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Research Article

THERAPEUTIC EFFICACY AND SAFETY OF SIDDHA FORMULATION NEERADIMUTHU VALLATHI MEZHUGU (INTERNALMEDICINE)

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ABSTRACT

Neeradimuthu Vallathi Mezhugu, a Traditional Siddha Herbo-mineral formulation is indicated for *Thandaga Vatham* disease. This pre-clinical research was carried out to ensure the safety and efficacy of this medicine. This Internal Medicine is to be given to patients in a dose of 1 gm twice a day for a period of 48 days. The trial medicines were prepared as per sastric literatures. Preclinical trial of this medicine for the safety (acute oral and repeated oral toxicity) and efficacy (analgesic and anti-inflammatory study) were done. Repeated oral toxicity study conducted for15days with the drug did not exhibit significant changes in blood counts and in Hb% . The test drug exhibited significant analgesic and anti-inflammatory activity in acute experimental inflammatory conditions in rats. The test drug showed maximum anti-inflammatory activity at the end of 4th hour after carrageenan challenge; the mechanism of anti-inflammatory activity of test drug may be attributed for its inhibitory activity on cyclooxygenase (COX) enzymes. Also, the phyto-chemical and antioxidant studies revealed its efficacy in treating the *Vatha* diseases and especially *Thandaga Vatham*.

Keywords: *Thandaga Vatham, Neeradimuthu Vallathi Mezhugu, preclinical, toxicity, anti-inflammatory.*

1. Introduction

Siddha system of medicine is one of the ancient traditional systems of medicine. Siddha system is a distinct science comprising of *Vatham,Vaithyam,Yogam* and *Gnanam*. *Siddhars* are divinely persons with supernatural powers. *Siddhars* were the first persons to employ metals and minerals as therapeutic agents. When Siddha medicines (prepared with ingredients of metals and minerals) are used, questions arises about its safety in human usage. Even though the medicines are invented by our great Siddhars who had deeper knowledge in science, it is mandatory to evaluate its safety in terms of scientific evidences.

Here, the Authors have taken *Neeradimuthu Vallathi Mezhugu* [1] for open clinical on *Thandagavatham*. This medicine is mentioned in *Anuboga Vaithyanavaneetham*, part-8. The test drug is indicated for Skin diseases and *Vatha* diseases according to indications mentioned in text. But according to *GUNAM* (Property) of the each ingredient, it may be very efficacious in treating the *Vatha* diseases.

2. MATERIALS AND METHODS

2.1 Medicine Preparation

The medicine mentioned in the *Anubhogavaidya Navaneetham* Part – VIII the major ingredients are:

1. *Neeradimuthu* (Soorty oil tree)- Hydnocarpus w*ightiana*
2. *Serankottai* (Marking nut) – Semecarpus anacardium
3. *Parangipattai* (Sarsaparilla) -Smilax china
4. *Amukkara,*(Indian ginseng)-Withania somnifera
5. *Vellerukkam ver* (king's crown) -Calotropis gigantea
6. *Senthatti* (Indian stinging nettle) - Tragia involucrata
7. *Surul pattai* (Red Creeper)- Ventilago madraspatana GAERTN.
8. *Rasam* – Mercury(Hg)
9. *Ghandagam* -Sulphur (S)
10. *Panai vellam (*Palm jaggery)- Borassus flabellifer

Medicine was prepared according to the *Mezhugu* consistency.

Method: Since it is indicated for Vatha diseases, it is necessary to evaluate the anti inflammatory, analgesic and anti oxidant properties. Since it is a metallic preparation it is mandatory to do safety and toxicity studies also.

2.2 Preliminary Basic, Acidic Radicals and Phyto-chemical Studies

The qualitative chemical analysis and acidic, basic radicals assay of the drugs showed the presence of phyto constituents and minerals.[2],[3], [4]

2.3 Toxicity Studies

2.3.1 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. Since the formulation is relatively non toxic in clinical practice the highest dose of 2000 mg/kg/p.o (as per OECD guidelines "Unclassified") was used in the acute toxicity study. The animals were observed closely for behavioural toxicity, if any by using FOB (Functional observation battery).

2.3.2 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 NMVM

Group I : Control animals received 1%CMC, 2 ml/kg/p.o. for 15days

Group II : NMVM at the dose Level of 500mg/kg/p.o. was given to rats for 15days

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 15days

treatment all the animals were sacrificed by over dosage of ether anaesthesia. Blood was collected and used for haematological studies. Section of liver, kidney, and heart were dissected out and kept in 10% formalin for histo-pathological studies.

NMVM at the dose of 2000mg/kg/po **did not exhibit** any mortality in rats. As per OECD 423 guidelines the dose is **Acute** said to be "Unclassified" under the toxicity scale. Hence further study with higher doses was not executed.

