

IN-VITRO THROMBOLYTIC POTENTIAL OF LEAF EXTRACT OF *ANOGEISSUS LATIFOLIA*

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ABSTRACT

Thrombotic disorders such as myocardial infarction and cerebral impediment are fatal blood clotting related diseases. The need of safer and reliable herbal medicines for targeted and better drugs is the need of the time, since the thrombosis may further lead to complex blood dyscrasias. Thus the current study is targeted at exploration of the thrombolytic potential of the hydro alcoholic leaf extract of indigenous herb *Anogeissus latifolia*. The plant has shown presence of phytoconstituents such as tannins, polyphenols which has been reported to show good cardio protective activity. Also, the plant has reported to show antihyperlipidemic activity, good anti-oxidant and anti-atherosclerosis activity. Hence the plant promises to show fair thrombolytic potential. Streptokinase was used as the standard drug for

comparison and the saline solution as vehicle control. The plant has shown significant activity compared to both the groups.

KEY CONSTITUENTS: Thrombolytic potential, Streptokinase, *Anogeissus latifolia*.

INTRODUCTION

Thrombosis is one of the cardiovascular disorders which may escalate into major diseases such as myocardial infarction or ischemia, deep vein thrombosis and pulmonary embolism. In India, Ayurveda has been noted as one of the most ancient traditional health care system. The drugs mentioned to be useful against the severe heart diseases have exhibited hypotensive, thrombolytic and hypocholesteremic activity. Scientific evidence has proved ayurvedic medicines to be useful. The recent synthetic drugs such as anistreplase, streptokinase, urokinase pose severe adverse effects such as bleeding complications such as systemic

fibrinogenolysis and lysis of normal hemostatic plugs.^[1] Thus the need for recent new and safer drugs is the need of the time. Herbal remedies are most notably preferred since they offer fairly significant activity with lesser chances of above mentioned blood dyscrasias. Also, the herbal remedies consist of various ingredients which may provide synergistic activity thus increasing its potency and efficacy.^[2]

Currently the incidence of stroke is higher in India than other countries. Few numbers of ischemic strokes are benefitted by the thrombolytic therapy. Thus there is a need of more emphasis on the thrombolytic activity of indigenous herbs. In India, Thrombosis is defined as the condition characterized by the formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system.^[3] Thrombosis is one of the most common preventable causes of hospital deaths in the western world,^[4] thus the emphasis on herbal medicines is more. The present study is majorly focused on the thrombolytic activity of the indigenous herb *Anogeissus latifolia*. The plant has been reported to have constituents such as β - sitosterol, leucocyanidin, gallotannins, polyphenols and many others.^[5] Also the plant has good antioxidant properties, anti-diabetic activity along with anti-hyperlipidemic activity.^{[6][7]} The patients with diabetes and cardiovascular disorders are more susceptible to suffer from atherosclerotic plaques, since these plaques are the major reason of the disease. Due to more fat accumulation which is the pathogenic condition in case of both diabetes and cardiovascular disorders, the plant may exhibit good thrombolytic activity.^[8]

MATERIALS AND METHOD

Reagents and chemicals

Streptokinase (SK) vials of 15,000 I.U. 8 blood (4 ml) samples drawn from healthy rats, Saline, Hydro-alcoholic leaf extract of the plant, Distilled water.

Preparation of the extract

The fresh leaf powder was subjected to Soxhlet extraction using Methanol:Water (70:30) ratio. This extract was suspended in saline solution and used for the study. Two concentrations 10 mg/ml and 20 mg/ml of the extract were evaluated for their activity.

Specimen

Whole blood (4 ml) was drawn from healthy rats. 500 μ l (0.5 ml) of blood was transferred to each of the previously weighed microcentrifuge tubes to form clots.

Procedure

4 ml blood was drawn from healthy rats which was distributed in different pre weighed sterile microcentrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To one microcentrifuge tube containing pre-weighed clot, 100 µl of hydroalcoholic extract of *Anogeissus latifolia* was added. As a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of saline was 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 released 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 experiment was repeated twice for better statistical results.

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

RESULTS

Table no. I: Values of the clot lysis experiment.

