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Research Article

Evaluation of thrombolytic properties of *Nigella sativa, Capsicum frutescens and Brassica oleracea*

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ABSTRACT

Thrombolytic drugs are widely used for the management of cerebral venous sinus thrombosis patients. Several in vitro models have been developed to study clot lysis activity of thrombolytic drugs, but all of these have certain limitations. The present study was carried out to investigate the thrombolytic activity, synergistic activity on thrombolysis in the aqueous extract of fruits of *Nigella sativa, Capsicum frutescens, Brassica oleracea.* An in vitro thrombolytic method was used to investigate the thrombolytic activity of plant extracts in blood sample from healthy human volunteers, along with streptokinase as a positive control and water as a negative control. Same method was also applied to assess the synergistic activity of plant extracts with streptokinase. Aqueous extract of *Nigella sativa, Capsicum frutescens, Brassica oleracea* showed 40.65%, 36.93%, 57.03% thrombolytic activity and also showed 60.46%, 80.00%, 72.50% synergistic activity with streptokinase respectively. Among the plant extracts studied *Capsicum frutescens* showed significant % of synergistic activity (80%) with compared to other plant extracts. The present study demonstrates that *Capsicum frutescens* extract showed synergistic activity with streptokinase on thrombolysis. Isolation and purification of the compound responsible for synergistic activity would be one of the best sources of herbal drugs for atherothrombotic diseases.

Keywords: Clot lysis; Herbal preparation; Streptokinase; Synergistic effect; Atherothrombotic disease.

INTRODUCTION

Atherothrombotic diseases such as myocardial or cerebral venous sinus thrombosis (CVST) are common disorders that are often accompanied by significant morbidity and mortality due to thrombus formed in blood vessel (Lee, 1995; Allroggen and Abbott, 2000; Watson RD *et al.*, 2002). Intravenous heparin is the first line drug for the treatment of Atherothrombotic diseases because of its efficacy, safety and feasibility (Watson *et al.*, 2002; Biousse and Newman, 2004). However, thrombolytic therapy, with its ability to produce rapid clot lysis, has long been considered an attractive alternative (Baruah *et al.*, 2006).

Atherothrombotic diseases are managed by thrombolytic drugs such as tissue plasminogen activator (t-PA), streptokinase (SK), urokinase (UK) etc. in all over the world (Sweta *et al*. 2006). Streptokinase (SK) and urokinase (UK) are commonly used in Bangladesh due to these drugs are easily available and cost effective. Though streptokinase and urokinase are "wonder drug" (Nikhil and Amit, 2007) but it has some severe drawbacks such as serious hemorrhage along with reocclusion and reinfraction, severe anaphylactic shock and non specificity as comparison with other thrombolytic agents (Patel and Mody, 1999; Haines and Bussey, 1995; Arcasoy and Kreit, 1999; Khalid, 2002). For these reasons, patients treated with streptokinase (SK) and Urokinase (UK) are carefully observed (Jennings, 1996). The immunogenic and hemorrhagic problems of the current thrombolytic agents lead to innovate improve recombinant variants of these drugs (Player *et al.,* 1992; Adams *et al.* 1991; Lijnen *et al.,* 1991; Marder *et al.,* 1993; Wu *et al,.* 2001)

So it is very much important to overcome the problem by our daily using or having food or by changing our lifestyle and food habit. Herbal preparations are used since ancient times to maintain health and regain healthy state of mind. Advances of phytochemistry and identification of plant compounds which are effective in curing certain diseases has renewed the herbal medicines. Herbs and their components possessing antithrombotic activity has been reported before; however

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herbs that could be used for thrombolysis has to be reported so far (Yamamoto *et al.* 2005).

The seeds of *Nigella sativa* Linn. (Ranunculaceae), commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhea and dyslipidaemia (Ali and Blunden, 2003). The spice Capsicum is the fruit of the cultivated species of the genus Capsicum (family, Solanaceae), *C. annuum* principally, and *C. frutescens L*. to a lesser extent. It has effect on the gastrointestinal tract, the cardiovascular system, the sensory system, thermoregulation and nutritional impacts. (Govindarajan and Sathyanarayana, 1991). The family Brassicaceae comprises a wide range of vegetable crops of economic and nutritional importance. *Brassica oleracea* L. is one of the most widely cultivated types of the vegetable (D'Antuono *et al*., 2007).

