

# Effects of garlic on haemostatic parameters and lipid profile in hyperlipidemic rats: antiatherogenic and antithrombotic effects

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**Abstract.** The effects of garlic on haemostatic parameters and lipid profile in hyperlipidemic rats were studied.

Thirty rats were allocated randomly into 3 groups, each of 10 rats. One group was put on normal chow diet and acted as normal control group. The other 2 groups were given high cholesterol diet for 8 weeks.

Both groups became hyperlipidemic after 8 weeks. One group received distilled water and acted as hyperlipidemic control while the other group received garlic (200mg/kg) for 4 weeks. Coagulation parameters and lipid profile were measured after 4 weeks of treatment with garlic. Blood samples were collected from all rats before and after commencement of the treatment to measure coagulation parameters, namely fibrinogen, activated partial thromboplastin time (APTT), prothrombin time (PT) and platelet count as well as serum lipid profile, namely total serum cholesterol (S.TC), serum triglyceride (S.TG), high density lipoprotein (HDL), and low density lipoprotein (LDL). These parameters were also measured after high cholesterol diet feeding. The results indicated that a significant reduction in fibrinogen, S.TC, S.TG, LDL ( $p < 0.01$ ); an increase in HDL ( $p < 0.01$ ); a prolongation in PT and APTT ( $p < 0.01$ ) and a significant decrease in platelet count ( $p < 0.01$ ) in hyperlipidemic rats after 4 weeks of treatment with garlic. Garlic may be recommended as an antiatherogenic and antithrombotic agent and is suggested to be used with food in patients with hyperlipidemia.

Key words: Garlic, hyperlipidemic, haemostatic parameters, antiatherogenic, antithrombotic

## 1. Introduction

Garlic is a plant member of the lily family closely related to onion. The important ingredient of garlic is alliin. The allinase, other ingredient of garlic, turn alliin to allicin which is responsible for garlic odor and plays a major role in lowering cholesterol (1,2). It was shown, in vitro studies, that garlic also contains s-allyl cysteine (SAC) and di-allyl-disulfide (DADS) which are potent inhibitors of cholesterol synthesis (3,4). It has been reported that garlic, either fresh or

extracted, normalize and remove to some extent the cardiovascular risk factor (5). Garlic enhances fibrinolytic activity and decreases plasma lipids, high blood pressure as well as high level of blood sugar (6).

The antihypertensive effect of garlic is attributed to its prostaglandin like effect that is leading to decrease in the peripheral vascular resistance. The gamma-glutamyl cysteines, one of the active ingredients of garlic, may decrease blood pressure by its ability to inhibit angiotensin converting enzyme (7) as well as elicits nitric oxide action and hence having pulmonary artery relaxant effect (8,9).

Garlic oil, di-allyl sulfide (DAS), (DADS), and di-allyltri-sulfide (DATS) play a role in modulation of antioxidant system in rats liver (10-12). Furthermore, garlic and its fraction

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(DAS) was proved in vitro of having antibacterial activities and to prevent or treat the nosocomial infection and antibiotic-resistant bacterial infection (13).

Studies on garlic and its effects on the different parameters have received a lot of attention. However, the effect of garlic on haemostatic parameters and serum lipid profile in hyperlipidemia have received little attention. In view of this deficiency and because garlic is considered as an important natural cardioprotective agent, the present work was undertaken in order to investigate the effects of garlic on haemostatic parameters, namely APTT, PT and platelet count and serum lipid profile, namely S.TC, S.TG, HDL and LDL in hyperlipidemic rats.

## 2. Material and method

400mg tablets, batch number 477, "Garlet" were supplied from Cosar Pharm. Co. The reagents and chemicals used in the measurement of S.TC; PT; APTT and S.TG were supplied by Bio-Merieux Company. The reagents used in the quantitative determination of plasma fibrinogen were supplied by Diagnostica Stago Company. The reagents used in the measurement of HDL-cholesterol were obtained from Joaquim Costa Company. All other chemicals used were of the highest grade available commercially.

### 2.1. Preparation of animals

Thirty male Sprague-Dawley rats (aged 16-18 weeks, weights 190-210 g) were supplied by the National Center for Drug and Research in Baghdad. The animals were housed in Kufa Medical College animal house and 12- hour light/dark cycles were provided. Food and water were given ad lib. Environmental conditions were maintained at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . The animals were left for 2 weeks without interference for acclimatization. Our investigations were performed after approval by our local ethical committee at Kufa University and in accordance with " Interdisciplinary Principles and Guidelines of the Use of Animals in Research".

