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A Blood Anticoagulant Substance from Garlic (*Allium Sativum*) I.

Its Preparation and Studies on its Anticoagulant Effect

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ABSTRACT

The blood anticoagulant factor (G. E.) in garlic was isolated. This substance was precipitated at a neutral pH as the calcium salt in water, and then the calcium salt was dissolved at a pH of 3.0. Calcium was removed by adding sodium oxalate. Then G. E. was precipitated by adding two volumes of 95% ethanol.

The effect of G. E. on blood coagulation was studied; prothrombin time, blood clotting time, antithrombin, anti-Ac-globulin and fibrinolysis. A half mg of G. E. completely inhibited one ml of blood from coagulating. The blood specimen containing G. E. showed a prolongation of the prothrombin time.

As the calcium ion concentration increased, the prothrombin time of the plasma containing G. E. was reduced, but not to that of the control(oxalated plasma). This indicated that G. E. inhibited the prothrombin time by precipitating calcium ions, and, in addition to this calcium precipitation, another means of G. E. inhibition may be present. G. E. showed fibrinolytic effects and, in the prothrombin time tests, the plasma containing G. E. always showed less fibrin formation than was shown with oxalated plasma.

G. E. showed inhibition of fibrin formation in experiments on its antithrombic effect. But this action may not be due to the antithrombin effect of G. E. but to the fibrinolytic effect of G. E.

In in-vivo experiments G. E. did not show any anticoagulant effect.

From these facts, it may be said that G. E. has an anticoagulant effect in at least two ways in vitro; first by precipitating calcium ions and secondly by causing fibrinolysis.

INTRODUCTION

Song et al. (1960) have isolated a blood anticoagulant substance from garlic and studied its physical and chemical properties.

This garlic extract (G. E.) is precipitated in a neutral sulution as the calcium salt and dissolved after adjusting the pH to 3.0 with hydrochloric acid.

Because of these chemical properties, G. E. was isolated more easily than before and its anticoagulant effect was still as active as a previously reported.

In this paper, this modified method of preparation of the anticoagulant factor from garlic is described and its anticoagulant effects are reported.

MATERIALS AND METHODS

Preparation: 200gm of garlic in 500 ml of distilled water were macerated in a Waring blendor and another 500 ml of distilled water were added to the garlic solution, which was filtered through gauze. Hydrochloric acid was added to the filtrate and mixed until a pH of 3.0 was obtained. Then the mixture stood for about 20 minutes, after which it was filtered to obtain a protein free filtrate, which was neutralized with sodium hydroxide. Next, 40 ml of 10% calcium chloride were mixed with filtrate. The precipitated G. E. calcium salt was collected by centrifuging. The precipitate was then dissolved in 30 or 40 ml of distilled water and adjusted to pH of 3.0 with hydrochloric acid and again centrifuged. 20ml of 3% sodium oxalate were added to the supernatant liquid and centrifuged. Under low pressure

this supernatant liquid was concentrated to a volume of 20 ml. Two volumes of 95% ethanol were added to the concentrate and kept in a refrigerator overnight. The precipitate was dissolved in 5ml of distilled water and undissolved materials removed by centrifuging.

The supernatant liquid was acidified with hydrochloric acid to pH of 3.0 Two volumes of 95% ethanol were added and kept in a refrigerator overnight. The precipitate was dried under low pressure and kept in a desiccator. The yield was about 150 mg from 200 gm. of garlic.

Plasma containing G. E.: Plasma containing G. E. was made by an adding 1.8ml of human blood to various amounts of the sodium salt of G. E.(1 to 30 mg range) dissolved in 0.2ml of 0.9% saline solution

Blood clotting time: To a series of small test tubes standing in a water bath of 37°C. 0.1 ml of various concentrations of G. E. dissolved in 0.9% sodium chloride solution was added. One ml of blood was added to each tube and mixed carefully for a few seconds. The clotting time was determined. For comparison, potassium oxalate was added in place of G. E. and the clotting time was determined.

Prothrombin time: Prothrombin time was carried out by Quick's method (1945). To the plasma containing G.E. 0.1 ml of various concentrations of calcium chloride (0.02–0.06M) was added, and the prothrombin time determined. For the control, oxalated plasma was used.

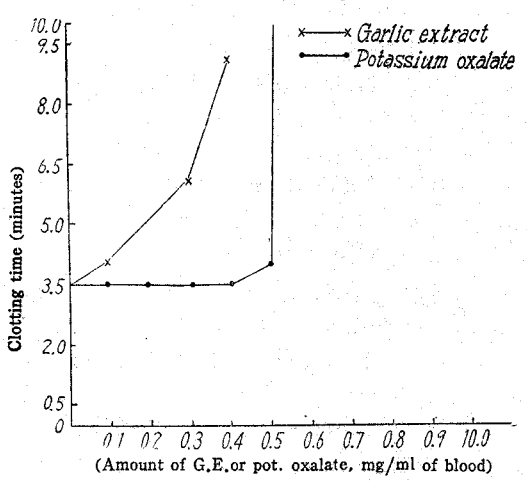


Fig. 1. Effect of G.E. on blood clotting time.

Fibrinolytic effect of G.E.: To a series of tubes containing 10mg (wet weight) of human fibrin in one ml of physiological saline, various amounts of G. E. (5 or 10, 20, 25mg) were added and the tubes were incubated at 37°C. for 24 hours.

Antithrombin effect: To 0.1ml of plasma containing various amount of G. E., 0.1ml of thrombin solution prepared by Eagle method(Hawk and Oser, 1947) was added, and the clotting time determined. Oxalated plasma was used as a control.

