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Thrombolytic potential of *Ocimum sanctum* L., *Curcuma longa* L., *Azadirachta indica* L. and *Anacardium occidentale* L.

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ABSTRACT

Atherothrombotic diseases such as myocardial or cerebral infarction are serious consequences of the thrombus formed in blood vessels. Thrombolytic agents are used to dissolve the already formed clots in the blood vessels; however, these drugs have certain limitations which cause serious and sometimes fatal consequences. Herbal preparations have been used since ancient times for the treatment of several diseases. The aim of this study was to investigate whether herbal preparations possess thrombolytic activity or not. An in vitro thrombolytic model was used to check the clot lysis effect of four aqueous herbal extracts viz., *O. sanctum*, *C. longa*, *A. indica*, *A. occidentale* along with Streptokinase as a positive control and water as a negative control. The percentage (%) clot lysis was statistically significant ($p < 0.0001$) when compared with vehicle control. Using an in vitro thrombolytic model, *O. sanctum*, *C. longa*, *A. indica* & *A. occidentale* showed moderate clot lysis activity ($30.01 \pm 6.168\%$, $32.94 \pm 3.663\%$, $27.47 \pm 6.943\%$, $33.79 \pm 2.926\%$ respectively) whereas standard streptokinase showed $86.2 \pm 10.7\%$ clot lysis effect. From our study we found that all the herbs showed reasonable % of clot lysis. These herbal extracts possess thrombolytic properties that could lyse blood clots in vitro; however, in vivo clot dissolving properties and active component(s) of these extracts for clot lysis are yet to be discovered.

KEYWORDS

O. sanctum, *C. longa*, *A. indica*, *A. occidentale*, thrombolysis

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INTRODUCTION

Thromboembolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks are the main causes of morbidity and mortality in developed countries. Thrombolytic therapy uses drugs called thrombolytic agents, such as alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator (TPA) to dissolve clots. Thrombolytic therapy is also used to dissolve blood clots that form in catheters or tubes put into people's bodies for medical treatments, such as dialysis or chemotherapy. However, the relatively weak substrate specificity of first generation agents (streptokinase and urokinase) can result in a state of systemic fibrinolysis and associated bleeding complications. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs [1-5]. Recently, preventive measures against thrombosis have been tried. Oral administration of the fibrinolytic enzyme nattokinase was one example, which has been reported to enhance fibrinolytic activity in plasma and the production of tPA [6].

Since ancient times, herbal preparations have been used for the treatment of several diseases. The leaves and/or twigs, stem, bark and underground parts of plants are most often used for traditional medicines. Herbal products are often perceived as safe because they are "natural" [7]. Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have antiplatelet [8, 9], anticoagulant [10, 11], antithrombotic [12], and thrombolytic activity. Epidemiologic studies have provided evidence that foods with experimentally proved antithrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported [13]. *Ocimum sanctum* belongs to Lamiaceae family and is known by a common name of "Tulsi" in India & Bangladesh. Fixed oil of *Ocimum sanctum* increases blood clotting [14] time and percentage increase was comparable to aspirin and could be due to inhibition of platelet aggregation. Turmeric (*Curcuma longa*) inhibits platelet aggregation [15].

The aim of our work was to investigate whether our selected herbal plants (aqueous extract of *Ocimum sanctum*, *Curcuma longa*, *Azadirachta indica* & *Anacardium occidentale*) possess thrombolytic activity or not by using an in-vitro procedure.

MATERIALS AND METHODS

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 μ l (30,000 I.U) was used for in vitro thrombolysis [16].

Specimen

Whole blood (5 ml) was drawn from healthy human volunteers ($n = 10$) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by the Institutional Ethics Committee of Central India Institute of Medical Sciences, Nagpur). 500 μ l of blood was transferred to each of the ten previously weighed alpine tubes to form clots.

Collection and extraction

Different parts of *Ocimum sanctum* (leaves), *Curcuma longa* (Rhizomes), *Azadirachta indica* (leaves) & *Anacardium occidentale* (Fruits/Nuts) was collected at their fully mature form, from Chittagong, Bangladesh. The plant parts were identified by Bangladesh forest research institute (BFRI), Chittagong. After cleaning, the plant parts of selected plant were taken and air dried for 10 days, and then kept in an oven at 45°C at 72 hours. Then the dried plant parts were grinded. After grinding the glass extractor was used for extraction process. 40gm of dried powder was taken in the glass extractor. Before placing, the extractor was washed properly and then dried. Then 500ml of solvent methanol was added gradually & extraction was done.

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Herbal Preparation

100 mg extract was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron syringe filter. 100 µl of this aqueous preparation of herbs was added to the alpine tube containing the clots to check thrombolytic activity [16].

Clot lysis

Experiments for clot lysis were carried as reported earlier [16]. Venous blood was drawn from healthy volunteers (n = 10) and transferred in different pre-weighed sterile alpine tube (500 µl/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 alpine tube containing clot was properly labeled and 100 µl of plant extract was added to the tubes. As a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. 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 obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The test was repeated ten times.

Statistical analysis

The significance of % clot lysis by herbal extracts by means of weight difference was tested by the paired t-test analysis. Data are expressed as mean ± standard deviation.

RESULTS

Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 86.2% clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (4.7%). The mean difference in clot lysis percentage between positive and negative control was very significant (p value < 0.0009). After treatment of clots with 100 µl of *Ocimum sanctum*, *Curcuma longa*, *Azadirachta indica* & *Anacardium occidentale* moderate clot lysis i.e., 30.01%, 32.94%, 27.47%, and 33.79% respectively was obtained and when compared with the negative control (water) the mean clot lysis % difference was significant (p value < 0.0001). Percent clot lysis obtained after treating clots with different herbs and appropriate controls is shown in Figure 1. Statistical representation of the effective clot lysis percentage by four herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is tabulated in Table 1.

