



Evaluation of Fibrinolytic Efficacy of *Averrhoa Bilimbi* Linn. by Using Euglobulin Lysis Time Method

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ABSTRACT

Background: An impaired fibrinolytic function had been reported in subjects suffering from venous thrombosis. The euglobulin clot lysis time is a test that reflects the overall fibrinolytic activity of plasma. **Objective:** To evaluate the fibrinolytic efficacy of ethanolic extract of *Averrhoa bilimbi* Linn. leaves by using Euglobulin lysis time method. **Methods:** Rats were divided into 3 groups. Each group was containing 6 rats. Group I: received vehicle (control) 0.9% saline solution; Group II: received 25 mg/kg ethanolic extract leaves of *Averrhoa bilimbi* Linn; Group III: received 50 mg/kg ethanolic extract leaves of *Averrhoa bilimbi* Linn. Blood (1.8 ml) was removed with a plastic syringe containing 0.2 ml 3.8% sodium citrate solution. The time interval between the addition of thrombin and the complete lysis of the clots was measured. The lysis time (min) was determined. **Conclusion:** We observed ethanolic extract of *Averrhoa bilimbi* Linn. leaves may increase the activators of the fibrinolysis. Further investigation is warranted.

Keywords: Euglobulin lysis time, Thrombin, Fibrinolysis, Blood clot, *Averrhoa bilimbi* Linn.

INTRODUCTION

The uncontrolled platelet aggregation may responsible for the thrombosis and cardiovascular diseases. The drugs which inhibit the platelet aggregation is called antiplatelet agents which prevents life-threatening diseases including cardiovascular events [1,2]. Euglobulin clot lysis time method is used to measure the plasma fibrinolytic activity [3]. *Averrhoa bilimbi* Linn. (Common name: bilimbi, cucumber tree) is a medicinal plant belonging to the family Oxalidaceae [4]. It is cultivated in many countries including India, Bangladesh, Malaysia, Indonesia, Philippines, Singapore, Thailand, and Myanmar [5]. The Chemical constituents found in bilimbi includes flavonoids, alkaloid, tannins, saponins, cardiac glycosides, glycosides, triterpenes, phenols, and carbohydrates [6]. Many pharmacological activities reported by many researchers to validate the traditional claim of bilimbi [7,8]. *Averrhoa bilimbi* has been traditionally used in the prevention and treatment of cardiovascular diseases, fibrinolytic efficacy of this plant still remains unknown. Thus, the aim of the study was to investigate the efficacy of *Averrhoa bilimbi* Linn. leaves extract on fibrinolytic activities in rat.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (10-15 week old, 150-180 g) were procured from the animal house of the institute. Six animals were housed per cage which contained sterile paddy husk as bedding and was allowed access to water and ad libitum food. Constant temperature ($23 \pm 1^\circ\text{C}$) and humidity ($55 \pm 5\%$) under a 12 h light/dark cycle were maintained. All experiments were conducted according to standard guidelines for care and use of laboratory animals, with the approval of the Institutional animal ethical committee. The animals were acclimatized in laboratory conditions prior to the experiment. (Institutional Ethical Committee Approval Number: SUCOM/LIRB/2019-03).

Drugs and Chemicals

All the chemicals, reagents and solvents required for the study were procured from Hi-media Private Ltd and were of analytical grade.

Plant Material

The fresh leaves of *Averrhoa bilimbi* Linn. were collected from the farm area and authenticated by the Botanist of the Institution and sample specimen were stored for further reference (Specimen Number: FAM 25).

Experimental Design

Rats were divided into 3 groups. Each group was containing 6 rats. Group I: received vehicle (control) 0.9% saline solution; Group II: received 25 mg/kg ethanolic extract leaves of *Averrhoa bilimbi* Linn; Group III: received 50 mg/kg ethanolic extract leaves of *Averrhoa bilimbi* Linn. Rats were anesthetized by intraperitoneal injection of 60 mg/kg Phenobarbital sodium and placed on a heating pad (37°C). At the same time, Group I received vehicle (control) and Group II and III was received test extracts of leaves of *Averrhoa bilimbi* Linn. 25 mg/kg and 50 mg/kg intraperitoneally respectively. After 25 minutes the animals received another intraperitoneal injection of 12 mg/kg Phenobarbital sodium to keep them in deep necrosis for 45 minutes.

Plasma preparation: After the control and test compound was absorbed, blood was withdrawn from the inferior vena cava vein exposed by the midline excision from each group animals. Blood (1.8 ml) was removed with plastic syringe containing 0.2 ml 3.8% sodium citrate solution. The sample was thoroughly mixed, transferred to a plastic tube and immediately immersed in ice. Plasma was prepared by centrifugation at 2000 g for 10 minutes at 2°C.

Euglobulin preparation: A 0.5 ml portion of plasma was added to 9.5 ml of ice-cold distilled water; the pH was brought to 5.3 by the addition of 0.13 ml of 1% acetic acid. The diluted plasma was kept on ice for 10 minutes and precipitated euglobulin fraction was collected by centrifugation at 2000 g for 10 minutes at 2°C. The supernatant was discharged and the remaining fluid was removed by drying the tube on filter paper for 1 minute. The Euglobulin precipitate was dissolved in 1 ml of 0.12 M sodium acetate solution.

Euglobulin lysis assay: Aliquots (0.45 ml) of the euglobulin solution was transferred to the test tubes and 0.05 ml thrombin (Test thrombin, Behring Werke) (25 U/ml) was added in each group animals. The tubes were transferred to the water bath at 37°C. The time interval between the addition of thrombin and the complete lysis of the clots was measured. The lysis time (min) was determined. ELT was shortened when activators of fibrinolysis were increased. Percent lysis time was calculated in different test dosage groups as compared to control [9].

