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ORIGINAL RESEARCH

In-Vitro Evaluation of Thrombolytic Activity of Five Medicinal Plants Available In Bangladesh

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ABSTRACT

The use of plants and plants derived substances increases day by day for the discovery of therapeutic agents. The current research is directed towards searching naturally occurring thrombolytic agents from plant origin since thrombolytic agents play a crucial role in developing various human diseases such as atherothrombotic diseases, pulmonary embolism, and myocardial infarction etc. The present study was designed to study the thrombolytic properties of five medicinal plants found in Bangladesh namely *Ixora nigricans, Amomum dealbatum Roxb, Zingiber montana, Ampelocissus barbata, Borreria laevis*. An in vitro thrombolytic model was used to evaluate the clot lysis effect of crude methanol extract of plants along with Streptokinase as a positive and water as a negative control. Using the in vitro thrombolytic mode, *Ixora nigricans, Amomum dealbatum Roxb, Zingiber montana, Ampelocissus barbata, Borreria laevis* demonstrated (35.07 ± 0.57)%, (19.21 ± 0.01)%, (18.89 ± 0.06)%, (25.32 ± 0.01)%, (19.46 ± 0.02)% clot lysis respectively. Among the plants studied *Ixora nigricans* and *Ampelocissus barbata* showed significant % of clot lysis (35.07 ± 0.57) % and (25.32 ± 0.01) % respectively with reference to Streptokinase (75.26 ± 0.48)%. Through our study it was observed that the studied plants possess thrombolytic properties that could lyse the blood clot in vitro; however, in vivo clot dissolving properties and active component(s) of these plants are yet to be explored. Once found they could be incorporated as a thrombolytic agent for the treatment of atherothrombotic diseases.

KEY WORDS: *Atherothrombotic, thrombolytic, clot lysis*

INTRODUCTION

A blood clot can be harmful in thrombosis, when clots obstruct blood flow through healthy blood vessels while recovering leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infarction, pulmonary embolism, at times leading to death (Lee, 1995). Different types of thrombolytic agents such as tissue plasminogen activator, Urokinase (UK), Streptokinase (SK) etc. are used globally for the treatment of these diseases as first-line clinical thrombolytic agents. Due to the lower cost of UK and SK are extensively used in Bangladesh, India and other developing countries (Rahman *et al*., 2013). In addition, exploitation of these drugs remains associated with complications, including intracranial haemorrhage, severe anaphylactic reaction, and lack of specificity (Simpson *et al*., 1982). Moreover, these drugs are not used in patients who had undergone surgery or those with a history of nervous lesions, gastrointestinal bleeding, or hypertension (Simpson *et al.*, 1982). Therefore, the quest for novel medicinal agents may let obtaining new drugs with enhanced pharmacological properties, extensive range of therapies, efficacy, and safety. Plants are the natural sources of medicinal agents include antimicrobial, anticancer

agents, analgesics and so on. Traditionally a large number of plant-derived medicines had been used without any adverse effects. A wide array of plants represents the natural source of valuable compounds that might provide as the lead for the development of novel drugs. It is, therefore, a great attention should be concentrated to introduce new medicinal agents to develop more effective and cheaper drugs. Thus, traditional medicine has been paid great consideration because they are economical, obtainable, and have minute side effects which argued WHO that around 80 % of the world population still rely mainly on plant-based drugs (Uddin *et al*., 2015).

Ixora nigricans R. Br. (Rubiaceae) is a large shrub which is found throughout Bangladesh, the forests of India and Indo-Malaysia (Barbhuiya *et al*., 2014). In local tribes of Bangladesh, it is known as Dikranga Chuillya (Chakma, Tripura), Rongma, Frareko (Marma). Extract of the root is used to treat diarrhoea and ear infections by the Chakma. A paste of the leaves is applied to affected areas for the treatment of boils, pills prepared from the paste of the leaves are taken thrice daily for dysentery by the Tanchangya. Extract prepared from leaf taken and paste prepared from the root is applied to the whole body as a remedy for unconsciousness of a little child and extract prepared from the root, taken one cupful four times daily for two days against vomiting over bleeding by the Marma [6]. It has been demonstrated that methanolic extract of *I. nigricans* leaf contains significant anti-arthritic activity and moderate cytotoxic activity (Alam *et al*., 2015). In addition, anti-inflammatory and anti-oxidative property of ethanolic extract of *Ixora nigricans* leaves were reported (Prawej Ansari Juthika Sarker, Sumonto Sen, Kallol Kanti Mondal, Zareen Tasnim Tapti, Sanjeeda Sarmin Badhan, 2015). However, the thrombolytic potentiality is not reported yet. *Ampelocissus barbata* is a wild plant and its tuberose is used traditionally for the relief of hernia pain (Majumdar and Datta, 2007).

