

Evaluation of In Vitro Thrombolytic Activity of Ethanolic Extract of *CURCUMA CAESIA* Rhizomes

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ABSTRACT

Thrombolytic agents dissolve blood clots and limit the damage caused by the blockage or occlusion of a blood vessel. These are widely used for the management of myocardial infarction, thromboembolic strokes, deep vein thrombosis and pulmonary embolism. Currently used synthetic drugs causes adverse effects such as major bleeding, cardiac arrhythmias, cerebrovascular hemorrhage and anaphylactic reaction, so there is a need to investigate some more safe natural thrombolytic agents. Present study is a preliminary work towards such endeavors. It was designed to investigate in vitro thrombolytic activity of ethanolic extract of *Curcuma caesia* rhizomes. An in vitro thrombolytic model was used to evaluate the clot lysis effect of ethanolic extract of *Curcuma caesia* rhizomes along with Streptokinase as a positive control and distilled water as a negative control. The ethanolic extract was found to have significant thrombolytic activity (49.18±3.41%) compared to the effect of Streptokinase (71.54±3.26%) used as a positive control and water (2.96±0.28%) used as a negative control. The current study refers the *Curcuma caesia* rhizomes as impressive thrombolytic agent for further used for the treatment of cardiovascular diseases. Therefore, steps should be taken to observe *in vivo* clot dissolving potential and to isolate active component(s) of these extracts which have Thrombolytic activity.

Keywords: *Curcuma caesia*, thrombolytic, streptokinase, clot lysis

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INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments [1].

Thrombosis is the formation of a potentially deadly blood clot inside a blood vessel artery (arterial thrombosis) or vein (venous thrombosis), obstructing the flow of blood through the circulatory system. Once formed, a clot can slow or block normal blood flow, and even break loose and travel to an organ. This can result in significant injury, including heart attack, stroke and venous thromboembolism the top three

cardiovascular killers [2]. Thrombolytic drugs are used to dissolve blood clots in a procedure termed thrombolysis. Antithrombotic drugs are used to dissolve blood clots and are mainly of three type's antiplatelet agents, fibrinolytic drugs and anticoagulants. Depending upon the thrombus formed, effective antithrombotic therapy can be instituted, i.e., arterial thrombosis is treated with antiplatelet agents and venous thrombosis can be treated with anticoagulants mainly but they do not cause clot lysis that has already been formed. They prevent thrombus extension, recurrence and embolic complications. For the lysis of already formed thrombus, fibrinolytic drugs are used (Streptokinase, Urokinase, Alteplase, Reteplase, Tenecteplase, etc.). But due to various side effects such as bleeding complications, Hemorrhagic stroke allergy and rarely

anaphylaxis researchers are bound to discover newer drugs [3]. Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need [4].

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Several plants such as *Ocimum sanctum*, *Curcuma longa*, *Azadirachta indica*, *Anacardium occidentale* [5], *Molineria recurvata*, *Terminalia bellerica*, *Tulbaghia violacea*, *Curcuma longa*, *Melastoma malabathricum*, *Gloriosa superba*, *Jatropha curcas*, *Porana volubilis*, *Synclisia scabrida*, *Allium sativum* *Allium cepa* [6] have been proved to possess thrombolytic activity and many such plants are yet to be scientifically investigated. In the present study ethanolic extract of *curcuma caesia* rhizomes which contains curcumene as one of its chemical constituent is used to evaluate the thrombolytic activity.

