Original Article

Study of garlic effect on fibrinolytic activity of the blood clot in vitro

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Abstract

Introduction

The main function of the fibrinolytic system is to dissolve fibrin clots in circulation. This system is composed of inactive precursor plasminogen which can be converted into plasmin by the proteolytic enzymes like tissue-type plasminogen activator (tPA). Fibrinolytic properties can be found in a variety of medicine plants and they could effectively prevent cardiovascular diseases (CVD). One of these medicine plants is Allium sativum, which was used for its antiplatlet and fibrinolytic effects in patients with CVD (garlic).
This study considers to find the fibrinolytic effect of the various concentration of garlic extract and the time of the most effect.

Methods

Garlic extracts were prepared using 70% ethanol, and labeling fibrinogen with fluorescent agent to create a labeled clot. Then 10, 25 and 50µg/µl of garlic extract were separately added to plasma environment and finally the labeled clots were inserted. At the end fluorescence intensity of the supernatant was measured. The data were analyzed using SPSS (version 16).

Results

The results indicated that garlic extract showed fibrinolytic effect significantly compared with the control group ($p<0.05$). Various concentrations of garlic extract showed significant different rise in the fibrinolytic activity of blood clot in different time ($p<0.05$). Desirable result obtained in the lower concentration of (10µg/µl) after 7 hours.

Conclusion

Garlic extract displayed the fibrinolytic activity which concentration and time factors of exposure influenced it but the minimum concentration and the maximum time showed the best result.

Key words

Fibrinolytic, Allium sativum (garlic), Hydro-ethanol, Extract

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Introduction

Fibrin is an insoluble protein that can be dissolved by two proteolytic enzymes; thrombin and plasmin. They could be activated by circulating plasma precursor plasminogen or prothrombin, which they could be activated by tissue-type plasminogen activator (tPA) or urokinase plasminogen activator (uPA) few days after wound repaired (2). Regulation of the fibrinolytic enzyme system is due to activity of Plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2). Unwanted accumulation of fibrin in the arterial can decrease fluidity of blood circulation and cause cardiovascular diseases (CVD) such as myocardial infarction and stroke (3). Current medication such as coumarin and warfarin are the cost-effective fibrinolysis treatment (4). Urokinase which was derived from human urine has been broadly employed for thrombosis therapy, but it has low specificity to fibrin and high cost (5).

Garlic (Allium sativum) consumption is an alternative thrombolysis medicine, which has been used for many years in different cultures (6). Allicin, one of the garlic components, could have a therapeutic effects, including anti-microbial effect, immunostimulating properties, improve fibrinolytic activity, inhibit platlet aggregation and adhesion and also reduce blood pressure (7).

Many studies showed garlic positive effect in cardiovascular disease treatment, but there are still some controversies about this function (7). The present study investigated the fibrinolytic effect of the garlic in vitro.

Materials and Methods

1) Method of extraction

Five grams of the pulverized sample was mixed with hydroalcoholic solution (70%ethanol) and kept at room temperature for 48 hours. The mixture was shaken for two hours before being filtered (8). The solution was reduced by one – third through evaporation. The extract was dried out at 37 ºC incubators overnight and then the dried powder dissolved in 5 ml ethyl alcohol and retained as the stock solution.

2) Labeling fibrinogen with FITC

Two percent Fibrinogen solutions (Green Cross, Korea) was prepared by adding 2 ml water to 0.04 grams of human fibrinogen. The solution was buffered using NaHCO3 (0.1M) buffer and it was labeled by adding 1mg Fluorescein isothiocyanate (FITC) (Sigma, Germany). After incubation at 4°C at dark place, the column chromatography containing G100 (Sigma, Germany) was used in order to remove unused FITC and purify fibrinogen-FITC complex (9).

3) Preparing labeled clots

Labeled clots were prepared by adding 10 µl Calcium Chloride (1M) to 0.5 ml plasma and 25 µl fibrinogen-FITC solution. The mixture was incubated for 80 minutes at 37ºC. The supernatant was discarded and the labeled clots were washed three times, using physiologic serum and centrifuged at 4000rpm for 15 minutes (10).

4) Fibrinolysis assay

Various volumes (10, 25, 50µl) of the garlic extract were added to 2 ml of plasma as positive controls and maintained at 4°C for 24 hours. A mixture containing 2ml plasma and particular volume of ethyl alchohol was utilized as a negative control. After inserting labeled clots to 50 µl plasma, samples were taken at 7 consecutive hours (between half an hour and every one hour) and enhanced to 2 ml by 0.1M PBS. The fluorescence intensity of the samples was measured in excitation of 495nm and emission of 515nm by a spectrophotofluorimeter. (Parkins-Elmer,Germany).

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5) Statistical Analyses

The data was analyzed using SPSS (version 16.0) software. Each test was conducted two times and the mean ± SD was determined. The mean differences were done by one-way ANOVA test. P value less than 0.05 was significant.

Results

The results showed that every concentration of the garlic extract increased the level of fibrinolysis in compared with control, but various concentrations did not have significant differences.Figure 1 illustrates the effect of the different garlic extract concentrations on fibrinolytic system.

Fibrinolytic effects were increased significantly from 40.83 in t 0 to 52.5 in t7 (Figure 2).

The intensity of fluorescence in various concentrations and consecutive time showed that the most desirable result was seen in 10 µg/µl concentration in the seventh hour (t7) (Figure3).

Discussion

The fibrinolytic enzyme prevent formation of fibrin clots in circulatory system(2). Some medicines like urokinase and streptokinase are widely used to inhibit homeostatic disorders, particularly thromboemboli(11). Previous studies have conducted to find the effects of herbal medicine in homeostatic disorders (12).

