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Research Article

THROMBOLYTIC ACTIVITY OF HERBAL MIXTURE CONTAINING AQUEOUS CRUDE EXTRACTS

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ABSTRACT

Objectives: Thrombolytics are used in the management of thrombosis. They all work by activating the plasminogen into plasmin which dissolves the clots. But the major drawbacks with them are bleeding and anaphylactic reactions. Natural plants and their products with thrombolytic potential and being economical and safe can be considered as an alternative for cardiovascular diseases associated with thrombus. The purpose of the present study was to evaluate thrombolytic activity of a herbal mixture containing aqueous crude extracts of pericarp of *Punica granatum*, rhizome of *Zingiber officinale* and fruits of *Phyllanthus emblica*.
Methods: The In-Vitro clot lysis activity of 100µl of various proportions of herbal mixtures was studied by comparing with 100µl streptokinase as a positive control and 100µl of isotonic normal saline as negative control.
Results: In our study streptokinase was able to dissolve 68.5% of the clot. The aqueous extracts of *Punica granatum*, *Zingiber officinale* and *Phyllanthus emblica* on an average were able to dissolve 37.42%, 30.03% and 34.31% of the clot respectively. And the herbal mixture at 1:1:1 of *Punica granatum*, *Zingiber officinale* and *Phyllanthus emblica* was able to dissolve 40.19% of the clot.
Conclusions: From the present investigation it can be concluded that the herbal mixture possessed thrombolytic properties to some extent and can be a subject for further studies in this field.
Keywords: Thrombus, thrombolytics, bleeding, plasminogen, Plasmin, Streptokinase, *Punica granatum*, *Zingiber officinale* and *Phyllanthus emblica*.

INTRODUCTION

Plants are important sources of new drug molecules. Almost every new drug which is available now in market is either a natural product or derived from them as a semi- synthetic derivative. The secondary metabolites of these plant sources have long been contributing to the development of therapeutic molecules due to the presence of combination of unique chemical features and potent bioactivities. Only about 10% of the existing higher plant species have been chemically characterized, so the vast diversity of the plant kingdom is an immense reservoir of molecules with potential pharmacological value[1]. In the estimation by W.H.O. about 80% of world population in the developing countries are totally dependent on medicinal plants for their primary healthcare. Over 25% of prescribed medicines in industrialized countries are directly or indirectly derived from plants (Vimalavady et al., 2012)[2].

The high mortality rate in many of the cardiovascular diseases is caused mainly due to the presence of thrombus (clot) inside a blood vessel which obstructs the flow of blood through the circulatory system. Thrombolytics when administered intravenously lyse the clot by activating the plasminogen a precursor into plasmin. Plasmin a natural fibrinolytic then lyses the clot by destroying the fibrin[3]. Though the currently available thrombolytics Alteplase, Anistreplase, Streptokinase, Urokinase and Reteplase[4] are wonderful clot lytics but still they have significant shortcomings, including the need for large doses, limited fibrin specificity, bleeding tendency, allergic reactions[5], and in some cases the thrombi have been proven to be resistant

to intravenous t-PA[6] .Therefore, currently researchers are shifting their focus on natural resources to find more effective alternatives. India is a country with a vast reserve of natural resources and a rich history of traditional medicine[7], so there is a huge scope for researchers to identify and isolate numerous biologically active compounds with clot lysis activity for helping us to improve the life and can prove to be a better alternative for the treatment of cardiovascular diseases involving a thrombus.

Punica granatum commonly known as Pomegranate belonging to the family Punicaceae contains alkaloids, flavanoids, tannins, carbohydrates, proteins, saponins, terpenoids, glycosides[8], anthocyaninis, proanthocyanidins, vitamin C, punicalagin, punicalin, catechin, ellagic acid and quercetin[9]. The fruits of the plants have various therapeutic benefits. It is used to treat stomach disorders, diabetes, cardiac diseases, haemorrhage, dental disorders, fever, piles, anaemia, sterility, cough and etc[10]. *Zingiber officinale* the ginger belonging to the family Zingiberaceae contains carbohydrates, vitamin C, proteins, flavanoids, tannins, fibres, zingerone, zingiberone and shogaol[11]. Its rhizome is used for the treatment of ulcers, oseteoarthritis, rheumatoid arthritis[12], heart diseases and lung diseases. It also relieves cough, cold and throat infections. It is useful for indigestion, dental problems, nausea and vomiting. Pharmacologically it is a hypolipidemic, antiemetic, chemoprotective, antiviral, anti-inflammatory, anti-ulcerogenic and analgesic[13]. *Phyllanthus emblica* commonly known as Indian gooseberry belongs to the family Euphorbiaceae. It contains alkaloids, anthocyanins, anthraquenones, carbohydrates, coumarins, flavanoids, flavanols, gallictannins, glycosides, saponins, steroids, triterpenoids, vitamin C and rutin [14]. Fruits of the plants are diuretic, carminative, astringent, improves appetite, tonic and laxative. It is useful in heart diseases, liver diseases, haemorrhoids, anemia, diarrhoea, jaundice, dyspnea and dysentry. Pharmacologically the fruits possesses anti-inflammatory, antimicrobial, antidiarrheal and analgesic activities[15].