DISCUSSION

Now-a-days, about 30% of the pharmaceuticals are prepared from plants worldwide [17]. A number of studies have been conducted by various researchers to find out the herbs and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect and there is evidence that consuming such food leads to prevention of coronary events and stroke [18-21]. There are several thrombolytic drugs obtained from various sources. Some are modified further with the use of recombinant technology in order to make these thrombolytic drugs more site specific and effective [20]. Side effects related to these drugs have been reported that lead to further complications [21]. Sometimes the patients die due to bleeding and embolism [22, 24-26].

Figure 1: Clot lysis by Streptokinase, water and various herbal preparations. Maximum clot lysis was observed in clot treated with streptokinase (SK). Water *Ocimum sanctum*, *Curcuma longa*, *Azadirachta indica* & *Anacardium occidentale* showed 30.01%, 32.94%, 27.47%, and 33.79% clot lysis respectively. Water (as a negative control) showed 4.7% clot lysis.

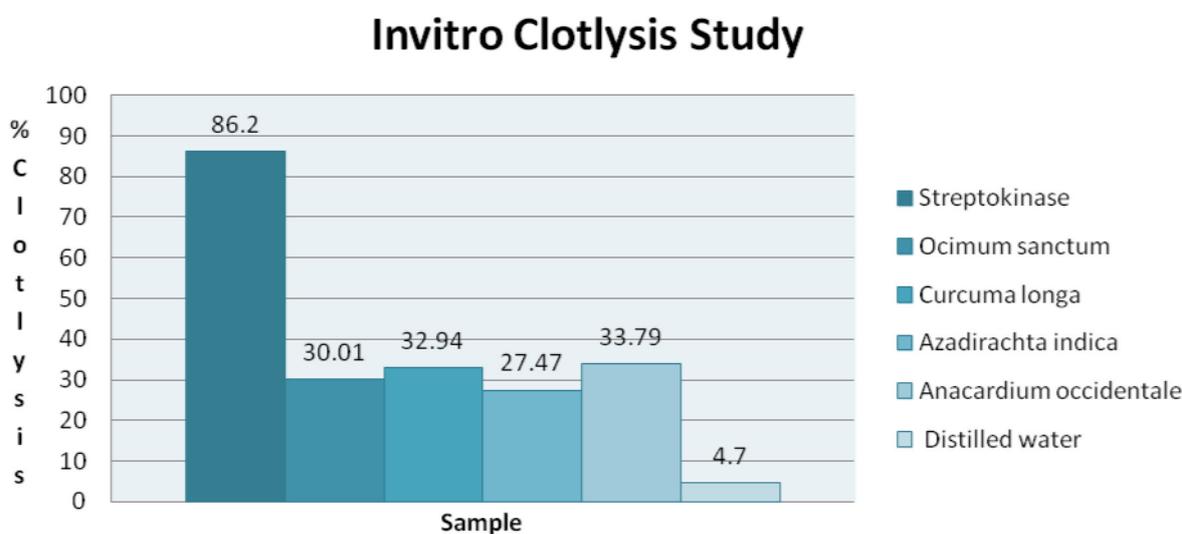


Table 1: Effect of herbal extracts on in vitro clot lysis.

Herb/Drug	% Clot lysis (mean ± S.D)	P value (Two-tailed) when compared to negative control (water)
Streptokinase	86.2 ± 10.7	< 0.0009
Ocimum sanctum	30.01 ± 6.168	< 0.0001
Curcuma longa	32.94 ± 3.663	< 0.0001
Azadirachta indica	27.47 ± 6.943	< 0.0001
Anacardium occidentale	33.79% ± 2.926	< 0.0001

Statistical representation of the effective clot lysis percentage by herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) done by paired t-test analysis; clot lysis % is represented as mean ± S.D. and p values of all Herbal preparations were < 0.05 was considered as significant.

In our study to evaluate thrombolytic properties of different plant extracts, we have tried *Ocimum sanctum*, *Curcuma longa*, *Azadirachta indica* & *Anacardium occidentale* preparations. These are used since ancient times for curing vascular diseases & many other diseases. For example, *Fagonia arabica* (Dhamasa) was reported to have antithrombotic activity [16]. There are few more plant extracts/products which have been identified to have fibrinolytic activity. These are *Lumbricus rubellus* [26], *Pleurotus ostreatus* [29], *Spirodela polyrhiza* [30], *Flammulina velutipes* [31], and *Ganoderma lucidum* [32], Ginger (*Zingiber officinale*) [33], Garlic (*Allium sativum*) [34].

In this study, Streptokinase (SK), a known thrombolytic drug is used as a positive control [27]. Water, on the other hand, was selected as a negative control. The comparison of positive control with negative control clearly demonstrated that clot dissolution does not occur when water was added to the clot. By comparing with this positive & negative control, a significant thrombolytic activity was observed after treating the clots with *Ocimum sanctum*, *Curcuma longa*, *Azadirachta indica* & *Anacardium occidentale* extracts with P value (Two-tailed) less than 0.0001.

CONCLUSION

From this experiment, it can be concluded that the extracts of *Ocimum sanctum*, *Curcuma longa*, *Azadirachta indica* & *Anacardium occidentale* showed moderate to good clot lysis activity. Once found these herbal preparations may be incorporated as a thrombolytic agent for the improvement of the patients suffering from Atherothrombotic diseases. This is only a preliminary study and to make final comment the extract should thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

CONFLICT OF INTEREST

There are no conflicts of interest.

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