International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Serological Evaluation of the Anti-coagulant Activity of the Classical Siddha Medicine: 'Veera Mezhugu' in Wistar Albino Rats

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Abstract

In setting the basis for the management of arterial thrombosis, cardiac ischemic changes followed by any severe coronary atherosclerotic changes or any venous thrombosis, there is a conventional use of Acute thrombolytic agents, Anticoagulants with antiplatelet medications in general.

These symptoms complex are mentioned as Thamaraga vaayu or Ruththira vaayu or Maaradaippu(Acute coronary syndrome) and Uthirakattu(thromboembolism) in siddha literatures.

Veera mezhugu is one of the Siddha formulations chosen from Anuboga vaithiya navaneetham part-4, which is indicated for Uthirakattu and Thamaragavaayu. The drug prepared as per the Siddha literature. The drug has undergone safety studies and the efficacy studies in Wistar albino rats where the serum of the different groups with different doses are compared with the placebo control to evaluate the anti-coagulant potential.

Coagulation profile of the three parameters of Activated partial thromboplastin time(APTT), Prothrombin time(PT) and International normalized ratio(INR) are taken for evaluation and had significant difference between the groups[$p<0.05$].Serological analysis of the Wistar rats shows significant anticoagulant activity in a dose dependent manner.

Keywords: Anti-coagulant, Complementary & Alternative medicine, Veera Mezhugu, Siddha Medicine, Drug development, Ethnopharmacology

INTRODUCTION

In setting the basis for the management of arterial thrombosis, cardiac ischemic changes followed by any severe coronary atherosclerotic changes or anyvenous thrombosis, there is a conventional use of Acute thrombolytic agents, Anticoagulants with antiplatelet medications in general.

These symptoms complex are mentioned as Thamaraga vaayu or Ruththiravaayu or Maaradaippu in siddha literatures with the analogous presentation as the Myocardial Infarction.

In the classical siddha text of Agathiyar gunavagadam published by Malaiyappa samy vaidhiyasaalai, Palani, there is evidence for the analogous symptomatic similarity for occlusive heart disorder especially mimicking MI under the topic of Maaradaippu. and also mentions a few acute medical interventions for thesymptomatic presentation of that syndrome. And all those suggetions of medical interventions is very much contributed to the acute thrombolytic interventional setup.

In the classical siddha text of Anuboga vaithiya navaneetham part 4 (p.no: 144)which also indicates for the disease of Ruththira vaayu (MI /Cardiac Ischemia), Udhira vaayu, Udhira chikkal(Thrombosis).

Based on this suggestion from the Agathiyar gunavagadam and AnubogaVaidhiya Navaneetham (part 4), I have taken the drug of Veera Mezhugu to scientifically validate whether the chosen drug helps in the amelioration of the mentioned symptoms.

AIM AND OBJECTIVE:

This study aimed to evaluate the anti-coagulant activity of 'Veera Mezhugu' through the in-vivo serological assays.

MATERIALS AND METHODS:

Approval of IAEC:

All the animals are housed and used for the experimentation after the approval in VPT, VCRI, Tirunelveli. [Proposal number:20/SA/IAEC/VPT, VCRI, TNI/2023]

Preparation Of Veera Mezhugu:

Process of preparation:

As mentioned in Anuboga Vaidhiya Navaneetham part 4 (p.no :144), All the following purified drugs are powdered and made into a paste by adding the Anda thailam frequently in a small quantity and grinded for sufficient duration and made into tablets..

1. Veeram (Hydragyrum perchloride) - 1 varaagan (3 ½ grams)
2. Navachaaram (Ammonium chloride) - 1 varaagan (3 ½ grams)
3. Rasa karpooram (Hydragyrum subchloride) - 1 varaagan (3 ½ grams)
4. Chukku (Zingiber officinalis) - 1 varaagan (3 ½ grams)
5. Milagu (Piper nigrum) - 1 varaagan (3 ½ grams)
6. Arisi thippili(Piper longum) - 1 varaagan (3 ½ grams)
7. Anda thailam(egg yolk oil) - Needed quantity

Experimental procedure:

A serological assay of the three parameters of APTT, PT& INR are evaluated by collecting the serum of rats of three groups pretreated orally with the trial drug of Veera Mezhugu with concentration of 2.5mg/kg, 5mg/kg & 10mg/kg respectively and a group of normal control without any intervention.

