

Research Article



Screening of Thrombolytic Activity of *Aegle marmelos* Linn Leaves Extract by *in vitro*

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ABSTRACT

Thrombotic 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. So, the aim of present work was to investigate whether our selected Extract of *Aegle marmelos* L. leaf possess thrombolytic and antithrombotic activity or not. During this study analysis of thrombolytic activity of *Aegle marmelos* leaf extract using a simple and quick in-vitro clot lysis assay was performed which exhibited maximum 84 % clot lysis at 800 µg/ml concentration in 90 mins of incubation at 37° C. Various concentrations of leaf extract i.e. 200µg/ml, 400µg/ml, and 800µg/ml were tested at time intervals of 90 mins and incubation at 37° C for observing maximum clot lysis. The result findings indicated that concentrations of leaf extract enhanced the percentage of clot lysis in dose dependent manner However; streptokinase a reference standard and water were used as a positive and Negative control showed clot lysis maximum 89% and 2% in 90 mins of incubation respectively.

Keywords: Thrombolytic activity, *Aegle marmelos*, Thrombotic disorders, streptokinase.

INTRODUCTION

A blood clot (thrombus) developed in the circulatory system due to the failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as acute myocardial or cerebral infarction, at times leading to death¹. Thrombolysis is a complex mechanism which interacts with clot components and surrounding plasma. In this interaction, plasmin, plasminogen, plasminogen activator and fibrin are involved². Commonly used thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (TPA) to dissolve clots³. 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 drugs⁴.

More 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 bleeding¹. Several third generation thrombolytic agents have been developed. Compared with the second generation agents (alteplase), third generation thrombolytic agents such as monoteplase, tenecteplase, reteplase, lanoteplase, pamiteplase, and staphylokinase result in a greater angiographic potency rate in patients with acute myocardial infarction, although, thus far, mortality rates

have been similar for those few drugs that have been studied in large - scale trials⁵.

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"⁶. Epidemiologic studies have provided evidence that foods with experimentally proved anti-thrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported⁷. Advances of Phytochemistry and identification of plant compounds which are effective in curing certain diseases has renewed the herbal medicines. Herbs and their components possessing anti-thrombotic activity has been reported before; however herbs that could be used for thrombolysis has to be reported so far⁸.

Aegle marmelos one such plant that was used quite often in traditional system of medicine. It belongs to the family Rutaceae, and popularly known as "Bael tree"^{9, 10}. It is indigenous to India and found wild all over the Sub Himalayan forests, in Central, and South India. The plant is reported to have multiple therapeutic properties such as antiinflammatory¹¹, antipyretic and analgesic¹², anti diabetic and hepatoprotective¹³ activity.

MATERIALS AND METHODS

Plant Material collection and extraction

Fresh green leaves of the plant were collected in sufficient quantity from the road city of Mumbai India. During the month of Jan 2014, some of the leaves were kept in sterile plastic bag duly sealed and was sent



Pharmacognosy department for the sake of authentication as a species of *Aegle marmelose* Linn.

Preparation of extract

Collected plant material were shade dried and pulverized into coarse powder using mortar and pestle. The dried powdered material was extracted with Ethanol by Maceration Process and filtered using filter Paper. The extract obtained from the solvent were evaporated to dryness at room temperature and suspended in sterile distilled water to make the final concentration.¹⁴

Streptokinase (SK) Solution Preparation

Commercially available lyophilized (Streptokinase) vial of 15, 00,000 I.U., was collected and then 5 ml of sterile distilled water was added and mixed properly. This suspension served as a stock from which 100µl was used for *in vitro* thrombolysis¹⁵.

In vitro Thrombolysis

Whole blood (3 ml) was drawn from healthy human volunteers (n = 5) by phlebotomist without a history of oral contraceptive or anticoagulant therapy. Collected blood was transferred to Five Eppendorf tubes and allowed to form a clot.

Experiment 1: Effect of leaf extract on clot lysis

Venous blood drawn from healthy volunteers (n = 5) was transferred in different pre weight sterile micro centrifuge 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) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). Add 100 µl of streptokinase as Positive control. Water was also added to one of the tubes containing clot and this serves as a negative thrombolytic control. 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.¹⁶

Maximum clot lysis observation with respect to concentration and incubation of time

Various concentrations of leaf methanol extracts viz; 200 µg/ml, 400 µg/ml and 800 µg/ml were tested against 90 mins duration of incubation at 37° C for maximum clot lysis.

Statistical Analysis

Data was analysed by one- way ANOVA by using student's t-test. Significance was set at p<0.05.

RESULTS AND DISCUSSION

Atherothrombotic diseases like myocardial or cerebral infarction occur owing to the development of thrombus that causes hindrance in the passage of vessels.¹⁷ Even,

sometimes in critical conditions, patients die due to embolism.¹⁸⁻¹⁹ A number of earlier conducted researches indicated that herbs and natural products possessed thrombolytic activity.²⁰ Thrombolytic agents that include tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) etc are used globally for the treatment of these diseases.²¹ Currently, various thrombolytic agents in practice are being used to dissolve the clots that have already formed in the blood vessels; but these drugs have certain limitations and can lead to potential fatal consequences in some cases. In this study, investigation of thrombolytic activity of *Aegle marmelos* leaf extract was carried out using a simple and rapid in-vitro clot lysis model. The results indicated maximum 80.34% clot lysis at 800 µg/ml concentration in 90min of incubation as mentioned in Table No-2. The results indicated clearly that concentrations of leaf extract enhanced the percentage of clot lysis. However; streptokinase SK a reference standard and water were used as a positive and negative control that showed clot lysis maximum 89% and 2% in 90mins of incubation respectively.