The aim of the study is to investigate whether herbal preparations (aqueous extract) from *Capsicum frutiescens* L, Family; Solanaceae, *Nigella sativa*, Family; Ranunculaceae, *Brassica oleracea* L, Family; Brassicaceae, possess thrombolytic activity or not, which may be beneficiary for the Atherothrombotic diseases. In addition, in vitro synergistic effect of herbal preparation with streptokinase (SK) was also investigated.

MATERIALS AND METHODS

Collection, purification and extraction

Commercially available mature *Nigella sativa, Capsicum frutescens and Brassica oleracea* were purchased from local market. The multiple solvent (methanol, isopropyl alcohol) extraction procedure was used to prepare the extract by the supplier. 100 mg extract was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight at room temperature to solubilization of water soluble part of the extract in aqueous medium and sediment the water insoluble part. After that supernatant aqueous part was separated through a paper filter (Whatman No.1). Then this solution became ready for in vitro thrombolysis (Sweta ***et al***. 2007).

Streptokinase (SK) solution preparation

To the Commercially available (in the local market) lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U) was used for in vitro thrombolysis.

Specimen

With all aseptic condition 5 ml of whole blood was drawn from healthy human volunteers (n=10) without a history of oral contraceptive or anticoagulant therapy. 500 µl of blood was transferred to each of the ten previously weighed eppendorf tubes to form clots.

In Vitro Thrombolysis Assay

Then, 5 ml venous blood drawn from healthy volunteers was distributed in ten different pre weighed sterile Eppendorf tubes (0.5 ml/tube) and incubated at 37°C for 45 minutes. 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) (Sweta *et al*. 2007).

To each eppendorf tube containing pre-weighed clot, 100 µl of aqueous extract of three herbs *Capsicum frutescens, brassica oleracea* and *Nigella sativa* were added separately. As a positive control, 100 µl of Streptokinase solution 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 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 repeated several times with the blood samples of volunteer.

Statistical analysis

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

RESULTS

In-vitro thrombolytic effect of *Capsicum frutescens*, *Nigella sativa* and *Brassica oleracea* extract

Extract of *Capsicum frutescens*, *Nigella sativa* and *Brassica oleracea* showed 40.65%, 36.93% and 57.03% clot lysis activity, respectively. In addition as positive control Streptokinase (Polamin Werk GmbH, Herdecke, Germany) showed 78.23% clot lysis activity (fig 1).

Addition of 100 µl of Streptokinase (a known thrombolytic drug) a positive control to the clots showed 78.23% clot lysis. On the other hand, clots when treated with 100µl of sterile distilled water (negative control) showed only negligible clot lysis activity which was only 3.52%, which was comparable with previously published result (4.7%).

The mean difference in clot lysis percentage between positive and negative control was very significant (***p value$<$ 0.0008).

Statistical representation (paired t-*test)* of the effective clot lysis percentage by three herbal preparations (*extract* of *Capsicum frutescens, Nigella sativa and Brassica oleracea*), positive control (Streptokinase) and nega-

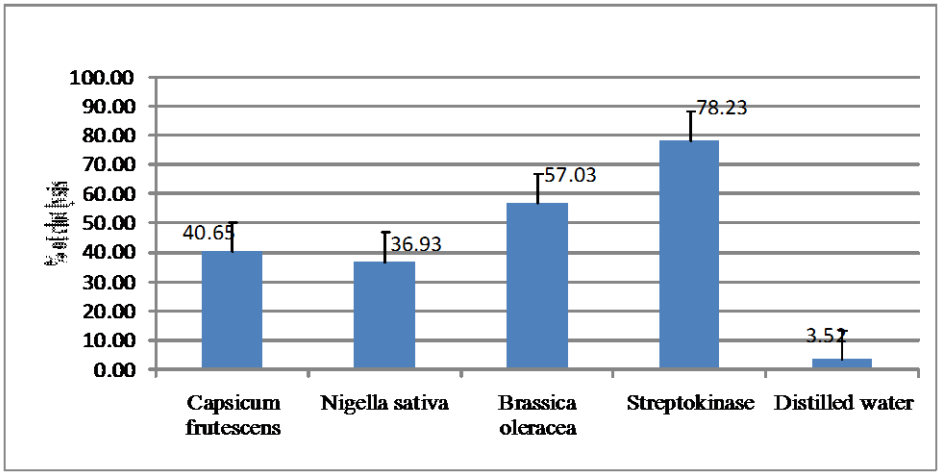

Figure 1: Clotlysis by Streptokinase, Distilled water and various herbal preparations

