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THROMBOLYTIC ACTIVITY OF *TINOSPORA SINENSIS*

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ABSTRACT

The methanol extract of *Tinospora sinensis* was estimated for thrombolytic effect. The extract demonstrated moderate thrombolytic activity which was 25.01 % whereas the thrombolytic activity of standard was found 88.23 %. Thrombolytic indicates that have potent clot lytic property and anti-oxidative activity. The obtained results support for the uses of this plant as traditional medicine.

INTRODUCTION

Many of the modern day's vital drugs and processed medicines are of plant origin. Medicinal plants contain different remedial agents which may have thrombolytic activity, antimicrobial activity, cytotoxic effect etc. Medicinal plants extract demonstrated that they can lyses thrombus as streptokinase [1]. Medicinal plants play a leading role in the treatment of varieties of human diseases from the dusk of human development [2]. *Tinospora sinensis* have been of medical interest due to their good therapeutic value in folk medicine [3]. Working with different medicinal plants extract showed that they can lyses thrombus as streptokinase [2, 4].

Thrombolysis is the breakdown (lysis) of blood clots [5] by pharmacological mean. It is colloquially referred to as clot busting for the reason. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator .The protein that normally activates plasmin. Thrombolysis requires the use of thrombolytic drugs, which are either derived from Streptokinase species. Some commonly used thrombolytics are: Streptokinase, Urokinase, Reteplase, Tenecteplase. Formation of blood clot lies at the basis of a number of serious diseases. By breaking down the clot, the diseases process can be arrested, or the complication reduced. While other anticoagulants (such as heparin) decrease the growth of a clot, thrombolytic agent actively reduces the size of the clot. Diseases where thrombolysis is used: Myocardial infarction, Stroke (ischemic stroke) [6], Massive pulmonary embolism, Acute limb ischemia.

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MATERIALS AND METHODS

Collection and identification of the plant sample

The fresh leaves & stems of *Tinospora sinensis* were collected from the Kurnool (Dt) and authenticated by Botanist, Dr. P. Jayaraman, Plant Anatomical Research Centre (PARC), Tambaram, Chennai and the voucher specimen was kept in the Department of Pharmacognosy, St.Mary's College of Pharmacy, Secunderabad, Andhra Pradesh, India. The leaves and stems were grind into coarse powder with the help of an attrition type of a grinder.

Extraction of leaves

About 250 gm of powdered leaves was taken in a clean flat –bottomed glass container and percolated with 3 liters of Methanol. The container with its content was sealed and kept for 7 days with occasional shaking and stirring .the mixture was the filtered successively through a piece of clean white cotton .The filtrate thus obtained are kept in a open air for the evaporation of the methanol. After 10 to 15 days all the methanol are evaporated and I got the extract of methanol.

THROMBOLYTIC EFFECT OF *TINOSPORA SINENSIS*

Preparation of extract solution

100 mg of extract is suspended in 10ml of distilled water.

- Then it kept overnight.
- The soluble supernatant is decanted and filtered.

Specimen preparation and *In Vitro* Thrombolytic Study

5 micro centrifuge tubes are taken, sterilized, weighed. 6 ml blood is drawn from each volunteer. The blood is distributed in 5 different pre weighed (W1) micro centrifuge tube, each tube contain 0.5 ml of blood. The blood specimen is centrifuged at 2500 rpm for 5 minutes. Experiments for clot lysis were carried as reported earlier [7].Then incubates the blood for 45 minutes 37º C. After clot formation i.e. incubation, the serum was completely removed by decantation, capillary absorption and by removing the serum from the inner surface of the tube. Kept the tubes at lying position on a tray for 6 minutes after first removal of serum and then remove the liquids of the tube surface by the cotton rod. Each tube was weighed (W_2) again. Weight of clot is found as-Weight of clot = weight of clot containing tube (W_2) – weight of tube alone (W_1). Result varies for inappropriate weighing so it is done very carefully. Then I add 100µl of aqueous extract of *Tinospora sinensis* plant extract to each micro centrifuge tube containing per-weighed clot. As a positive control, 100 µl of streptokinase is added to clot of standard tube. As a negative control, 100 µl of water is added to clot of blank tube. All the tubes are incubated at 37º c for 90 minutes and observed if clot lysis had occurred.

