

IN-VITRO THROMBOLYTIC ACTIVITY OF HERBAL ANTI-ARTHEROSCLEROSIS FORMULATION

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Abstract

In recent study, the Herbal Anti-atherosclerosis Formulation comprising of Lemon, Garlic, Ginger and Apple fruit extract vinegar have been generally recognized as agents for prevention and treatment of cardiovascular and other metabolic ailments, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes and cholesterol bringing down impact. Accordingly, the present study was intended to investigate thrombolytic properties of Herbal Anti-atherosclerosis Formulation. Natural Anti-atherosclerosis Formulation of demonstrated exceptionally huge ($P < 0.001$) clot lytic properties in different blood samples. The percent clot lytic activity was compared with negative control (water) and standard enzyme positive control (streptokinase). The mean % of clot lysis for water and streptokinase was discovered 4.71% and 86.21% individually. Then again the mean percent clot lytic activity of Herbal Anti-atherosclerosis Formulation was discovered 29.51%, which is huge contrast and the positive and negative control. The present research recommends that Herbal Anti-atherosclerosis

Formulation has significant thrombolytic action. Consequently the detailing may be a wellspring of powerful natural medication.

Keywords: Thrombolytic activity, Streptokinase, Herbal Anti-atherosclerosis Formulation

Introduction

Herbal medicine is utilized to treat numerous conditions, for example, asthma, dermatitis, premenstrual disorder, rheumatoid joint pain, headache, menopausal manifestations, unending weariness, fractious inside disorder, and growth, among others [1]. In many situations, researchers aren't sure what particular ingredient in a specific herb attempts to treat a condition or ailment. Entire herbs contain numerous ingredients, and they may cooperate to deliver a valuable effect. Frequently, herbs may be utilized together on the grounds that the mixture is more effective and may have less effects [2]. The point of the present research is to find out the thrombolytic effect of Herbal Anti-atherosclerosis Formulation. Therefore the formulation may be a source of powerful natural medication.

A blood clot (thrombus) produce in the blood circulatory system due to the failure of homeostasis reason vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as acute myocardial or cerebral infarction, at times prompting to death. Usually utilized thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) to dissolve clots [3]. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be vast effective, limited fibrin specificity and bleeding tendency. In view of the inadequacies comings of the available thrombolytic drugs, attempts are in progress to create enhanced recombinant variations of these medications [8]. Heparin and Aspirin are just decently efficient for acceleration of lysis and prevention of reocclusion, however are safe. More particular thrombin inhibitors and antiplatelet agents are more powerful, yet their safety stays to be affirmed [4]. Proceeded investigation in this area will provide new insights and advance advancement toward the improvement of the perfect thrombolytic treatment, characterized by maximized stable coronary arterial thrombolysis with minimal bleeding [5].

Materials and Method

Reagents and chemicals

Streptokinase(SK) vials of 15, 00, 000 I.U.10 blood (5ml) sample drawn from healthy human volunteers, Herbal antiatherosclerosis formulation, Distilled Water.

Apparatus

Micro centrifuge tube (0.5ml/tube), Micropipette, Vortex mixer, 0.22-micron syringe filter, Beaker, Electric Balance, Incubator.

Experimental procedure

Streptokinase (SK)

To the commercially available lyophilized SK vial (Durakinase, Dongkook Pharma. Co. Ltd. South Korea) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 μ l (30,000 I.U.) was used for in vitro thrombolysis.

Specimen

Whole blood (4 ml) was drawn from healthy human volunteers (n = 20) 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 μ l (0.5 ml) of blood was transferred to each of the three previously weighed microcentrifuge tubes to form clots.

Herbal preparation

100 mg Herbal Antiatherosclerosis formulation was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22-micron syringe filter. 100 μ l of this aqueous preparation of herb was added to the microcentrifuge tubes containing the clots to check thrombolytic activity^[7].

Clot lysis

4 ml venous blood drawn from healthy volunteers was distributed in three different pre weighed sterile microcentrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 minutes. 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 – weight of tube alone). To one microcentrifuge tube containing pre-weighed clot, 100 μ l of aqueous extract of Herbal Anti-atherosclerosis Formulation was added. As a positive control, 100 μ l of SK and as a negative non thrombolytic control, 100 μ l of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 minutes 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. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot

lysis. The experiment was repeated 20 times with the blood samples of 20 volunteers [10, 11, 12, 13, 14, and 15].

$$\% \text{ Clot lysis} = \frac{2\text{nd wt.} - 3\text{rd wt.} \times 100}{2\text{nd wt.} - 1\text{st wt.}} [15]$$



Figure 1: Micro centrifuge tubes used in the experiment.
Result and Observation

Effect of Herbal Anti-atherosclerosis Formulation on *in vitro* thrombolysis:

Blood sample	% of Clot lysis		
	Control (water)	Streptokinase	Formulation
1	4.20	87.20	37.22
2	5.73	85.00	35.22
3	4.58	86.80	30.24
4	4.89	87.36	24.31
5	5.26	84.60	33.41
6	4.13	85.10	25.26
7	4.27	84.18	26.31
8	4.00	86.16	27.34
9	5.65	85.12	28.66
10	5.28	86.2	27.22
Mean	4.706	86.2075	29.519

Table 1: Comparing the data of % of Clot lysis using SPSS 11.5

Group Statistics (Control vs. Streptokinase)

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Control	10	4.7990	.65113	.20590
	Streptokinase	10	85.7720	1.12211	.35484

Group Statistics (Control vs. Formulation)

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Control	10	4.7990	.65113	.20590
	Formulation	10	29.5190	4.39455	1.38968

Group Statistics (Formulation vs. Streptokinase)

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Streptokinase	10	85.7720	1.12211	.35484
	Formulation	10	29.5190	4.39455	1.38968

Here, all values are expressed as MEAN ± SEM (n=20).