The animals were randomly divided into 3 groups, each of 10 rats. Ten rats were put on normal standard chow for 8 weeks and acted as control group (group I ). Twenty rats were put on cholesterol rich diet (4% cholesterol), made by addition of cholesterol powder to standard chow diet, for 8 weeks. After 8 weeks, blood samples were taken from the animals and analyzed for

lipid profile. The hyperlipidemic rats were randomly divided into 2 groups each of 10 rats. One group received distilled water and acted as hyperlipidemic control (group II) and the other group received garlic for 4 weeks (group III). Before commencing the experiment, the normal values of all required parameters were taken and acted as normal control values. These control values were used for comparison with the values after treatment (self control).

### 2.2. Preparation of the drug

A concentration of 40mg/ml of garlic was prepared. This was achieved by dissolving a 400mg Garlet tablet in 10 ml of distilled water. A dose of 200 mg /kg was used (14). The appropriate volume of the preparation was given once daily through stomach tube.

### 2.3. Preparation of the sample

From each rat, 3 ml of blood was collected from caudal artery without the use of heparin after an overnight fasting. The blood sample was taken before and after 8 weeks of high cholesterol diet treatment as well as 4 weeks after garlic treatment for each group.

The sample was divided into 3 parts. The first 1 ml of blood was placed in a tube containing sodium citrate as anticoagulant in a ratio 9:1 (9 volume of blood :1 volume of anticoagulant). The plasma was prepared via centrifugation at 2500 rpm for 10 minutes for determination of fibrinogen, APTT and PT. Another 0.5 ml of blood was placed in a tube containing EDTA as anticoagulant in 9:1. Blood film was prepared for counting platelets. The last 1.5 ml of blood was placed in serum tube and left to stand for 30 minutes. The serum was prepared via centrifugation at 3000 rpm for 15 minutes. 0.6 ml of serum was obtained for determination of lipid profile

### 2.4. Reading of the samples

Quantitative determination of fibrinogen, measurement of APTT, PT and platelet count were carried out using the methods described by Dacie and Lewis (15). Measurements of S.TC, LDL and VLDL were carried out according to procedures of Friedwald et al. (16); S.TG by Assmann (17); and HDL-C by Tietz (18).

### 2.5. Statistical Analysis

All data expressed as mean  $\pm$  standard error of the mean (SEM) unless otherwise stated.

Independent sample t-test was employed to compare the statistical significance between control and experimental groups.

Table 1

Mean values of haemostatic parameters and serum lipid profile in normal control and hyperlipidemic control groups after 8 weeks of study.

Parameters	Normal Control	Hyperlipidemic control
Fibrinogen level (mg/dL)	179.9 <sup>*</sup> ±1.6	375.6±4.2
APTT (sec.)	24.9 <sup>*</sup> ±0.2	20.8±0.1
PT (sec.)	11.8 <sup>*</sup> ±0.1	8.6±0.1
Platelet count 1000/cmm	179.9 <sup>*</sup> ±2.9	286.5±2.2
S.TC (mg/dL)	103 <sup>*</sup> ±0.7	198.6±0.6
S.TG (mg/dL)	100.1 <sup>*</sup> ±1.6	209.4±0.6
HDL (mg/dL)	30.8 <sup>*</sup> ±0.7	35.4±0.4
LDL (mg/dL)	52.9 <sup>*</sup> ±0.6	121.8±0.9

Values are reported as mean ±SEM. n = 10, \* P < 0.01 between normal control and hyperlipidemic control groups for each parameter.

### 3.Results

#### 3.1.Establishment of hyperlipidemic model

After 8 weeks of cholesterol rich diet treatment, a significant changes were found in the haemostatic parameters and serum lipid profiles in the hyperlipidemic group compared with those in normal diet group. APTT and PT were significantly decreased (p<0.01) while fibrinogen, platelets count, S.TC, S.TG, HDL and LDL were significantly increased (p<0.01) (Table 1). The hyperlipidemic group were then acted as hyperlipidemic control for treatment group.

#### 3.2.Effects on haemostatic parameters

Table 2 shows that haemostatic parameters, namely fibrinogen level, APTT, PT and platelets count, were not changed significantly (p>0.05) in hyperlipidemic group after 4 weeks of treatment. However, these parameters were significantly changed (p<0.01) when hyperlipidemic group were treated with 200 mg/kg garlic for 4 weeks.

#### 3.3.Effects on serum lipid profiles

No significant difference (p>0.05) was detected in serum lipid profiles, namely S.TC, S.TG, HDL and LDL in hyperlipidemic group after 4 weeks of treatment (Table 2). A significant decrease in

S.TC, S.TG and LDL and a significant increase in HDL (p<0.01) was present when hyperlipidemic rats were treated with 200 mg/kg for 4 weeks (Table 2).