Table 1: Effect of herbal extracts on in vitro clotlysis

Herb/Drug	Mean ± S.D. (Clot lysis %)	P value when compared to negative control (water)
Streptokinase	78.23±16.85	0.0008
Capsicum frutescens	40.65 ± 10.12	0.0250
Nigella sativa	36.93 ± 3.38	0.0098
Brassica oleracea	57.03 ± 6.943	0.0001

tive control (sterile distilled water) is tabulated in Table 1.

Data strongly suggests that only *Brassica oleracea* have higher clot lysis activity than other herbs.

100µl of aqueous extract of three herbs & 100µl (30,000 I.U) of Streptokinase & 100µl of Distilled water were added to blood clot, incubated at 37ºC for 90 minutes and observed the clotlysis. Maximum clotlysis activity was observed in clot treated with streptokinase (SK). Among herbal preparations *Capsicum frutescens,* showed 40.65% clotlysis. *Nigella sativa* showed 36.93% clotlysis. *Brassica oleracea* showed 57.03% clotlysis. Water (as a negative control) showed 3.52% clotlysis activity.

Statistical representation of the effective clotlysis percentage by three herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) done by paired t-test analysis; clotlysis % is represented as mean ± S.D. and a P value < 0.05 was considered as significant.

In-vitro Synergistic effect of Methanol extract of Capsicum frutescens and Ethanol extract of *Nigella sativa* and *Brassica oleracea* with Streptokinase

To explore, whether the plant extract of *Capsicum frutescens, Nigella sativa* and *Brassica oleracea* has any synergistic effect with Streptokinase or not, clotlysis activity of plant extracts along with Streptokinase was carried out.

100µl of *Capsicum frutescens* herbal extract along with 100 µl streptokinase (a), 100µl of *Nigella sativa* extract

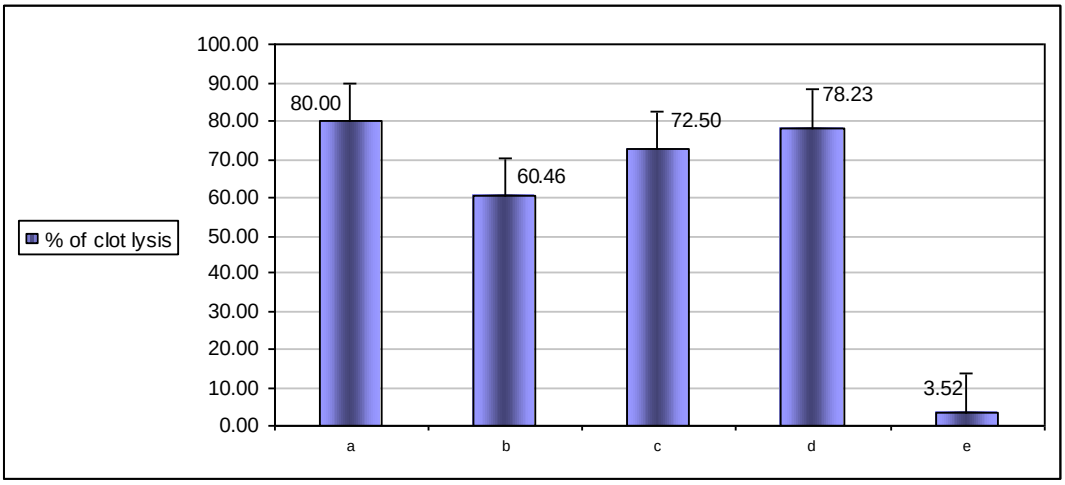

Figure 2: Synergistic effects on clotlysis by various herbal extracts with Streptokinase

along with 100µl of streptokinase (b) and 100µl of *Brassica oleracea* with 100µl of streptokinase (c) respectively showed 80%, 60.46% and 72.50% clot lysis activity (Fig: 2). Streptokinase alone showed 78.23% (d) clotlysis which is lower than concurrently with Capsicum frutescens (80%).

