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Research Article

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In vitro thrombolytic activity of Chanthira Prakasa Mathirai

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Abstract

Hemiplegia is the commonest manifestation of a “Stroke” with neurological defect affecting the face, limbs and trunks on one side or either side of the body. Impulses for voluntary movement are transmitted by the pyramidal tract or upper motor neurons. Damage to these pyramidal tracts due to any lesion, trauma, ischemia or haemorrhage produce paralysis. In our siddha system Hemiplegic may be compared to *Paarisa vayu* or *patchavatham*. *PATHCAM* means half, since it cause paralysis of one half of the body it is called as *Patcha vatham. S*troke is the third most common cause of death after cancer and ischemic heart disease and most common cause of severe physical disability. According to the world Health organization 15 million people suffer from stroke worldwide each year. Of these 5 million die and another 5 million are permanently disabled. Those who with high blood pressure, diabetes, high blood fat (Cholesterol) are specially at risk. In the indigenous system of medicine, the *Chanthira prakasa mathirai* (CPM) is reported to be useful in the treatment of Hemiplegia. Accordingly, the present study was intended to investigate invitro thrombolytic activity of *Chanthira prakasa mathirai* a demonstrated very significant (P<0.001) clot lytic properties in different blood samples. The present clot lytic activity was compared with water (Negative control) and standard enzyme streptokinase (Positive control). The mean % of clot lysis for water and streptoinasge was found 3.8% and 72% separately. Then again the mean percent clot lytic activity of CPM was found 31.60% which is significant compare with positive and negative control.

Keywords: Hemiplegia, *Patcha vatham.,Chanthira prakasa mathirai,* clot lytic activity

Introduction

Siddha system is considered to be one of the ancient system of medicine in the world. Siddha is a complete holistic medical system that has been practiced in India for more than thousands of years. In our Siddha system Hemiplegia may be compared to *paarisa vayu. Chanthira prakasa mathirai* is a herbomineral preparation said in our siddha literature is very effective and traditionally used in the treatment of *paariasa vayu (*Hemiplegia*).* It is less expensive and it has no complications. In this study, Thrombolytic activity of CPM is analysed in human blood sample.

Materials and Methods

Chanthira Prakasa mathirai is a herbo mineral formulation which indicated as a drug in Siddha sastric text “Veeramamunivar Vagada Thirattu – Pg.No.83” for treatment of Hemiplegia, Periarthritis and joint disease ect. The ingredients of *Chanthira prakasa mathirai* are *Aconitum ferox, Piper nigrum, Zingiber officinale* and *Sodium Biborate*. The drug was prepared as per the text.

Pharmacological analysis

Thrombolytic activity

Reagents and chemicals

Streptokinase (SK) vials of 15, 00, 000 I.U.10 blood (5ml) sample drawn from healthy human volunteers, *Chanthira Prakasa Mathirai* , Distilled Water.

Apparatus

Micro centrifuge tube (0.5ml/tube), Micropipette, Vortex mixer, 0.22-micron syringe filter, Beaker, Electric Balance, Incubator.

Experimental procedure

Streptokinase (SK)

To the commercially available lyophilized SK vial (Polamin Werk GmbH Herdecke, Germany) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U.) was used for in vitro thrombolysis.

Specimen:

Whole blood (5 ml) was drawn from healthy human volunteers (n=10) without a history of oral contraceptive or anticoagulant therapy. 500 µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots.

Sample preparation:

The siddha formulation was suspended in 10 ml distilled water and shaken vigorously on a vortex mixer. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a filter paper. The solution was then ready for in vitro evaluation of clot lysis activity.

Streptokinase (SK) solution preparation:

To the commercially available lyophilized SK vial (PolaminWerk GmbH, Herdecke, Germany) of 15,00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl 30,000I.U) was used for in vitro thrombolysis.

Thrombolytic assay:

Experiments for clot lysis were carried as reported earlier (1). Venous blood drawn from healthy volunteers was transferred in different pre- weighed sterile eppendorf tube (500µl/tube) and incubated at 37 °C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube-weight of tube alone). Each eppendorf tube containing clot was properly labeled and 100 µl of Chanthira Prakasa Mathirai was added to the tubes. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight taken before and after clot lysis was expressed as percentage of clot lysis(2).

Streptokinase and water were used as positive and negative control, respectively. The experiment was repeated several times with the blood samples of different volunteers.

% clot lysis = (Weight of the lysis clot /Weight of clot before lysis) × 100.

