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COMPARATIVE ANALYSIS OF ANTITHROMBOTIC EFFECT OF ETHANOL EXTRACT AND ITS DIFFERENT FRACTIONS OF *STERCULIA CORDATA* LEAVES.

Syed Md. Abdul Kader[1,2], Md. Imdadul Haque[1,2*], Shababa Wajida Nihar[2], Mahmudul Hasan[2,3], Abid Newaz Shihab[2,4], Fatema Akhter[4], Nazia Hassan[4] and Tajmira Zaman[4]

[1]Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh.
[2]GUSTO A Research Group, Chittagong, Bangladesh.
[3]Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka, Bangladesh.
[4]Department of Pharmacy, University of Science and Technology Chittagong, Chittagong, Bangladesh.

***Corresponding Author: Md. Imdadul Haque**
Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh.
Mail ID: Imdad_ph@yahoo.com

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ABSTRACT

Background: The purpose of the investigation was to ascertain whether the leaf extract and fractions of *Sterculia cordata* holds any significant antithrombotic properties. **Methods:** Leaves of *Sterculia cordata* was extracted with pure ethanol (EESC) then ethanol extract fractioned with methanol (MFEESC), n-hexane (NHFEESC) and chloroform (CHFEESC). The antithrombotic activity of the extracts and fractions were evaluated by clot lyses test using the standard streptokinase on human blood. **Results:** Using an *in vitro* antithrombotic model, all extracts, fractions, and Streptokinase exhibited significant ($P < 0.0001$) clot lysis. EESS showed the highest significant ($62.04 \pm 2.10\%$) clot lysis activity among all the extracts ($P > 0.0001$ compared to negative control and $P > 0.001$ compared to positive control), which is highest compared with other sample treatment. **Conclusions:** The overall results of the study indicated significant antithrombotic activity of different extracts and fractions of leaves of *Sterculia cordata*. Furthermore, this plant deserves further investigation for other paramount pharmacological activities and comprehensive research and isolation of the active constituents responsible for these activities and establishes the mechanism of action.

KEYWORDS: *Sterculia cordata*, antithrombotic, extract, fraction.

INTRODUCTION

Therapeutic plants are always very promising for the development of new drugs. To distinguish any plant owning medicinal quality, proper clinical screening is essential. Typically different plants are known to will vary usefulness for treating various kinds of diseases. If the right plant is known for healing a particular disease, attempts should be made to isolate the bioactive lead molecules from the plant.[1]

Arterial and venous thrombosis is a major cause of morbidity and mortality. Arterial thrombosis is a recurrent cause of myocardial infarction (MI), ischemic stroke, and limb gangrene; venous thrombosis includes DVT, which is often complicated by the postthrombotic symptoms and pulmonary embolism (PE), that can be fatal or can lead to chronic thromboembolic pulmonary hypertension.[2] This disease due to the formation of thrombus or embolus which hinders the blood flow by blocking the blood vessel, therefore, depriving tissues of normal blood flow and oxygen. These leads to necrosis of the tissue in that area. 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.[3-5] Limitations of existing antithrombotic drugs have prompted a search for novel agents. Focusing on new drugs for the prevention and treatment of arterial and venous thrombosis. And we are searching the drug from natural sources.

Sterculia cordata Blume, Bijdr. belongs to the family "Malvaceae". Sterculia cordata is a deciduous tree growing up to 46 metres tall. The bole can be 76cm in diameter. The large seeds of many species in this genus are used for food. Usually cooked, they are rich in oil and have a flavour described by some as like peanuts. This plant distributed at Bangladesh, Thailand, Peninsular Malaysia, Sumatra, Java, Borneo, Philippines.[6][7] The wood is harvested from the wild for commercial usage. There is no previous systemic pharmacological investigation done on this plant.

The main objective of the study was to investigate the antithrombotic effect of the ethanol extract of *Sterculia cordata* leaves and its different fractions.

MATERIALS AND METHODS

Plant materials

The leaves of *S. cordata* were collected from Bandarban, Bangladesh in March 2015 at a mature stage. The leaves were cut into small pieces and then dried in shade at 21-30ºC for 7 days. Then the materials were dried in an oven at low temperature to improve grinding. Then the pieces were ground by a mechanical grinder and then passed through a size 60 mesh screen to obtain a fine powder of the leaf material. This was stored in an air-tight container.

Preparation of sample

The fine powder of leaves of *S. cordata* (800 g) was taken in a clean round-bottom flask (5 L) and soaked in 4 L of Ethanol for 15 days at room temperature with occasional shaking and stirring. Then the mixture was first filtered with cotton plug followed by Whatman No. 1 filter paper. The filtrate is evaporated to dryness in Heidorph rotary evaporator at 45ºC to obtain a concentrated extract. This was then air dried to obtain a solid residue. Thus the Ethanol extract of the leaves of *S. cordata* was prepared and then four solvents chloroform, n-hexane, ethyl acetate and methanol was used for solvent-solvent partitioning from ethanol solution. To prepare test samples and the standards were suspended in distilled water using Tween 80 for *in vivo* test.

