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Investigation of *in vitro* thrombolytic potential of ethanolic extract of *Momordica charantia* fruits: An anti-diabetic medicinal plant

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ABSTRACT

The study was carried out to investigate the thrombolytic potential of crude ethanolic extract of fruits of Momordica charantia, an anti-diabetic medicinal plant, using an in vitro assay method. The thrombolytic investigation involved five young Bangladeshi volunteers to get different nature of blood samples. Five concentrations of the crude extract were used for the study, with a standard (Streptokinase), and negative control (sterilized distilled water) to validate the method. The thrombolytic nature of the plant was found significant ($p < 0.01$), except for the concentration 4 mg/ml, when compared with the negative control (water) at different doses. The plant showed mild clot lysis, i.e. 2.16±0.723%, 5.06±1.058%, 8.60±0.626%, 11.64±0.747% , and 15.18±1.691% at 2, 4, 6, 8, and 10 mg/ml concentrations respectively, while the standard (streptokinase) showed 47.22±2.738% clot lysis. Therefore, the obtained results suggest that the crude ethanolic extract of Momordica charantia fruits possess light thrombolytic activity in vitro; however, in vivo thrombolytic potentiality and active component(s) of the extract for clot lysis are yet to be discovered.

Keywords: *Momordica charantia*, ethanolic extract, thrombolytic, streptokinase, clot lysis

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INTRODUCTION

Thrombosis is a fatal disease which is characterized by the development of a blood clot (thrombus) in the circulatory system because of the imbalance of homeostatic system of the body. It leads to vascular blockade and while recovering causes fatal consequences, such as myocardial or cerebral infarction and even death [1]. Thrombosis underlies some acute coronary disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks and these are the main causes of morbidity and mortality in developed countries [2, 3, 4]. Therefore, anticoagulation therapy is the basis of management, and the proper choice of thrombolytic drugs to decrease platelet aggregation or interfere with the clotting process can be critical [2]. Intravenous heparin, the first line of treatment for cerebral venous sinus thrombosis (CVST), is used in the anticoagulation therapy, because it is safe, effective and feasible [5]. With the development of modern pharmaceuticals many drugs have been developed with the purpose of dissolving clots, such as alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen (TPA) etc. [3, 6]. Among these drugs streptokinase and urokinase are widely used because of their cost effectiveness as compared to other thrombolytic drugs [7, 8]. But due to the weak substrate specificity of these first generation drugs (streptokinase and urokinase), they lead to systemic fibrinolysis, anaphylactic reaction and bleeding complications (hemorrhage) [9]. Again, multiple treatments with streptokinase are restricted in a given patient, as a result of immunogenicity [10].

Because of the shortcomings associated with the thrombolytic drugs, it is necessary to find an attractive alternative and to develop improved recombinant of these drugs [1, 11, 12, 13].

Because of the safety of the herbal products they are being used successfully to treat all types of diseases, by our traditional system of medicine and folklore, since the immemorial [14]. Numerous efforts have been taken to discover and develop herbal products from various plant and animal sources, having antiplatelet [15, 16], anticoagulant [17, 18], antithrombotic [19] and thrombolytic activity. Several studies were proved to be successful and provided evidence that some herbs possess significant thrombolytic activity [20]. *Momordica charantia* (Family-Cucurbitaceae), commonly known as bitter gourd or bitter melon in English, is used as a tropical vegetable as well as used in ayurvedic and unani system of medicines for the treatment of many diseases [21]. It is a very common herb having various medicinal properties for the treatment of different kind of disease, viz. antifungal, wound healing and antidiabetic agents [22, 23]. The earlier reports showed that the plant also has anti- malarial, anti-plasmodial properties [24, 25] and insecticidal activity against mustered saw fly [26]. Thus the present study focuses on screening of ethanolic extract of *Momordica charantia* fruits, for its clot lysis property (thrombolytic activity) by using an *in vitro* assay method.

MATERIALS AND METHODS

Plant collection and extraction

The fruits of *Momordica charantia* were collected by the authors from the surrounding area of Noakhali, a coastal region of Bangladesh in September, 2012. The plant was identified and authenticated by expert botanist of Bangladesh National Herbarium (DACB Accession no. 37656), Mirpur, Dhaka.

500 mg of the dried and powdered sample was soaked in 2500 ml of 99.8% ethanol (Merck KGaA, Darmstadt, Germany). After 15 days the solution was filtered using filter cloth and Whatman® filter paper No. 1. The resulting filtrates were then evaporated in water bath maintained at 45°c to dryness and thus a blackish-green semisolid mass of the extract was obtained.