Test drug NMVM at the dose of 500 mg/kg/po when administered orally for 15 days in rats did not exhibit toxicity in hematopoietic system, liver and kidney. (Tables 2 and 3).

2.4 Histopathological Studies

Animals were sacrificed at the end of repeated oral toxicity and tissues were processed for histo-pathological studies. NMVM at the dose of 500 mg/kg/po daily administered for 15days did not show evidence of pathological lesions in the tissues tested (Plate 1)[5]

2.4.1 Biochemical Studies

Here estimation of Aspartate aminotransferase (AST), Alanine amino tranferase(ALT), Alkaline phosphatase (ALP), Cholesterol, Urea and Uric acid levels are noted in specified kits, routine Haematological studies were done.[6],[7],[8],[9]

2.5 Analgesic Activity

The analgesic activity of the medicine is analyzed by Tail Flick method in Wistar rats

Group-1 Control animals received 1% CMC 10ml/Kg/po

Group 2 Test drug at the dose of 500mg/kg/po

NMVM at the dose of 500 mg/kg/p.o showed significant analgesic activity in rats.

2.6 Anti Inflammatory Activity

Anti inflammatory activity was evaluated in acute model of inflammation

Group-1 Control group received CMC 10ml/kg/po

Group-2. Received Carrageenan (0.1% solution) and served as positive control

Group-3 Received test drug (NMVM) at the dose of 500mg/kg/po

Group-4 received standard drug Diclofenac sodium (5mg/kg/po)

The anti-inflammatory activity of the medicine is evaluated by Carrageenan induced Hind paw edema. NMVM exhibited significant anti-inflammatory activity in Carrageenan induced hind paw edema (acute inflammation model) in rats. The results of present study was comparable to that of the standard NSAID Diclofenac sodium (5 mg/kg/p.o)

2.7 In Vivo Antioxidant Study

Samples of serum collected from rats treated with test drugs were assayed for GSH (Moron *et al*, 1979) and LPO (Yagi, 1976) and the results were compared with control group. [10]

3. RESULTS

3.1 Preliminary Basic, Acidic Radicals and Phytochemical Studies

The qualitative chemical analysis and acidic, basic radicals' assay of the drugs showed the presence of phyto constituents and minerals as depicted in (Table 1).

Table 1. Preliminary acid, basic radicals and phyto-chemical screening

S.No.	Constituents	NMVM
1.	Calcium	+
2.	Iron (Ferric)	-
3.	Iron (Ferrous)	+
4.	Sulphate	+
5.	Chloride	+
6.	Carbonate	-
7.	Starch	+
8.	Phosphate	+
9.	Tannic acid	-
10.	Unsaturated	+

11.	Reducing Sugar	+
12.	Alkaloids	+
13.	Steroids	+
14.	Protein	+
15.	Tannins	+
16.	Phenols	+
17.	Flavanoids	+
18.	Saponins	+
19.	Amino acid	+
20.	Glycosides	+
21	Sterols	+

(+) - Present, (-) –Absent, **NMVM-**Neeradi Muthu Vallathi Mezhugu

3.2 Acute Oral Toxicity Study

NMVM at the dose of 2000mg/kg/po 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 executed.

3.3 Repeated Oral Toxicity for 15 Days

Test drug NMVM at the dose of 500 mg/kg/po when administered orally for 15 days in rats did not exhibit toxicity in haematopoietic system, liver and kidney. (Tables 2 and 3)

Table 2. Effect of Siddha Formulations (NMVM) on Haematological parameters after 15 days repeated oral dosing (500 mg/kg)

Groups	**Hb (gm/100ml)**	**RBC (millions/cu.mm)**	**WBC (cells/cu.mm)**	**Differential leucocyte count (%)**		
				Lympho Cytes	**Mono cytes**	**Granulo cytes**

Normal	14.0 ± 0.354	5.51 ± 0.635	5785 ± 9.434	75.06 ± 3.829	6.30 ± 1.904	18.70 ± 4.627
NMVM (500mg/kg/p.o)	13.88 ± 0.710 ns	5.88 ± 0.732 ns	5986.66 ± 3.343ns	77.67 ± 3.382 ns	6.16 ± 1.7 ns	17.66 ± 3.474 ns

n=6; Values are expressed as mean ± S.D followed by Students Paired 'T' Test

ns – Non significant when compared to control groups

Table 3. Effect of Siddha formulation (NMVM) on Biochemical markers of liver and kidney after 15 days repeated oral dosing (500 mg/kg/po) in rats

Groups	**AST (IU/L)**	**ALT (IU/L)**	**Cholestrol (mg/dl)**	**Urea (mg/100ml)**	**Uric acid (mg/ 100ml)**
Normal	70.48±0.273	30.40 ± 0.831	45.09 ± 0.397	24.56 ± 0.637	1.98 ± 0.650
NMVM (500mg/kg/p.o)	82.65±5.952 ns	34.61 ± 2.267 ns	41.59 ± 0.627ns	23.30 ± 0.59 ns	2.41 ± 0.735 ns

N=6; Values are expressed as mean ± S.D followed by Students Paired 'T' Test

Ns – non significant when compared to control groups

3.4 Histopathological Study

NMVM at the dose of 500 mg/kg/po daily administered for 15days did not show evidence of pathological lesions in the tissues tested (Plate 1).