The plant *Zingiber montana* is grown in tropical Asia. The rhizomes are very popular for the treatment of gastric ulcer as a folk medicine at Rangpur Division of Bangladesh (Al-Amin *et al.*, 2012). Besides, it is also reported to be used in folk medicine for several ailments such as inflammation, colic, diarrhoea, vermifuge, stimulant, pain, sprains, wounds, and asthma (Kimiaki *et al.*, 1989; Pithayanukul *et al*., 2007). Although several pharmacological studies of this plant such as anti-inflammatory, antimicrobial, antioxidant (Habsah *et al*., 2000), antiulcer (Al-Amin *et al.,* 2012) activities have been reported, yet there is no report of thrombolytic activity of this plant.

Amomum dealbatum Roxb. belongs to Zingiberaceae family and its rhizome is used in the treatment of Septic abscess in aboriginal people of Chittagong Hill Tracts region (Rahman, 2010). *Borreria laevis* (Lam.) Griseb. is a small herb found in the tropical regions of Asia and also occurs in Mexico, where a decoction of the leaves is used to treat kidney pain and prevent menstruation while the entire plant in admixture with *Cuscuta* L. and *Zebrina pendula* Schum is used for amenorrhea in Jamaica and West India (Conserva and Ferreira, 2012). However, no thrombus degrading properties is reported yet.

These five medicinal plants of Bangladesh is widely used in the different parts of the country particularly in Hill tracts region, and to our knowledge, there is no report yet on thrombolysis potentiality of these plants. Therefore, the aim of this study was to evaluate the clot lysis (thrombolytic activity) properties of methanol extract of these five plants by using in vitro models.

RESULTS

Thrombolytic activity

The thrombolytic properties of all extracts of selected plants were assessed and the results are presented in Table. In this paper, the highest percentage of thrombolytic activity exhibited by the Streptokinase (75.26 ± 0.4832) % while crude methanol extract of *Ixora nigricans* exhibited a (35.07 ± 0.5785) % clot disruption which is statistically highly significant (p value <0.0001) when compared with negative control. In addition, significant thrombolytic activity was demonstrated by *Ampelocissus barbata* (25.32 ± 0.01764) % which is also statistically significant (p value <0.0001). However, there was comparatively moderate clot dissolution was found by *Zingiber montana* (19.21 ± 0.01202) % and *Borreria levis* (19.46 ± 0.02028) % respectively. The percent clot lysis obtained after treating clots with different plants preparation and appropriate controls is shown in Figure. The mean difference between positive and negative controls in

clot lysis percentage was found statistically significant. Statistical calculations of the effective clot lysis in percentage by all plant extract preparations, positive control (Streptokinase) and negative control (sterile distilled water) done by t-test analysis. Percent of clot dissolution is represented as mean±SEM and p values of all the plants were considered as significant (p value<0.05).

Table: Thrombolytic activity of five different plants extracts

Plants/Drugs	Mean ± SEM. (Clot lysis %)
Streptokinase	75.26 ± 0.4832
Ixora nigricans	35.07 ± 0.5785
Zingiber montana	19.21 ± 0.01202
Amomum dealbatum Roxb	18.89 ± 0.06173
Ampelocissus barbata	25.32 ± 0.01764
Borreria laevis	19.46 ± 0.02028
Water	7.543 ± 0.1184

Note: Values are expressed as mean±SEM (n = 3). $p < 0.05$, significantly different from control.

DISCUSSION

Medicinal derivatives from plants have a long history of use for the prevention and management of human diseases and plants serve as the prominent source of the potentially bioactive compounds for the discovery and development of drugs (Prasad *et al.*, 2007). Globally, around 30% of the pharmaceuticals are chiefly prepared from plant sources, and it is considered that the bioactive phytoconstituents are accountable for the remedial value of plant (Mollik *et al*., 2010; Rahman *et al.*, 2012). The bucolic population of Bangladesh still largely relies on the traditional system of medicine for their health-related issues(Rahman *et al*., 2013). In the present study of thrombolysis, we have investigated the five different plants preparation which is traditionally used for the treatment of various diseases to explore the thrombolytic properties of these plants. The majority of the thrombolytic agents exert their useful outcome by activating the enzyme plasminogen, which solubilizes the cross-linked fibrin mesh to restore blood flow in blocked blood vessels (Simpson *et al.*, 1982).