### 4.Discussion

Garlic reduced fibrinogen level in hyperlipidemic rats, after 4 weeks of treatment, compared with hyperlipidemic control group (Table 2). This reduction was highly significant (p<0.01) and would suggest that garlic has a potent fibrinolytic activity. The present results seem to conflict with a previous study (19) which reported that garlic has no significant effect on fibrinogen level. However, the latter findings were performed on normal healthy human subjects while the present study was done on hyperlipidemic rats and the biological variation could be a factor as well. The disturbance in coagulation-fibrinolytic systems may be an important factor leading to development of thrombosis and ischemia. The fibrinolytic activity of garlic, therefore, is a favorable antithrombotic effect.

The prolongation of APTT and PT in hyperlipidemic rats after 4 weeks of treatment with garlic (200 mg/kg) is highly significant (p<0.01) (Table 2). These results are supported by other studies (20,21). However, no mention was made regarding platelet aggregation and platelet count in the last two studies. The present study showed a significant inhibition of platelet aggregation as well as lowering of platelet count. A possible explanation for the present results is that garlic inhibits adenosine diphosphate (ADP), collagen, arachidonate, epinephrine, calcium ionophore as well as inhibits the formation of thromboxane, phospholipase and lipooxygenase formed in the platelets (22). Supporting evidence for this suggestion arises from the fact that allicin (one of the garlic's ingredients) inhibits platelet aggregation in vitro (23). Garlic may have direct interaction with the putative fibrinogen receptor (GpIIb/IIa) (22,24).

Garlic decreased the S.TC and S.TG levels in hyperlipidemic rats compared with hyperlipidemic control group and this lowering effect is highly significant (p<0.05). It is suggested that this effect may be due to the action of s-allyl cystein (SAC) and di-allyl-di Sulfide (DADS) present in garlic oil. These ingredients are potent inhibitors of monoxygenase enzyme.

Table 2  
Effects of garlic (200 mg/kg) on haemostatic parameters and serum lipids profile after 4 weeks of treatment

Group	Fibrinogen level (mg/dL)		APTT (sec)		PT (sec)		Platelets count (1000 cmm)		S.TC (mg/dL)		S.TG (mg/dL)		HDL (mg/dL)		LDL (mg/dL)	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after
Hyperlipidemic Control	375**±4.2	379±3.3	21**±0.2	20±0.1	9**±0.1	8±0.5	286**±2.2	282±2.1	198**±0.6	196±1.0	209**±0.6	209±0.7	35**±0.4	34±0.4	121**±0.9	120±0.8
Garlic	364*±6.4	229±2.2	21*±0.2	26±0.3	9*±0.2	17±0.2	283*±2.5	205±1.9	192*±5.3	122±1.2	208*±0.8	100±0.8	35*±0.4	61±1.4	120*±0.6	45±1.8

Values are reported as mean ±SEM. n = 10, \*P < 0.01 for each parameter, \*\* P > 0.05 for each parameter.

Inhibition of monooxygenase enzyme inhibits cholesterol synthesis (3,4,25). The lowering effect of garlic on S.TC may also be attributed to the fact that garlic increases the excretion of cholesterol. The latter effect is manifested by enhanced excretion of acidic and neutral steroids after garlic feeding (26). Furthermore, garlic depresses the hepatic activities of lipogenic and cholesterologenic enzymes, such as malic enzyme, fatty acid synthase, glucose 6-phosphate dehydrogenase (G6PD) and HMG-CoA reductase (4) which provide a possible explanation for the reduction effect of garlic on S.TG. Garlic caused a significant elevation in HDL and a significant reduction in LDL ( $p < 0.01$ ) after 4 weeks of treatment of hyperlipidemic rats. The significant reduction in LDL by garlic may be due to the suppression of LDL oxidation (27).

There is an inverse relationship between the concentration of HDL and the development of coronary heart disease (CHD) and vice versa for LDL. Based on this fact, the present results may suggest that garlic can be used as antiatherogenic agent by elevation of HDL and reduction of LDL.

The present results seem to conflict with a previous study which demonstrated that garlic shows an insignificant effect on S.TC, S.TG, HDL and LDL (28). This conflicting results may be due to the fact that the latter study was performed on children with hyperlipidemia. It could, therefore, be that the decreased sample size and biological variation would be behind this conflicting results.

## 5. Conclusion

It can be concluded that garlic Reduces fibrinogen, platelets count, serum TC, TG, LDL and increases APTT, PT and HDL Can be it useful as antithrombotic agent by reducing the fibrinogen level and as antiatherogenic agent by elevation of HDL and reduction of LDL.

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