036**	1.086±0.041**	0.933±0.058**
Standard (Dic.Sodium 5 mg/kg/p.o.)	0.835±0.065^{ns}	1.315±0.069**	1.128±0.049**	1.011±0.056**	0.896±0.048**

n=6; Values are expressed as mean ± S.D followed by One Way ANOVA –Dunnett's multiple comparison test.
ns - Non significant as compared with control;
P<0.01 (**) as compared with control

Table-04: Anti - inflammatory activity of VRM in Cotton Pellet Granuloma

Groups	Cotton pellet Granuloma method
	Dry Weight (mg)
Control	115.87 ± 15.42
'VRM'	70.75 ± 8.44***
Standard (Dic.Sodium 5 mg/kg/p.o.)	70.00 ± 7.42***

n=6; Values are expressed as mean ± S.D followed by Students Paired 'T' Test***P<0.001 as compared with that of control.

CONCLUSION

In the present study the formula was found to be safe at the dose of 2000 mg/kg when tested for acute toxicity study. On repeated oral administration for 14 days the drug did not exhibit alteration in the liver function tests and hematopoietic parameters. However the drug showed no significant alterations in the kidney function after 14 days treatment. A significant reduction in the edema volume and granuloma formation was observed with the use of **'VRM'** in experimental model of inflammation. The obtained results will help for the global recognition of the formulation and its usage amidst the contemporary medicine with fewer side effects.

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