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Study on in-vitro thrombolytic activity of chloroform extract of leaves of *Dioscorea bulbifera*

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Abstract
The present study was designed to investigate the thrombolytic activity of chloroform extract of leaves of *Dioscorea bulbifera*. Thrombolytic effect of the fraction was investigated in clot lysis experiment. The extract exerted 31.30% lysis of the blood clot in thrombolytic activity test while 85.77%. And 4.71% lyses were obtained for positive control (streptokinase) and negative control respectively. So, the extract possessed considerable thrombolytic activity which compounds are responsible for the present pharmacological actions and to know their mechanism of action, extensive pharmacological and phytochemical experiments are essential.

Keywords: Thrombolytic activity, Streptokinase, *Dioscorea bulbifera*

Introduction
Given that ancient times plants have served being a natural source of treatments and therapies for instance aspirin, quinine, and coffee. Today, scientists are using these renewable resources to generate a new generation of therapeutic solutions. Plants improved with the use of biotechnology can produce the essential protein for innovative treatments for diseases for instance cancer, HIV, heart disease, diabetes, Alzheimer's condition, kidney disease, Crohn's disease, cystic fibrosis, numerous sclerosis, spinal cord injuries, Hepatitis Chemical, chronic obstructive pulmonary disorder (COPD), weight problems, arthritis and iron deficiency. The usage of natural products with therapeutic properties can be as ancient as human civilization and, for some time, mineral, plant and animal products were the key sources of drugs [1]. The vast most these cannot yet be synthesized economically and so are still obtained from wild or discerning plants. Natural compounds can be direct compounds, allowing the design and realistic planning of new drugs, biomimetic synthesis development as well as the discovery of new therapeutic properties not yet caused by known compounds [2]. In addition, compounds for instance muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and also phorbol esters, all obtained from crops, are important tools used in pharmacological, physiological and biochemical studies [3]. Thrombolysis could be the breakdown (lysis) of blood clots simply by pharmacological means. It is colloquially called elot busting for this reason. It functions by stimulating fibrinolysis by plasmin through infusion regarding analogs of tissue plasminogen activator (tPA), the particular protein that normally activates plasmin. Thrombolytic therapy is the usage of drugs to break up or break up blood clots, which are the main reason behind both heart attacks and stroke. Thrombolytic medications are approved for your immediate treatment of stroke and also heart attack. One of the most commonly used drug for thrombolytic remedy is tissue plasminogen activator (tPA), but other drugs can do a similar thing [5].

Materials and Method
Collection of Plant Materials
The particular leaves of *Dioscorea bulbifera* were collected coming from Chittagong local forest area; the leaves regarding Dioscorea bulbifera were collected at their totally mature form. After cleaning, the results in were taken and splitting the peal, next air dried for 8 days, and kept in an oven at 45 °C with 72 hours. 250 gm. of dehydrated powder was cold extracted with Methanol. Dried powder soaked with chloroform for 1 week. Then filtered to take the

targeted extract, extract containing beaker was added to the water bath (at 40 °C-45 °C) to evaporate the solvent from your extract [6].

Preparation of Extraction

The extract is served by cold extraction process. In this process the coarse powdered was submerged in chloroform (95%) since chloroform is the most frequent solvent for extracting most of the constituents within herbal materials. Amber glass bottle were used for this specific purpose, which were kept at room temperature and allowed to stand for 7 days with infrequent shaking and stirring. When the solvent became concentrated the contents were first decanted through the use of cotton and then filtered through Whatman Simply no. 1 filter paper. The filtrate so obtained was then concentrated to dryness from the evaporation of solvent using rotary evaporator. Ultimately we got the concentrated semi-solid remove. The concentrated were then used since crude extract of respective test studies. In our present investigation, we employed chloroform extract for thrombolytic action [7].

In-Vitro Thrombolytic Study

Thrombolysis is the breakdown (lysis) of blood clots by pharmacological means. It is colloquially referred to as clot busting for this reason. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator (tPA), the protein that normally activates plasmin.

Preparation of Extract Solution for Thrombolytic Test

10 mg of the extract was suspended in 10ml 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 (Whatman No. 1). The solution, was then ready for in vitro evaluation of clot lysis activity [7].

Preparation of Streptokinase (SK) Solution

To the commercially available lyophilized SK vial (PolaminWerk GmbH, Herdecke, Germany) of 1,500,000 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 [7].

Specimen of Thrombolytic Test

3ml blood was drawn from healthy human volunteers 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 [7].

Test Procedure for Thrombolytic test

Experiments for clot lysis were carried as reported earlier [7]. Venous blood drawn from healthy volunteers was transferred in different pre-weighed sterile Epen drop tube (500µl/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 Epen drop 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 obtained in weight taken before and after clot lysis was expressed as percentage. Thrombolytic Activity of chloroform extract of Dioscorea bulbifera leaves clot lysis. Streptokinase and water were used as a positive and negative (non-thrombolytic) control respectively. The experiment was repeated several times with the blood samples of different volunteers.

