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## BENEFICIAL EFFECTS OF *ALLIUM SATIVUM* LINN IN EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN CHICKEN. *Part II:* Curative Effects

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Curative effects of garlic were established in a chicken model of atherosclerosis. Atherosclerosis was produced in chickens by feeding them cholesterol-enriched feed for a period of 12 weeks and later administered twice weekly alongwith garlic for another 12 weeks. At the end of 24 weeks, the animals were sacrificed and their dorsal aortae were removed to examine the atherosclerotic lesions. The results of studies on blood showed that garlic administration leads to significant decrease in plasma levels of triglycerides, total cholesterol and LDL(+VLDL)-cholesterol. The aortic damage was also significantly lower in the garlic-administered group as compared to the model group fed on cholesterol-enriched feed alone. In addition, aggregation studies showed a marked inhibitory effect of the aqueous extract of garlic on platelet aggregation *In vitro*. Thus, it may be inferred that administration of garlic provides a significant protection against hyperlipidemia and possibly thrombus formation and therefore has a potential to protect against cardio-vascular anomalies.

***Key words:*** *Allium sativum,* Hypolipidemia, Chicken.

### Introduction

Garlic has long been used in the traditional system of medicine for a number of ailments [1]. However, its most significant beneficial effect is claimed to be in the cardiac conditions [2-4] and it is reputed for its cholesterol lowering and fibrinolysis activating effects [5]. Recently, it has again become the focus of attention and considerable work is appearing in the literature on its components and the mechanism of anti-aggregatory action [6-10]. Furthermore, its beneficial effects have been reported in infectious diseases [11], heavy metal intoxication [12], free radical damage [13], tumour formation [14,15] and immune deficiency diseases [16]. In addition to this it has been elucidated that raw garlic extract elevates the activities of Lactate dehydrogenase and trans ketolase in the liver and red cells of the rats [16]. As mentioned in the previous paper [17], hypolipidemic effects of garlic have been reported in cholesterol or lard fed rabbits and rats [18,22]. However, the lipoproteins of these animals are not as closely related to human lipoproteins as those of Chicken [19]. Therefore, chicken, was chosen as a model for studying the beneficial effects of garlic in experimental atherosclerosis. In this context, in a previous study, we reported the protective effects of garlic in cholesterol-fed chickens by simultaneous feeding of garlic [17]. In the present study, garlic was fed after producing a model of atherosclerosis by feeding cholesterol-enriched feed to chickens for 12 weeks and then administering garlic to see amelioration in their condition. For this purpose plasma lipid levels were assayed and the extent of damage to the dorsal aorta was evaluated by examining the lipid infiltration in the intima. Another group of animals was allowed to revert to normal feed but was not fed with garlic to assess the autoreversal of the disease. Platelet aggregation studies were also undertaken to assess the antithrombotic effects of garlic.

### Materials and Methods

One day old, male layer chickens (star cross) were used in these studies. The animals were fed on commercial feed [17] and had free access to feed and water. At the age of eight weeks, the animals were randomly divided into three groups, each comprising of 6 animals. Group I was fed on cholesterol-enriched feed for 12 weeks and thereafter reverted to normal feed but received cholesterol-enriched feed twice in a week. This group served as a control of atherosclerotic model. Group II animals were fed cholesterol-enriched feed for 12 weeks and then given garlic paste for another 12 weeks to see the curative effect. These animals also received cholesterol-enriched feed twice in a week. Group III animals were reverted to normal feed after 12 weeks of feeding on cholesterol-enriched feed to see the autoreversal of the symptoms. Biochemical analyses were carried out fortnightly for 12 weeks and then the animals were sacrificed to assess the aortic damage. The composition of the feed and details of incorporation of cholesterol (1%) in the feed have been reported previously [17]. Garlic paste was prepared by blending peeled cloves in a blender without addition of water. It was administered after addition of water (1:1) at the time of feeding with the help of a feeding tube. Each animal received 4 g of the paste daily in the afternoon.

Blood was withdrawn at 14 day's intervals from the wing vein as previously reported [17]. Triglycerides, total cholesterol, HDL-, and LDL(+VLDL) - bound cholesterol were determined in the plasma using commercial diagnostic kits obtained from E. Merck, Darmstadt, W. Germany. At the age of 32 weeks, (12 weeks of feeding cholesterol-enriched feed started at the age of 8 weeks and then giving garlic for a period of 12 weeks) the animals were sacrificed and their dorsal aortae were removed carefully for the examination of atherosclerotic lesions as reported previously [17].

