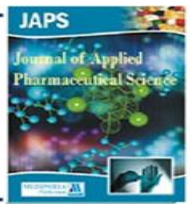
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 02-09-2011
Revised on: 08-09-2011
Accepted on: 12-09-2011

Assessment of Thrombolytic & Cytotoxic Activity of Herbal Preparations Originated from Botanical Source of Bangladesh

Md. Sheikh Anwar, Irfan Newaz Khan, Sabuj Barua, A T M Mostafa Kamal, S M Zahid Hosen and Md. Hassan Kawsar

Md. Sheikh Anwar,
Irfan Newaz Khan,
Sabuj Barua
Department of Pharmacy,
University of Science & Technology Chittagong (USTC), Bangladesh

A T M Mostafa Kamal
Department of Pharmacy,
International Islamic University Chittagong, Bangladesh

S M Zahid Hosen
Department of Pharmacy,
BGC Trust University Bangladesh, Chittagong, Bangladesh

Md. Hassan Kawsar
Department of Pharmacy,
Dhaka International University (DIU), Dhaka, Bangladesh

For Correspondence:
Irfan Newaz Khan
Assistant Professor
Department of Pharmacy
Faculty of Basic Medical & Pharmaceutical Science
University of Science and Technology Chittagong (USTC)
Foy's Lake, Chittagong - 4202, Bangladesh.
Tel: + 88-659070-1, Ext-124 (office), Mobile: + 88-01717026373

ABSTRACT

An *in vitro* thrombolytic model was used to check the clot lysis effect of four herbal extracts viz., Honey, *Nigella sativa, Capsicum frutescens, Brassica oleracea,* combination of Honey & *Nigella sativa* and Honey & *Capsicum frutescens* along with Streptokinase as a positive control and water as a negative control. And also brine shrimp lethality bio-assay was done using brine shrimp Nauplii and 5% of DMSO as a solvent for the ethanol extracts of *Nigella sativa* & *Capsicum frutescens* and Honey. Using an *in vitro* thrombolytic model, Honey, *Nigella sativa, Capsicum frutescens, Brassica oleracea,* combination of Honey & *Nigella sativa* and Honey & *Capsicum frutescens* showed 26.82%, 47.13%, 57.40%, 62.44%, 56.58% and 44.54% clot lysis effect respectively. From our study we found that *Brassica oleracea, Capsicum frutescens,* and combination of Honey & *Nigella sativa* showed significant % of clot lysis effect with reference to Streptokinase. Again from *in vitro* brine shrimp lethality bio-assay, we found that the LC_{50} of Honey, *Capsicum frutescens* & *Nigella sativa* were 129.62 µg/ml, 83.33 µg/ml & 45.45 µg/ml respectively.

Key words Honey, *Nigella sativa, Capsicum frutescens, Brassica oleracea,* thrombolysis, brine shrimp lethality bio-assay.

INTRODUCTION

A blood clot (thrombus) developed in the circulatory system due to the failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as acute myocardial or cerebral infarction, at times leading to death. Commonly used thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) to dissolve clots **(**Collen, 1990**)**. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs **(**Marder, 1993**)**. Heparin and Aspirin are only moderately efficient for acceleration of lysis and prevention of reocclusion, but are safe. More selective thrombin inhibitors and antiplatelet agents are more potent, but their safety remains to be confirmed. Continued investigation in this area will provide new insights and promote progress toward the development of the ideal thrombolytic therapy, characterized by maximized stable coronary arterial thrombolysis with minimal bleeding **(**Collen, 1990**)**. Several third generation thrombolytic agents have been developed. Compared with the second generation agents (alteplase), third generation

thrombolytic agents such as monoteplase, tenecteplase, reteplase, lanoteplase, pamiteplase, and staphylokinase result in a greater angiographic potency rate in patients with acute myocardial infarction, although, thus far, mortality rates have been similar for those few drugs that have been studied in large-scale trials. Bleeding risk, however, may be greater **(**Verstraete, 2000**)**. Recently, preventive measures against thrombosis have been tried. Oral administration of the fibrinolytic enzyme nattokinase was one example, which has been reported to enhance fibrinolytic activity in plasma and the production of tPA **(**Gesler, 1992**)**.

Since ancient times, herbal preparations have been used for the treatment of several diseases. Herbal products are often perceived as safe because they are "natural" **(**Demrow, et al., 1995**)**. Epidemiologic studies have provided evidence that foods with experimentally proved anti-thrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported **(**Basta et al., 2004**)**. 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 anti-thrombotic activity has been reported before; however herbs that could be used for thrombolysis has to be reported so far **(**Yamamoto et al., 2005**)**. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose, & toxicology is simply pharmacology at a higher dose. Cytotoxic properties of plant samples were carried out by brine shrimp lethality bioassay technique against a simple zoological organism, brine shrimp nauplii **(**Jerry et al., 1998**)**.

