ORIGINAL ARTICLE

In-vitro anti-atherothrombosis activity of four Bangladeshi plants

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Thrombous formation inside the blood vessels obstructs blood flow through the circulatory system leading hypertension, stroke to the heart, anoxia and so on. The complete deprivation of oxygen and infarction is a mode of cell death. Crude biologicals and their components possessing anti-thrombotic activity have been reported before. This study was aimed to investigate thrombolytic activity of ethanol extracts of four traditionally used medicinal plants. For this an *in-vitro* thrombolytic study was carried out along with streptokinase, and ethanol was taken as reference standard and negative control, respectively. The ethanol extracts (5 µg/µl) of *Alpinia conchigera*, *Lannea grandis*, *Aglaonema hookerianum* and *Tridax procumbens* 24.50%, 13.31%, 11.18%, and 8.70% clot lysis, respectively. Among the extracts studied *Alpinia conchigera* showed significant percent of clot lysis (24.50%) with reference to streptokinase (81.08%). Preliminary chemical group identification revealed the presence of alkaloids, glycosides, steroids, terpenoids, tannins and reducing sugars important secondary metabolites.

Key words: Crude extracts, streptokinase, thrombolytic, traditional medicine

INTRODUCTION

Bangladesh is a developing country. More than 75% people here are fully dependent upon the traditional medicine practioners (*Kabiraj*) for the treatment of various infectious and other life-threatening diseases. A clot inside the blood vessel called atherothrombosis is one of the major consequences of cardiovascular (CVS) diseases. 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 problems. At present streptokinase (SK) and urokinase (UK) are widely used for the treatment of thrombosis. Due to higher cost and unavailability, most of the poor people of various countries are still at risk to combat the thrombosis. Moreover, the SK and UK as compared to other thrombolytic drugs, their use is associated with hyper risk of haemorrhage,[1] severe anaphylactic reaction and lacks specificity. So, not only newer but also safest thrombolytic agents are essential to manage the present situation.

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Many reports are available for antimicrobial, anticancer, hepatoprotective, nephroprotective and other pharmacological activities of the suspected plant species. *A. hookerianum* and *L. grandis* revealed the presence of anticancer and antibacterial activities.[2] Significant antimicrobial and liver protective activity were observed in case of *T. procumbens*.[3,4] The aqueous extract of *O. corniculata* revealed the presence of nephroprotective activity and it can be used to treat myocardial infarction.[5,6] The ethanol extract of *A. conchigera* was found to be the presence of antinociceptive and anti-inflammatory activity. Moreover, the plant *A. conchigera* possessed anticancer activity.[7,8]

Herbal products are often perceived as safe because they are "natural".[9] Considerable efforts have been directed towards the discovery and development of natural products from biological sources. The aim of the present study was to screen the crude ethanol extracts of four (4) traditionally used plants viz., *Alpinia conchigera* Griffith. (Local name: Khatranga; Fam: Zingiberaceae), *Lannea grandis* Engl. (Local name: Jiyal, Bati; Fam: Anacardiaceae), *Aglaonema hookerianum* Schott. (Local name: Patabahar; Fam: Araceae) and *Tridax procumbens* L. (Local name: Tridara; Fam: Compositae) for their clot lysis (thrombolytic) property using an *in-vitro* procedure.[10]

MATERIALS AND METHODS

Plant Materials

The aerial parts of *Alpinia conchigera*, *Lannea grandis*,

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Aglaonema hookerianum and *Tridax procumbens* were collected from various regions of Chittagong, Bangladesh. The plant species were identified by the botanist, as CU-4331, BFRIH-0852, BFRIH-095 and RAJ-6205 for *A. conchigera*, *L. grandis*, *A. hookerianum* and *T. procumbens*, respectively, from Bangladesh. The plant materials were shade dried for 14 d and then pulverized.

Preparation of Extracts

After drying the plant materials were crushed into fine particles (powder) using a grinder. The powdered materials (200 g each) were packed in four separate amber coloured glass container and subjected to cold extraction (maceration) for 5 d using ethanol (97%) as solvent. Extracts obtained were passed through the Whattman filter paper no.1 and the crude extracts were concentrated by air cooler (at room temperature) and dried in a dessicator.

Identification Tests for Active Compounds

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannins by the following procedure.

Alkaloids

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added.[11] Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent.[12] The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Glycosides

Glycosides are compounds, which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown colouration at the junction of two layers and bluish green colour in the upper layer.[11]

Terpenoids and Steroids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then, concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids.[11]

Flavonoids

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5 - 6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones.[11]

Tannins

To 0.5 ml of extract solution, 1 ml of water and 1 - 2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.[13]

Reducing Sugar

To 0.5 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

In-vitro Thrombolytic Activity Test

Experiments for clot lysis were carried as reported earlier.[10] In brief, 4 ml venous blood drawn from healthy volunteers and was distributed in six different (4 for 4 test extracts, 1 for reference standard and 1 for negative control) pre-weighed sterile microcentrifuge (alpin/ ephendorf's) tubes (0.5 ml/tube) and incubated at 37°C for 45 min. 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 each micro-centrifuge tube containing pre-weighed clot, 100 µl (5µg/µl) of ethanol extract of four plants were added separately. As a positive control, 100 µl of streptokinase (DURAKINASE Inj. Dong Kook, Streptokinase powder for reconstitution; 1,500,000 unit / vial) and as a negative control, 100 µl of ethanol were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 min 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 duplicated at different days with the blood samples of 10 healthy volunteers (both male and female have no using contraceptives and anticoagulants).