Chemicals and reagents

The chemicals used were: ethanol, methanol, n-hexane, chloroform (Merck, Germany). Lyophilized streptokinase's (SK) vial (Square Pharmaceuticals Ltd. Bangladesh). All chemicals used were analytical grade.

In vitro antithrombotic activity

Blood specimen

Whole blood (3.5 ml) was drawn from healthy human volunteers (n = 12) without a history of oral contraceptive or anticoagulant therapy. A new consent, approved by Mohammed Abu Sayeed, Assistant Professor & Head of Department of Pharmacy, International Islamic University Chittagong (IIUC), Bangladesh, for the collection of blood samples from Human volunteers. Blood collection was conducted by Md. Shariful Islam (Lab technician, Department of Pharmacy, IIUC) and preservation were conducted by Abdul Karim (Lab technician, Department of Pharmacy, IIUC), who stored the clot containing Eppendorf tube in the refrigerator in Microbiology lab, Department of Pharmacy, IIUC. A 500 µL of blood was transferred to each of the 12 previously weighed Eppendorf tube tubes to form clots.

Statement on informed consent of the donors

The volunteer donors were supplied a consent form which informed the title of the research project, name and detail contact of investigators as well as purpose of the research. Description of the research mentioning step-by-step brief of the proposed research, inclusion and exclusion criteria of the donors, whether donors will receive any therapy or not, the volume of blood to be taken, possible discomfort at the puncture sites, the time required for the blood sampling. Benefits of the volunteer described. It was indicated to the consent form that the volunteers might refuse to donate blood at any time. The donor, whether could withdraw his sample data, was disclosed. The sample was restricted to that individual study, not for future research projects was presented in the consent form. Potential harm, injuries, discomforts or inconvenience associated with donors in this study was added as informed consent statement. If there was known harm to the donors, the potential harm, current knowledge regarding the probability of the occurrence of the harm, clinical importance of the harm; and any relevant knowledge regarding the probability of reversibility. Treatment alternative and possibility of the research was described. Confidentiality statement was included in the consent form in the way that "confidentiality will be respected and no information that discloses the identity of the participant will be released or published without consent unless required by law of states. Finally, identification of investigators was provided in case of further query. The consent form was concluded with major questions on above disclosures in Yes/NO form followed by the signature (with date) of the donor.

In vitro antithrombotic study procedure

Experiments for clot lysis were carried as reported earlier. Briefly, 3.5 mL venous blood drawn from the healthy volunteers was distributed in 7 different pre-weighed sterile Eppendorf tube (0.5 mLtube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube - the weight of tube alone).[8] To each Eppendorf tube containing pre-weighed clot, 100 µL of different extracts and fractions of *S. cordata* leaves were added separately.[9] As a positive control, 100 µL of SK and as a negative non-antithrombotic 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 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption.[10] The difference obtained in weight taken before and after clot lysis was expressed as a percentage of clot lysis.[11] The experiment was repeated with the blood samples of the 12 volunteers.

Statistical Analysis

All results are expressed as Mean ± Standard error of the mean (SEM). The results were statistically analyzed using repeated measures analysis of variance with Tukey test for antithrombotic effect. $P<0.05$, $P<0.01$ and

P<0.001 were considered as statistically significant. Statistical programs used were SPSS (Statistical Package for Social Science, version 22.0, IBM Corporation, NY) and for graphical presentation GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) were used.

RESULTS

In vitro antithrombotic activity assay

In an antithrombotic approach with a human blood sample, the addition of 100 μL streptokinase (a positive control), to the clots and subsequent incubation for 90 minutes at 37°C 79.8 ± 1.38% clot lysis. On the other hand, distilled water was treated as negative control which showed negligible 6.60 ± 1.84% clot lysis. EESS showed the highest significant (62.04 ± 2.10%) clot lysis activity among all the extracts (P > 0.0001 compared to negative control and P > 0.001 compared to positive control), which is highest compared with other sample treatment. Percentages of clot lysis obtained after treating the clots with different extracts, fractions, and appropriate controls are shown in Table 1 and their comparison was represented in Figure 1.

Table 1: *In vitro* clot lysis activity of the extracts and fractions of *S. cordata* leaves and Streptokinase on human blood.