***P<0.001 significant compared to negative control.

Effect of Clot lysis

Sample	Result
Streptokinase (STD)	86.21.56%
Distilled water (control)	4.71 %
Sample (Herbal anti-atherosclerosis formulation)	29.51 %

% Clot lysis activity of herbal formulation:

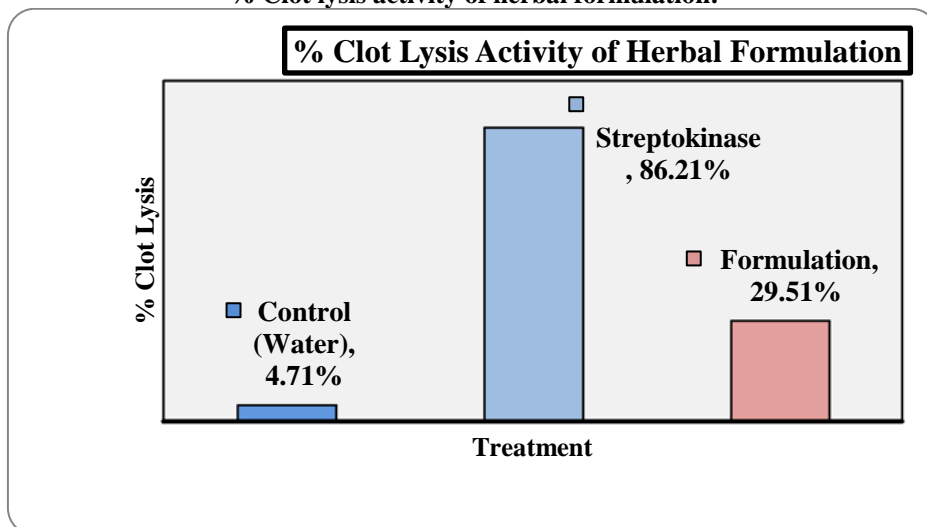


Figure 2: Comparative % in vitro thrombolytic effect of formulation, streptokinase and water (negative control).

Discussion

Addition of 100µl Streptokinase (Durakinase, Dongkook Phama. Co. Ltd, South Korea) , a positive control (30,000I.U.) to the clots along with 90 minutes incubation at 37°C, showed 86.21% clot lysis. On the other hand, clots when treated with 100µl sterile distilled water (negative control) showed only negligible clot lysis which was only 4.71%.The mean difference in clot lysis percentage between positive and negative control was very significant (**p value<0.001).But when 100µl Herbal Anti-atherosclerosis Formulation was added to 20 different clots, 29.51% clot lysis were obtained and when compared with the negative control(water) the mean clot lysis percentage differences was significant (**P value<0.001).Percent clot lysis obtained after treating with water, streptokinase and Herbal Anti-atherosclerosis Formulation shown in Fig.4.35 .Statistical representation(*Student's t-test*) of the effective clot lysis percentage by Herbal anti-atherosclerosis formulation, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is tabulated in Table 4.17. Percentage of clot lysis of 20 different blood samples after treated with water, streptokinase and Herbal Anti-atherosclerosis Formulation is shown in Table.

Here, Clot weight = weight of clot containing tube – weight of tube alone.

All the tubes were incubated at 37°C for 90 minutes and observe for clot lysis. After Incubation fluids released was removed and tubes were again weighted to observe the difference in weight after clot disruption. Differences obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis.

Conclusion

Atherosclerosis-induced heart attacks and strokes are leading reasons of morbidity and mortality. Current essential and auxiliary prevention strategies emphasize control of different atherosclerotic danger components, including smoking, hypertension, hypercholesterolemia, diabetes mellitus, weight, irritation, and homocysteine^[16]. Current pharmacological studies recommend remedial estimations of these natural preparations, including lowering of blood pressure and lipids, antioxidation, thrombolytic activity and the promotion of microcirculation. There is a requirement for more goal and scientific approaches to authenticate individual herbs to identify chemical constituents, detect adulteration or contamination of herbs, and screen the quality of herbs and herbal medicines^[17]. There is also a need to check the consistency of different batches of herbs utilized as a part of this study and to distinguish bioactive parts in herbs reported to have physiological effects.

Under this study, Herbal Anti-atherosclerosis Formulation of demonstrated very significant ($P < 0.001$) clot lytic properties in different blood samples. The percent clot lytic activity was compared with water (positive control) and standard enzyme streptokinase (negative control). The mean % of clot lysis for water and streptokinase was found 4.71% and 86.21% separately. Then again the mean percent clot lytic activity of Herbal Anti-atherosclerosis Formulation was found 29.51%, which is significant compare with the positive and negative control. So, the present research proposes that, the Herbal Anti-atherosclerosis Formulation has significant thrombolytic activity. Thus the formulation may be a source of effective herbal drug.

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