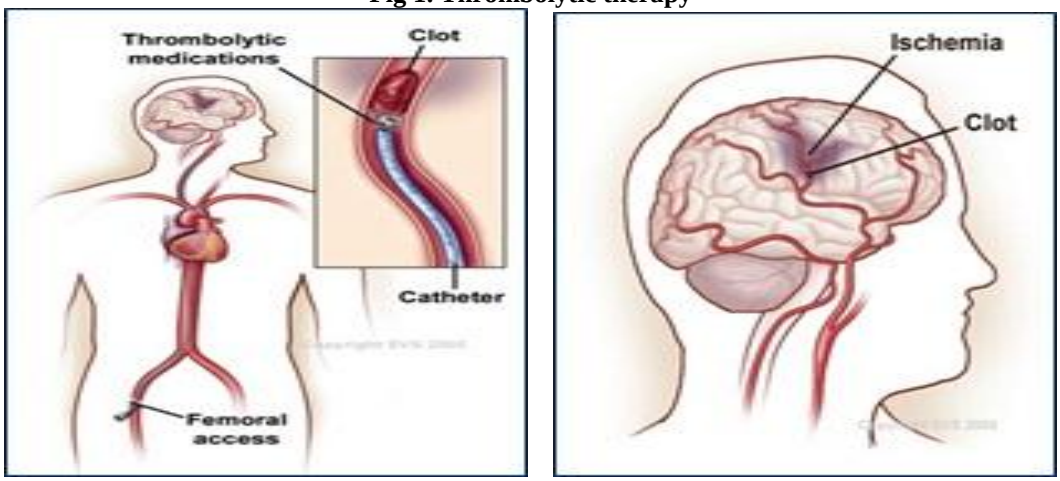
After 90 minutes of incubation, the released fluid is completely removed by decantation, capillary absorption and by removing the dissolved clot containing liquid from the inner surface of the tube carefully by cotton bar or by use of cotton tightly bound at top of a glass rod without disrupting the clot. The tubes are then weighed again. I have to ensure complete removal of released fluid, or the result will be erroneous. Keep the tubes at lying position on a tray for 6 minutes after first removal of released clot and then remove the liquids of the tube surface by the cotton rod. Weigh the tubes (W_3) very carefully, please weigh very carefully, result varies for unsuitable weighing. The difference obtained in weight taken before and after clot lysis is expressed as percentage of clot lysis [7].

% of clot lysis = (wt of released clot /clot wt) × 100
= (W2 – W3 / W2 – W1) × 100

Table 1. Data Analysis (Experiment)

Sample No.	W_1 (gm)	W_2 (gm)	W_3 (gm)	Sample No.	% of clot lysis
Tube 1	6.16	6.69	6.59	Tube-1	24.52
Tube 2	6.09	6.58	6.45	Tube-2	26.53
Tube 3	6.35	6.4	6.34	Tube-3	24
Standard	6.03	6.2	6.05	Standard	88.23
Blank	6.35	6.74	6.68	Blank	15.38

Fig 1. Thrombolytic therapy



RESULTS AND DISCUSSION

The thrombolytic activity of standard was found 88.23% and for Crude extract, n-Hexane, and CCl_4, respectively 24.52%, 26.53%, 24.00 % which indicates mild thrombolytic activity of *Tinospora sinensis*. Atherothrombotic diseases occur as serious impacts of the thrombus formed in blood vessels. Various thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels; but these drugs are not above limitations and can lead to serious and sometimes fatal consequences. Thrombolytic technique was used to

examine the thrombolytic activity of Plant extracts in blood sample from healthy human volunteers, along with streptokinase as a positive control and water as a negative control [7]. SK, a known thrombolytic drug is used as a positive control [8].

The comparison of positive control with negative clearly demonstrated that clot dissolution does not occur when water was added to the clot. On the basis of the result obtained in this present study we can say that the extract has moderate thrombolytic activity compared to negative control (water). However further research is necessary to find out the thrombolytic activity of the active compound.

One of the major causes of blood circulation problem is the formation of blood clots. Thrombi or emboli can lodge in a blood vessel and block the flow of blood in that location depriving tissues of normal blood flow and oxygen. This can result in damage, destruction (infarction), or even death of the tissues (necrosis) in that area. Fribrinolytic drugs has been used to dissolve thrombi in acutely occluded coronary arteries there by to restore blood supply to ischemic myocardium, to limit necrosis and to improve prognosis [9].

Streptokinase is an antigenic thrombolytic agent used for the treatment of acute myocardial infarction. It reduces mortality as effectively as the nonantigenic altreplase in most infarct patients while having the advantages of being much less expensive. Tissue-type Plasminogen activator (tPA) is generally preferred as being effective and safer than either urokinase or streptokinase type activators. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs [10, 11].

CONCLUSION

From this experiment, in summary, pharmacological evaluation of methanol extract of *Tinospora sinensis* has got the very good potential as a candidate for future thrombolytic. This is only a preliminary study and to make final comment the extract should methodically investigated phytochemically and pharmacologically to develop their medicinal and pharmaceutical potentialities.

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