	Weight of empty tube	Weight of clot before adding drug	Weight of clot after adding drug	Weight of clot before lysis	Weight of clot after lysis	% clot lysis
Negative control (0.1 ml saline)	1016.7	1039.1	1033.0	22.4	3.2	14%
	984.5	1003.2	9987.4	18.7	2.9	15.5%
Drug extract (10mg/ml)	949.7	1006.1	985.5	56.4	23.5	41.67%
	982.9	1030.5	1005.1	47.6	22.2	46.63%
Drug extract (20mg/ml)	993.8	1052.3	1028.2	58.5	34.4	58.80%
	940.8	993.0	977.0	52.2	36.2	69.34%
Standard drug streptokinase	987.4	1062.3	1043.9	74.9	56.5	75.43%
	1005.6	1040.7	1034.5	35.1	28.9	82.33%

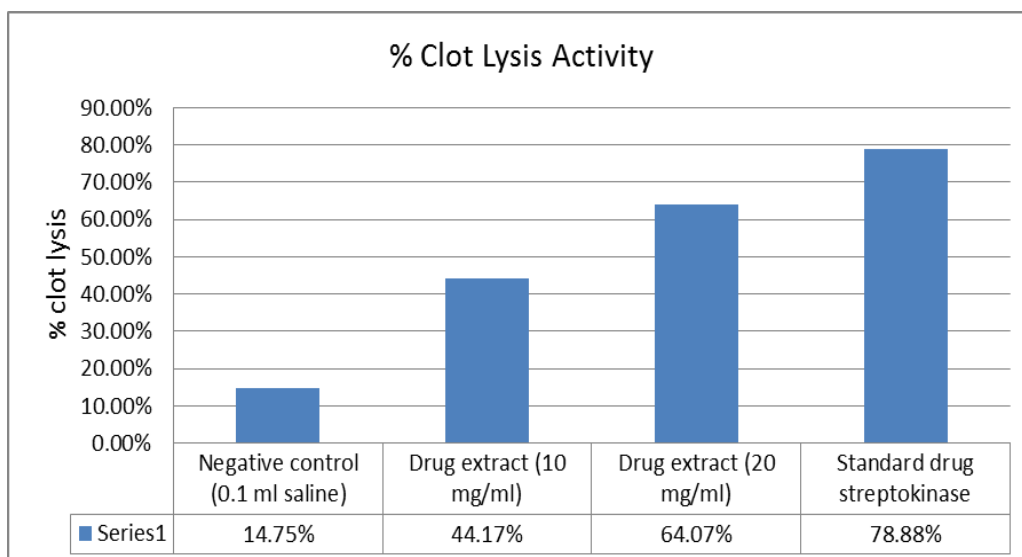


Figure 1: Clot lysis by streptokinase, saline solution and *Anogeissus latifolia*.

DISCUSSION

Several herbal drugs are being evaluated for their thrombolytic activity.^{[9][10][11]} Since the formation of thrombus leads to fatal diseases such as venous thrombosis, Portal vein thrombosis, Paget-Schroetter disease, Budd-Chiari syndrome.^[3]

In this study, investigation of thrombolytic activity of *Anogeissus latifolia* leaf extract was carried out using a simple and rapid *in-vitro* clot lysis model.^[13] This model is a reliable and sensitive technique. From the results it is evident that the drug has significant thrombolytic activity. The plant shows 44.17% and 64.07% thrombolytic activity in the concentrations of 10 mg/ml and 20 mg/ml respectively as seen in Figure 1. However; streptokinase SK a reference standard and saline were used as a positive and negative control that showed clot lysis maximum 78.88% and 14.75% in 72 hrs of incubation respectively. The plant may have shown good activity since the leaf has constituents such as gallotannins, polyphenols have been responsible for anti-diabetic and anithyperlipidemic activity. Furthermore, the future aspects may be considered as the isolation of the main constituents of the plant. The evaluation of the isolates may elaborate more on the nature and efficacy of the thrombolytic activity. Thus encouraging furthermore use in other complex thrombosis related disorders. Further research in this field may provide better results statistically.

There are also other sophisticated methods available for finding out the clot lysis activity of the plant such as quantitation of D-dimer photometrically by immunoturbidimetric method, morphology of fibrin loss and confocal microscopy.^[14]

CONCLUSION

The *In vitro* clot lysis method was used for evaluation of the thrombolytic activity of the plant extract. Increase in the thrombolytic activity with increase in the concentration of the extract has been noted. The activity may be considered as significant compared to the activity of the positive control Streptokinase. The plant extract may prove as an effective herbal medicine for the thrombolytic disorders.

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