Curcuma caesia, black turmeric or black zedoary, Family: Zingiberaceae is a perennial herb with therapeutic significance which is bluish-black rhizome, native to North-East and Central India. It is also sparsely found in the Papi Hills of East Godavari, West Godavari of Andhra Pradesh, Khammam district of Telangana, the root hills of the Himalayas and North Hill forest of Sikkim [7]. Traditionally, the rhizomes are reported to have valuable in treating leucoderma, asthma, tumours, piles, bronchitis, bruises, rheumatic pains etc. [8]. A preliminary phytochemical study on the rhizomes of *Curcuma caesia* revealed presence of carbohydrate, flavonoid, steroid, phenol, alkaloid, tannin, amino acid, terpenoids and glycoside compounds as major constituents [9]. The research on the volatile oil of *C. caesia* rhizomes resulted in the identification of 30 components, representing 97.48% of the oil, with camphor (28.3%), *ar*-turmerone (12.3%), (*Z*)- β ocimene (8.2%), *ar*-curcumene (6.8%), 1,8-cineole (5.3%), β -elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%) and γ -curcumene (2.82%) as the major constituents [10]. From the literature available is evident that rhizomes of

Curcuma caesia has pharmacological functions including analgesic [11], anti-inflammatory [11], antiulcer [12], antifungal [13], antioxidant [14], antiasthmatic [15], smooth muscle relaxant activity [15], anticonvulsant [16], locomotor depressant [16], skeletal muscle relaxant activity [16], antibacterial [17], antipyretic [18] and hepatoprotective [19] activity. The present study has been carried out to explore the Sthrombolytic activity of ethanolic extract of *curcuma caesia* rhizomes.

MATERIALS AND METHOD

Collection of Plant Material:

The rhizomes of *Curcuma caesia* were collected from the local medicinal plant supplier and were authenticated by Dr. P. Veera Reddy, Professor, Government Ayurvedic College, Warangal, Telangana, India.

Preparation of Standard:

Commercially available lyophilized Streptokinase vial (Beacon pharmaceutical Ltd.) of 15, 00,000 I.U. was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 μ l (30,000 I.U) was used for *in vitro* thrombolysis.

Preparation of Plant Extract:

The rhizomes of *Curcuma caesia* were shade dried. The dried roots were powdered in an electrical processor and then the powder was separated. 100 gram of dried powder material was extracted in a soxhlet apparatus with 500 ml. of absolute alcohol. The ethanolic extract was then distilled, evaporated and dried in vacuum. All the extracts were kept in desiccator and stored in a refrigerator for pharmacological experiment.

Methodology:

Healthy volunteers who are not on any type of medication for past 7 to 10 days were selected for the study. 4ml of venous blood was withdrawn from each human volunteer and transferred to different pre weighed sterile alpine tubes tube (0.5ml/tube). The tubes were now incubated at 37 $^{\circ}$ C for 45 minutes. After clot formation serum was aspirated out without disturbing the clot formed. Each tube having the clot was again weighed to estimate the clot weight.

Clot Weight= Weight of clot formed in the tube-weight of empty tube

To each alpine tube containing clot appropriate labeling was done and 100 μ l of ethanolic extract of rhizomes of *Curcuma caesia* was added. To positive control tube Streptokinase was added and distilled water was added to negative control alpine tube. All the tubes were then incubated at 37°C for 90 minutes and observed for clot

lysis. After incubation the supernatant fluid released was removed slowly and the tubes were weighed again to observe the difference in weight after clot disruption.

Difference between weight taken before and after clot lysis was plot as ratio to obtain the percent of clot lysis [20].

$$\% \text{ Of Clot Lysis} = \frac{\text{Weight of Lysis}}{\text{Weight of clot before Lysis}} \times 100$$

The test was repeated ten times with the blood samples of ten healthy volunteers.

STATISTICAL ANALYSIS

The % of clot lysis is expressed as Mean \pm standard deviation. Paired Student's t-test was used to analyze the level of significance. The significance of % clot lysis was assessed using one way analysis of variance (ANOVA). The test followed by the paired t-test p values less than 0.05 were considered as statistically significant [21].

RESULT

The graphical representation of effect of ethanolic extract of rhizomes of *Curcuma*

caesia is given in Figure 1. The extract showed 49.18 \pm 3.41% of clot lysis, addition of 100 μ l Streptokinase has 71.54 \pm 3.26% of clot lysis, which is taken as positive control whereas distilled water which was taken as negative control showed negligible clot lysis of 2.96 \pm 0.28% which was incubated for 90 minutes at 37°C. The percentage of clot lysis between positive and negative control was found to be significant statistically. The in vitro thrombolytic activity study revealed ethanolic extract of rhizomes of *Curcuma caesia* exhibits significant thrombolytic activity when compared with negative control.

