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Thrombolytic and cytotoxic activity of methanolic extract of *Commelina benghalensis* (Family: Commelinaceae) Leaves

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Abstract

This study was subjected to investigate thrombolytic and cytotoxic properties of *Commelina benghalensis* methanol extract. The cytotoxicity had been assessed while using brine shrimp lethality bioassay and also thrombolytic impact with individual blood. The brine shrimp lethality bioassay result was (LC_{50}= 278.69 µg/ml) compared with standard vincristine sulphate (LC_{50}=0.512 µg/ml). It has significant thrombolytic activity (40.94%) compared with standard streptokinase (75%).

Keywords: *Commelina benghalensis*, Thrombolytic, Cytotoxic, Clot lysis.

Introduction

Thrombosis is the formation or presence of a blood clot in a blood vessel. Mainly two types of thrombosis based according to the site of clot formation and these are-Venous thrombosis and Arterial thrombosis. Thrombolytic therapy uses drugs called thrombolytic agents, such as alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator (TPA) to dissolve clots. Thrombolytic therapy is also used to dissolve blood clots that form in catheters or tubes put into people's bodies for medical treatments, such as dialysis or chemotherapy. However, the relatively weak substrate specificity of first generation agents (streptokinase and urokinase) can result in a state of systemic fibrinolytic and associated bleeding complications. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs.[1-5] 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.[6] The clot itself is termed thrombus.[7-8] Medicinal plants play a dominant role in the treatment of varieties of human diseases from the twilight of the human civilization.[9] The Parliamentary Health Select Committee heard in 2005 that the annual rate of death due to thrombosis was 25,000, with at least 50% of these being hospital-acquired.[10] Obsession on modern medicinal system leads people to an alternative approach to improve and maintain good health is increased tremens dously by using medicinal herb over the last centuries. Many of the modern days important drugs and processed medicines are of plant origin.[11] Medicinal plants contain different therapeutic agents which may have thrombolytic activity, cytotoxic effect etc. Working with different medicinal plants extract showed that they can lyses thrombus as streptokinase.[12] Some of the plant extracts also increases lethality of the cell due to their known cytotoxic effect. Cytotoxicity is performed for evaluating the level of toxicity. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay.[13] Streptokinase (SK) belongs to a group of medications known as fibrinolytic, and complexes of streptokinase with human plasminogen can hydrolytically activate other unbound plasminogen by activating through bond cleavage to produce plasmin. SK is used as an effective and inexpensive thrombolysis medication in some cases of myocardial infarction (heart attack)[14] and pulmonary embolism[15]. There are few more plant extracts/ products which have been identified to have fibrinolytic activity. These are *Lumbricus rubellus*, *Pleurotus ostreatus*, *Spirodela polyrhiza*, *Flammulina velutipes* and *Ganoderma lucidum*, Ginger (*Zingiber officinale*), Garlic (*Allium sativum*).[16-21]

C. benghalensis is an annual or perennial herb. A plant can produce 1,600 Seeds. Leaves are 3 cm long

and 2 cm wide. *C. benghalensis* known as the Bengal dayflower (Family: Commelinaceae*)*, is a perennial herb native to tropical Asia and Africa. It has been widely introduced to areas outside its native range, including to the neotropics, Hawaii, the West Indies and to both coasts of North America. Flowering May to October, fruiting July to December. It flowers from spring into the fall and is often associated with disturbed soils. There it can be found from near sea level up to 2300 meters. It is useful in diuretic, febrigual and anti-inflammatory effects, cure swellings of the skin, leprosy, laxative, sore eyes, sore throats leaves are used as human food, as medicine for infected wounds.[22] Herbal preparations have been used as a potential source of medicine since ancient times to maintain health and regain healthy state of mind. Herbs showing thrombolytic activity have been studied and some significant observations have been reported.[23]

The aim of the present study was to identify thrombolytic activity, and cytotoxicity of Commelina benghalensis

Materials and Methods

Plant collection

Then leaves *C. benghalensis* was collected from Chittagong, Bangladesh in the month October, 2014. The plant was taxonomically identified by Dr. Shaikh Bokhtear Uddin (Associate Professor, Department of Botany, and University of Chittagong, Bangladesh).

Extracts preparation

The collected plant was washed thoroughly with water and air dried for a week at 35 to 40°C and pulverized in an electric grinder. The obtained powder was successively added to methanol with vigorous shaking at 55 to 60°C temperature. The extracts were made to dry by using a rotary evaporator under reduced pressure.

Chemicals and drugs

Lyophilized streptokinase vial (1500000 IU) was purchased from Square Pharmaceuticals Ltd, dimethyl sulfoxide (DMSO) and Vincristine sulfate (2 mg/vial; Techno Drugs Limited Bangladesh). All other chemicals and reagents were of analytical grade.