The objective of the present study is to investigate thrombolytic activity of herbal mixture containing aqueous crude extracts of pericarp of *Punica granatum*, fruits of *Phyllanthus emblica* and rhizome of *Zingiber officinale* so that it can be considered to be a better alternative and be a subject for further studies.

EXPERIMENTAL WORK

Materials: Blood from healthy volunteer, electronic balance, syringe, eppendrof tubes, microcentrifuge, isotonic normal saline, streptrokinase (SK).

Collection of Plant materials: The fruits of Phyllanthus emblica, rhizome of Zingiber officinale and the pericarp of Punica granatum were collected from local market. The materials were thoroughly cleaned with water and shade dried.

Preparation of plant extract

Fruits of *Phyllanthus emblica* were separated from its seeds. All the three plant materials were then cut into small pieces and shade dried and pulverized to powder with a mechanical grinder. The powders were then extracted individually with distilled water by soxhlet extraction apparatus and then concentrated by air drying. The aqueous extracts of these plant materials were then stored in separate air tight containers.

Thrombolytic activity

In vitro thrombolytic activity was conducted by testing the clot lysis activity of these aqueous extracts against the standard streptokinase. Streptokinase (SK), a standard clot lysis agent is used as a positive control and isotonic normal saline as a negative control.

Streptokinase (SK)

To the commercially available lyophilized SK vial of 15, 00,000 I.U., 5 ml isotonic normal saline was added and mixed properly. This suspension was then used as a stock from which 100µl was used for the study.

Sample preparation

100 mg of the crude extracts were suspended in 10 ml of isotonic normal saline. The suspension was left for an overnight and decanted to remove the soluble supernatant portion, which was then filtered through a filter paper. 100 µl from the clear solution so obtained was then used for the evaluation of clot lysis activity.

Thrombolytic study

Whole blood was drawn from a healthy human volunteer without any history of oral contraceptive or anticoagulant therapy and then transferred in different pre-weighed sterile eppendrof tubes (0.5 ml/tube) and incubated at 37°C

for 45 minutes. After clot formation the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (W2). To each eppendrof tube containing pre-weighed clot, 100 µl of these aqueous crude extracts and different proportions of mixtures containing these crude extracts were added to the eppendrof tubes containing the clot. As a positive control 100 µl of SK and as a negative control 100 µl of isotonic normal saline were added to the tubes separately to the clot containing tubes. All the tubes were then incubated at 37°C for 90 minutes. After incubation, the released fluid was removed completely and the tubes were again weighed (W3) to observe the difference in weight after clot disruption[16]. Differences obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis[17].

$$\% \text{ of clot lysis} = (\text{wt. of released clot /clot wt.}) \times 100$$

$$= (W_R/W_C) \times 100$$

W_1 = weight of empty eppendrof

W_2 = weight of eppendrof with clot

$W_C = W_2 - W_1$

W_3 = weight of eppendrof after lysis

$W_R = W_3 - W_2$

RESULTS

In the present study Streptokinase is used as standard thrombolytic agent. The average clot lysis by streptokinase is 68.5%. Aqueous crude extracts of Punica granatum, Zingiber officinale and Phyllanthus emblica at 10mg/ml concentration have exhibited 37.42%, 30.03%, 34.31% of clot lysis activity respectively (table 1).

Four mixtures of varying proportions were prepared by using the three aqueous crude extracts. From the above results (table 2) it is observed that the M1 at 1:1:1 ratio of *Punica granatum, Zingiber officinale and Phyllanthus emblica* has shown better clot lytic action than the other three mixtures. This indicates that the three herbs are acting synergistically.

DISCUSSIONS

A thrombus within a blood vessel that breaks away from the vessel wall and freely circulates will become fatal if it obstructs the oxygen supply to a vital tissue. If thrombus is found in arterial system then it can lead to stoke, myocardial infarction, pulmonary embolism and angina pectoris. If it is seen in venous system then it can lead to renal vein thrombosis, deep vein thrombosis, portal vein thrombosis[18].