Statistics

The significance between %-clot lysis by streptokinase and herbal extracts by means of weight difference was tested by the paired *t*-test analysis. Data are expressed as mean ± standard error.

RESULTS

In the phytochemical screening test, the crude ethanol extracts of the plant species revealed the presence of tannins and reducing sugars (almost all extracts), alkaloids (EEAC, EEAH and EETP), terpenoids and steroids (EEAC, EELG and EETP) and flavonoids (EEAH only) Table 1.

Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots along with 90 min of incubation at 37°C, showed 81.08% clot lysis. Clots when treated with 100 µl ethanol (negative control) showed only negligible clot lysis (2.47%). The mean difference in clot lysis percentage between positive and negative control was very significant (*P* value < 0.001). After treatment of clots with 100 µl of *Tridax procumbens* and *Aglaonema hookerianum* negligible clot lysis, i.e., 8.80% and 11.18%, respectively, were obtained but mean of percentage of clot lysis was more than ethanol alone. Lyses by the other two extractives viz *Alpinia conchigera* and *Lannea grandis* were 24.50% and 13.31%, respectively, and when compared with the negative control the mean clot lysis %difference was significant (*P* value < 0.001). Percent clot lysis obtained after treating clots with different herbs and appropriate controls is shown in Figure 1. Statistical representation of the effective clot lysis percentage by five herbal preparations, positive thrombolytic control (streptokinase) and negative control (ethanol) is tabulated in Table 2.

Table 1: Preliminary phytochemical presumption

Chemical groups	Crude extracts			
	EEAC	EELG	EEAH	EETP
Alkaloids	+	-	+	+
Glycosides	-	-	+	+
Terpenoids and steroids	+	+	-	+
Tannins	+	+	+	+
Flavonoids	-	-	+	-
Reducing sugars	+	+	+	+

+: Presence; -: Absence; EEAC – Ethanol extract of *Alpinia conchigera*; EELG – Ethanol extract of *Lannea grandis*; EEAH – Ethanol extract of *Aglaonema hookerianum*; EETP – Ethanol extract of *Tridax procumbens*

Table 2: Effect of four crude extracts (5 mg/ml) on *in vitro* clot lysis

Drugs / crude extracts	Clot lysis % (Mean ± SE)
SK	81.08±0.009*
EEAC	24.50±0.019*
EELG	13.31±0.028*
EEAH	11.18±0.014*
EETP	8.80±0.014*

**P*<0.001; SK – Streptokinase; EEAC – Ethanol extract of *Alpinia conchigera*; EELG – Ethanol extract of *Lannea grandis*; EEAH – Ethanol extract of *Aglaonema hookerianum*; EETP – Ethanol extract of *Tridax procumbens*

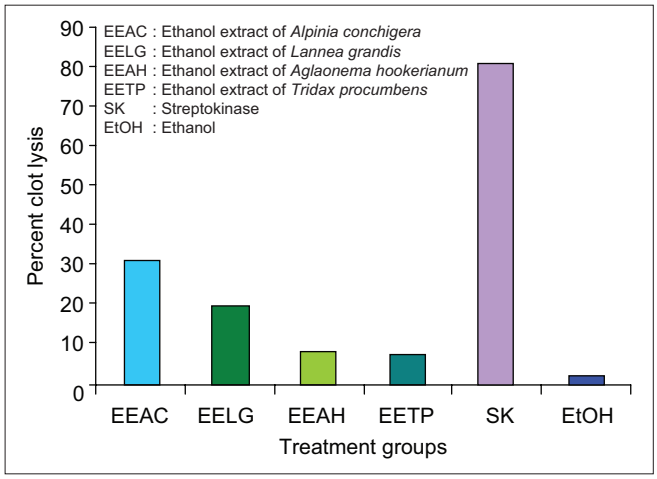

Figure 1: Thrombolytic activity by different treatment groups

DISCUSSION

A number of studies have been conducted by various researchers to find out the herbs and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect and there is evidence that consuming such food leads to prevention of coronary events and stroke.[14-17]

There are several thrombolytic drugs obtained from various sources. Some are modified further with the use of recombinant technology[18] in order to make these thrombolytic drugs more site specific and effective. Side effects related to these drugs have been reported that lead to further complications.[19]

Herbal preparations, if taken in appropriate dose, can lead to a better option for curing various ailments. Due to the inappropriate dosing, sometimes the crude naturals may lead acute and chronic ailments to the consumers. Toxicity of plant extract could be beneficial to mankind, however, if taken in higher/lethal dose plant extracts could be harmful.[20,21]

From the traditional history, we have tried five herbal preparations Table 2. In context of the above discussion it would be interesting to investigate the causative components as well as the possible mechanism(s) for clot lysis by them with respect to their toxicity and other possible pharmacological effects.

CONCLUSION

In *in-vitro* preliminary clot lysis test, the four crude ethanolic extractives revealed the presence of anti-thrombolytic activity, however, *in-vivo* clot dissolving property and active component(s) are yet to be found out. Once found they may be incorporated as a thrombolytic agent for the improvement of patients suffering from atherothrombotic diseases.

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