Standard drug: Streptokinase (SK)

To the commercially available lyophilized S-Kinase™ (Streptokinase) vial (Batch no: VEH 09, Popular Pharmaceutical Ltd., Bangladesh) of 15, 00,000 I.U., 5 ml 0.9% sodium chloride (NaCl) was added and mixed properly. This solution was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis assay [27].

Specimen

Whole blood (7 ml) was drawn from healthy Bangladeshi human volunteers (n = 5) (aged 20-23 years) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by Institutional Ethics Committee). 1 ml of blood was transferred to each of the 7 previously weighed sterilized micro-centrifuge tubes to form clots.

Preparation of test sample

Five different test solutions were used to evaluate the thrombolytic activity of the plant extract. The plant extract was dissolved in methanol and shaken vigorously on a vortex mixer to prepare different concentrations (2, 4, 6, 8 and 10 mg/ml respectively) of the test sample. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron syringe filter. 100 µl of the ethanolic preparations of the plant were added to the micro-centrifuge tube containing the clots to check thrombolytic activity [27].

Anti-thrombotic assay

In vitro clot lysis activity of *Momordica charantia* was carried out according to the method of [27]. 7 ml of venous blood was drawn from healthy volunteers (n = 5) and transferred to different pre weighed sterile micro-centrifuge tube (1 ml/tube). The micro-centrifuged tubes were subjected to incubation at 37°C for 45 minutes. After the formation of clot, serum was completely removed from the tubes (carried out without disturbing the clot formed) and each tube having clot was again weighed to determine the weight of the clot (clot weight = weight of clot containing tube – weight of tube alone).

Each micro-centrifuge tube containing clot was properly labeled and 100 µl of the plant extract with various concentrations (2, 4, 6, 8 and 10 mg/ml respectively) was added to the tubes accordingly. As a positive control, 100

µl of streptokinase and as a negative non thrombolytic control, 100 µl of sterilized distilled water were separately added to the control tubes numbered. Then all the tubes were incubated again at 37°C for 90 minutes and observed for clot lysis. After incubation, the obtained fluid was removed from the tubes and they were again weighed to observe the difference in weight after clot disruption. At last, difference obtained in weight was calculated and the result was expressed as percentage of clot lysis following the underneath equation.

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

Statistical analysis

The significance of percent clot lysis by herbal extracts (with different concentrations) and streptokinase was compared with water using paired t-test analysis. Data are expressed as mean ± standard deviation (SD). A p value < 0.05 was considered to be statistically significant.

RESULTS

Table 1: Effect of different concentrations of the test sample and the controls on *in vitro* clot lysis

Treatment	% Clot lysis (mean ± S.D)	P value (Two-tailed) when compared to negative control (water)
Extract (2 mg/ml)	2.16±0.723	< 0.01
Extract (4 mg/ml)	5.06±1.058	< 0.30
Extract (6 mg/ml)	8.60±0.626	< 0.01
Extract (8 mg/ml)	11.64±0.747	< 0.01
Extract (10 mg/ml)	15.18±1.691	< 0.01
Streptokinase (standard)	47.22±2.738	< 0.01

