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In Vitro Comparative Cardioprotective Activity of Methanol Extract of Caesalpinia Digyna (Rottl.) Stems and Senna Sophera (L.) Roxb. Stems

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Abstract

The present study was meant to explore the thrombolytic action of methanol extract of *Senna sophera* stems (MESS) and *Caesalpinia digyna* stems (MECD) alongside streptokinase as positive control and saline water as negative control. In the In vitro thrombolytic model, the MESS stems and MECD stems has been indicated 32.79±4.51% and 9.19±0.80% clot lysis individually. Between two extracts, MESS stems. has been demonstrated the noteworthy percent of clot lysis (32.79±4.51%) with reference to streptokinase (66.09±2.37%). From our investigation it has been uncovered that between the plants, MESS stems uncovered moderate thrombolytic action. In this way, steps ought to be taken to search *in vitro* clot dissolving potential and to isolate dynamic segments of MESS Stem for clot lysis are proposed to found. After found, MESS Stem could be recommended as a clot lysis agent in the treatment of patients experiencing disease related with blood clot (Myocardial Infraction, Hypertension, atherosclerosis, Peripheral artery sickness, heart attack and so forth)".

Keywords: Clot lysis; Streptokinase*; Caesalpinia Digyna*; *Senna sophera*; Human blood.

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1. Introduction

At present days development of clot has turned into an extreme issue of blood course. “The blood coagulation produce in the blood circulatory framework because of disappointment of homeostasis reason, vascular blockage and while leads to serious accordance in disease , for example, intense myocardial or cerebral infraction, on occasion elevating to death. Factors involved in the pathogenesis of atherosclerosis, thrombosis and vasoconstriction [1]. Hindrance of blood flow takes place due to presence of thrombus or embolus by blocking the blood vessel therefore disowning tissues of usual blood supply as well as oxygen [2]. Thrombin formed blood clot from fibrinogen and is lysed by plasmin, which is activated from plasminogen by tissue plasminogen activator (tPA). The purpose of a fibrinolytic drug is to dissolve thrombin in acutely occluded coronary arteries thereby to restore blood supply to ischemic myocardium, to limit necrosis and to improve prognosis [3]. Thrombolytic agent is a treatment to dissolve dangerous clot in blood vessels, improve blood flow, and prevent damage to tissue and organs. For the treatment of myocardial infarction and other clot related disease, many thrombolytic agents are used. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue Plasminogen Activator (tPA), the protein that normally activates plasmin. Thrombolytic agents that include tissue Plasminogen Activator (t PA), Urokinase (UK), Streptokinase (SK) etc., are used all over the world for the treatment of athero thrombotic diseases such as myocardial or cerebral infarction, at times leading to death [4]. New thrombolytic agents under development for pulmonary embolism include reteplase, saruplase, and recombinant staphylokinase. Future clinical trials will require multicenter collaboration and focus on clinically relevant endpoints such as reduction of mortality and recurrent venous thromboembolism [5]. Among them, streptokinase is remarkable and widely used. Moreover, Tissue-type Plasminogen activator is more effective and safer than either urokinase or streptokinase type activators. It is noted that all available thrombolytic agents still have significant deficiencies, including the necessity of large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency. Therefore, steps are taken to develop improved recombinant variants of these drugs in order to minimize deficiencies of the available thrombolytic drugs [2]. Now a day in Bangladesh, there has been increasing research on traditional ayurvedic herbal medicines on the basis of their known effectiveness for the treatment of ailments for which they have been traditionally applied. Herbal medicines are assumed to be of great importance in the primary healthcare of individuals and communities in many developing countries [6] Herbal products are often perceived as safe because they are natural” [7]. *Senna sophera* (L.) Roxb. and *Caesalpinia digyna* (Rottl.) are shrub in the family of Caesalpiniaceae and Legumes individually. Local name of S. sophera is kolkasunda is a yearly undershrub, conceivably starting in Bangladesh and most topical nations. It is most commonly seen on waste grounds, on roadsides and in the woodlands. All the parts of the plant are traditionally utilized for different restorative properties. The entire plant is a laxative and febrifuge. Customarily the leaves are utilized in skin disorder as outer applications and roots are utilized as diuretic. This plant has been utilized by ancient Indian physicians for its activity in respiratory issue. *C. digyna* is also called Teri pod is an enormous scandant sparingly prickly shrub, This plant is broadly dispersed in Assam, Bengal, Chittagong, Myanmar, Malay peninsula and Archipelago. As, there is no scientific report accessible concerning the thrombolytic action of stems extract of *S.sophera* and *C.digyna* consequently we expected to experiment the thrombolytic action of methanol extract of stems of *S.sophera* and *C.digyna* ought to discover the cardioprotective agents inside the plant stems.

