Short report

Thrombolytic activity of some spices and plants available in Bangladesh

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Abstract:

Thrombolytic activities of some plants, namely *Tamarindus indica* (Fabaceae), *Flemingia congesta* (Fabaceae), *Lawsonia inermis* (Lythraceae), *Mesua nagassarium* (Clusiaceae, and spices, namely *Coriandrum sativum* (Apiaceae), *Curcuma longa* (Zingiberaceae), *Cinnamomum tamala* (Lauraceae), *Nigella sativa* (Ranunculaceae), *Eugenia aromaticum* (Myrtaceae), available in Bangladesh, were evaluated using an *in vitro* model. The thrombolytic activity in terms of percentage of weight loss of *in vitro* formed clots were found as *C. sativum* 43.25 ± 7.18%, *C. longa* 53.32 ± 4.96%, *C. tamala* 22.10 ± 3.18%, *N. sativa* 28.49 ± 3.72%, *E. aromaticum* 32.18 ± 3.10%, *T. indica* 28.91 ± 2.29%, *F. congesta* 35.27 ± 7.35%, *L. inermis* 62.40 ± 5.04%, *M. nagassarium* bark 39.54 ± 7.15% and *M. nagassarium* leaf 46.75 ± 3.97% with reference to the negative control distilled water 8.37 ± 1.18% and positive control streptokinase 84.63 ± 1.03%. Through our study, it was found that *L. inermis* and *C. longa* possess thrombolytic property that could lyse blood clots *in vitro.*

Keywords: Thrombolytic activity; *Lawsonia inermis; Curcuma longa;* Bangladesh

Introduction

Atherothrombotic diseases such as myocardial or cerebral infarction are serious consequences of the thrombus formed in blood vessels [1, 2]. Thrombolytic agents such as tissue plasminogen activator, urokinase, streptokinase (SK), etc are used to dissolve the already formed clots in the blood vessels [3-5]. However, these drugs have certain limitations which cause serious and sometimes fatal consequences including hemorrhage, severe anaphylactic reaction, lacked specificity, etc. Moreover, as a result of immunogenicity multiple treatments with SK in a given patient are restricted [6].

Agents from plant source are expected to be less antigenic and cheaper. Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have antiplatelet [7, 8], anticoagulant [9-11], antithrombotic and thrombolytic activities [12]. Epidemiologic studies have provided evidence that foods with experimentally proved antithrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported [11]. With this view we studied four plants and five species available in Bangladesh to find out if they posses any thrombolytic activity. In these study, we have screened the aqueous soluble extracts of some plants viz., *Tamarindus indica* (F. Fabaceae; local name Tetul; English name Tamarind), *Flemingia congesta* (F. Fabaceae; local name Baro shalpani), *Lawsonia inermis* (F. Lythraceae; local name Mehendi; English name Henna), *Mesua nagassarium* (F. Clusiaceae; local name Nageshar; English name Indian rose chestnut or Iron wood tree); and some spices viz., *Coriandrum sativum* (F. Apiaceae; local name Dhonia; English name Coriander), *Curcuma longa* (F. Zingiberaceae; local name, Holud; English name, Turmeric), *Cinnamomum tamala* (F. Lauraceae; local name Tejpata; English name Bay leaf), *Nigella sativa* (F. Ranunculaceae; local name kalojira; English name Black cumin), and *Eugenia aromaticum* (F. Myrtaceae; local name, Labanga; English name Clove) for their clot lysis property (thrombolytic activity) by using an *in vitro* procedure [10].

These plants and herbs are widely used in traditional medicine or as spices. The organic solvent soluble extracts of these plants or spices are reported to have different pharmacological, biological and microbiological activities [13]; but no such significant reports on the cardio-protective or thrombolytic activity of the water soluble fractions of these plant and herbs have been investigated so far. Among these plants or spices *C. longa* is reported to inhibit platelet aggregation [14] and possesses cardio-protective activity [15, 16].