The aim of our work was to investigate whether our selected herbal preparations of Honey, *Nigella sativa, Capsicum frutescens, Brassica oleracea,* combination of Honey & *Nigella sativa* and Honey & *Capsicum frutescens* possess thrombolytic activity or not by using an in-vitro procedure and also to see the cytotoxic activity of the herbal extracts of *Capsicum frutescens, Nigella sativa* and Honey.

MATERIALS AND METHODS

Herbal Extracts

The plant samples were collected at their fully mature form, from local market. The parts of plants were identified by Dr. Mohammed Yousuf, Taxonomist, Bangladesh Council of Science and Industrial Research (BCSIR), Chittagong, Bangladesh. After cleaning, the plant parts of selected plant were taken and air dried for 10 days, and then kept in an oven at 45°C for 72 hours. The glass extractor was used for extraction process. 40 gm of dried powder was taken in the glass extractor. Before placing, the extractor was washed properly and then dried. Then 500 ml of solvent ethanol was added gradually & extraction was done.

Herbal preparation for Individual / Combined Thrombolytic Activity study of Extracts

For individual study 100 mg extract (*Capsicum frutescens or Nigella sativa* or *Brassica oleracea* or Honey) and incase of combined study, 1:1 ratio (i.e. 50mg of each) extract 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 through a 0.22 micron syringe filter. Then this solution is ready for the study to check *in vitro* thrombolytic activity (Prasad S. et al., 2007).

In vitro Clot lysis study

Streptokinase (SK)

To the commercially available 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 100 µl (30,000 I.U) was used for *in vitro* thrombolysis (Prasad S. et al., 2007).

Specimen

Whole blood (5 ml) was drawn from healthy human volunteers (n = 10) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by the Institutional Ethics Committee of Central India Institute of Medical Sciences, Nagpur). 500 µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots (Prasad S. et al., 2007).

Clot lysis

Experiments for clot lysis were carried as reported earlier (Prasad S. et al., 2007). Venous blood drawn from healthy volunteers (n = 10) was transferred in different pre-weighed sterile alpine tube (500 µl/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone). Each alpine tube containing clot was properly labeled and 100 µl of plant extract was added to the tubes. As a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained 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 test was repeated ten times.

Statistical analysis

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

Brine Shrimp Lethality Bio-assay (Cytotoxic Study)

The cytotoxicity of the extracts was tested on brine shrimp nauplii (Artemia salina Leach) according brine shrimp lethality bioassay (Meyer BN. et al., 1982). For hatching eggs were

kept in brine with a constant oxygen supply for 48 hours. The matured nauplii were then used in the experiment. Test sample was applied at different concentrations and the number of viable organisms was applied was counted after 24 hours for determination of LC50 values. DMSO was used as a solvent and also as negative control test was one in triplicate.

RESULTS & DISCUSSION

In vitro Clot lysis study

Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 84.48% clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (4.04%). The mean difference in clot lysis percentage between positive and negative control was very significant (p value < 0.0009). The *in vitro* thrombolytic activity study revealed that Honey, *Nigella sativa*, *Capsicum frutescens*, *Brassica oleracea*, combination of Honey & *Nigella sativa* and Honey *&* *Capsicum frutescens* showed 26.82%, 47.13%, 57.40%, 62.44%, 56.58% and 44.54% clot lysis respectively and compared with the negative control (water) the mean clot lysis % difference was significant (p value < 0.0001). % 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 four herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is tabulated in Table 1.

Table 1: Effect of herbal extracts on *in vitro* clot lysis.

Herb/Drug	Mean ± S.D. (Clot lysis %)	P value when compared to negative control (water)
Streptokinase	84.48 ± 3.10	0.0001
Brassica oleracea	62.44 ± 02.35	0.00014
Capsicum frutescens	57.40 ± 2.37	0.00015
Nigella sativa	47.13 ± 0.86	0.00013
Honey	26.82 ± 0.84	0.0004
Nigella sativa & Honey	56.58 ± 0.95	0.00023
Capsicum frutescens & Honey	44.54 ± 1.46	0.000115

***Statistical representation of the effective clot lysis percentage by herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) done by paired t-test analysis; % clot lysis is represented as mean ± S.D. and p values of all Herbal preparations were < 0.001 was considered as significant.

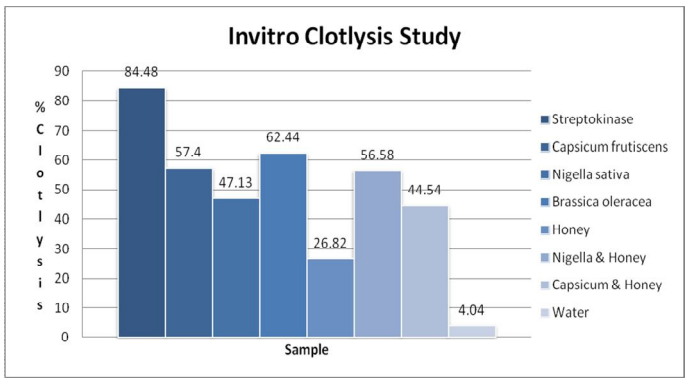


Fig 1: Clot lysis by Streptokinase, water and various herbal preparations.

