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Study on in-vitro thrombolytic activity of methanolic extract of *Mesua ferrea* leaves

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Abstract

Objective: The present study was designed to investigate the thrombolytic activity of methanolic extract of leaves of *Mesua ferrea*.

Method: Methanolic extract of *Mesua ferrea*. Was used to evaluate various phytochemical methods. Thrombolytic effect of the fraction was investigated in clot lysis experiment.

Result: The extract exerted 36.32% lysis of the blood clot in thrombolytic activity test while 85.45% and 7.5% lysis were obtained for positive control (streptokinase) and negative control respectively. So, the extract possessed considerable thrombolytic activity.

Conclusion: Our present investigations indicate that methanolic extract of leaves of *Mesua ferrea* exhibited considerable thrombolytic activity. There may be many pharmacologically active constituents in the fraction. So, extensive pharmacological and phytochemical experiments are essential.

Keywords: *Mesua ferrea*, Thrombolytic activity, Streptokinase.

1. Introduction

A pill are widely used not as they are inexpensive but in addition greater cultural acceptability, better compatibility while using skin and minimal side consequences. Herbal medicine remains be your mainstay of about 75-80% on the globe population, mainly in your establishing countries for primary health. However one of several estimated 400, 000-400, 000 plant life species, only 6% are actually examined for biological activity and about 15% have been investigated phytochemicals [1]. The trend of using natural products has increased together with active plant extracts are normally for new drug discoveries [2]. Plants have been the time frame of countless traditional medicine systems across the world for thousands of years and continue and offer people with brand-new cures. Plant based medicines initially furnished available as crude drugs including tinctures, green tea, poultices, powders, and also other plant-based formulations, now serve while foundation of novel drug breakthrough discovery [3]. New drugs derived from pure sources has available throughout the last couple involving years. These new drugs have obtained approval for the management involving cancer, neurological diseases, infectious problems, cardiovascular and metabolic diseases, immunological, inflammatory as well as related diseases, and genetic problems, which encompass many of a normal human diseases. Besides new drugs launched available from 2000 as of yet, there are many of brand-new chemical entities via natural solutions undergoing clinical trial offers, [4]. Nearly all thrombolytic providers work However, the potential points about herbal medicines could lie with their high acceptance by patients, performance, relative safety and low fees [5]. Thus documentation of indigenous knowledge on use of plants and providing products involving useful plants from local flora generally is a great help for right employing traditional medicines. Identification and isolation inside active constituents from traditionally used phyto-therapy might make sure this care. In supplement, herbal drugs can even always be scientifically modified for better medicinal activity and to put together effective and safe drugs.

Thrombolysis stands out as the breakdown (lysis) of blood clots by just pharmacological means [6]. It is colloquially mentioned as clot busting for this evidence. It works by stimulating fibrinolysis by just plasmin through infusion of analogs affecting tissue plasminogen activator (tPA), one's protein that normally activates plasmin [7]. Thrombolysis suggests the usage of

thrombolytic drugs, which are either that, is generated by *Streptococcus* species or more simply just lately, using recombinant biotechnology whereby tPA is usually manufactured by bacteria, resulting in a recombinant tissue plasminogen activator together with rtPA. Formation of blood clots is place for the basis of many serious disorders [8]. By extracting the clot, the disease process might possibly be arrested, or the complications receded. While other anticoagulants (such though heparin) decrease the "growth" of any clot, thrombolytic agents actively minimize the dimensions of by activating the enzyme plasminogen, which clears the cross-linked fibrin mesh (the backbone of any clot). This makes the clot soluble and grow subject to further proteolysis by means of other digestive support enzymes, and restores circulation over occluded arteries and thrombolytic drugs dissolve blood clots by just activating plasminogen, which forms the latest cleaved product called plasmin. Plasmin may be a proteolytic enzyme that is competent at breaking cross-links between fibrin things, which give the structural loyalty of blood clots. Because connected with such actions, thrombolytic drugs are otherwise often known as "plasminogen activator" and "fibrinolytic drug treatments". You will discover three significant classes of fibrinolytic prescription drugs: structure plasminogen activator (tPA), streptokinase (SK), in conjunction with urokinase (UK). While drugs in these three classes all seem to effectively dissolve blood clots, they differ in their detailed mechanisms in ways that can alter their selectivity for fibrin clots [9]. Derivatives of tPA add some most commonly used thrombolytic prescriptions, especially for coronary and cerebral vascular clots, with regards to relative selectivity for activating fibrin-bound plasminogen. Thrombolytic therapy is the usage of drugs to break up together with dissolve blood clots, which add some main cause of both coronary heart attacks and stroke [10].