Data strongly indicated that only *Capsicum frutescens* had synergistic effects with streptokinase to increase the clotlysis activity and the clotlysis action of *Capsicum frutescens* became double compared to alone action of *Capsicum frutescens*.

Addition of aqueous extracts of three herbs i.e. *Capsicum frutescens, Nigella sativa* and *Brassica oleracea* along with 100 µl (30,000 I.U) of Streptokinase were added to blood clots, incubated at 37°C for 90 minutes and observed the clotlysis. Some synergistic effect 80% (a) was observed in clot treated with *Capsicum frutescens*. Other herbal preparations showed 60.46% (b) clotlysis by *Nigella sativa* and Brassica *oleracea* showed 72.50% (c) clotlysis with streptokinase. Water (as a negative control) showed 3.52% (e) clotlysis.

DISCUSSION

To maintain health and regain healthy state of mind, herbal preparations are widely used since ancient times. Herbal medicines are still now popular due to its less or no adverse effects. Isolation and identification of medicinal plants by using advance technology of phytochemistry deliver the specific crude drug for specific diseases which is safe and effective (Anwar *et al.* 1979). Within these plants, some plants have antithrombotic effect. The interesting thing is we use these plants as food supplement, which also prevent the atherothrombotic diseases(Gillman *et al* 1995; Joshipura *et al.* 1999; Liu *et al.* 2000; Bazzano *et al.* 2002)

Different types of thrombolytic drugs obtained from different sources. Within this, some are modified further with the use of recombinant in order to make these thrombolytic drugs more site specific, reduce adverse effects and effective technology (Verstraete, 2000). Bleeding and embolism are adverse effects of these drugs which sometimes cause death of the patients (Baruah *et al.* 2006; Gallus, 1998; Wardlaw *et al.* 2004; Capstick and Henry, 2005)

The aim of this study was to determine that these extracts have thrombolytic activity or not. Streptokinase is used as a positive control. Water, on the other hand, was selected as a negative control. The comparison of positive control with negative control clearly demonstrated that clot dissolution does not occur when water was added to the clot. The percentage of clot lysis by both these controls differs significantly as the p value was < 0.0008.

The comparison between the extracts of *Capsicum frutescens, Nigella sativa, Brassica oleracea* and water (negative control) with the percentages of in vitro clotlysis is a significant thrombolytic activity observed after treating with the clots, specially with *Brassica oleracea* extract (57.03%; p value <0.0001).

Another study was watch out for the plant extract of *Capsicum frutescens, Nigella sativa* and *Brassica oleracea* whether they have any synergistic effect with Streptokinase or not. For this reason, clot lysis activity of plant extracts along with Streptokinase was carried out.

Capsicum frutescens herbal extract along with streptokinase, *Nigella sativa* extract along with streptokinase and *Brassica oleracea* with streptokinase showed 80%, 60.46% and 72.50% clotlysis respectively. Streptokinase alone showed 78.23% clotlysis which is lower than the combined with *Capsicum frutescens* (80%). Although Streptokinase alone has a higher clot lytic activity (Sweta *et al*. 2007) but this value might be due to the storage of local provider.

Data strongly indicated that only *Capsicum frutescens* had synergistic effects with streptokinase to increase the clotlysis activity and antithrombotic action of *Capsicum frutescens* became double compared to alone action of *Capsicum frutescens* on clotlysis.

Another finding is, Streptokinase has individual effect on clotlysis and it showed 78.23% activity but along with *Nigella sativa and Brassica oleracea* showed 60.46% and 72.50% respectively. So, these extracts may have some inhibitory action to the streptokinase activity.

CONCLUSION

At the end, It can be said that *Capsicum frutescens*, *Nigella sativa* and *Brassica oleracea* has good clotlysis activity. So the fruit of these plants might be used for clot lysis of Atherothrombotic diseases.

The mechanism of action and active compounds of *Capsicum frutescens, Nigella sativa* and *Brassica oleracea* is unknown. In future, by using phytochemical study we may isolate the active compounds responsible for clotlysis and using pharmacological study we can determine its mechanism of action.

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