2. Materials and methods

2.1. Collection and identification of plant material

The various parts of *Senna sophera* (L.) Roxb. Stem. and *Caesalpinia digyna* (Rottl.) stem. were gathered from Prashanti lake, kaptai, Rangamati, Bangladesh in the period of September, 2018 at that point recognized by Dr. Sheik Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh and has been saved in the Herberium of the university of Chittagong for future reference.

2.2. Preparation of extract

"The collected stem *Senna sophera* (L.) Roxb. and *Caesalpinia digyna* (Rottl.) were washed completely with distill water and then cleaved, air dried for a two weeks and pulverized in electric blender (Miyako 3 out of One blender, Miyako, China). 500 g of powdered material was soaked in 2.5 liter of methanol at room temperature for 10 days with occasional shaking and mixing. The mixture was separated through cotton fibre pursued by whatman No. 1 filter paper. The filtrated supernatant was evaporated to dry utilizing a rotating evaporator (RE200, BB Sterling, UK) at 45º C and under diminished pressure to get the crude methanol extract of *Senna sophera* stems and *Caesalpinia digyna* stems".

2.3. Thrombolytic Activity

2.3.1. Drugs and chemicals Reagents and chemicals

"Methanol of analytical grade was given by the Department of Pharmacy, International Islamic University Chittagong. Streptokinase (Incepta pharmaceuticals Ltd) was utilized as positive control and saline water as negative control for in vitro thrombolytic test".

2.3.2. Preparation of test doses

The crude extract (0.01 g) was suspended in 10 ml distilled water and shaken overwhelmingly on a vortex blender. At that point the suspension was kept over-night and decanted to expel the soluble supernatant, which was separated through a filter paper. 100 µL of this aqueous preparation of methanol extract was added to the eppendorf tubes containing the clots to check thrombolytic action.

2.3.4. Streptokinase (SK) solution preparation

"To the commercially accessible lyophilized SK vial (Sanofi Aventis Bangladesh Ltd) of 15,00,000 I.U., 5 ml sterile distilled water was included and mixed appropriately. This suspension was used as a stock preparation from which 100 µl (30,000I.U) was utilized for in vitro thrombolysis as a positive control".[8]

2.3.5. Ethical consideration

"The study protocol was commissioned by the Department of Pharmacy, International Islamic University

Chittagong, Bangladesh. Blood specimens were aggregated from the volunteer of the Department of Pharmacy, International Islamic University Chittagong".

2.3.6. Determination of Thrombolytic activity

The clot lysis investigation was carried out by the method prescribed before [9] For each herbal extract (MESS & MECD), whole blood from healthy individuals (n = 10) who has not taken oral contraceptives or anticoagulants for most recent 10 days, was permitted to form clot in 15 pre-weighted (W1) sterile eppendorf tubes. To each properly labeled tube, 0.5 ml of freshly collected blood was added and placed to clot formation by incubating at 37°C for 45 mins. Serum was separated completely from each tube without stirring clot after its development and weighted (W2). clot weight was determined from the distinction of above weights (W2-W1).The equation for calculating weight of clot is given below:

Clot weight = (Weight of the Eppendorf with clot - weight of empty Eppendorf)