Materials and Methods

Collection of Plant materials

The particular leaves of *Mesua ferrea* have been collected from Saraf-bhata hill, Rangunia, alongside Bandarban, Bangladesh and authenticated from the expert of Department of Botany, Asst. Mentor Dr. Shaikh Bokhtear Uddin, school of Chittagong, Bangladesh. The results in of *Mesua ferrea* were accumulated at their fully mature kind, from Chittagong hill Tract. Right after cleaning, the leaves were obtained and air dried for 10 nights, and then kept in a great oven at 45 °C at 72 hours. 250gm of dried powdered was cold extracted with Methanol. Dried powder soaked with methanol for 1 week. Then filtered to take the particular concentrated extract, extract containing beaker was added to the water bath (at 40 °C-45 °C) to evaporate the solvent from your extract [11].

Preparation of Extraction

This extract is served by wintry extraction process. In this process the coarse powder was submerged in ethanol (95%) since ethanol would be the most frequent solvent for extracting the vast majority of constituents present in organic products. Amber glass bottle were used for this purpose, of kept at room temperature and allowed to stand for many days (5-7) having occasional shaking and stirring. When the solvent became centered the contents were first decanted by making use of cotton and then filtered as a result of (Whatman No. 1) filter forms. The filtrate so obtained seemed to be then concentrated to dryness on the evaporation of solvent using rotary evaporator. Finally we got the exact concentrated semi-solid

extract. The centered were then used as medieval extract of respective test trials. In our present investigation, every one of us used methanolic extract for cytotoxic in addition to thrombolytic activity [12].

Thrombolytic Test

Thrombolysis could be the breakdown (lysis) of blood clots simply by pharmacological means. It is colloquially termed as clot busting that is why. It works by stimulating fibrinolysis by plasmin through infusion of analogs regarding tissue plasminogen activator (tPA), the protein that normally activates plasmin.

Preparation of Extract solution for thrombolytic Test

10g with the extract was suspended in 10ml distilled water and shaken vigorously over a vortex mixer. Then the suspension was kept overnight and decanted to eliminate the soluble supernatant, which was filtered through filter papers (Whatman No. 1). The solution was then ready for inside vitro evaluation of clot lysis activity [12].

Preparation of Streptokinase (SK) Solution

On the commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of just one, 500,000 I. U., 5 ml clean distilled water was added along with mixed properly. This suspension was used as being a stock from which 100 µl (30,000 my spouse and i.U) was used for throughout vitro thrombolysis [12].

Specimen for 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 [12].

Test procedure for thrombolytic test

Reports for clot lysis were maintained as reported earlier [5]. Venous blood drawn from healthy volunteers was transferred in a number of pre-weighed sterile Epen drop tv (500µl/tube) and incubated at 37 °C with regards to 45 minutes. After clot enhancement, serum was completely removed (aspirated around without disturbing the clot formed). Each tube having clot was again weighed to look for the clot weight. (Clot weight = bodyweight of clot containing tube - weight of tube alone). Every single Epen drop tv containing clog was properly described and 100 µl of plant extract was and also the tubes. All the hoses were then incubated at 37 °C associated with 90 minutes and observed associated with clot lysis. After incubation, fluid obtained was removed as well as tubes were again weighed to observe the difference in weight right after clot disruption. Difference obtained throughout weight taken before and right after clot lysis was expressed even though percentage. Thrombolytic activity of methanolic purchase of *Mesua ferrea* Leaves block up lysis. Streptokinase and water were used being a positive and negative (non-thrombolytic) demand respectively. The experiment was repeated a few times with the blood samples regarding different volunteers. % clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100

Result and Discussion

Thrombolytic Activity

This particular methanolic extract of *Mesua ferrea* simply leaves is exerted 38. 1208% lysis from the blood clot in

thrombolytic activity test while 75. 18% as well as 15. 82% lysis were being acquired for positive control (streptokinase) and negative control respectively which frequently shown in table 1 as well as figure 1. So, this draw out possessed considerable thrombolytic activity.

