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Evaluation of Thrombolytic Activity of Different Fractions of *Viburnum foetidum* L

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Abstract: Plant is one of the richest sources of promising versatile chemical compounds and medicinal values. So the demand in study of plants is growing constantly throughout the world during the last few decades. This study was designed to evaluate the thrombolytic activity of six different fractions of crude methanolic extract of leaves of *Viburnum foetidum* L. The thrombolytic potential was determined against human blood. The extract shows the good clot lysis effect as compared to standard streptokinase (70.00%). Among them Petroleum Ether (PetE) fraction shows highest activity (62.70%) than others five fractions n-Hexane (60.10%), Chloroform (49.40%), Aqueous (48.25%), Methanol (43.25%), Ethyl acetate (38.71%).

Key words: Thrombolytic · Clot lysis · *Viburnum foetidum* L

INTRODUCTION

Plant could play a great role in exploring new thesaurus against the threats of new and recent diseases. Because of their potent pharmacological activity, low toxicity and economic feasibility, research of the properties of plant has been performed [1].

Blood clot is one of the vital reasons of blood circulation problem [2]. In the patient admitted to hospital, thrombosis is a major cause for complications and occasionally death. In UK, for instance, 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 [3]. Thrombosis is the formation of a blood clot inside a blood vessel; block the flow of blood throughout the circulatory system. When a blood vessel is injured, the body uses platelets (thrombocytes) and fibrin to form a blood clot to avoid blood loss. When a blood vessel is not injured, blood clots may form in the body under certain conditions [4]. Thrombi or emboli can lodge in a blood vessel and obstruct the flow of blood in that location depriving tissues of regular blood flow and oxygen. This can result in injure, damage, destruction (infarction), or even death of the tissues (necrosis) in that area [2]. A blood clot (thrombus) is formed from fibrinogen by thrombin and is lysed by plasmin which is activated from plasminogen by tissue plasminogen activator (tPA). Fribrinolytic drugs have been used to lyse thrombi in acutely occluded coronary arteries there by restoring blood supply to ischemic myocardium to limit necrosis and to improve prognosis [5]. Streptokinase (SK) belongs to a group of medications known as fibrinolytics and complexes of streptokinase with human plasminogen can hydrolytically stimulate other unbound plasminogen by activating through bond cleavage to produce plasmin [6] and pulmonary embolism [7]. Streptokinase is an antigenic thrombolytic agent used for the healing of acute myocardial infarction. It reduces mortality as effectively as the nonantigenic altreplase in most infarct patients while having the advantages of being cheap. Tissue-type plasminogen activator (tPA) is commonly preferred as being effective and safer than either urokinase or streptokinase type activators. All available thrombolytic agents still have major shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency. Because of the shortcomings of the existing thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs [8-12]. The plant represents a vast reservoir of biologically active compounds with a range of chemical structures and protective/disease preventive properties [13]. Almost 50% of drugs used in medicine are of plant origin and only a

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tiny proportion of plants with medicinal activity have been assayed. Therefore much modern research has been devoted to the phytochemical investigation of higher plants which have ethno-botanical information associated with them. The phytochemicals isolated are then screened for various kinds of biological activity like thrombolytic potentials [14]. Herbal preparations are used as 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 remarks have been reported [15].

Viburnum foetidum is a medicinal plant belongs to the family of Adoxaceae, (Bengali name- Khailzakhori, Motmoti) is an evergreen small sized tree, distributed mostly in hilly areas, deciduous and semi-evergreen forests in Bangladesh, native to tropical China, Burma, Taiwan, Himalayas. Leaves can be rhombic-ovoid (narrow at the base and wider toward the tip) with no serrations or they may have 3 lobes in the upper half of the leaf. Flowers are white in colour, formed at the top of stem, small, 3–5 mm across. Tubers may occur near the soil surface or underground. The fruit is a spherical, oval, or somewhat flattened drupe, red to purple, blue, or black and containing a single seed [16]. *Viburnum* is well recognized in folk medicine for their spasmolytic, sedative and anti-asthmatic properties [17]. *Viburnum* species are used in treatment of different diseases, such as diarrhoea, rheumatoid arthritis and tumefaction [18]. In addition, anti-diabetic, anti-oxidant, anti-bacterial and anti-cancer activity has been reported *Viburnum* [19].

A few Viburnum species, such as *Viburnum acuminatum* [20], *Viburnum awabukii* [21], *Viburnum nervosum* [22], *Viburnum luzonicum* [23] and *Viburnum chinshanense* [24] have been already investigated and reported in literatures for their medicinal values. But it may best suit to state that the phytochemical based pharmacology of those species is very inconsistent or doubtful. Hence, it may essential to create a profile in regards to their identification and then standardization which may lead to further scientific investigations. Since there is no scientific report for thrombolytic potential of *Viburnum foetidum* extract, the present study was an attempt to evaluate the thrombolytic effect by *in vitro* analysis.

MATERIALS AND METHODS

Collection and Extraction: The whole plant of *Viburnum foetidum* was collected at their fully matured form from hill area of Syhlet district in September 2014 and was identified by taxonomist Dr. A. H. M. Mahbubur Rahman, Associate Professor, Department of Botany, University of Rajshahi, Bangladesh. Adequate amount of whole parts of plants were air dried for 10 days and then kept in an oven at 45°C for 72 hours. After completion of drying, the dried leaves were grounded in a grinder to make powder. The amount of 500gm of dried powder was cold extracted with ethanol. Dried powder soaked with methanol for 7 days. Then filtered concentrated extract contained in a beaker was placed on the water bath (at 40°C-50°C) to evaporate the solvent from the extract. Then this crude methanolic extract (CME) was subjected to fractionation in five different solvents such as Petroleum Ether (PetE), n-Hexane (n-HF), Aqueous (AqF), Chloroform (CHF) and Ethyl Acetate (EAF).

Sample Preparation: The six different fractions of crude extract were suspended separately in six test tubes containing 10 ml distilled water each 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: The 5 ml of sterile distilled water was added and mixed properly with commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15,00,000 I.U. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for in vitro thrombolysis.

Specimen: The amount of 5 ml blood was drawn from healthy human volunteers (n = 05) without a history of oral contraceptive or anticoagulant therapy. Then 500 µl of blood was transferred to each of the five previously weighed alpine tubes to form clots. This method was same for the different fractions of the extract.

Method: The experiments for clot lysis were carried as reported by Sweta *et al*. [25]. This method also concurs with method as used by Chowdhury *et al*. [26] Kamal *et al.*[27]. Venous blood drawn from healthy volunteers was transferred in different pre-weighed sterile Eppendorf 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 eppendorf tube containing

clot was properly labelled 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. The difference in weight taken before and after clot lysis was expressed as percentage of clot lysis. The Streptokinase sample and water were used as positive and negative control respectively. The experiment was repeated two times with the blood samples of different volunteers. Then the percentage (%) of lysis was calculated by the following equation.

% clot lysis = (Weight of the lysis clot /Weight of clot before lysis) × 100.

This study was conducted in compliance with the Good Clinical Practice (GMP) standards and principles of the declaration of Helsinki. The study protocol was approved by the ethics committee of the International Islamic University Chittagong, Bangladesh.

RESULTS

The amount of 100 µl SK as a positive control (30,000 I.U.) was added to the clots along with 90 minutes of incubation at 37°C, showed 70% clot lysis. The formed clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (2.8%). The in vitro thrombolytic activity study revealed that *V. foetidum* showed medium clot lysis activity. The percentage of weight loss of clot after application of extract solution was taken as the functional indication of thrombolytic activity. % Clot lysis obtained after treating clots with six different fractions of extract- PetE (62.30%), n-HF (60.10%), CHF (49.40%), AqF (48.25%), CME (43.25%), EAF (38.71%). These results were shown in Table 1 & Figure 1. Among the six fractions of CME the petE fraction showed highest percent of clot lysis 62.30 followed by n-HF 60.10, CHF 49.40, AqF 48.25, CME 43.25 and EAF 38.71 respectively. Hence, the order of highest activity is as following:

Streptokinase > PetE > n-HF > CHF > AqF > CME > EAF.