Materials and Methods

Streptokinase

The commercially available lyophilized streptokinase (SK) (Durakinase®, Dongkook Pharma Co. Ltd., South Korea) of 1,500,000 I.U per vial were used as positive control. The whole lyophilized powder of the vial was to be dissolved in 100 ml water to get 1,500,000 I.U. of SK solution, which is the recommended dose for myocardial patients. We took one tenth of the powder in 10 ml of water for each time to make 1,500,000 I.U. of SK from where 100 µl was used for *in vitro* thrombolysis.

Preparation of clots

Six milliliters of blood were withdrawn from healthy human volunteers (n=9) irrespective of gender following the guidelines set up by the local ethical committee. The blood was distributed into 12 previously weighed microcentrifuge tubes (0.5 ml to each centrifuge tube) to form clots, and they were then centrifuged at 2000 rpm for 5 min to let the serum separate above for easy removal from the centrifuge tube. The centrifuge tubes were then incubated in simulated body temperature i.e. at 37°C for 45 min in heat controlled incubator.

Preparation of extractives

The plants and spices used in this study were collected, identified by the taxonomist by the Department of Botany, University of Dhaka; and then dried under shed if necessary and ground into powder. The powder of each plant and spices were soaked in methanol for 5 d with successive shaking and the extracts were collected and dried at low temperature employing vacuum.

100 mg dried extracts of each was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered. A hundred µl of this aqueous preparation from each type was added to the microcentrifuge tubes containing the clots to check thrombolytic activity.

Clot lysis

The thrombolytic activity in terms of in vitro clot lysis was carried as reported earlier [10]. Briefly, 6 ml of blood was withdrawn from healthy human volunteers. The blood was distributed into 12 pre-weighed centrifuge tubes (0.5 ml to each centrifuge tube) and they were then centrifuged at 2,000 rpm for 5 min. The centrifuge-tubes were then incubated in simulated body temperature i.e. at 37°C for 45 min in heat controlled incubator. After 45 min, blood clot was formed at the bottom of each centrifuge tube. The serum was removed from each centrifuge tube above the clot carefully and completely without disrupting the clot. After removing the serum, the tubes that contained clot (but no serum) were weighed again. One hundred µl of each aqueous extractive was added in each microcentrifuge tubes, where streptokinase and distilled water were applied as positive and negative control respectively. All the 12 centrifuge-tubes were then again incubated at 37°C for 90 min to see their clot dissolving capacity. After 90 min the centrifuge-tubes were taken out of the incubator and the dissolved clot along with the applied agents (extract solution or streptokinase preparation or distilled water) were removed carefully and completely from the centrifuge tubes. Then the centrifuge tubes that contained undissolved clot were weighed. The weights of the clot before and after the clot lysed by the applied extract solutions, streptokinase and distilled water were calculated from the differences in the weights. The weight loss due to thrombolytic activity of each applied agent was calculated in percentage. The ability of the extracts to dissolve clot in percentage of weight loss were compared with that of standard and blank. The experiment was repeated 9 times with the blood samples of 9 volunteers. The equivalent doses of streptokinase for the highest activity showed by the plants and spices were also measured. For these, streptokinase was added at concentration of 1,500,000 I.U., 1,400,000 I.U., 1,300,000 I.U, 1,200,000 IU and 1,000,000 I.U. to the clot and the clot lysis activity was measured according to the procedure described above.

Statistical analysis

The values were calculated as mean ± SEM and expressed as percentages. The significance between % clot lysis by streptokinase and herbal extract by means of difference in weight was tested by the paired *t*-test analysis.

Results

Addition of 100 µl SK as a positive control (1,500,000 I.U.) to the clots along with 90 min of incubation at 37°C showed 84.6% clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (8.40%). Among the plants *L. inermis* leaf, *M. nagassarium* leaf, *M. nagassarium* bark, *F. congesta* flower, and *T. indica* bark gave thrombolytic activity of 62.40%, 46.75%, 39.54%, 35.27%, and 28.91%, respectively (Table 1). Whereas among the spices *C. longa, C. sativum, E. aromaticum, N. sativa,* and *C. tamala* gave the thrombolytic activity of 53.32%, 43.25%, 32.18%, 28.49%, and 22.10%, respectively (Table 2). Clearly *L. inermis* leaf and *C. longa* showed potent *in vitro* clot lysis activity and are seemed to be potent sources for further investigation to find the responsible lead compounds for the thrombolytic activity. The equivalent doses of SK for the activity of *L. inermis* and *C. longa* were also observed and were found to 1,320,000 IU (approx.) and 1,140,000 IU (approx.), respectively.

