THROMBOLYTIC ACTIVITY OF PHYLLANTHUS AMARUS

Prabodh Shukla *1, Padmini Shukla 1 and Shashi Alok 2

Pharmacy College Saifai 1, UPUMS, Saifai, Etawah, Uttar Pradesh, India.
Institute of Pharmacy 2, Bundelkhand University, Jhansi, Uttar Pradesh, India.

ABSTRACT: Objective: Phyllanthus amarus (Euphorbiaceae) is an erect annual herb of not more than one and half feet tall and has small leaves. Plant contains many important phytoconstituent which are responsible for various pharmacological activity like to treat jaundice, diabetes, gonorrhea and arthritis. The plant is rich source of polyphenol and some particular types of polyphenol are responsible for thrombolytic activity. So in current study the plant is selected to screen its thrombolytic activity. Method: The thrombolytic activity was screened by in-vitro clot lysis model. In this model the blood sample (500μl/tube) from human volunteer has been collected in microcentrifuge tubes. After incubation the standard drug and different dilutions of test drug were added whereas water is taken as control. Percentage of Clot lysis was calculated on the basis of the weight difference of microcentrifuge tubes obtained before and after clot lysis. Result: Using an in-vitro clot lysis model, Phyllanthus amarus at a dose of 10mg/100 μl and 20mg/100 μl showed 18.99%, 39.8% clot lysis respectively. The result shows that Phyllanthus amarus possess significant % of clot lysis with reference to Streptokinase (62.15%). Conclusion: The present study indicated that Phyllanthus amarus possesses positive thrombolytic properties that could lysis blood clots. Further studies using in vivo models are required to carry out and establish the effectiveness and pharmacological rationale for the use of Phyllanthus amarus as a thrombolytic drug. The drug may be further explored for its phychochemical profile to identify the active constituent responsible for thrombolytic activity.

INTRODUCTION: The plants of the genus Phyllanthus (Euphorbiaceae) are widely distributed in most tropical and subtropical countries. It is a very large genus consisting of approximately 550 to 750 species and is subdivided into 11 subgenera. Phyllanthus genera contain many important phytoconstituent which are responsible for various type of pharmacological activity like very effective hepatoprotective agents in the Indian indigenous systems of medicine and are considered bitter, astringent, stomachic, diuretic, febrifuge, deobstruant and antiseptic 1.

Among the popular species, P. niruri, P. urinaria, P.emblica, P. flexuosus, and P. amarus, have received the most attention.

Phyllanthus amarus known as “Bhui amla” (Family – euphorbiaceae) widely distributed throughout the rainforest region of Northern India. It is an annual herb grows up to 15-60 cm high have an erect stem. The plant is most commonly used in the Indian Ayurvedic system of medicine in problems of stomach, genitourinary system, liver, kidney and spleen. P. amarus have different classes of organic compounds of medicinal importance including alkaloids, flavonoids, hydrolysable tannins (Ellagitannins), major lignans, polyphenols, triterpenes, sterols and volatile oil 2. The mentioned plant material is rich source of polyphenolic compounds and some typical types of polyphenolic are responsible for thrombolytic activity 3.
Thrombosis is the formation of a clot or thrombus inside a blood vessel, obstructing the flow of blood through the circulatory system. So the proposed study is aimed to check the thrombolytic activity of the selected species of the genus Phyllanthus.

**MATERIAL AND METHOD:**

**Collection of plant material:** The *Phyllanthus amarus* was collected from Chandrashekhar Azad agriculture university, Kanpur in the month of July-August. The plant material was authenticated by Prof J. P. Shukla, Department of Botany, D.B.S College, Kanpur where a voucher specimen (PH/PA/20) is deposited for further reference.

**Extraction and isolation:** The air dried aerial part of *Phyllanthus amarus* was successively extracted with benzene, chloroform and acetone-water (7:3) fraction. Among these only acetone-water fraction was taken and after eliminating the acetone by distillation the pigment and lipid are further removed from aqueous solution by solvent extraction with dichloromethane and ethyl acetate. The extract was concentrated and evaporated to dryness. This extract will be taken for the thrombolytic activity.

**Thrombolytic activity:** The thrombolytic activity was performed by in-vitro model. The human volunteers (n=20) without the history of oral contraceptives and anticoagulant therapy has been selected for the collection of venous blood sample. The protocol was approved by our institutional ethical committee (IAEC/PSIT/1273/ac/09). The blood was transferred in different pre-weighed sterile micro centrifuge tube (500μl/tube) and incubated at 37°C for 45 min and allowed to stand. The serum was completely removed (aspirated out without disturbing the clot formed) after clot formation. Each tube having clot was again weighed to determine the clot weight -

Clot weight = weight of clot containing tube – weight of tube alone

Each microcentrifuge tube containing clot was properly labeled and 100 μl of streptokinase (standard drug), 10mg/100μl and 20mg/100μl of extract of *Phyllanthus amarus* and distilled water (as control) was added. 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 six times with all different dilution of the isolated constituents, standard drug and control.

**Preparation of standard drug dilution:** The commercially available lyophilized streptokinase vial (15 lac I.U.) was added in 5 ml phosphate saline buffer and mixed properly. This suspension was used as a stock solution.

**Statistical analysis:** The mean clot lysis percent of streptokinase and isolated constituent was compared with water using the Tukey post test. Data are expressed as mean ± standard deviation. The p value less than 0.05 was considered to be statistically significant.

**RESULT:** The negligible clot lysis was observed when water (100μl) was added to the control clot microcentrifuge tube whereas tubes to which different dilution of extract of *Phyllanthus amarus* and streptokinase was added shows varying degree of clot lysis. With water very less weight difference was seen. The extract of *Phyllanthus amarus* at 10mg/100μl and 20mg/100μl shows the clot lysis 18.99%, 39.88% respectively While 100 μl of standard drug streptokinase shows 62.15% clot lysis. The graph was plotted between percent clot lysis and different dilutions of extract.
CONCLUSION: In India though Streptokinase and urokinase are commonly used as thrombolytic drugs but these are the drugs with some serious adverse effect like they have short half life, increase the bleeding time, higher dose. So in the current study it was tried to screen the thrombolytic drug from natural origins. Some polyphenolic constituents show the clot lysis activity in dose dependent manner. Phyllanthus amarus is also rich source of polyphenolic compounds. The extract of the drug also show the positive results for thrombolytic activity. The plants from the same genera or family have generally similar biosynthetic pathways and they produce secondary metabolites which are also found in the other plants of same genera or family. So there may be chances that the other plant of the phyllanthus genera may also show the positive activity. Further we can screen the thrombolytic activity to other plants of same genera by in-vitro and in-vivo methods as well as can try to find out the phytoconstituent which are responsible for the activity.

REFERENCES:


