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EVALUATION OF THROMBOLYTIC ACTIVITY OF DIFFERENT MARKETED POLYHERBAL FORMULATIONS

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ABSTRACT: Thrombosis remains a leading cause of cardiovascular morbidity and death, with complications including myocardial infarction and stroke. It is characterized by the development of thrombi, or blood clots, inside blood vessels. Herbal remedies are increasingly being studied as safer, complementary alternatives to conventional thrombolytics like streptokinase. A human blood clot lysis model was used to investigate the *in-vitro* thrombolytic efficacy of two Bangladeshi polyherbal formulations, Rohitakarishta (Hepatolin) and Sharbat Chylosin (Hepa-10). Ten healthy people's venous blood samples were placed under incubation at 37°C to cause clot formation. Thrombolytic activity of the formulations was evaluated at 100 µL and 200 µL concentrations using distilled water as the negative control and streptokinase as the positive control. The standard, streptokinase, showed maximum clot lysis (58.52% ± 2.02), while the negative control caused minimal clot lysis (2.8% ± 0.48). At 200 µL, Hepatolin caused the most clot lysis (51.52% ± 0.43), followed by Hepa-10 (42.30% ± 1.64). At 100 µL, the lysis rates of Hepatolin and Hepa-10 were (26.11% ± 2.09) and (24.36% ± 1.93), respectively. According to these findings, Hepatolin has a significant thrombolytic effect at higher dosages that is comparable to standard treatment. Even though these first findings are promising for treating thromboembolic disorders, additional *in-vivo* studies and phytochemical characterization are needed to clarify the underlying processes and maximize therapeutic effectiveness.

INTRODUCTION: Blood clot formation has been a serious issue with blood circulation [1]. Thrombus is the primary cause of cardiovascular diseases (CVD), which can result in stroke, embolism, ischemia, deep vein thrombosis, and other complications. When a blood clot forms, the condition is known as thrombosis [2]. Impaired thrombolysis is one of the factors that contribute to the development of cardiovascular disease [3]. The process of dissolving blood clots is called thrombolysis [4]. Patients with thrombosis are treated with thrombolytic medicines, which break up clots [5].

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Numerous lives have been saved by thrombolytic agents, sometimes referred to as clot busters [6]. The purpose of thrombolytic therapy is to restore function to the damaged area by removing problems caused by blood clots or thrombus [7]. The mechanism behind thrombolytic action is the activation of plasminogen, which results in the production of plasmin. When the ensuing plasmin cleaves the fibrin, the clot is ultimately broken up [3].

Since ancient times, a wide range of diseases have been treated with herbal treatments. Because herbal products are "natural," they are often seen as harmless [8]. Natural resources are widely regarded as safe and potentially more effective. It is possible to lower the risk of thrombosis by using plants that have fibrinolytic, thrombolytic, and antiplatelet properties [9]. Chinese medicine has been using herb-herb combinations, sometimes referred to as polyherbal therapy, for thousands of years [10].

Synergism allows polyherbalism to offer some advantages that aren't possible with a single herbal mixture [11]. The combined action of several phytoconstituents in the herbs produces the therapeutic impact of polyherbal medications, and these effects are further enhanced when they are formulated in a solution that is compatible with one another [12]. The purpose of this study is to examine the *in-vitro* thrombolytic efficacy of two Bangladeshi polyherbal formulations: Rohitakarishta (Hepatolin) and *Sharbat* Chylosin (Hepa-10). A significant amount of death and disability worldwide is caused by ischaemic heart disease, ischaemic stroke, and venous thromboembolism, all of which have thrombosis as their underlying pathology [13]. Thrombolytic therapy is a crucial technique for quickly re-establishing blood flow, but its practical application is still constrained by hemorrhagic risk, contraindications, and the requirement for cautious patient selection [14]. Because of this, research for safer and easier-to-access thrombolytic alternatives is still ongoing.

Because traditional medical systems have long mixed several herbs to enhance therapeutic outcomes through synergistic action, polyherbal compositions are particularly interesting [15]. Such combinations are valued in Ayurvedic and other traditional methods because one herb may enhance the activity of another, increasing efficacy and occasionally lowering toxicity [16]. Additionally, recent evaluations indicate that thrombolytic medicinal plants could be promising sources of natural clot-dissolving chemicals, particularly when combined or included in a manufactured product [17]. The thrombolytic potential of commercialized polyherbal preparations merits a thorough scientific assessment because they are widely used and frequently regarded as natural medicines. Therefore, evaluating the *in-vitro* thrombolytic activity of Sharbat Chylosin (Hepa-10) and Rohitakarishta (Hepatolin) may help determine whether these formulations play any significant role as natural alternatives in the treatment of thrombosis.