Discussion

This study evaluated the thrombolytic potential of some plants and herbs available in Bangladesh. Herbal preparations are used since ancient times for the treatment of diseases. Phytopharmacological investigation has lead to discovery plant derived drugs, which are effective in remedial of certain diseases, and renewed the interest in herbal medicines. About 30% of the

Table 1 Thrombolytic activity of the aqueous soluble fractions of plants

Name of plants	Family	% of wt. loss of clot[a]
Tamarindus indica	Fabaceae	28.91 ± 2.29
Flemingia congesta	Fabaceae	35.27 ± 7.35
Lawsonia inermis	Lythraceae	62.40[b] ± 5.04
Mesua nagassarium (Bark)	Clusiaceae	39.54 ± 7.15
Mesua nagassarium (Leaf)	Clusiaceae	46.75 ± 3.97
Distilled water	--	8.37 ± 1.18
Streptokinase	--	84.63[b] ± 1.03

[a]Values are mean ± SEM (n=5); [b]$P < 0.001$ vs control.

Table 2 Thrombolytic activity of the aqueous soluble fractions of spice

Name of spices	Family	% of wt. loss of clot[a]
Coriandrum sativum	Apiaceae	43.25 ± 7.18
Curcuma longa	Zingiberaceae	53.32[b] ± 4.96
Cinnamonum tamala	Lauraceae	22.10 ± 3.18
Nigella sativa	Ranunculaceae	28.49 ± 3.72
Eugenia aromaticum	Myrtaceae	32.18 ± 3.10
Distilled water	--	8.37 ± 1.17
Streptokinase	--	84.63[b] ± 1.03

[a]Values are mean ± SEM (n=5); [b]$P < 0.001$ vs control.

pharmaceuticals are prepared from plants worldwide [6,12]. 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 [17-20]. Although there are several thrombolytic drugs including those obtained by recombinant DNA technology, but side effects related to some of these drugs that lead to further complications have been reported [21-24].

However, herbal preparations on the other hand, if taken in appropriate doses, can lead to an alternative and better option for curing various ailments. Although, toxicities of many plant extract are major concern, but due to the development of methods for lethality assay has been successfully used to biomonitor the cytotoxicity of plant materials [25, 26]. We have assessed the cytotoxicities of the investigated plants and spices (data not shown here). Our studies showed that *L. inermis* showed potent toxicity against brine shrimp nauplii. These suggest that extractives of this plant should not be wise to be used as a remedial of heart diseases as it traditional forms. However, further approaches are awaited to isolate the active principles responsible for the thrombolytic activity.

Conclusion

Although the beneficial effects of thrombolytic therapy are now well established [26] and the biochemical mechanisms of thrombolytic therapy have been elucidated, but the search for alternative and complimentary therapy is still continuing due to some reasons including availability and diversity of natural resources, easy access and affordability. Our present studies showed that among the plants, *L. inermis* and *M. nagassarium* showed significant thrombolytic activity. The percentage of weight loss due to clot lysis induced by *L. inermis* was 62.40 ± 5.04% and that of, *M. nagassarium* was 39.54 ± 7.15% (bark) and 46.75 ± 3.97% (leaf). These

two plants are seemed to potent sources for further investigation to find the responsible lead compounds for thrombolytic activity. We do not suggest them to be used as food supplement, since *L. inermis* is toxic (as found by brine shrimp cytotoxicity test) and the toxicity of *M. nagassarium* is still unknown. On the other hand, among the spices, *C. longa* and *C. sativum* have significant thrombolytic activity, having the percentage of weight loss 53.32 ± 4.96%, 43.25 ± 7.18%, respectively. Based on these results we suggest that to take *C. longa* and *C. sativum* as preventive treatment by prospective atherothrombotic patients in crude form is safe, since they are already in use as spices, and no toxic effect is reported and thus can contribute in improvement of the patients suffering from atherothrombotic diseases.

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