Bangladesh Pharmaceutical Journal ***17****(1): 67-69, 2014*

Thrombolytic Activity of Methanolic Extracts of *Desmodium paniculatum* (L.) and *Sarcochlamys pulcherrima* (Roxb.)

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Abstract

An *in vitro* thrombolytic model was used to check the clot lysis effect of two herbal extracts viz., *Desmodium paniculatum* (L.) and *Sarchochlamys pulcherrima* (Roxb.) by using Streptokinase as positive control and water as negative control. *D. paniculatum* and *S. pulcherrima* showed 31.92 ± 8.09% and 36.12 ± 6.81% clot lysis, respectively. From our study we found that *D. paniculatum* and *S. pulcherrima* showed significant % of clot lysis effect with reference to Streptokinase (72.54 ± 6.03%). and water (3.48 ± 0.84%).

Key words: *Desmodium paniculatum*, *Sarchochlamys pulcherrima*, Thrombolytic, % of Clot lysis.

Introduction

A blood clot (thrombus) developed in the circulatory system due to the failure of hemostasis causes vascular blockage and leads to serious consequences in atherothrombotic diseases such as acute myocardial or cerebral infarction, at times leading to death. Commonly used thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) to dissolve clots (Collen, 1990). All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs (Marder, 1993). Heparin and Aspirin are only moderately efficient for acceleration of lysis and prevention of reocclusion, but are safe. Selective thrombin inhibitors and antiplatelet agents are more potent, but their safety remains to be confirmed. Continued investigation in this area will provide new insights and promote progress toward the development of the ideal thrombolytic therapy, characterized by maximized stable coronary arterial thrombolysis with minimal bleeding (Collen, 1990). Several third generation thrombolytic agents have been developed. Compared to the second generation agents (alteplase), the third generation thrombolytic agents such as monoteplase, tenecteplase, reteplase, lanoteplase, pamiteplase result in a greater angiographic potency in patients with acute myocardial infarction, although, so far, mortality rates have been similar to those few drugs that have been studied in large-scale trials. Bleeding risk, however, may be greater (Verstraete, 2000). Recently, preventive measures against thrombosis have been tried. Oral administration of the fibrinolytic enzyme nattokinase is an example, which has been reported to enhance fibrinolytic activity in plasma and the production of tPA (Gesler, 1992).

Since ancient times, herbal preparations have been used for the treatment of several diseases. Herbal products are often perceived as safe because they are "natural" (Demrow *et al.,* 1995). Epidemiologic studies have provided evidence that foods with experimentally proven anti-thrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported (Basta *et al.,* 2004).

The aim of our present work was to investigate whether our selected methanolic extracts of *D. paniculatum* (L.) and *S. pulcherrima* (Roxb.) possess thrombolytic activity or not by using an *in vitro* procedure (Prasad *et al.,* 2007).

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Materials and Methods

Plant materials: Adequate amount fresh leaves of *D. paniculatum* and *S. pulcherrima* for this study were collected from the hill tracts of Cox's Bazar area, Chittagong, Bangladesh and were authenticated by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, and University of Chittagong, Bangladesh. The leaves were dried at room temperature in shade for 5 days and in hot air oven for 2 days.

Extraction: The dried leaves were coarsely powdered and extracted with methanol for 7 days. The sediments were filtered and the filtrates were evaporated to dryness at 40ºC. The solvent was completely removed and the dried crude extract was used for the experiment.

In vitro clot lysis study

Streptokinase (SK): About 5 ml sterile distilled water was added to the commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15, 00,000 I.U. and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis (Prasad *et al.*, 2007) study.

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 µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots (Prasad *et al.*, 2007).

Clot lysis: Experiments for clot lysis were carried as reported earlier (Prasad *et al.*, 2007). Venous blood drawn from healthy volunteers (n = 10) was 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. 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 between % clot lysis by herbal extract by means of weight difference was tested by the paired t-test analysis. Data are expressed as mean ± standard deviation.

Results and Discussion

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 72.54 % clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (3.48%). The mean difference in clot lysis percentage between positive and negative control was very significant (p value < 0.0001). The *in vitro* thrombolytic activity study revealed that the methanolic extracts of *D. paniculatum* and *S. pulcherrima* showed 31.92% and 36.123% clot lysis, respectively and when compared with the negative control (water) the mean clot lysis % difference was significant (p value <0.0001). % Clot lysis obtained after treating clots with these two extracts and appropriate controls is shown in Figure 1. Statistical representation of the effective clot lysis percentage by two herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) has been shown in Table 1.

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

Extracts/ Drugs	Mean ± S.D (% of clot lysis)
Water (negative control)	3.48 ± 0.84%
Streptokinase (positive control)	72.54 ± 6.03%*
Methanol extract of *D. paniculatum*	31.92 ± 8.09%*
Methanol extract of *S. pulcherrima*	36.12 ± 6.813%*

*p < 0.0001, significant compared to control.

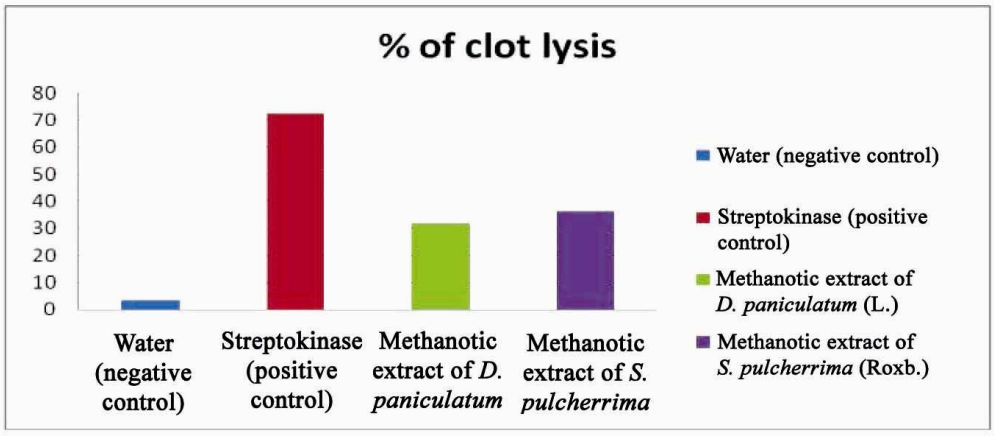

Figure 1. Clot lysis by water, Streptokinase, Methanol extract of *D. paniculatum* and *S. pulcherrima*.

Conclusion

From the *in vitro* clot lysis study, we demonstrated that *D. paniculatum* (L.) and *S. pulcherrima* (Roxb.) have very good clot lysis activity. It may be assumed that these extracts can be considered as potential sources of natural thrombolytic agents. This is only a preliminary study and the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potential.

Acknowledgement

Authors wish to thanks Dr. Shaikh Bokhtear Uddin, Associate professor, Department of Botany, University of Chittagong for identification the plant.

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