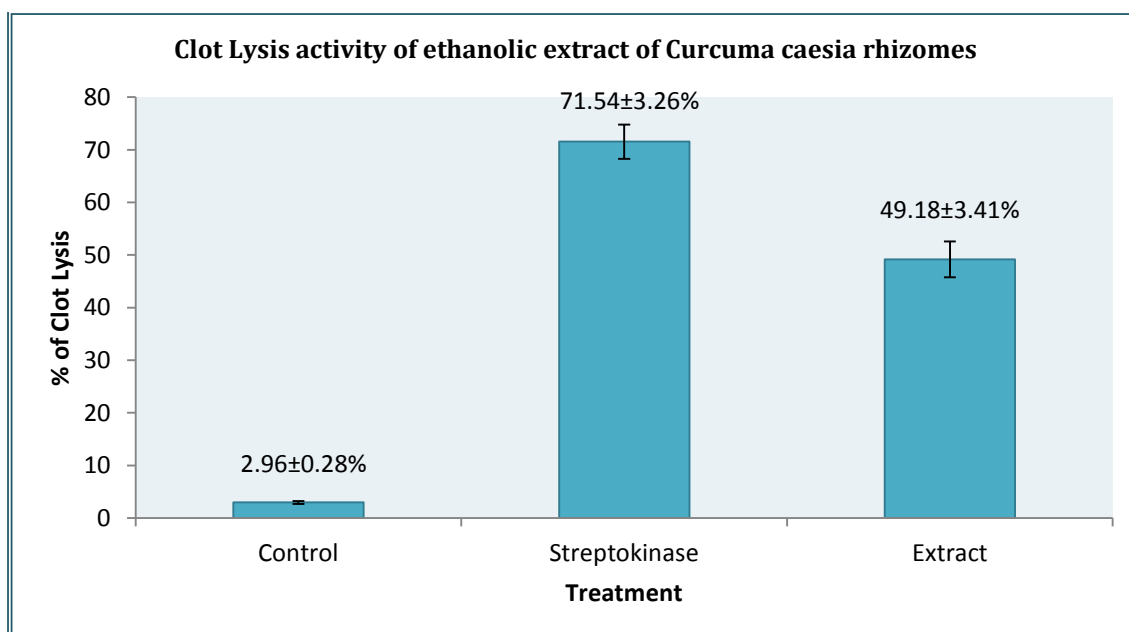


Figure 1: Clot lysis activity of control (Negative control), Streptokinase (Positive Control) and ethanolic extract of rhizomes of *Curcuma caesia*.

DISCUSSION

Nowadays phytopharmacological investigation has created a new field to discovery plant derivative drugs, which are effective

in remedial of certain diseases, and renewed the attention in herbal medicines.

It is estimated that about 30% of the pharmaceuticals are prepared from plants derivatives [22]. A failure of hemostasis and

consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infarction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and streptokinase. A number of research works have been conducted to discover the plants and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect and there is indication that consuming such food leads to prevention of coronary events and stroke [23].

In the present study ethanolic extract of rhizomes of *Curcuma caesia* showed significant thrombolytic activity this effect may be possibly due to phytoconstituents present in the plant extracts affecting activation of plasminogen both by fibrin-dependent and fibrin-independent mechanisms similar to Streptokinase which causes extra production of plasmin which breaks down fibrin the major constituent of thrombi, to dissolve unwanted blood clots.

CONCLUSION

The present study was conducted to evaluate the ethanolic extract of *curcuma caesia* rhizomes by in-vitro thrombolytic activity. The extract showed significant clot dissolution activity may be due to activation of plasminogen. The above result suggests that the application of *curcuma caesia* rhizomes may be assessable for the treatment of ischemic myocardium or thromboembolic disorders. However, the exact mechanism and the active principle by which this extract exert its action remain unclear. Further studies are required to study precise mechanism of actions.

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