Fibrinolytic effects of garlic have been extensively studied in vivo. One Indian study showed that intake of garlic in a regular diet could remove fibrin clots and reduce the incidence of CVD (13).

Almost all human researches on fibrinolytic activity of garlic have been found to have positive effect in fibrinolysis (14-16). Other study showed antiplatelet garlic activity, and inhibiting platlet thromboxan formation (17). One Meta analysis has been suggested possible short-term benefits of garlic to reduce blood lipid and have antiplatelet effect (18).

An animal study showed that feeding rabbits for 3 months with cholesrol decreased plasma fibrinolytic activity (19).

In the present study, the fluorescence intensity indicated significant rise over the time. Garlic extracts increased blood clot lysis in compared with control group which demonstrates fibrinolysis effect of garlic hydro-alcohol extracts. Garlic components, adenosine and allicin, both could raise fibrinolysis effect as previous studies (20), which might prevent platelet aggregation without any impact on arachidonic acid metabolites such as cyclooxygenase and lipoxygenase. Trisulfides could induce new lipoxygenase metabolites through synthesizing of thromboxane and inhibiting of platelet aggregation.

Our study also demonstrated that increasing the concentration of garlic extract does not have a notable influence on the fluorescence intensity, therefore the lysis of blood clots could be related to a lack of additional plasminogen in the environment. In fact the concentration of 10µg/µl is the best concentration. Our results suggested that the concentration of 10µg/µl in the longest time (t7) accounted for the highest rate of blood clot lysis.

Although the current study examined fibrinolytic effect of garlic in vitro, a novel and simple method i.e. Fluorescence assay is introduced for further and future studies.

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Fig 1) Comparison of fibrinolytic activity of different concentrations of garlic extract with control group

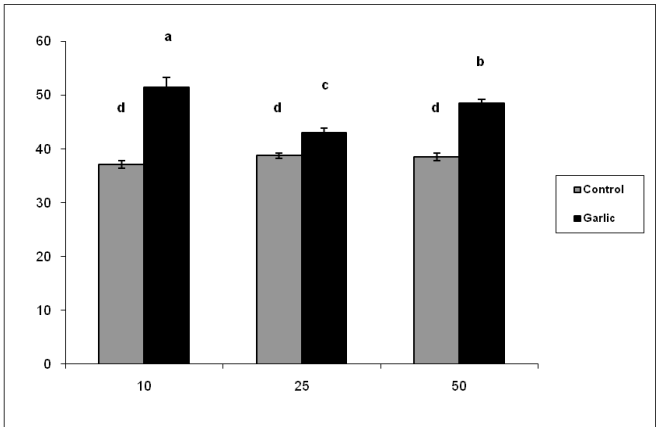


Fig 2) Effect of time on fibrinolytic activity of garlic extract

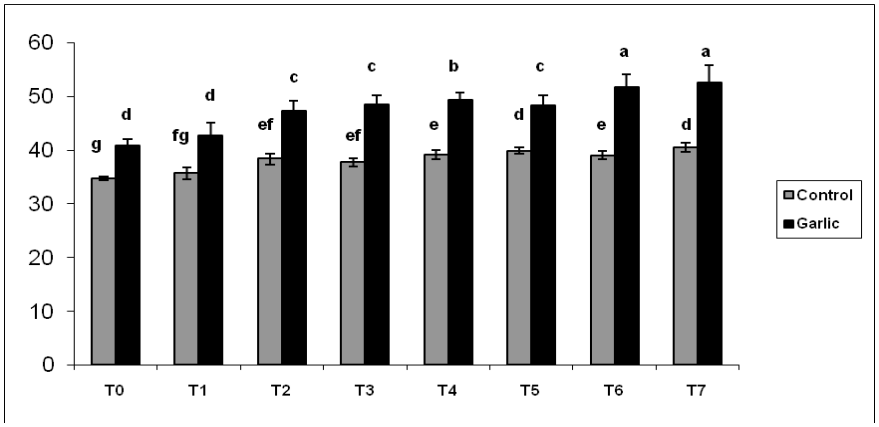
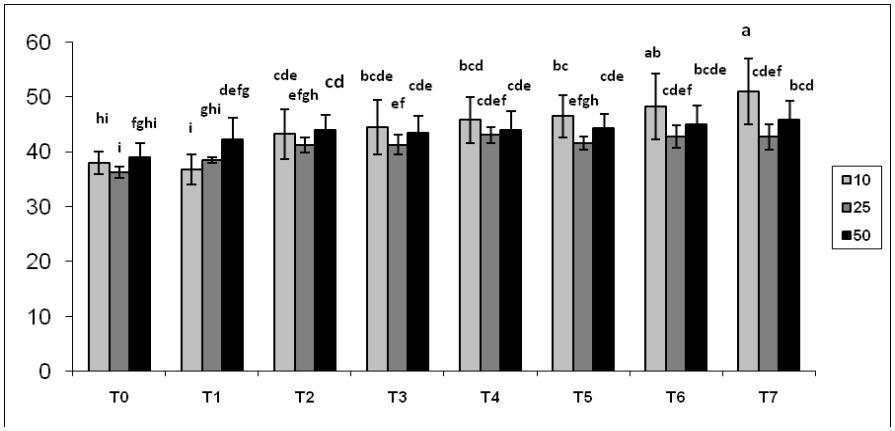


Fig 3) Interaction of garlic extract concentration and time on fibrinolytic activity



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