Table 1: Thrombolytic activity of aqueous crude extracts of herbs.

Name of Extract	Wt of empty Eppendrof (W1) gm	Wt of Eppendrof with clot (W2) gm	Wt of clot (W2-W1) gm	Wt of Eppendrof after clot lysis (W3) gm	Wt of released clot (W2- W3) gm	% of Clot lysis	Average
Blank 1	1.033	2.006	0.973	1.983	0.023	2.3	2.3%
SK 1	1.033	2.098	1.065	1.352	0.746	70	
SK 2	1.033	2.014	0.981	1.388	0.626	63.8	68.5%
SK 3	1.033	2.093	1.06	1.334	0.759	71.6	
APG 1	0.979	1.634	0.655	1.345	0.289	44.12	
APG 2	1.033	1.738	0.705	1.475	0.263	37.3	37.42%
APG 3	1.043	1.714	0.671	1.507	0.207	30.84	
AZO 1	1.012	1.736	0.724	1.473	0.263	36.32	
AZO 2	1.033	1.674	0.641	1.499	0.175	27.30	30.03%
AZO 3	1.033	1.656	0.623	1.491	0.165	26.48	
APE 1	0.926	1.625	0.699	1.370	0.255	36.48	
APE 2	1.033	1.647	0.614	1.468	0.179	29.15	34.31%
APE 3	1.033	1.663	0.63	1.428	0.235	37.30	

SK= Streptokinase, APG= aqueous extract of *Punica granatum*, AZO= aqueous extract of *Zingiber officinale*, APE= aqueous extract of *Phyllanthus emblica.*

Table 2: Thrombolytic activity of different proportions of mixtures containing *Punica granatum, Zingiber officinale and Phyllanthus emblica*

Name of Mixture (M)	Wt of empty Eppendrof (W_1) gm	Wt of Eppendrof with clot (W_2) gm	Wt of clot (W_2-W_1) gm	Wt of Eppendrof after clot lysis (W_3) gm	Wt of released clot (W_2- W_3) gm	% of Clot lysis	Average
M1	1.033	1.738	0.705	1.4102	0.328	46.52	40.19 %
	1.033	1.467	0.434	1.320	0.147	33.87	
M2	1.033	1.390	0.357	1.290	0.1	28.01	28.36%
	1.033	1.576	0.543	1.420	0.156	28.72	
M3	1.033	1.674	0.641	1.394	0.28	43.681	37.58%
	1.033	1.341	0.308	1.244	0.097	31.493	
M4	1.033	1.434	0.401	1.315	0.119	29.675	30.03%
	1.033	1.339	0.306	1.246	0.093	30.392	

M= (APG: AZO: APE), M_1=1:1:1, M_2= 2:1:1, M_3= 1:2:1, M_4= 1:1:2

Almost all the thrombolytics work similarly by activating the plasminogen activator into plasmin which then lyses the clot.

Pomegranate peels containing anthocyanins have beneficial effects in cardiovascular diseases[9]. Catechin and quercetin treatment have shown to modulate endothelial cell fibrinolytic protein (t-PA, u-PA) expression at the cellular, molecular and gene levels indicating their fibrinolytic activity[19]. Vitamin C which is present in the fruits of *Phyllanthus emblica*, appears to lower plasma fibrinogen levels (Khaw 1995; Wannamethee 2006). This fruit also contain rutin which is a natural thrombolytic agent inhibits either platelet aggregation or fibrin generation and it also blocks earliest stages of thrombus formation by inhibiting protein disulfide isomerase (PDI)[20]. Ginger contains gingerols, shogaols and paradols which inhibit arachidonic acid induced platelet aggregation as well as COX and thromboxane activity, indicating its beneficial activity in heart diseases[21, 22,23]. Aqueous extracts of *Punica granatum, Zingiber officinale and Phyllanthus emblica* exhibited 37.42%, 30.03%, 34.31% of average clot lysis suggesting they are equally potent. Herbal mixture containing a selective 1:1:1 ratio of these three aqueous crude extracts was able to lyse 40.19 % of the clot, suggesting the three herbs are acting synergistically. Thrombolytic activity of this herbal mixture might be due to the collective effort of the chemical constituents present in these extracts.

Thus it can be concluded that the herbal mixture containing aqueous crude extracts of *Punica granatum, Zingiber officinale and Phyllanthus emblica* possessed thrombolytic activity to some extent. Further studies should be conducted on this mixture with isolated fibrinolytic active chemical constituents for more precise results. By considering the advantage of cost effective and safety properties of herbs a formulation with better patient compliance can be formulated using this herbal mixture which can be an alternative thrombolytic.

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