To each tube 100 µL of concentrate (100mg/10ml) preparations (MESS and MECD), streptokinase (positive control) and distill water (negative control) were included independently and hatched at 37°C for 90 min and expelled clot lysis and weighed again for getting the weight variety among the primary weight and final weight that was accomplished for clot lysis (thrombolysis). The experiments were repeated three times with same blood sample of five volunteers. The level of clot lysis was determined utilizing the accompanying equation:

Clot lysis (%) = (Weight of the eppendorf with clot after lysis / Weight of the clot) × 100

3. Statistical analysis

The significance between level of clot lysis by streptokinase and herbal preparations by means of weight distinction was carried out by one way ANOVA pursued by Dunnett's test method. Information is represented as Mean ± SEM with p value <0.001. The statistical analysis was done by GraphPad Prism 5.

4. Result

"Following 90 minutes incubation of streptokinase (positive control) with clots indicated significantly ($P<0.001$) clot lysis of 66.09±2.37% whereas saline water (negative control) treated-clots demonstrated just 3.98±0.30% clot lysis which is very negligible. MESS stem extract demonstrated the significant ($P<0.05$) clot lysis activity (32.79±4.51%) contrasted with another methanol concentrate of MECD stem which indicated just (9.19±0.80%) of clot lysis. Percent of clot lysis has been gotten by treatment of blood clot with Streptokinase, MESS stem, MECD stem and saline water. Graphical representation of all the investigational values has been appeared in Figure-1. Statistical representation of the effective clot lysis rate by negative control (Saline water), positive control (Streptokinase) and two herbal preparations has been appeared in Table: 1 & 2.

Table 1: Thrombolytic activity study of methanol extract of *Senna sophera* stems.

Number of Eppendorf	Weight of the empty Eppendorf (A) gm	Weight of the Eppendorf with clot (B) gm	Weight of the clot. (C). C=B-A	Weight of the Eppendorf with clot after lysis(D) gm	Weight of lysis (E). (E)=B-D	% of clot lysis	Average % of clot lysis
1	0.79	1.16	0.37	1.00	0.16	43.24	32.79±4.51
2	0.80	1.09	0.29	1.02	0.07	24.14	
3	0.79	1.18	0.39	1.09	0.09	23.08	
4	0.81	1.18	0.37	1.07	0.11	29.72	
5	0.81	1.13	0.32	0.99	0.14	43.75	

Each value is presented as the mean ± SEM (n=5); One-way analysis of variance (ANOVA) suggested by Dunnett's test.

Table 2: Clot lysis activity study of extract C*aesalpinia digyna* (Rottl.) stems.

Number of Eppendorf	Weight of the empty Eppendorf (A) gm	Weight of the Eppendorf with clot (B) gm	Weight of the clot. (C). C=B-A	Weight of the Eppendorf with clot after lysis(D) gm	Weight of lysis (E). (E)=B-D	% of clot lysis	Average % of clot lysis
1	0.793	1.293	0.500	1.253	0.040	8.00	9.19±0.80
2	0.783	1.240	0.457	1.203	0.037	8.46	
3	0.784	1.213	0.429	1.173	0.040	9.15	
4	0.800	1.231	0.431	1.178	0.053	12.29	
5	0.793	1.178	0.385	1.147	0.031	8.05	

Each value is presented as the mean±SEM (n=5); One-way analysis of variance (ANOVA) suggested by Dunnett's test.

Table 3: Average percent of clot lysis activity of streptokinase, MESS stems, MECD stems & saline water.