Result and Discussion

Thrombolytic Activity

The chloroform extract of *Dioscorea bulbifera* leaves is exerted 31.30% lysis of the blood clot in thrombolytic activity test while 85.77% were obtained for positive control (streptokinase) and 4.70% were obtained for negative control respectively which has shown in table 1. So, the extract possessed considerable thrombolytic activity.

Blood sample	% of Clot lysis		
	Control (water)	Streptokinase	*Dioscorea bulbifera* (Chloroform extract)
1	4.20	87.20	32.08
2	5.73	85.00	40.64
3	4.58	86.80	23.05
4	4.89	87.36	34.50
5	5.26	84.60	21.37
6	4.13	85.10	36.61
7	4.27	84.18	34.09
8	4.00	86.16	54.98
9	5.65	85.12	24.12
10	5.28	86.2	11.59
Mean	4.70	85.77	31.30

Comparing the data of % of Clot lysis using SPSS 11.5

Group Statistics (Control vs. Streptokinase)

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Control	10	4.7990	.65113	.20590
	Streptokinase	10	85.7720	1.12211	.35484

Group Statistics (Control vs. Extract)

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Control	10	4.7990	.65113	.20590
	Extract	10	31.3030	12.0254	3.8027

Group Statistics (Extract vs. Streptokinase)

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Streptokinase	10	85.7720	1.12211	.35484
	Extract	10	31.3030	12.0254	3.80279

Here, all values are expressed as MEAN ± SEM (n=20). ***P<0.001 significant compared to negative control.

Effect of Clot lysis

Sample	Result
Streptokinase (STD)	85.77%
Distilled water (control)	4.71 %
Sample (Extract)	31.30 %

% Clot lysis activity of Extract formulation

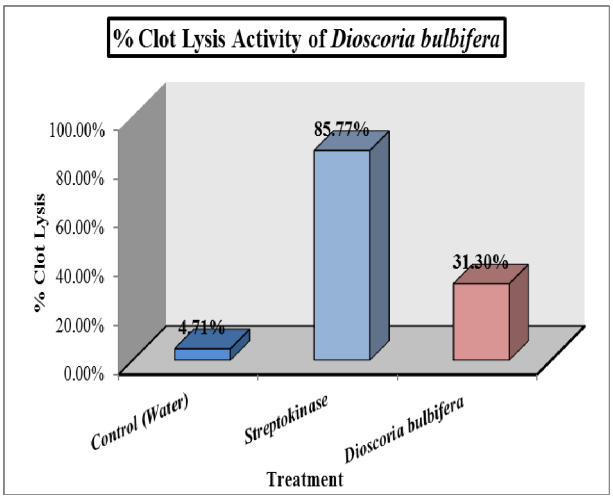

Fig 1: Comparative % in vitro thrombolytic effect of formulation, streptokinase and water (negative control).

Discussion

Plant-derived medicines have a long history of usage for the prevention and treatment involving human diseases. Today, many pharmaceuticals currently approved with the Food and Drug Administration (FDA) get origins to plant sources. A major role for plant-derived compounds using the reported immune modulatory effects has emerged these days and has led to the rigorous scientific examination to discover efficacy and safety. A number of plants source especially several leaves and vegetables are actually studied for their supplements having anticoagulant, antiplatelet and fibrinolytic activity and there exists evidence that consuming such food brings about prevention of coronary events and cerebrovascular event. Some of these plant products are generally modified further with recombinant technology to generate them more effective and site distinct. In our thrombolytic assay, the comparison of positive control using negative control clearly demonstrated that clot dissolution won't occur when water was added on the clot. When compared with the clog lysis percentage obtained through SK along with water, an extremely significant thrombolytic task was observed after treating the clots using C. arborea, chloroform fraction. Cell floor bound plasminogen is easily activated for you to plasmin, which could lead to fibrinolysis. Bacterial plasminogen activator: staphylokinase, streptokinase, act as cofactor molecules that help with exosite formation and enhance the substrate presentation on the enzyme. Staphylokinase activates plasminogen to melt clots, also destroys the extracellular matrix along with fibrin fibers that hold cells jointly. However, the extremely significant effect of *Dioscorea bulbifera* demonstrates it to get the best thrombolytic component for even more processing.

Conclusion

From the above study it can be concluded that the chloroform extract of *Dioscorea bulbifera* may be a potential candidate for future thrombolytic agent. Furthermore study and isolation is needed to obtain site specific and more potent agent that causing this effect. The test was made under full concentration to develop a new compound. I found that the extract I choose, was quite good in use. At the conclusion I can recommend that this plant part is useful for further use and isolation. The thrombolytic study was close to the standard used. The thrombolytic potency of *Dioscorea bulbifera* is found 31.30% and the standard have 85.77%. It seems good result or may be said significant as the extract was the mixture of many phytochemical, it shows nearby percent of clot lysis.

Here experimental studies of leaves extract exhibited considerable thrombolytic activity. So, further comprehensive pharmacological and phytochemical investigations are needed to elucidate the specific chemical compounds responsible for cytotoxic and thrombolytic activities and their mode of actions. The long term toxic effect and its protective effects should also be elucidated.

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