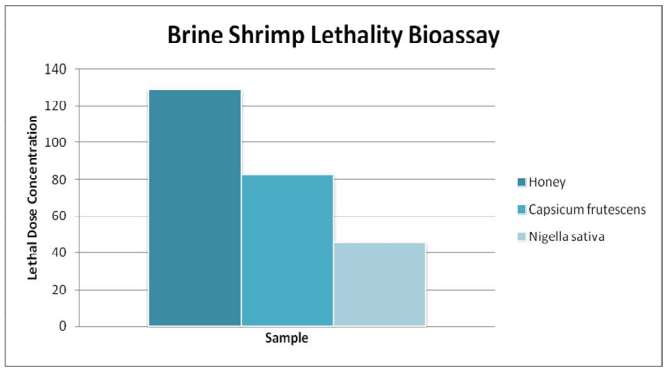


Fig 2: Cytotoxicity Study of Honey, *Capsicum frutescens* & *Nigella sativa* showed LC_{50} at 129.62 µg/ml, 83.33 µg/ml & 45.45 µg/ml respectively on Brine Shrimp Lethality Bioassay.

Table 2: LC_{50} (µg/ml) value of Honey, *Capsicum frutescens* & *Nigella sativa*.

Herb/Drug	Log Concentration (Log C)	LC_{50} (µg/ml)
Capsicum frutescens	2.70	83.33
Nigella sativa	2.39	45.45
Honey	2.94	129.62

The comparison among the extracts of Honey, *Nigella sativa*, *Capsicum frutescens*, *Brassica oleracea*, combination of Honey & *Nigella sativa* and Honey *&* *Capsicum frutescens* revealed a significant variation in thrombolytic activity observed after treating with the clots, Especially, *Brassica oleracea extract* (62.44%; p value < 0.00014), *Capsicum frutescens* (57.40%; p value < 0.00015) and combination of Honey & *Nigella sativa* (56.58%; p value < 0.00023) showed significant thrombolytic effect. Honey along with *Nigella sativa* had synergistic activity when these two administered concurrently. But, *Capsicum frutescens &* Honey showed no synergistic effect.

Brine Shrimp Lethality Bio-assay

The ethanol extracts of *Nigella sativa* & *Capsicum frutescens* and Honey were tested for brine shrimp lethality bioassay using brine shrimp Nauplii and 5% of DMSO as a solvent. The LC_{50} (Lethal Concentration for 50% population) value for the extracts and Honey was obtained. The ethanol extracts of *Nigella sativa* & *Capsicum frutescens* and Honey showed positive result on brine shrimp lethality bioassay. So they are pharmacologically active. But particularly *Nigella sativa* indicates very good cytotoxic effect. Control was used to see whether DMSO had any effect on brine shrimp lethality. The control group of brine shrimp Nauplii with and without DMSO exhibited no mortality. The results is shown in the figure 2 & table 2.

CONCLUSION

From *In vitro* Clot lysis study, we demonstrated that *Capsicum frutescens*, *Brassica oleracea* and combination of Honey & *Nigella sativa* have very good clot lysis activity. So that, we may assume that these extracts can be considered as a potential source of natural thrombolytic agent. As apparent from our results of brine shrimp lethality bioassay it can be revealed that the ethanol extracts

of *Nigella sativa* & *Capsicum frutescens* and Honey has cytotoxic activity and among them *Nigella sativa* showed highest cytotoxic effect. In context of the above discussion it would be interesting to investigate the causative components/mechanism for clot lysis by these plant extracts. This is only a preliminary study and to make final comment the extract should thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

REFERENCES

Collen D. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator *Ann Intern Med.*; 1990; 112:529–538.

Demrow HS, Slane PR, Folts JD. Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation.* 1995;91: 1182–1188.

Gesler WM. Therapeutic landscapes: medical issues in light of the new cultural geography. *Soc Sci Med*. 1992;34:735–746.

Giuseppina Basta , Cristiana Lupi , Guido Lazzerini , Piero Chiarelli , Antonio L'Abbate , Daniele Rovai. An *in vitro* study on blood of normal subjects and patients with coronary artery disease. Thromb Haemost. 2004;91:1078-1083.

Jerry L. Mclaughlin, Lingling L. Rogers & Jon E. Anderson. The use of Biological assays to evaluate Botanicals. Drug information journal. 1998;32:513-524.

Marder VJ. Recombinant streptokinase – opportunity for an improved agent. *Blood Coagul Fibrinolysis*. 1993; 4: 1039–1040.

Meyer BN, Ferringni NR, Puam JE, Lacobsen LB, Nicols DE, McLaughilin JL. Brine Shrimp: A convenient general bioassay for active constituents. Planta Med. 1982;45: 31-32.

Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of Fagonia Arabica (Dhamasa) on *in vitro* thrombolysis. BMC Complementary and Alternative Medicine. 2007;7:36.

Verstraete M. Third Generation thrombolytic drugs. Am J Med. 2000;109(1):52-58.

Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R. Testing various herbs for antithrombotic effect. *Nutrition.* 2005;21: 580–587.