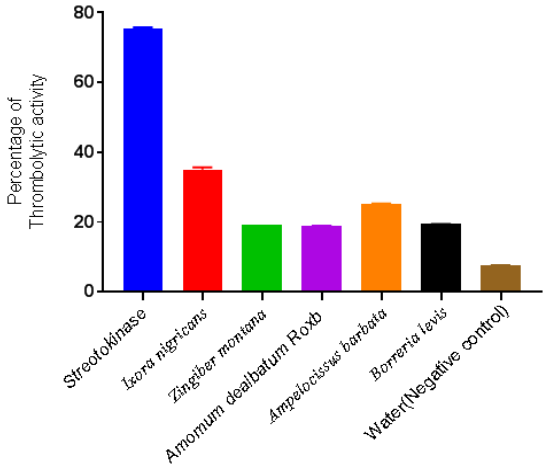

Figure: Clot lysis by Streptokinase, water and various plants extracts. Maximum clot lysis (75.26 ± 0.4832) % was observed in clot treated with Streptokinase (SK) which served as positive control. Among different plants, *Ixora nigricans* showed (35.07 ± 0.5785) % clot lysis and *Ampelocissus barbata* showed (25.32 ± 0.01764)% clot lysis. Water as a negative control showed (7.543 ± 0.1184) % clot lysis. With other plants, moderate clot lysis was observed. Values are expressed as mean±SEM (n = 3). $p < 0.05$, statistically significant.

Dissolution of clots, therefore, is useful for the treatment of clot-related disorders, including myocardial infarction, thromboembolic strokes, deep vein thrombosis, and pulmonary embolism, to clear a blocked artery that circumvents stable damage to the respective tissues (Prasad *et al*., 2007). The comparison between positive and negative controls clearly showed that there was no clot dissolution when water was added to the clot. SK, a known thrombolytic drug (Mucklow, 1995) was used as a positive control. The percentage of clot lysis by both of these controls differ significantly as the p value was <0.0001. When compared with the clot lysis percentage obtained through negative control, a significant thrombolytic activity was observed after treating the clots with *Ixora nigricans,* and *Ampelocissus barbata* also showed significant clot lysis. The lysis of clot by crude methanol extract of five plants compared to the

controls demonstrates its potential use in clot-related disorders.

CONCLUSION

It can be concluded that the extracts of all the above five different plants methanol crude extracts can be used to design different anti-thrombotic agents which will have significant implications in cardiovascular health due to its moderate thrombolytic activity. Further work is needed to isolate and characterize the compounds responsible for thrombolytic activity and study thoroughly for more precise and accurate activity.

MATERIALS AND METHODS

Collection of Plant Sample and Extraction

The leaves of five plants were collected from Chittagong Hill Tract region of Bangladesh. The collected plant leaves were shade desiccated and powdered. The powdered plants (950 g) were subjected to extraction with methanol at room temperature for 7 days with sporadic shaking. The extractive solution was filtered and concentrated under vacuum in a rotary evaporator at 45 °C to yield 17 g of the crude extract.

Streptokinase (SK)

Commercially available lyophilized Streptokinase vial (Beacon pharmaceutical Ltd., Mymensingh, Bangladesh) of 15, 00,000 I.U., was collected. 5 ml sterile distilled water was mixed properly. This suspension served as a stock from which 100µl (30,000 I.U) was used for *in vitro* thrombolysis.

Blood Specimen and Thrombolytic Activity

The thrombolytic potentiality of the five plants was evaluated by the method explained by Prasad et al (Prasad *et al.*, 2007) using an approved protocol by research project committee (2016-207/4829) of Islamic University, Kushtia, Bangladesh for collection of blood samples from human volunteers. A consent form was supplied to all the volunteer donors, which informed the title of the research project as well as the purpose of research. A 4 ml venous blood drawn from the healthy volunteers without the history of oral contraceptives and anticoagulant therapy since two weeks, was dispersed in different pre weighed sterile microcentrifuge tube (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). Each of the five plants methanol crude extracts (100 µl) were transferred to each microcentrifuge tube separately containing pre-weighed clot. As a positive control, 100 µl of Streptokinase (SK) and as a negative non-thrombolytic control, 100 µl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37°C for 90 minutes and observed dissolution of the clot. After incubation, fluid released was removed and tubes were again weighed to study the difference in weight after clot disruption. The difference obtained in weight taken before and after the clot lysis was expressed as the percentage of clot lysis.

Statistical analysis

All values of thrombolytic activity were calculated as mean±SEM and evaluated using t-test implemented by GraphPad Prism Data Editor for Windows, version 7.0 (GraphPad Software Inc., San Diego, CA, USA). p-values <0.05 were regarded as statistically significant

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AUTHOR CONTRIBUTIONS

M.R.R., H.M.F. conceived and designed the experiments; M.R.R, S.A. , K.A, A.M. and T.I. performed the experiments; M.R.R., T.I., and H.M.F analyzed the data; K.A, A.M., S.A. and T.I. contributed reagents/materials/analysis tools; M.R.R. wrote the paper.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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