Statistical Analysis

All results are expressed as Mean \pm SEM (standard error of the mean). Statistical analyses were performed using one-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$. All statistical analyses of the data were performed by using Graph Prism Pad software version 5.0.

RESULTS

Euglobulin lysis time in different groups is shown in Table 1 and Figure 1.

Table 1 Euglobulin lysis time in different groups

Groups	ELT (Min)	Percent lysis time
I: Control (saline)	186 \pm 6	100
II: 25 mg/kg EEAB	143 \pm 5	76.88
III: 50 mg/kg EEAB	137 \pm 6	73.65

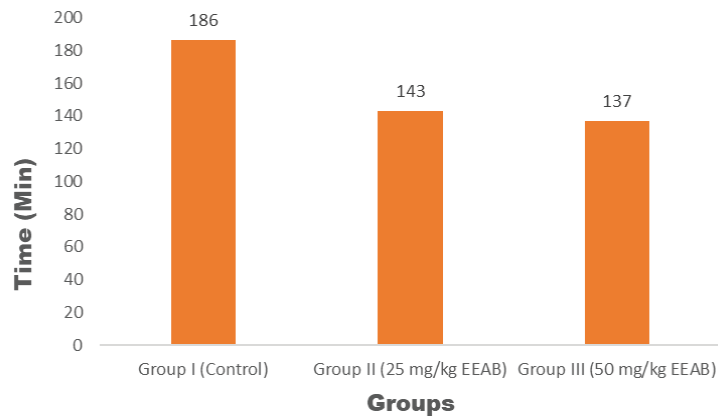


Figure 1 Euglobulin lysis time in different groups

DISCUSSION

The Euglobulin lysis time (ELT) test measures fibrinolysis. The test was performed by mixing plasma with acid in a glass test tube. The acidification makes the precipitation of clotting factors called the euglobulin fraction. The euglobulin fraction contains fibrinolytic factors [10].

The Euglobulin lysis time is used as an indicator for the influence of the compounds on the fibrinolytic activity in the rat blood. The Euglobulin fraction of plasma was separated from inhibitors of fibrinolysis by acid precipitation and centrifugation. Euglobulin predominantly consists of plasmin, plasminogen, plasminogen activator, and fibrinogen. By the addition of thrombin to this fraction, fibrin clots were formed. The lysis time these clots were determined as a measurement of activity of activators of fibrinolysis (e.g. plasminogen activators) [9].

In this experiment, animal Group III treated with 50 mg/kg ethanolic extract of *Averrhoa bilimbi* Linn. leaves showed reduction in Euglobulin lysis time (137 ± 6 min) as compared to Group II treated with 25 mg/kg ethanolic extract of *Averrhoa bilimbi* Linn. leaves (143 ± 5 min) and Group I control (186 ± 6 min). Animal Group III treated with 50 mg/kg ethanolic extract of *Averrhoa bilimbi* Linn. leaves showed 73.65% lysis time as compared to control Group I.

CONCLUSION

We observed 50 mg/kg ethanolic extract of *Averrhoa bilimbi* Linn. leaves showed a reduction in Euglobulin lysis time and reduction in percent lysis time as compared to control group. From this study we can conclude that ethanolic extract of *Averrhoa bilimbi* Linn. leaves may increase the activators of the fibrinolysis. Further investigation is warranted.

DECLARATIONS

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Conflict of Interest

The authors declared there are no known conflicts of interest associated with this publication.

REFERENCES

- [1] De Meyer, Simon F., et al. "Antiplatelet drugs." *British Journal of Haematology*, Vol. 142, No. 4, 2008, pp. 515-28.
- [2] Klafke, Jonatas Zeni, et al. "Antiplatelet, antithrombotic, and fibrinolytic activities of *Campomanesia xanthocarpa*." *Evidence-Based Complementary and Alternative Medicine*, 2012, pp. 1-8.
- [3] Smith, Amy A., et al. "A new euglobulin clot lysis assay for global fibrinolysis." *Thrombosis Research*, Vol. 112, No. 5-6, 2003, pp. 329-37.
- [4] Quattrocchi, Umberto. *CRC World Dictionary of Plant Names: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology*. Vol. 3. New York, USA: CRC Press, 1999.

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- [5] Orwa, C., et al. "Agroforestry database: A tree reference and selection guide version 4.0." *World Agroforestry Centre, Kenya*, 2009.
- [6] Gunawan, Cordeno Arlene, and Anamy Paano. "Structure elucidation of two new phytol derivatives, a new phenolic compound and other metabolites of *Averrhoa bilimbi*." *Research Congress*, 2013, pp. 1-8.
- [7] Ramjan, Ali, et al. "Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds." *Avicenna Journal of Phytomedicine*, Vol. 4, No. 6, 2014, pp. 430-36.
- [8] Alhassan, Alhassan Muhammad, and Qamar Uddin Ahmed. "*Averrhoa bilimbi* Linn.: A review of its ethnomedicinal uses, phytochemistry, and pharmacology." *Journal of Pharmacy and Bioallied Sciences*, Vol. 8, No. 4, 2016, pp. 265-71.
- [9] Vogel, Hans G. *Drug discovery and evaluation: Pharmacological assays*. 2nd ed., Springer Science and Business Media, 2002.
- [10] Kowalski, E., M. Kopeć, and S. Niewiarowski. "An evaluation of the euglobulin method for the determination of fibrinolysis." *Journal of Clinical Pathology*, Vol. 12, No. 3, 1959, p. 215.