Sample preparation

The crude extract was suspended in 10 ml distilled water and shaken vigorously on a vortex mixer. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a filter paper. The solution was then ready for in vitro evaluation of clot lysis activity.

Streptokinase (SK) solution preparation

To the commercially available lyophilized SK vial (Polamin Werk GmbH, Hedrick, 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 (30,000I.U) was used for in vitro thrombolysis. In this study, Streptokinase (SK), a known thrombolytic drug is used as a positive control.[24]

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 Chittagong University, faculty of medicine. 500µl of blood was transferred to each of the ten previously weighed eppendorf tubes to form clots. Different countries volunteers are used for collection of sample (Nigeria, Somalia, and Nepal).

Brine shrimp lethality bioassay

For the preparation of sea water 38 g of sodium chloride was weighed, dissolved in distilled water to make 1 liter solution and then filtered off to get clear solution. This simulated sea water was used for hatching of brine shrimp. The shrimp was allowed for two days to hatch and mature as nauplii (larvae). In a small beaker, measured amount of the sample was accurately weighed and dissolved in DMSO (Dimethyl sulfoxide) to give a final concentration of 10mg/ml (10 µg/µl). From the test tube containing brine shrimp nauplii, 10 test tubes were taken for the sample where each contained 5ml of seawater and 10 nauplii. These test tubes were marked from 1 to 10 for the sample. To these test tubes different concentrations (1000 µg/ml, 800 µg/ml, 500 µg/ml, 300µg/ml, 200 µg/ml, 150 µg/ml, 100 µg/ml, 50 µg/ml, 20 µg/ml and 10 µg/ml) of the sample were added. Then the samples were subjected to brine shrimp lethality evaluation.[13] In this case, only 50 µl DMSO was added in 5 ml sea water containing 10 nauplii. No extract was added to prepare control solution. Measured amount of the vincristine sulphate (Techno Drugs Ltd., Bangladesh) was used dissolved in DMSO to get an initial concentration of o.512 µg/ml.9 test tubes for the standard sample were taken where each contained 5 ml of seawater and 10 nauplii .

Thrombolytic assay

Experiments for clot lysis were carried as reported earlier [12]. Venous blood drawn from healthy volunteers was transferred in different pre-weighed sterile eppendorf 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 the clot containing tube – weight of tube alone). Each eppendorf tube containing clot was properly labeled and 100 µl of plant extract was added to the tubes. 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 in weight taken before and after clot lysis was expressed as percentage of clot lysis. Streptokinase and water were used as positive and negative control, respectively. The experiment was repeated several times with the blood samples of different volunteers. % clot lysis = (Weight of the lysis clot /Weight of clot before lysis) × 100.

Statistical analysis

The data were analyzed statistically using ANOVA followed by student't' test with Graph Pad Prism Data Editor for Windows, Version 6.0 (Graph Pad software Inc., San Diego, CA). Values were expressed as mean ± Standard error for mean (± SEM). $P < 0.05$ - 0.01 were considered as statistically significant.

Result

Addition of 100 µl SK, a positive control (15,00,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed (75± 0.09 %) clot lysis. Clots when treated with 100µl sterile distilled water (negative control) showed only negligible clot lysis (4.19±0.12%). The in vitro thrombolytic activity study revealed that *C. benghalensis* showed 40.94±0.78%.Statistical representation of the effective clot lysis percentage by our herbal preparation, positive thrombolytic control Streptokinase) and negative control (sterile distilled water) is tabulated

in (Table1 and Figure 1). In brine shrimp lethality bioassay using brine shrimp nauplii, the methanol extract of *C. benghalensis* leaves showed positive result in comparison with the positive control Vincristine Sulphate & that's why it can be assumed that extract is pharmacologically active. By plotting different concentration versus percent (%) of mortality for all test samples showed an approximate linear correlation. From the graph, the median lethal concentration LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii were determined. The crude methanol extract of *C. benghalensis* showed significant cytotoxic activity against brine shrimp nauplii and LC_{50} value was 278.69 µg/ml (Table 2 & Figure 2). As positive control Vincristine Sulphate was used & as negative control DMSO was used to validate the test method.