Table 1: Results of Thrombolytic activity of various fractions of *V. foetidum*

Extract	No. of Sample	Weight of empty tube (A) g	Weight of tube with clot (B) g	Weight of clot C (B-A)g	Weight of tube with reagent (D)g	Weight of lysis E (B-D)g	% of clot lysis (E/C)%	Mean of % of clot lysis
CME	1	0.799	1.250	0.451	1.074	0.176	39.024	43.25
	2	0.794	1.303	0.509	1.061	0.242	47.544	
	3	0.790	1.254	0.464	1.057	0.197	42.456	
	4	0.783	1.281	0.468	1.068	0.213	45.512	
	5	0.809	1.257	0.448	1.070	0.187	41.741	
Pet E	1	0.780	1.250	0.470	1.007	0.243	51.700	62.30
	2	0.794	1.250	0.546	1.082	0.168	15.563	
	3	0.790	1.233	0.443	0.980	0.249	30.76	
	4	0.784	1.257	0.473	1.150	0.784	165.75	
	5	0.790	1.212	0.422	1.011	0.201	47.630	
n-HF	1	0.804	1.204	0.400	0.964	0.140	35.000	60.10
	2	0.824	1.220	0.395	0.950	0.269	68.100	
	3	0.844	1.200	0.355	0.960	0.240	67.600	
	4	0.834	1.204	0.369	0.980	0.223	60.430	
	5	0.820	1.150	0.330	0.942	0.229	69.390	
CHF	1	0.824	1.280	0.456	0.984	0.296	64.910	49.40
	2	0.814	1.220	0.406	0.990	0.229	56.400	
	3	0.804	1.260	0.458	1.050	0.210	45.850	
	4	0.780	1.334	0.554	1.120	0.214	38.620	
	5	0.812	1.280	0.468	1.087	0.193	41.230	
EAF	1	0.780	1.242	0.462	1.049	0.193	41.770	38.71
	2	0.804	1.280	0.476	1.108	0.171	35.920	
	3	0.805	1.280	0.474	1.130	0.149	29.740	
	4	0.801	1.294	0.493	1.053	0.241	48.880	
	5	0.790	1.255	0.464	1.082	0.173	37.280	
AqF	1	0.794	1.210	0.416	1.004	0.206	49.510	48.25
	2	0.826	1.220	0.397	1.083	0.137	34.500	
	3	0.823	1.180	0.357	0.980	0.199	55.740	
	4	0.790	1.340	0.550	1.007	0.335	60.900	
	5	0.785	1.115	0.330	0.980	0.134	40.600	

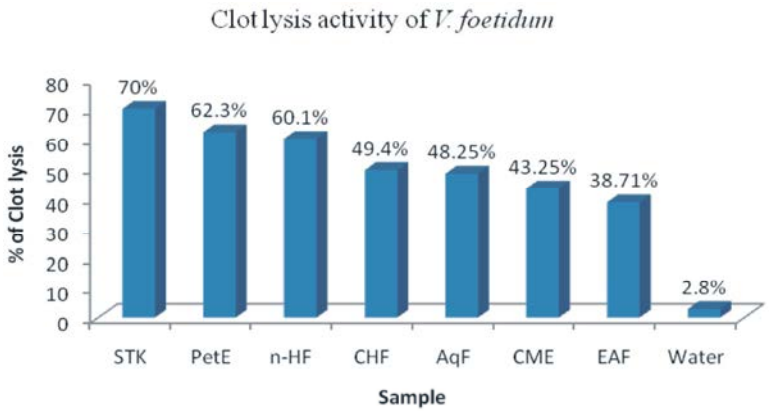

Fig. 1: Comparison of Thrombolytic activity of Streptokinase, Water and various extracts of *V. foetidum*.

DISCUSSION

The three main components of a blood clot are platelets, thrombin and fibrin; each of these components is a key therapeutic target. Thrombolytic drugs are used to dissolve dangerous clots in blood vessels, improve blood flow and prevent damage to tissues and organs [28]. It may involve the injection of clot-busting drugs through an intravenous line or through a long catheter that delivers drugs directly to the site of the blockage. Thrombolytic therapy is used for the treatment of acute myocardial infarction, deep vein thrombosis, pulmonary embolism, [29] acute ischemic stroke, acute peripheral arterial occlusion. The most commonly used clot-busting drugs also known as thrombolytic agents include: Eminase (anistreplase), Retavase (reteplase), Streptase (streptokinase, kabikinase), t-PA (class of drugs that includes Activase), TNKase (tenecteplase), Abbokinase, Kinlytic (rokinase). Glycoprotein (GP) IIb/IIIa inhibitors and clopidogrel have an inhibitory effect on platelet activation and aggregation. Plasminogen gathers in the fibrin matrix. Fibrin-bound plasminogen will be converted by thrombolytic drugs to plasmin, the rate-limiting step in thrombolysis.

The agent having the ability to disrupt clots might be present in the crude extract of leaves of *Viburnum foetidum*. However, this is very preliminary study; so further attempt like compound isolation followed by clinical trial may be the good prospect for getting the thrombolytic compounds.

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