Coagulation assay of Veera Mezhugu

For the *in vivo* coagulation assay, the APTT, PT & INR levels of plasma were determined using commercial diagnostic kits, following the instructions of the manufacturers. Briefly, after 2 hours of the oral dosage, blood samples were collected from the retro-orbital vein of Veera Mezhugu-treated (2.5mg/kg,5mg/kg &10mg/kg) & control group (untreated) rats in microfuge tubes containing 3.8% sodium citrate solution (9:1, v/v). The platelet poor plasma (PPP) was obtained by centrifugation of the blood at 2000*g* for 10 minutes at 4°C. For each experiment, 100 µL of PPP was used, and the coagulation time (APTT, PT and INR) of the treated and the control PPP groups was recorded in a coagulometer.

Statistical Analysis

The data were statistically analyzed by One way- ANOVA test, using SPSS software for Windows (version 10.0). A *P* value of <.05 was considered as the significant difference between the values.

Results and Discussion:

To evaluate the *in-vivo* anticoagulant potential of the 'Veera Mezhugu' in Wistar albino rats we have taken the serum of the rats after 2 hours of oral treatment and then undergone the serological coagulation assay. The dose-dependent treatment of Veera Mezhugu shows prolongation of APTT& PT compared to the normal control group of rats. Also, the INR value of the Veera Mezhugu treated group have shown significant increase compared to the normal control group. From this we have arrived that the drug acts on both common pathways of intrinsic as well as extrinsic pathway.

Group	APTT (Mean)	PT (Mean)	INR (Mean)
Control	26±1.01	14±1.03	0.9±0.07
VM(2.5mg/kg)	27±1.72	14±1.22	1±0.09
VM(5mg/kg)	29±1.02	15±1.3	1±0.07
VM(10mg/kg)	31±1.05	16±1.2	1.2±0.08

F-statistic and p-value of APTT – 12.05 & 0.0003 respectively
F-statistic and p-value of PT – 2.58 & 0.094 respectively
F-statistic and p-value of INR – 10.93 & 0.0006 respectively[1]

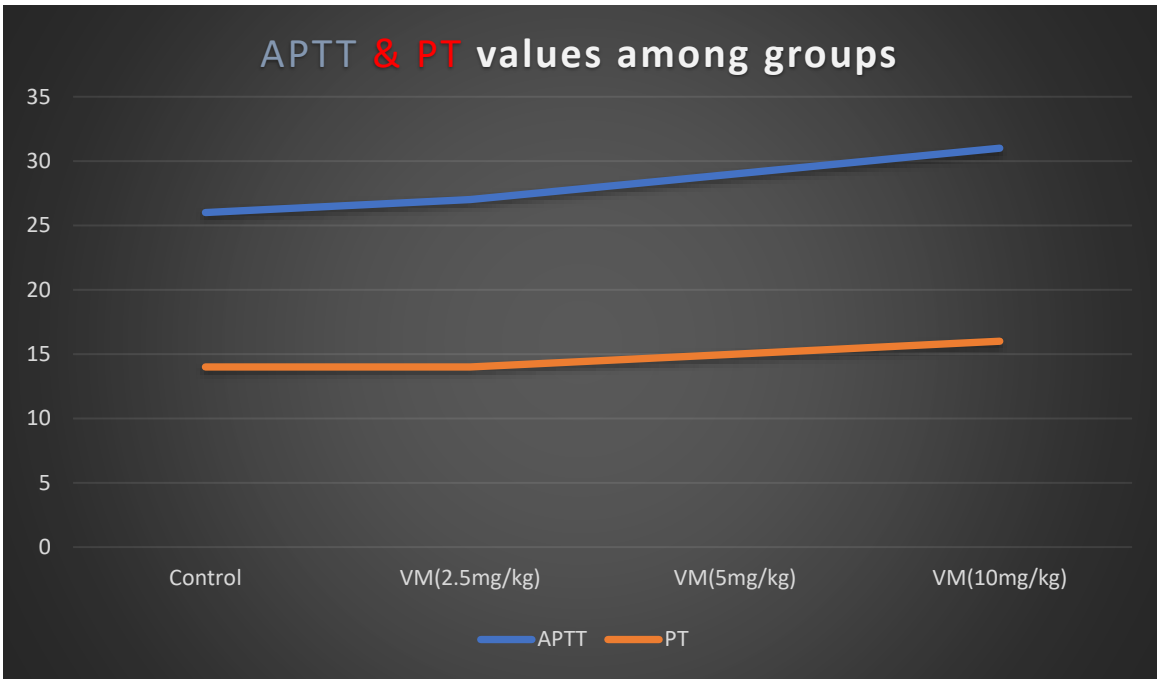

Figure-1 , Activated partial thromboplastin time (APTT) and prothrombin time (PT) of Veera Mezhugu (2.5mg/kg,5mg/kg,10mg/kg 2 hours after oral administration) was measured against citrated platelet poor rat plasma in a coagulometer at 37°C. Data represent mean ± SD of quadruplicate experiments. Significance of difference with respect to normal control group of rats, *P* < .05.