Test group	Average % of clot lysis
Streptokinase	66.09±2.37
MESS	32.79±4.51
MECD	9.19±0.08
Saline Water	3.98±0.30

Each value is presented as the mean ± SEM (n=5); One-way analysis of variance (ANOVA) suggested by Dunnett's test. MESS=methanol extract of *Senna sophera* (L.) Roxb. stems and MECD = methanol extract of

C*aesalpinia digyna* (Rottl.) stems

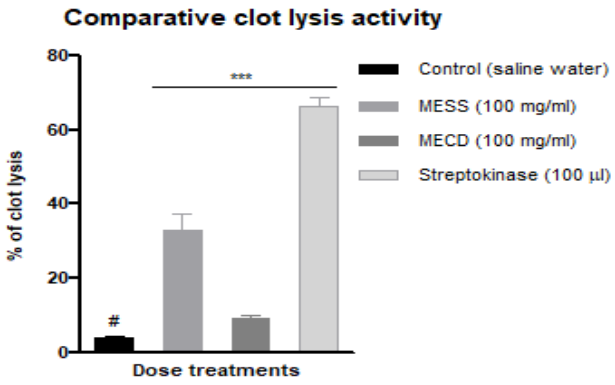

Figure 1: Comparative Clot lysis by Water, Streptokinase, MECD stems & MESS stems.

Values are represented as mean ± SEM (n=5). All the test group were significantly analysed in One-way analysis of variance (ANOVA) followed by Dunnett's test***p<0.001. The control was designated by #".

5. Discussion

This study evaluated the thrombolytic potentiality of two shrubs available in Bangladesh. "Herbal preparations are used since ancient times for the treatment of diseases. A number of research works have been conducted to discover the plants and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect and there is indication that consuming such food leads to prevention of coronary events and stroke [10, 11]. Phyto-pharmacological investigation has leads to discovery plant derived drugs, which are effective in remedial of certain diseases, and renewed the interest in herbal medicines. About 30% of the pharmaceuticals are prepared from plants worldwide [2, 12]. A number of studies have been conducted by various researchers to find out the herbs and natural food sources and their supplements having anti thrombotic (anticoagulant and antiplatelet) effect and there is evidence that consuming such food leads to prevention of coronary events and stroke[13]. It is clear that, several thrombolytic drugs obtained from various sources. Some are modified further with the use of recombinant technology in order to make these thrombolytic drugs more site specific and effective [14]. Platelets play a significant role in the development of atherothrombosis as well as damage the regions of endothelial surface (produced by reactive oxygen species). The stimulated platelets form platelets to platelets bonds, binds also to leucocytes carrying them into an intricate process of plaque development and progression [15]. Plasmin, a natural fibrinolytic agent, lyses clot by breaking down the fibrinogen and fibrin contained in a clot. Streptokinase forms a 1:1 stoichiometric complex with plasminogen that can convert additional plasminogen to plasmin [16]. Despite the fact that there are a few thrombolytic medications including those acquired by recombinant DNA innovation, yet side effects identified with a portion of these medications that lead to facilitate confusions have been accounted for. Nonetheless, natural arrangements then again, whenever taken in fitting dosages, can prompt an option and better choice for restoring different afflictions. Herbal preparations, if taken in appropriate dose, can lead to a better option for curing various ailments. Toxicity of plant extract is a major concern of scientists and medical practitioners. Among

several methods lethality test has been successfully used to bio-monitor the isolation of cytotoxic, antimalarial, insecticidal and antifeedants compounds from plant extracts [17]. The present examination was completed to research thrombolytic action of two plants accessible in Bangladesh. Streptokinase (SK), a known thrombolytic drug is used as a positive control" [18].In our present in vitro clot lysis test affirmed that, MESS demonstrated the moderate thrombolytic activity where MECD indicated very mild thrombolytic action at the prepared dose which recommend that extractives of MECD ought not be wise to be utilized as a therapeutic of heart maladies as it conventional structures. Be that as it may, further methodologies are anticipated to confine the active constituents of MESS in charge of the thrombolytic action. In near future it might be executed as thrombolytic agent for the improvement of patients experiencing coronary cardiac disease.

6. Conclusion

It may be inferred that *Senna sophera* (L.) Roxb. stems has the potential as a possibility for future thrombolytic agent and *Caesalpinia digyna* (Rottl.) stems is not as significant as *Senna sophera* (L.) Roxb. This investigation was a preliminary study; further study is required to exploit their therapeutic and pharmaceutical possibilities.

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