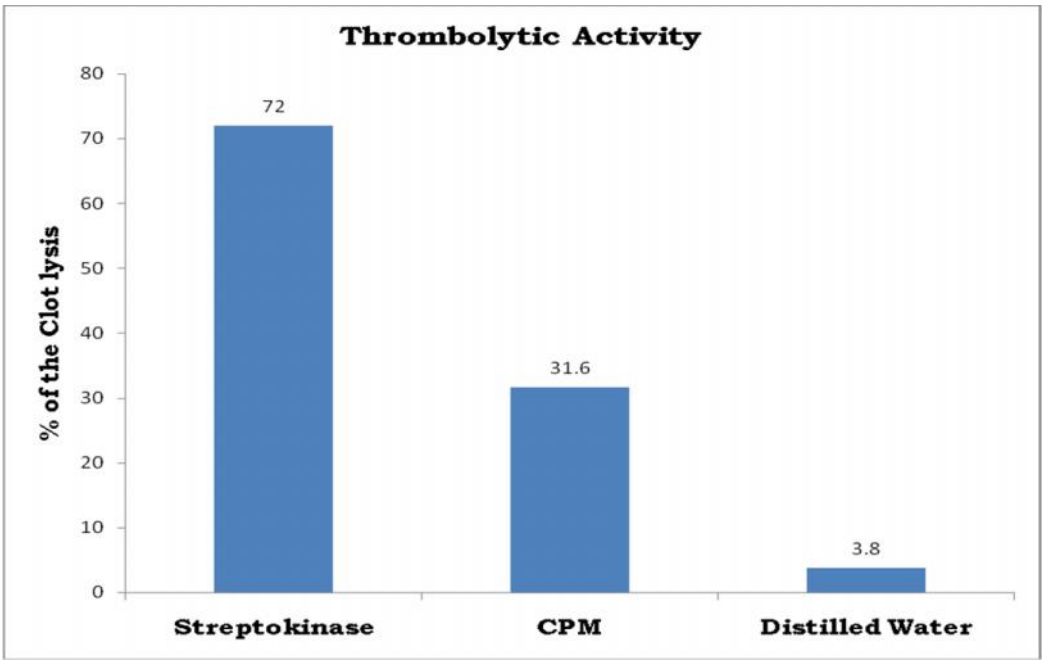
Results

Thrombolytic activity assay

Addition of 100 µl SK, a positive control (15,00,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 72% ± 1.95 % clot lysis. Clots when treated with 100µl sterile distilled water (negative control) showed only negligible clot lysis (3.80%). The in vitro thrombolytic activity study revealed that Chanthira Prakasa Mathirai showed 31.60%.Statistical representation of the effective clot lysis percentage by our siddha preparation, positive thrombolytic control Streptokinase) and negative control (sterile distilled water) is tabulated in (Table 1)

Table 1: Thrombolytic Activity of Chanthira Prakasa Mathirai

No	Weight of the empty tube(A)gm	Weight of the tube with clot(B)gm	Weight of clot.(C) C=BA	Weight of the tube with clot after lysis(D)gm	Weight of lysis(E) (B-D)	% of clot lysis	Average % of clot lysis
1	0.82	1.09	0.27	1.02	0.7	23.24	
2	0.84	1.12	0.28	1.03	0.9	39.30	
3	0.84	1.13	0.29	1.01	0.12	31.24	
4	0.79	1.18	0.39	1.09	0.9	37.50	31.60%
5	0.81	1.08	0.27	0.98	0.10	29.61	
6	0.83	1.16	0.33	1.05	0.09	32.34	
7	0.76	1.18	0.42	1.07	0.11	23.08	
8	0.78	1.20	0.42	1.12	0.08	32.36	
9	0.79	1.21	0.42	1.03	0.18	36.12	
10	0.82	1.29	0.47	1.16	0.13	31.10	



Discussion

The present study was undertaken to evaluate the thrombolytic activity of Chanthira Prakasa Mathirai denticulata. In the thrombolytic bioassay result suggested that the Chanthira Prakasa Mathirai showed very significant activity. The Chanthira Prakasa Mathirai can be evaluated to further research for thrombolytic activity to a specific disease.

Atherosclerosis-induced heart attacks and strokes are leading reasons of morbidity and mortality. Current essential and auxiliary prevention strategies emphasize control of different atherosclerotic danger components, including smoking, hypertension, hypercholesterolemia, diabetes mellitus, weight, irritation, and homocysteine . Current pharmacological studies recommend remedial estimations of these natural preparations, including lowering of blood pressure and lipids, antioxidation, thrombolytic activity and the promotion of microcirculation. There is a requirement for more goal and scientific approaches to authenticate individual herbs to identify chemical constituents, detect adulteration or contamination of herbs, and screen the quality of herbs and herbal medicines.

There is also a need to check the consistency of different batches of herbs utilized as a part of this study and to distinguish bioactive parts in herbs reported to have physiological effects.

Conclusion

Under this study, *Test drug Chanthira Prakasa Mathirai* of demonstrated moderate (P <0.001) clot lytic properties in different blood samples. The percent clot lytic activity was compared with water (positive control) and standard enzyme streptokinase (negative control). The mean % of clot lysis for water and streptokinase was found 3.8% and 72% separately. Then again the mean percent clot lytic activity of *Test drug Chanthira Prakasa Mathirai* was found 31.60%, which is significant compare with the positive and negative control. So, the present research proposes that, the *Test drug Chanthira Prakasa Mathirai* has moderate thrombolytic activity. Thus the formulation may be a source of effective herbal drug.

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