Effect of G. E. on Ac-globulin: Human blood was kept at room temperature overnihgt. The separated serum was used as a crude Ac-globulin. Prothrombin time was determined on samples of plasma containing G. E. plus Ac-globulin, and on samples of plasma containing G. E. plus saline as a control.

Effect of G. E. in Vivo: 100–150mg of G. E. dissolved in three or four ml of 0.9% saline was administered slowly through the vein of rabbit ear, and the prothrombin time and clotting time determined. 70mg of G. E. were injected intravenously into a rabbit and the prothrombin time and blood clotting time determined at intervals. Five rabbits (body weight 1.5–2.5kg) were used in these experiments.

RESULTS

The effect of G. E. on blood clotting time is shown in Fig 1. Its effect was compared with that of potassium oxalate. 0.5mg of G. E. completely inhibited one ml of blood from coagulation. The inhibiting effect of G. E. on blood clotting was almost the same as that of potassium oxalate.

G. E. showed a prolongation of the prothrombin time as compared with that of sodium oxalate (Table 1). 5mg of G. E. dissolved in one ml of blood completely inhibited fibrin formation. Even though the amount of G. E. was reduced to only 1.5 mg, the test prothrombin time was still longer than the control.

The effect of G. E. on prothrombin time at various concentrations of calcium ions is shown in Table. 2. As the concentration of calcium ions was increased, the prothrombin time of plasma containing G. E. became reduced but not down to that of

Table 1. Effect of G. E. on prothrombin time

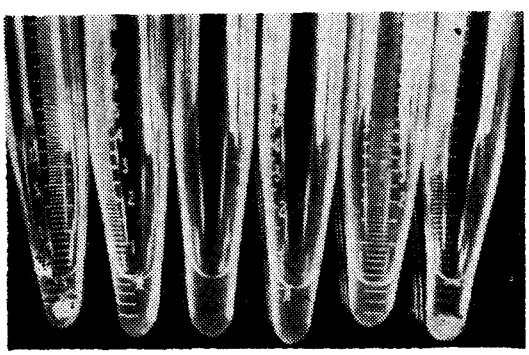
Amount of G. E. in ml of blood (mg)	0.5	1.5	2.5	5.0	7.0	Oxalated plasma
Prothrombin time(sec.)	13	24	26	>180	>180	13

Table 2. Relation to the concentration of calcium ion

Calcium chloride(M)		0.02	0.025	0.03	0.035	0.04	0.06	0.08
Prothrombin time(seconds) Amount of G. E. in 1 ml of blood(mg)	0.5	—	13	—	—	—	—	—
	1.5	24	24	26	—	26	—	81
	2.5	25	26	30	29	30	32	122
	5.0	—	>180	49	38	43	59	121
	7.5	—	—	—	>180	60	71	67
	10.0	—	—	—	—	—	—	>180
Oxalateed blood	13.4	13	13	13	20	22	31	36

Table 3. Antithrombin effect of G. E.

Amount of G. E. in ml of blood (mg)	2.5	5.0	7.5	15.0	20.0	25.0	Oxalated plasma
Clotting time (seconds)	7	9	105	125	>180	>180	7



※ 5 10 15 20 25 0
※ mg of G.E./ml and each tube contains 10mg of fibrin

Fig. 2. Fibrinolytic effect of garlic extract.

the control (oxalated plasma). Also, as amount of G. E. was increased the prothrombin time was pro-longed. At a level of 1.5 or 2.5mg of G. E. dissolved in one ml of blood even though the concentration of calcium ions was increased there was no change in the prothrombin time. Plasma containing G. E. showed a prolonged prothrombin time in the antithrombin experiment as is shown in Table 3.

The fibrinolytic effect of G. E. is shown in Fig.2 Fibrin in the tubes containing 10~25mg of G. E. was dissolved completely. 5mg of G. E. dissolved the fibrin partially while, in the tube containing no G. E., the fibrin remained undissolved.

In measuring the prothrombin time it was noted that the amount of fibrin formation always was less in the plasma containing G. E. than in the oxalated plasma control.

G. E. did not show any anti-Ac-globulin effect.

When 100-150mg of G. E. were administered intravenously to rabbits, the rabbits always died. The blood from heart of the dead rabbits did not coagulate or had either a normal or a prolongation of the clotting time. However, the prothrombin time was always within normal limits. When 70 mg of G. E. were injected intravenously there were no changes in the clotting time nor in the prothrombin time.

DISCUSSION

G. E. showed an anticoagulant effect. A half mg of G. E. completely inhibited one ml of blood from coagulating. The inhibiting effect of G. E. on blood clotting was almost the same as that of potassium oxalate. The plasma containing G. E. showed a prolongation of prothrombin time as compared with that of oxalated plasma. As the concentration of calcium ions was increased, the prothrombin time was reduced but not down to that of the control (oxalated plasma). G. E. has a fibrinolytic action as shown in Fig. 2 and in performing the prothrombin time tests, the plasma containing G. E. always showed less fibrin formation than was shown with oxalated plasma control.

Plasma containing G. E. showed a prolonged time for fibrin formation in the antithrombin experiment as shown in Table 3. This result may not be due to the antithrombin effect of G. E. but to the fibrinolytic effect of G. E. Anti-Ac-globulin effect of G. E. was not revealed. In vivo, neither the blood clotting time nor the prothrombin time inhibited by G. E. The amount of G. E. considered to be the inhibiting dosage in vitro experiments on blood clotting time always killed the rabbits. Non-toxic lower doses did not show any anticoagulant effect in vivo.

From these facts, it may be said that G. E. have an anticoagulant effect in at least two ways in vitro experiment; first by precipitating calcium ions and secondly by causing fibrinolysis.

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