The percentage of fat decrease of clot after application associated with extract solution was taken because functional indication of thrombolytic exercise. The average value of percentage of fat loss was calculated and shown upon table. *Mesua ferrea* actually simply leaves have significant thrombolytic activity. Numerous plants source especially several fruits and vegetables have been studied for their own supplements having anticoagulant, antiplatelet and fibrinolytic activity and there might be evidence that consuming such food results in prevention connected with coronary occasions and stroke. Some of these plant items are modified further with recombinant technology to make them more effective and website specific. In our thrombolytic assay, the comparison of positive manage with negative control clearly shown that clot dissolution isn't likely to occur when water was put into the clot. As compared towards the clot lysis percentage obtained via SK and mineral water, an incredibly significant thrombolytic activity was noticed after healing the clots along with *Mesua ferrea*. Cell surface bound plasminogen is generally easily activated to plasmin, which can lead to fibrinolysis. Microbial plasminogen activator: staphylokinase, streptokinase, behave as cofactor molecules that promote exosite formation and boost the substrate presentation to this enzyme. Staphylokinase triggers plasminogen to dissolve

clots, also kills the extracellular matrix as well as fibrin fibers that hold solar panels together. So the extract is going to be processed as drug products within thrombolytic uses. However, the very significant consequence of *Mesua ferrea*, demonstrates it to become the beneficial thrombolytic component with regard to further processing.

Table 1: Thrombolytic Activity of methanolic extract of *Mesua ferrea*

Group	Percent of lysis
Streptokinase	85.45%
<i>Mesua ferrea</i>	36.32%
water	7.5%

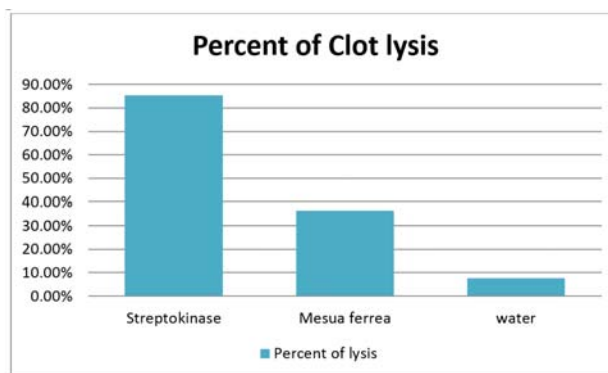


Fig 1: Clot lysis by water, Streptokinase, Methanol extract of *Mesua ferrea*

Table 2: Results of clot lysis of methanol extract of *Mesua ferrea*

Sl No volunteer	Weight of tube	Weight of tube with clot	Weight of tube clot after lysis	Weight of clot	Weight of lysis	% of lysis	Avg % of lysis
1	0.8256	1.3195	1.2667	0.4939	0.0528	10.69	36.32
2	0.7886	1.2515	1.2055	0.4629	0.046	11.41	
3	0.8138	1.1709	1.1128	0.3571	0.0581	16.26	
4	0.7976	1.1973	1.0152	0.3997	0.1821	45.55	
5	0.7707	1.1924	1.1631	0.3924	0.0293	7.46	
6	0.7944	1.2245	0.9631	0.4301	0.2614	60.77	
7	0.8254	1.3497	1.0695	0.5243	0.2802	53.44	
8	0.8042	1.3068	0.9995	0.5026	0.3073	61.14	
9	0.8288	1.3743	1.0437	0.5455	0.3306	60.6	
10	0.7990	1.3345	1.0848	0.5355	0.2497	46.629	

Conflict of interest

Authors declare that there is no conflict of interest.

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Conclusion

From the above study it can be concluded that the methanolic extract of *Mesua ferrea* 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 and cytotoxic study was close to the standard used. The

thrombolytic potency of *Mesua ferrea* is found 36.32% and the standard have 85.45%. It seems good result or may be said significant as the extract was the mixture of many phytochemical, it shows nearly percent of clot lysis. At present scientist give their best regard in developing a more potent and site specific drug in the treatment of cancer. Nature could be a great source in this purpose. Most of potent drugs are using came from nature, either directly or in their derived form. In this regard my study can help to find a new lead compound for future drug discovery. Here experimental studies of leaves extract exhibited considerable thrombolytic and cytotoxic activity and moderate 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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