[1] P-value <0.05 for all serum coagulation parameters among the Veera Mezhugu treated and placebo control groups.

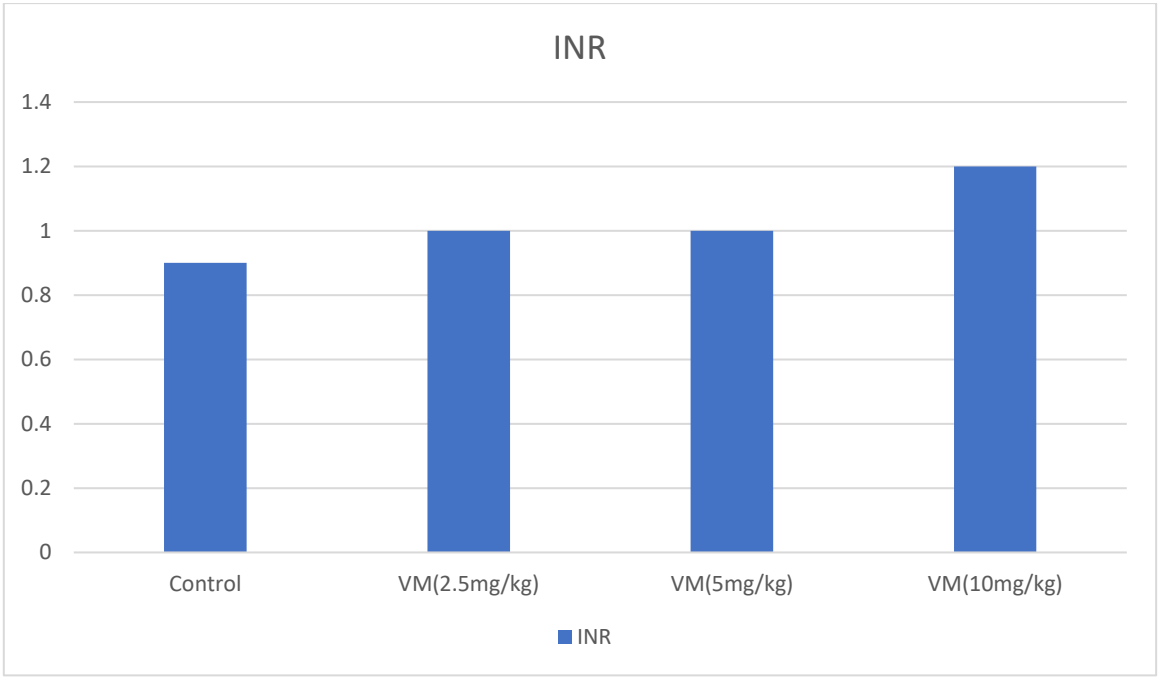

Figure-2. INR value with respect to normal control and Veera Mezhugu-treated groups of rats. The experiment was done as described in the text. Data represent mean ± standard deviation (SD) of quadruplicate determinations. Significance of difference with respect to control group of rats, *$P < 0.005$

The advent of novel anticoagulant drugs is essential as the already commercialized conventional drugs like heparin have a narrow therapeutic index. Hence in this way the study of drugs from the ethnopharmacological background should be encouraged and the above drug shows some significance potential for further development in this anticoagulant pharmacology.

Conclusion:

The results of the present study proves the significant anticoagulant activity for the Siddha Medicine 'Veera Mezhugu' compared with the placebo controlled rats by the prolongation of APTT, PT time and increased INR in a dose-dependent manner. This study further have to go through the pharmacokinetics and pharmacodynamics for the Phase-II studies to study its potential. We have to further look into the ethnopharmacological & traditional medicinal literature data as the lacuna for the novel drug development is widening.

ACKNOWLEDGMENTS

The author is thankful to Prof Dr.G.Esakky Pandian ,MD(S), Government Siddha Medical College, Palayamkottai for his guidance.

Conflict of Interest: None

Source of Funding: None

Ethical Approval: Approved

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