MATERIALS AND METHODS:

Collection and Identification: The hepatoprotective polyherbal formulations 1. Hepatolin syrup (manufactured by ACME Laboratories Ltd) and 2. HEPA–10 (manufactured by Jyson Natural Products Ltd, Unani division) are derived from the unrefined pharmaceuticals of particular medicinal plants. On February 1st, 2026, the crude drugs were bought from Lazz Pharmacy, a reputable vendor in the model pharmacy industry. The medications were thoroughly inspected at the time of purchase to make sure they were sterile, well-dried, and free of adulteration, fungus, and insect contamination.

Streptokinase: For the *in-vitro* thrombolytic test, the streptokinase stock solution was prepared. To measure thrombolytic activity, streptokinase (STK), a popular and traditional thrombolytic medication, was used as a positive control. A commercially available STK injection (lyophilized powder, 15,00,000 I.U., Incepta Pharmaceutical, Jirabo, Savar, Bangladesh) was reconstituted by adding 5 mL of sterile distilled water and gently mixing the mixture until it dissolved completely. 100µL, or 30,000 I.U., of this stock solution was used for the thrombolytic test.

Formulation Preparation: Thrombolytic activity of various commercially available polyherbal formulations (Hepatolin syrup and Hepa-10) employing Sharbat chylosin and Rohitakarista as direct samples. Two concentrations were used for each formulation. A 100 µL and a 200 µL concentration were used as low dose and high dose.

TABLE 1: TWO MARKETED POLYHERBAL FORMULATIONS (LIQUID) USED IN CURRENT INVESTIGATION (COMPILED FROM THE MANUFACTURER'S INSTRUCTIONS)

Formulation	Principle Ingredients	Purpose	Standard Dose
Hepatolin syrup (ACME Laboratories LTD., Dhaka, Bangladesh)	Each 5 ml contains aqueous extract of *Cinnamomum zylanicum* 15.24 g *Aphanamixis polystachya* 1.52 g, *Emblica officinalis* 15.24 g, Piper longum (Root) 15.24 g, *Terminalia chebula* 15.24 g, *Elettaria cardamomum* 15.24 g, *Terminalia belerica* 15.24 g, *Woodfordia fructicosa* 0.24 g, *Zingiber officinale* 15.24 g, *Piper longum* (Seed) 15.24 g,	Splenomegaly (enlargement of the spleen), Malaria splenomegaly, Anemia linked to splenic enlargement, Abdominal distension or bloating, IBS, or irritable bowel syndrome, Anorexia, Hepatomegaly or enlarged liver, Persistent appetite loss, Piles, Leucorrhea, Syphilis,	Adults 10 to 30 ml Maximum possible dosage 60 ml per day (in divided doses)

Hepa 10 (Jyson Natural Product LTD., Unani division, 231 Tejgaon, I/A Dhaka, Bangladesh)	*Cinnamomum tamala* 15.24 g, Each 5 mL contains extracts of *Foeniculum vulgare* 100 mg, *Apium graveolens* seeds 150 mg, *Apium graveolens roots* 200 mg, *Cichorium endivia* 150 mg, *Cassia fistula* 150 mg, *Astercantha longifolia* 100 mg, *Trianthema portulacastrum* 100 mg, *Andrographis paniculata* 100 mg, *Terminalia chebula* 100 mg, *Artemisia absinthium* 100 mg, *Leonurus cardiaca* 100 mg & other ingredients q.s.	Gout Hepatitis, Obstructive jaundice, Weakness of the Liver, Dropsy, *Acne vulgaris* due to liver disturbance, Constipation, Chronic and drug induced G.I. troubles, Hepatic fever, Anorexia.	Adults: mix with water and take 2-4 teaspoonfuls twice a day, half an hour before meals.

Blood Sampling: Venous blood samples were collected from normal human volunteers (n = 10) who were fasting and had no record of contraceptive use or anticoagulant treatment. Strict sterile conditions were used for every surgery. A sterile syringe was used to extract 5 mL of blood from each volunteer. Following that, 500µL of each blood sample was meticulously moved into Eppendorf microcentrifuge tubes that had been previously weighed in order to facilitate coagulation. The ethics committee of Primeasia University's pharmacy department in Banani, Dhaka, Bangladesh, gave its approval to the study protocol. Before blood collection, each participant provided written informed consent.

Thrombolytic Activity: To allow for the growth of clots, fresh blood samples were placed in sterile Eppendorf tubes that had been previously weighed and incubated at 37 °C for 45 minutes. The tubes were centrifuged for 10 minutes at 2,000 rpm to extract the serum without upsetting the clots once they were fully formed. Following the safe disposal of the serum, every single tube holding the clot was weighed to determine the original clot weight.