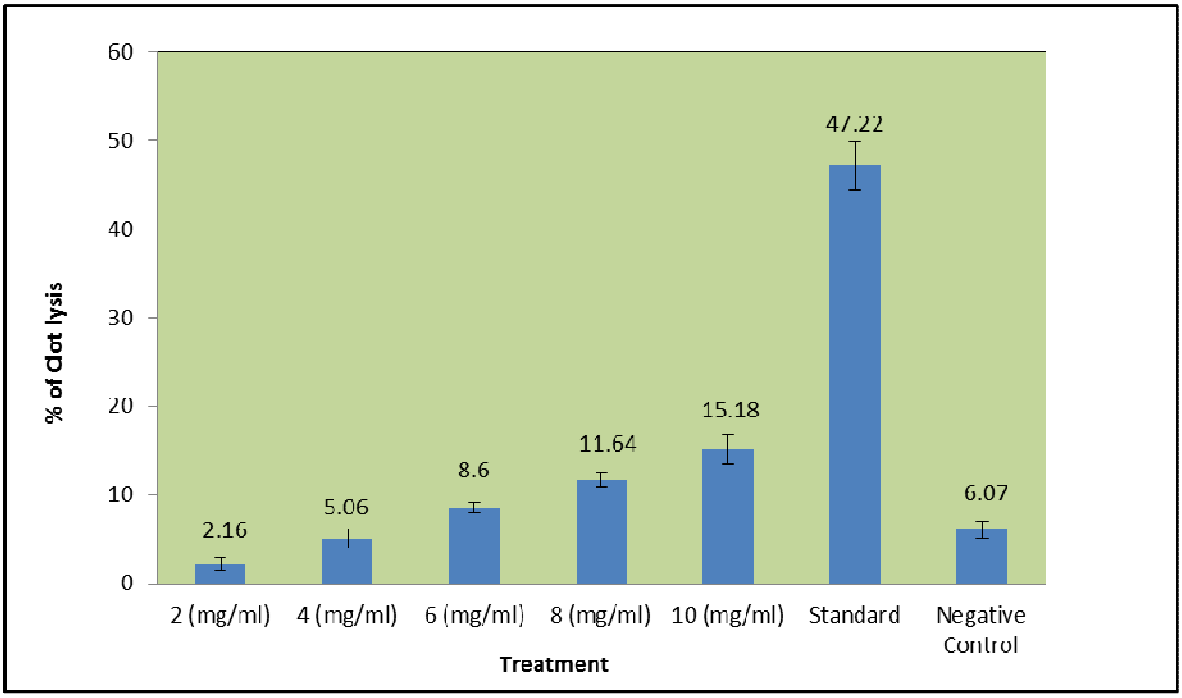

Figure 1: Thrombolytic activity of crude extract of *Momordica charantia*, negative control and Streptokinase by clot lysis activity. Results are mean±SD of five parallel measurements

Statistical representation of the effective clot lysis percentage by five different concentrations of the plant extract, positive thrombolytic control (Streptokinase) and negative control (water) is tabulated in Table 1. From table 1, it is evident that the percentage of clot lysis was 48.67% when 100 µl of streptokinase (30,000 I.U.) was used as a positive control, while in case of water (negative control) the percentage of clot lysis was negligible (6.07%). The mean difference in clot lysis percentage between positive and negative control was very significant ($p < 0.01$). When

clots were treated with 100 µl each of different concentrations (2, 4, 6, 8 & 10 mg/ml respectively) of the test sample moderate clot lysis activity, i.e., 2.16%, 5.06%, 8.60%, 11.64%, and 15.18% respectively, was observed and when compared with the negative control (water) the mean of percentage (%) of clot lysis was significant for all the concentrations except for the dose of 4 mg/ml ($p < 0.3$) i.e., at concentration of 4mg/ml, the percent of clot lysis is insignificant compared with negative control (water). Percentage of clot lysis after treatment with different concentrations of the ethanolic extract and appropriate controls is shown in Figure 1.

DISCUSSION

Thrombus is formed by the adhering of the damaged regions (caused by reactive oxygen species) of the endothelial cell surface, where platelets plays a vital role in the formation process. The process of thrombosis is initiated when the activated platelets form platelets to platelets bonds and also bind to the leucocytes and bring them into a complex process of plaque formation and growth [28]. Plasmin, a fibrinolytic agent by nature, lyses clot by disrupting the fibrinogen and fibrin contained in a clot. Again, cell surface bound plasminogen is easily activated to plasmin, which could lead to fibrinolysis [29]. A 1:1 stochiometric complex of Streptokinase (a bacterial plasminogen activator) with plasminogen is capable of converting additional plasminogen to plasmin [30]. However phlorotannin (oligomers of phloroglucinol), which is isolated from a natural product namely marine brown algae, have a unique property in promotion of dissolution of intravascular blood clot via antiplasmin inhibition [27]. Several thrombolytic drugs from various sources have been discovered by the scientists and some of them have been modified with recombinant technology in order to make those thrombolytic drugs more site specific and effective [31]. Unfortunately, these drugs are shown to have several adverse effects related to such as bleeding and embolism, which lead to further complications [32, 33, 34]. In order to discover new sources of herbs and natural foods and their supplements having antithrombotic effect (anticoagulant and antiplatelet) a number of studies have been conducted by various researchers and the study confirmed that consuming such food leads to prevention of coronary events and stroke [31, 35, 36, 37].

Our present study was an attempt to find if the herbal preparation of *Momordica charantia* possesses clot lysis potentiality or not. The comparison of the positive control (streptokinase) with negative control clearly demonstrated that clot dissolution does not occur when water was added to the clot. Encouraged by the result of the positive control, we compared five different concentrations of the test sample in the same manner with the negative control and observed significant thrombolytic activity. It was also observed from the study that the percentage of clot lysis was proportional to the concentration, although at the concentration of 4 mg/ml the clot lysis by the extract was almost similar to the percentage of clot lysis by the water.

CONCLUSION

In the context of the above discussion it can be concluded that the ethanolic extract of *Momordica charantia* possesses very mild thrombolytic activity and the activity increases with the increasing of concentrations. In fine, it would be interesting to investigate the *in vivo* thrombolytic activity, and the causative component(s), and mechanism for clot lysis by *Momordica charantia*.

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