3.5 Analgesic, Anti inflammatory Studies

NMVM at the dose of 500 mg/kg/p.o showed significant analgesic activity in rats(Table-4).NMVM also exhibited significant anti-inflammatory activity in carrageenan induced hind paw edema (acute inflammation model) in rats. The results of present study was comparable to that of the standard NSAID Diclofenac sodium (5 mg/kg/p.o) (Table-5).

Table 4. Analgesic activity of NMVM using Tail flick Method

Control	**Paw licking response (Sec)**			
	0 min (Sec)	**30 min (Sec)**	**60 min (Sec)**	**120 min (Sec)**
Control	5.56 ± 0.96	5.86 ± 0.96	5.76 ± 0.67	5.86 ± 0.53
NMVM (500mg/kg. p.o.,)	5.66 ± 0.206 ns	7.12 ± 0.258 ***	9.26 ± 0.516 ***	9.71 ± 1.394 ns

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.

Table 5. Anti inflammatory activity of NMVM in Caarrageenan induced hind paw edema in rats

Groups	**Paw volume (ml) by mercury Displacement at regular interval of time**					
	0min	**30min**	**60min**	**120min**	**240min**	**4thhrs**
Control	1.233 ± 0.338	1.733± 0..225	1.766± 0.286	2.200 ± 0.236	2.25 ± 0.273	2.266± 0.236
NMVM (500mg/kg. p.o.,)	1.533 ± 0.638 ns	1.90 ± 0.236 ns	2.25 ± 0.966 ns	3.932± 0.616 ***	3.347 ± 0.672 ***	1.691 ± 0.174 ***
Standard (Dic.Sodium 5 mg/kg/po)	0.835 ± 0.065 ns	1.315 ± 0.069 ns	1.128 ± 0.049 ns	1.011 ± 0.056 ***	0.896 ± 0.048 ***	0. 85 ± 0.054 ***

n=6; Values are expressed as mean ± S.D followed by student paired T- test.

ns - Non *significant* as compared with control; P< 0.001 (***) as compared with control

3.6 Antioxidant Activity

At the end of 15 days repeated oral toxicity study when the plasma of drug treated animals was examined for GSH activity, the level of GSH activity was increased significantly ($p>0.001$) in test groups. On the other hand LPO activity of treated animals was increased when compared to control (Table-6).

Table 6 .Anti oxidant activity of Siddha Formulation (NMVM) After 15 days repeated oral dosing (500 mg/kg)

Groups	**LPO (µmols/g.protein)**	**GSH (µmols/g.protein)**
Control	0.60 ± 2.637	45.28 ± 2.31
NMVM (500mg/kg/p.o)	0.83 ± 5.96***	100.831 ± 1.35***

N=6; Values are expressed as mean ± S.D followed by Student T- Test.

***P<0.001 as compared with control.

4. DISCUSSION

The preliminary phytochemical study revealed the presence of several phyto-constituents. The test drug answered for the presence of calcium, ferrous iron, sulphate, and chloride. Repeated oral toxicity study conducted for15days with the drug did not exhibit significant changes in blood counts and in Hb% .The biochemical markers of liver function and kidney function tests did not show evidence of liver and kidney toxicity. There was no significant changes in biochemical parameters like blood Cholesterol, body weight, food, water intake and behavioural parameters. The test drug exhibited significant analgesic and anti-inflammatory activity in acute experimental inflammatory conditions in rats. The test drug showed maximum anti-inflammatory activity at the end of 4^{th} hour after carrageenan challenge; the mechanism of anti-inflammatory activity of test drug may be attributed for its inhibitory activity on cyclooxygenase (COX) enzymes.

Oral administration of NMVM for 15 days at the dose of 500mg/kg/po showed an increase in LPO in serum. Likewise GSH also showed an increase in the serum of the drug treated animals. The free ferrous iron present in the preparation may be responsible for the enhanced lipid peroxidation and the tissue synthesis higher amount of GSH in defence against LPO, can also be accounted for this observation.

5. CONCLUSION

As per our literatures, medicine is prepared with standard raw drugs, proper purification methods, proper consistency, accurate dosage with adequate adjuvant and proper duration there is no doubt about the safety and toxicity of the medicine which contains metals and minerals. Also, the anti inflammatory, analgesic and antioxidant studies revealed its efficacy in treating the *Vatha* diseases and especially *Thandaga Vatham*.

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