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Research Article

CHEMICAL ANALYSIS AND ANALGESIC ACTIVITY FOR A SIDDHA FORMULATION 'VITHU RASA MEZHUGU'

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Abstract: Vithu Rasa Mezhugu is a Siddha formulation used for vatha diseases. The formulation is subjected for qualitataive phytochemical analysis including evaluation of inorganic radicals and heavy metal. The formulation was also attempted to check its analgesic activity using tail flick method against paracetamol as standard. The formulation showed the presence of some potent biochemical and radicals like sodium, potassium, iron, alkaloid, saponin, tannin which may claim for its activity. Most of the heavy metals were found to be within limit. The formulation also showed good analgesic action against paracetamol as standard. In this paper attempt was made for analyzing the chemicals as a parameter of standardization of herbo-mineral drug like VRM.

Key words: VRM, biochemical, Perkin Elmer, mercury, paracetamol, analgesic activity.

INTRODUCTION

Siddha literature prescribes lot of formulations and drugs for curing diseases. One such preparation Vithu Rasa Mezhugu (VRM) used for arthritic pain was taken for screening phytochemicals, inorganic radicals and heavy metal evaluation. VRM consists of *Semicarpus anacardium* seeds and purified *mercury* as major ingredients. The formulation was given at a dose of 65 mg twice a day for the treatment of arthritic pains[1,2].

MATERIALS AND METHODS

The phytochemical and inorganic qualitative analysis was carried out using standard texts [3,4]. The heavy metals and radicals were evaluated using Perkin Elmer Optima 5300 DV ICP-OES in IITM, Chennai using standard procedures[5,6,7].

Fine chemicals used 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 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).

Preparation of drug for dosing

VRM was 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.

Dose calculation:

The human dose 130mg (ie., 65mg bd) 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 x 5 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.

The acute toxicity was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at the 2000 mg/kg Doses, so the calculated dose 12 mg/kg was selected.

Statistical analysis

The statistical data are expressed as Mean ± Standard deviation (SD). Student paired T-test was followed for analysis. P<0.001 considered as significant.

Evaluation of Analgesic activity

Tail Flick method

Wistar rats of either sex (200-250g) were divided into different groups with 6 animals in each group.

Group I :Control animals received 1% CMC 10ml/kg/p.o.

Group II :Test drug at the dose of 12mg/kg/p.o.

Withdrawal of tail (Tail Flick) for noxious thermal (radiant heat) can be used for screening drugs with analgesic activity[8,9,10]. Radiant heat can be generated by passing electrical current through nichrome wire mounted in an analgesiometer. The base of the tail of the test rats is placed on a nicrome wire. The tail withdrawal for the radiant heat (flicking response) is taken as the end point. Normally the rats and mice withdraw their tails within 3 – 5 secs. A cutoff time of 10 – 12 secs is used to prevent damage to the tail. Any animal failing to withdraw its tail in 3-5 secs is rejected from the study.

The reaction time of test drug, standard and control are taken at intervals of 30, 60 and 120 mts. A reaction time (withdrawal time) increment of 2-5 secs more than the control animals can be considered for analgesic activity of the drug.

RESULTS AND DISCUSSION

The phytochemical and inorganic qualitative analysis of the formulation showed the presence of sodium, calcium, iron, sulphate, chloride, carbonate, phosphate, alkaloid, saponin, tannic acid and the result is depicted in Table- 01.

Table- 01:Biochemical screening of (VRM)

S.No.	Constituents	VRM
1.	Calcium	+
2	Sodium	+
3.	Iron	+
4.	Sulphate	+
5.	Chloride	+
6.	Carbonate	+
7.	Phosphate	+
8.	Starch	-
9.	Tannic acid	+
10.	Sugar	-
11.	Alkaloids	+
12.	Sterols	-
13.	Protein	+
14.	Phenols	+
15.	Flavanoids	-
16.	Saponins	+
17.	Glycosides	-

(+) – Present, **(-)** - Absent

Table – 02: Evaluation of heavy metals and radicals in the formulation using Perkin Elmer optima 5300 DV ICP – OES

S.NO.	ANALYTE	MEAN
1.	As 193.696	BDL
2.	Cd 226.502	BDL
3.	Hg 253.652	3.075 mg/L
4.	Pb 230.204	BDL
5.	Ca 317.933	8.092 mg/L
6.	Fe 238.204	0.472 mg/L
7.	K 766.490	7.25 mg/L
8.	P 213.617	4.08 mg/L
9.	S 181.975	15.321 mg/L

The formulation proved that the heavy metals (arsenic, cadmium and lead) checked were in permissible limit. Mercury was found to be above the limit as per ISM standards[11]. Calcium, iron, potassium, phosphorous and sulphur were also evaluated. (Table - 02).

VRM at the dose of 12mg/kg/p.o. exhibited analgesic activity in tail flick method and the activity can be compared to that of paracetamol

at the end of 60mts (Table - 03). 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. It may be because of the presence of amount of mercury.

Table 03: Analgesic activity of VRM using Tail flick Method

Groups	Paw licking response (Sec)			
	0 min (Sec)	30 min (Sec)	60 min (Sec)	120 min (Sec)
Control	2.56 ± 0.396	2.61 ± 0.96	2.76 ± 0.67	2.46 ± 0.53
Test (12mg/kg.p.o.)	2.96 ± 0.626^{ns}	3.133 ± 0.258***	5.966 ± 0.646***	5.33 ± 1.734***
Paracetamol 45mg/kg/p.o.	3.01± 0.675ns	3.27 ± 0.312***	6.12 ± 0.430***	5.98 ± 0.975***

n=6, Values are expressed as mean ± S.D using followed by student paired T – test, ns- non significance
***P<0.001 as compared with control.

Other essential elements present, acts as additional supplement and help in increasing the efficacy which needs further validation. Hence an extensive research will through a better understanding about the complete pharmacokinetic and pharmacodynamic studies of this drug. Further the chemicals should be analyzed completely for establishing the mercurial/metal containing drugs as bhasmas and metal amalgamated formulations (Poorna chandhrodhayam) exist though having more amount of mercury (even 141.7600mg/g). Several macro/trace elements found to be present in different amount make it bio-available and enhance its activity and reduced the toxicity of the compounds present in them[12].

However the formulation is a useful medicine. It should be further validated for complete standardization and development in processing considering in mind about heavy metals in mercurial formulations like the trial drug for its global recognition as metal drugs and herbo-mineral drugs are well accepted in Indian system of medicine.

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