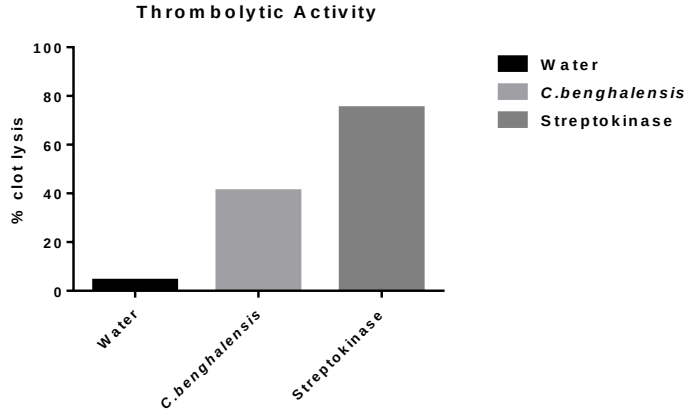

Figure 1: Clot lysis by Streptokinase, water and *C.benghalensis*

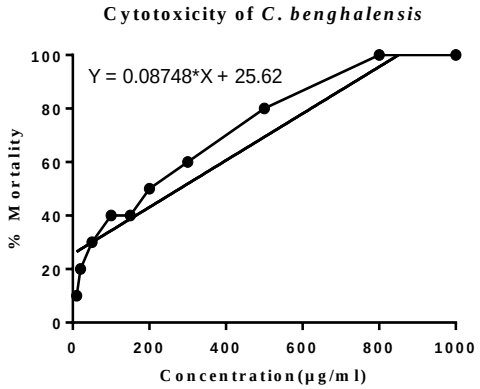

Figure 2: Determination of LC_{50} value for extract of *C .benghalensis* from linear correlation between log C versus % of mortality

Table 1: Thrombolytic Activity of *C. benghalensis*

No.	Weight of Empty tube (A) gm	Weight of tube with clot (B)gm	Weight of clot (C) (B-A) gm	Weight of Tube with clot after lysis (D) gm	Weight of lysis (E) (B-D)	% of clot lysis	Average % of clot lysis
1	0.8015	1.2945	0.493	1.053	0.2415	48.9858	40.93284
2	0.799	1.2505	0.4515	1.074	0.1765	39.0919	
3	0.8055	1.28	0.4745	1.1305	0.1495	31.5068	
4	0.794	1.303	0.509	1.061	0.242	47.5442	
5	0.79	1.2545	0.4645	1.057	0.1975	42.5188	
6	0.7905	1.255	0.4645	1.082	0.173	37.2443	
7	0.78	1.2425	0.4625	1.049	0.1935	41.8378	
8	0.783	1.2815	0.4985	1.068	0.2135	42.8284	
9	0.8095	1.2575	0.448	1.0705	0.187	41.7410	
10	0.804	1.28	0.476	1.1085	0.1715	36.0294	

Table 2: Cytotoxicity of methanol extract of *C. benghalensis*

Concentration	Log C	Total nauplii	No. of nauplii dead	No. of nauplii live	% of mortality	LC_{50} µg/ml
10	1	10	1	9	10	278.69
20	1.30103	10	2	8	20	
50	1.69898	10	3	7	30	
100	2	10	4	6	40	
150	2.17610	10	4	6	40	
200	2.30103	10	5	5	50	
300	2.47713	10	6	4	60	
500	2.69898	10	8	2	80	
800	2.90309	10	10	0	100	
1000	3	10	10	0	100	

Discussion

Plant-based drugs have a long history of utilization for the prevention and treatment of human illnesses. Today, numerous pharmaceuticals at present sanction by the Food and Drug Administration (FDA) have inceptions to plant sources. A major role for plant-derived compounds based on the reported immunemodulatory effects has emerged in recent times and has prompted the thorough experimental examination to focus viability and wellbeing.

The results of the present study showed that *C. benghalensis* leaves extract possesses thrombolytic property, as it significantly shows cytotoxic effect. The extract at a concentration range of 1000-100 µg/ml shows 100% mortality. Toxicity of plant materials is a major concern to scientists and medical practitioners and therefore cytotoxic assay was conducted in this study to determine the toxicity profile of the plant extracts through the Brine Shrimp Lethality (LC_{50}, 24 h) test. Cytotoxicity screening model provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work.[25] Brine shrimp lethality test is carried out in order to reveal new anticancer compounds. Thrombolytic agents are used to disrupt already formed blood clots in clinical settings where ischemia may be fatal (acute myocardial infarction, pulmonary embolism, ischemic stroke, and arterial thrombosis). Thrombolytic drugs dissolve blood clots by activating plasminogen, which forms a cleaved product called plasmin.

Conclusion

It can be concluded that *C. benghalensis* has got the potential as a candidate for future thrombolytic agent. It can also be investigated as a possible source of antitumor drugs. This is only a preliminary study and investigated be phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentials.

Conflict of interest statement

We declare that we have no conflict of interest.

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Author's contribution

This work was carried out in collaboration between all authors. Authors TAC and AH collected the plant leaves and prepared the extract. MSHK designed the study, wrote the protocol. Author MSHK performed the statistical analysis and Author AH wrote the first draft of the manuscript. TAC, AH, MJ, MMH, MSH and AM performed the experiment. Author ATMMK managed the literature searches. All authors read and approved the final manuscript.

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