Drug/Extracts	% of clot lysis
Water	6.60±1.84
Streptokinase	79.8 ±1.38[a]
EESC	62.04 ± 2.10[a, b]
NHFEESC	48.24 ± 2.28[a, b]
CHFEESC	52.14 ± 2.06[a,b]
EAFEESC	43.36 ± 1.93[a,b]
MFEESC	55.62 ± 2.34[a,b]

Values are mean ± SEM (n = 12); [a]P<0.0001, Tukey test as compared to negative control, [b]P< 0.001, compared to positive control. Statistical representation of the effective clot lysis percentage by extracts and fractions preparations, positive antithrombotic control (streptokinase) and negative control (sterile distilled water) processed by Tukey test by using SPSC for windows, version 22.0.

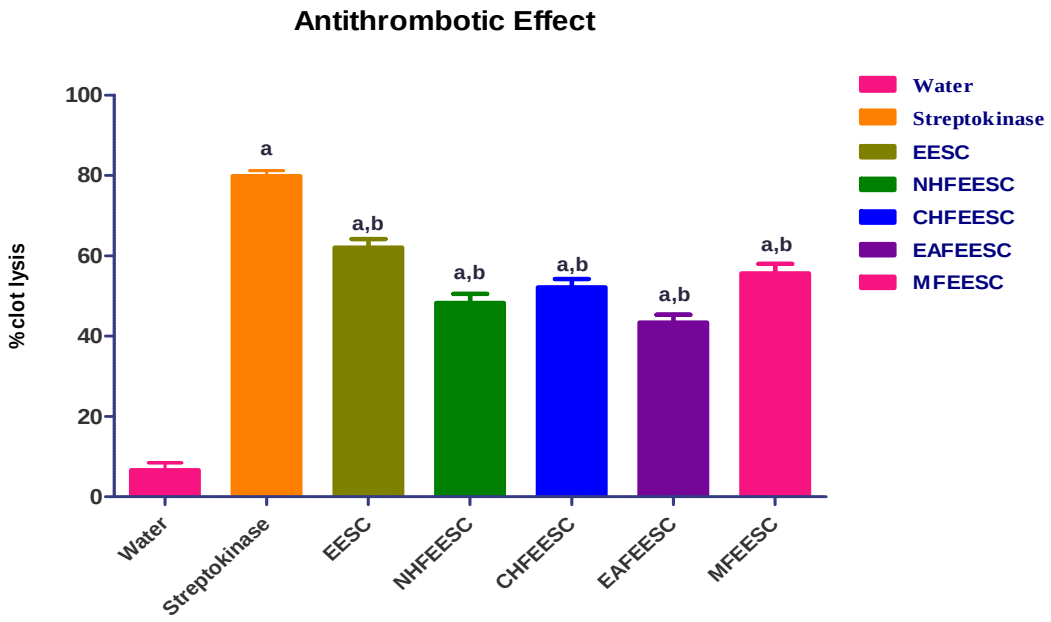

Figure 1: *In vitro* clot lysis activity of the extracts and fractions of *S. cordata* leaves and Streptokinase on human blood.

Values are mean ± SEM (n = 12); [a]P< 0.0001, Tukey test as compared to negative control, [b]P< 0.001, compared to positive control. Statistical representation of the effective clot lysis percentage by extracts and fractions preparations, positive antithrombotic control (streptokinase), and negative control (sterile distilled water) processed by Tukey test by using SPSC for windows, version 22.0.

DISCUSSION

The antithrombotic activity of various fractions of ethanolic extract of *S. cordata* was measured and compared with streptokinase (the positive control) and sterile distilled water (the negative control). In the study, MFEESC fraction was found to provide the maximum antithrombotic activity. However, the percentage of clot lysis produced by the commercially available positive control was greater than those produced by the fractions. The presence of a minor amount of bioactive antithrombotic compound in the extracts may contribute to the weak result. All extracts exhibited statistically significant antithrombotic activity (P<0.001).[12] These herbal preparations may be incorporated as an

antithrombotic agent for the improvement of the patients suffering from atherothrombotic diseases.[13] This is a preliminary study and the extracts should thoroughly be investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

CONCLUSIONS

In conclusion, this well-informed study evaluated significant antithrombotic activity of Ethanol extract and its different fractions of *S. cordata* leaves. It can be expected that distinctive dynamic secondary metabolites are available in this concentrate and maybe some of these mixes may work in a synergistic way. On the other hand, further studies are important to illustrate the component lying with these impacts. On the other hand, this is the first write about this example and it may serve as a stride with respect to the natural and pharmacological exercises of this specimen.

Abbreviations

EESC = Ethanolic extract; NHFEESC = n-hexane fraction of ethanolic extract; CFEESC = Chloroform fraction; EAFEESC = Ethyl acetate fraction; MFEESC = Methanol fraction of ethanolic extract of *S. cordata* leaves; µg: Microgram; L= liter; mL= Milliliter; µL= Micro liter; µg/mL= Microgram per Milliliter; *etss al*.= et alliori (and others); SEM: Standard error for mean.

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Competing interests

The authors declare that they have no competing interests.

Employment or leadership: None declared.

Honorarium: None declared.

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