Table 1: *In vitro* - thrombolytic activity of control (water) and standard (Streptokinase)

	Incubation time	Clot Lysis
Standard	90 mins	89%
Control	90 mins	2%

Table 2: *In vitro* - thrombolytic activity of crude extract of *Aegle marmelos*

Concentration of crude leaf extract	Incubation Time	Clot Lysis
200 µg/ml	90 mins	40.53
400µg/ml	90 mins	60.11
800 µg/ml	90 mins	80.34

Some studies indicate that thrombolytic activity probably due to the diverse composition of plant extracts like phytoconstituents including rich sources of alkaloids, flavonoids, tannins and terpenoids.

With further research on cell viability tests and in vivo studies, this finding may have important implications in the treatment of cardiovascular diseases which is increasing at an alarming rate. Since the drugs used for the cardio vascular diseases are not economical and not accessible to the greater section of the society, application of this study may be a soon for them.

CONCLUSION

This is however; a preliminary study yet indeed a valuable addition in the list of natural plant products that have been screened lately for thrombolytic activity. As, presently the entire world is in search of and designing of such molecules that could have therapeutic implication in the atherothrombotic diseases like myocardial or cerebral infarction. However; further studies ought to be



conducted later to isolate and characterize the molecule that have clot lysis potential.

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REFERENCES

- Collen D. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator, *Annals of Internal Medicine*, 112, 1990, 529–538.
- Alkjaersig N, Fletcher AP, Sherry S, The mechanism of clot dissolution by plasmin, *Journal of Clinical Investigation*, 38, 1959, 1086.
- Gesler WM. Therapeutic landscapes: medical issues in light of the new cultural geography, *Social Science & Medicine*, 34, 1992, 735–746.
- Marder VJ, Recombinant streptokinase – opportunity for an improved agent, *Blood Coagulation and Fibrinolysis*, 4, 1993, 1039–1040
- Verstraete M. Third Generation thrombolytic drugs. *Am J Med.*, 109(1), 2000, 52-58.
- Demrow HS, Slane PR, Folts JD. Administration of wine and grape juice inhibits *in vivo* platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation.*, 91, 1995, 1182–1188.
- Giuseppina Basta, Cristiana Lupi, Guido Lazzarini, Piero Chiarelli, Antonio L'Abbate, Daniele Rovai. Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis. An *in vitro* study on blood of normal subjects and patients with coronary artery disease. *ThrombHaemost.*, 91, 2004, 1078-1083.
- Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R. Testing various herbs for antithrombotic effect. *Nutrition.*, 21, 2005, 580–587.
- Gamble, J.S, 1993. *Flora of the Presidency of Madras, Vol I-III*, Bishen Singh Mahendra Pal Singh, Dehra Dun, India.
- Mathew, K.W, 1985. *The Flora of Tamil Nadu Carnatic, The Rapinant Herbarium, St. Josephs College, Tiruchirapalli, India.*
- Arul, S., Miyazaki and Dhananjayan, R, 2005. Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos*, *J. Ethnopharmacol.*, 96, 159–163
- Shankarananth, V., Balakrishnan, N., Suresh, G., Sureshpandian, E., Edwin and Sheej, E, 2007. Analgesic activity of methanol extract of *Aegle marmelos* leaves, *Fitoterapia*, 78, 258–259.
- Singh, R. and Rao, H S, 2008. Hepatoprotective effect of the pulp/seed of *Aegle marmelos* Correa ex Roxb against carbon tetrachloride induced liver damage in rats, *Int. J. Green Pharm.*, 2, 232–234
- Vanphawng Lalremruta and Gurunath S. Prasanna Evaluation of protective effect of *Aegle marmelos* Corr. in an animal model of chronic fatigue syndrome *Indian J Pharmacol.*, 2012 May-Jun, 44(3), 351–356.
- Chowdhury SN, Badrul AM, Haque ASMT, Zahan R, Mazumder ME and Haque ME, In vitro Free Radical Scavenging and Thrombolytic Activities of Bangladeshi Aquatic Plant *Aponogeto nundulatus* Roxb, *Global Journal of Pharmacology*, 5(1), 2011, 27-32.
- Sweta Prasad, Rajpal S Kashyap, Jayant Y Deopujari, Hemant J Purohit, Girdhar M Taori¹ and Hatim F Daginawala Development of an in vitro model to study clot lysis activity of thrombolytic drugs *Thrombosis Journal*, 2006, 4,14.
- Apu AS, Chowdhury FA, Khatun F, Jamaluddin ATM, Pathan A and Pal A, Phytochemical Screening and *In vitro* Evaluation of Pharmacological Activities of *Aphanamixis polystachya* (Wall) Parker Fruit Extracts, *Tropical Journal of Pharmaceutical Research*, 12(1), 2013, 111-116.
- Wardlaw JM, Berge E, Zoppo DG, Yamaguchi T, Thrombolysis for acute ischemic stroke, *Stroke*, 35, 2004, 2914-2915.
- Capstick T, Henry MT, Efficacy of thrombolytic agents in the treatment of PE, *European Respiratory Journal*, 26, 2005, 864-874.
- Giuseppina B, Cristiana L, Guido L, Piero C, Antonio LA, Daniele R, Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis. An *in vitro* study on blood of normal subjects and patients with coronary artery disease. *Journal of Thrombosis and Haemostasis*, 91, 2004, 1078-1083
- Ansari AV, Siddiqui HH, Singh SP, Antithrombotic and Thrombolytic activity of *Terminalia bellerica* fruit extracts, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 3(2), 2012, 471.

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