The thrombolytic activity was evaluated using various concentrations of the test formulation, Hepatolin and Hepa-10. Separate additions of low concentration (100 µL) and higher concentration (200 µL) were made to the tubes containing clots. Streptokinase was used as the positive control and sterile distilled water as the negative control. All tubes were then incubated for 90 minutes at 37 °C to promote clot lysis. After incubation, the fluid that had been produced from the lysed clots was carefully extracted using a micropipette so as not to harm the remaining clot. The final clot weight was determined by weighing the tubes again. The percentage of clot lysis was calculated using the following equation:

Thrombolysis percentage (%) = The difference in the weight of the thrombus between before and after the addition of the sample / Weight of the thrombus before the addition × 100

Heptolin and Hepa 10's thrombolytic activity was evaluated using this technique in contrast to standard and control therapies at both low (100 and 200 µL) and high doses.

RESULT:

TABLE 2: RESULT OF THROMBOLYTIC ACTIVITY OF HEPATOLIN AND HEPA 10

Sample	Mean ± SEM (Clot lysis %)
Negative Control	2.8 ± 0.48
Standard Streptokinase	58.52 ± 2.02
Hepatolin (100 µL)	26.11 ± 2.09****
Hepatolin (200 µL)	51.52 ± 0.43*
Hepa 10 (100 µL)	24.36 ± 1.93****
Hepa 10 (200 µL)	42.30 ± 1.64***

The results of this study are presented as mean ± SEM (n = 3). When compared to the standard Streptokinase, statistical significance was indicated by ****p< 0.0001, ***p < 0.001, and *p < 0.05. In comparison to streptokinase, Hepatolin showed significant thrombolytic activity ($p < 0.0001$ at 100 µL and $p < 0.05$ at 200 µL). Hepa 10 also demonstrated significant action at both concentrations, with $p < 0.0001$ at 100 µL and $p < 0.001$ at 200 µL.

DISCUSSION: Currently, a large number of medications that have received FDA approval come from plant sources. According to the documented immunomodulatory effects, plant-derived chemicals have recently taken on a more significant role, which has prompted a thorough scientific analysis to ascertain their safety and effectiveness [18-19]. Two distinct hepatoprotective polyherbal formulations showed thrombolytic efficacy in our current investigation. Streptokinase (SK) was used as a reference standard medication to evaluate the thrombolytic activity. The thrombolytic activity of several polyherbal formulations was assessed and compared by using standard Streptokinase **Table 2.** The results indicate potential thrombolytic properties since the tested formulations

demonstrated varying degrees of clot lysis. Streptokinase, the positive control, demonstrated the strongest thrombolytic effect, as evidenced by its (58.52% ± 2.02) clot lysis rate. However, the negative control (distilled water) demonstrated very little clot breakdown (2.8% ± 0.48) due to the lack of fibrinolytic properties. Hepa 10 (100 µL) had a clot lysis rate of (24.36% ± 1.93), much greater than the negative control but significantly lower than the positive control. Hepa 10 (200 µL) had a clot lysis rate of (42.30% ± 1.64), suggesting a dose-dependent effect where a higher concentration led to better clot dissolution. In comparison to Hepa 10 (100 µL), Hepatolin (100 µL) demonstrated a clot lysis rate of (26.11% ± 2.09), which is lower than Hepa 10 (200 µL). The highest clot lysis rate among the herbal formulations and the one that was closest to the positive control was (51.52% ± 0.43) for Hepatolin (200 µL). According to these findings, Hepatolin has the highest thrombolytic impact of all the investigated polyherbal formulations, especially at 200 µL.

Despite the encouraging outcomes, there are certain restrictions. The research was carried out *in-vitro*, which might not accurately represent the intricacy of blood clot development in a real system, which highlights the absence of *in-vivo* testing. Besides, it was not possible to pinpoint the precise phytochemicals that caused the clot lysis activity, which causes the phytochemicals to be unidentified.

CONCLUSION: The results of this study show that the chosen polyherbal formulations have significant *in-vitro* thrombolytic activity, suggesting that they may be useful for managing thrombus. Hepatolin at a greater concentration (200 µL) demonstrated the most noticeable clot destruction among the studied samples, with activity similar to that of the conventional thrombolytic agent. Both formulations showed a definite dose-dependent impact, indicating that their fibrinolytic capacity improves at higher concentrations. These results provide evidence for the hypothesis that polyherbal preparations may aid in the successful breakdown of clots because of their combined phytoconstituents. The observed action provides a scientific foundation for the traditional use of these mixtures and emphasizes their therapeutic potential.

Overall, this study shows that the polyherbal compounds under investigation are physiologically active and might be good candidates for thrombolytic treatment research, promoting more in-depth investigation of their pharmacological potential.

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CONFLICT OF INTEREST: Nil

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