Besides the hypolipidemic effects, studies were also undertaken on the antiaggregatory properties of garlic. Blood was withdrawn from normal human volunteers (aged 18-30 years) by venipuncture. The volunteers were free of medication for one week prior to blood collection. Blood samples were mixed with 3.8% sodium citrate solution (9:1) and centrifuged at 260 x g for 15 min at 20°C to obtain platelet rich plasma (PRP). The remaining blood was centrifuged at 1000 x g to get platelet poor plasma (PPP). Platelet count was obtained with the help of a phase contrast microscope.

All aggregation studies were carried out at 37°C with PRP having platelet counts between 2.5 - 3.0 x $10^6$/ml and within 3 min after PRP recovery. Aggregation was monitored on a LUMI-aggregometer (Model 400, Chronolog Corporation, Chicago, USA) using 0.45 ml aliquots of PRP. The mixture was preincubated with the test substance for 1 min before being challenged with the aggregating agent. Following aggregating agents were used at concentrations noted against them. Adrenaline (200 µM), adenosine diphosphate (2.2 µM) and sodium arachidonate (1.7 µM). The resulting aggregation was recorded and expressed as percent inhibition with respect to control at 4 mins after challenge. The garlic extract (aqueous) was tested at 3-4 concentrations in triplicate. Differences between control and extract-treated preparations were statistically determined by students 't' test.

Dorsal aortae were removed after autopsy as described before [17]. They were classified into normal, stage I or stage II of atherosclerotic lesions on the basis of visual examination according to W.H.O. classification [20].

## Results and Discussion

Tables 1-4 show the changes in the levels of triglycerides, total cholesterol, HDL- and LDL(+VLDL)-bound cholesterol in the plasma of chickens belonging to groups I, II and III maintained respectively on cholesterol-enriched feed (atherosclerosis model control), cholesterol-enriched feed for 12 weeks and then fed with garlic for another 12 weeks (curative effect) and cholesterol-enriched feed for 12 weeks and then allowed to revert to normal feed to see the autoreversal of the symptoms (autoreversal effect). The data presented therein show that after stopping administration of cholesterol-enriched feed, the levels of triglycerides, total, and LDL(+VLDL)-bound cholesterol started decreasing in all the three groups (I,

TABLE 1. CURATIVE EFFECT, LEVEL OF TRIGLYCERIDES, TOTAL-, AND LDL+ (VLDL)- BOUND CHOLESTEROL (mg/dl) IN THE PLASMA OF CHICKENS FED ON CHOLESTEROL-ENRICHED FEED (MEAN ± S.E.M.).

| Animal Group | Parameter | 2nd week levels | 4th week levels | 6th week levels |
|---|---|---|---|---|
| *Group-I* (Atherosclerosis model) | Triglycerides | 64±5.3 | 71±4.8 | 79±5.6 |
| | total cholesterol | 580±23.7 | 718±32.5 | 741±29.3 |
| | HDL-cholesterol | 98±7.1 | 107±9.7 | 104±8.8 |
| | LDL+(VLDL)-cholesterol | 480±18.4 | 613±21.8 | 635±16.9 |
| *Group-II* (Curative/therapeutic effect) | Triglycerides | 79±4.9 | 129±7.7 | 148±6.9 |
| | total cholesterol | 611±22.1 | 974±32.4 | 965±44.3 |
| | HDL-cholesterol | 91±5.8 | 96±3.6 | 93±3.7 |
| | LDL+(VLDL)-cholesterol | 518±20.3 | 880±26.4 | 874±28.3 |
| *Group-III* (Autoreversal effect | Triglycerides | 94±6.1 | 100±7.3 | 117±8.3 |
| | Total cholesterol | 715±27.8 | 730±31.2 | 771±24.5 |
| | HDL-cholesterol | 85±4.3 | 91±4.9 | 99±3.8 |
| | LDL+(VLDL)-cholesterol | 632±21.8 | 636±26.6 | 673±30.7 |

All the groups (I, II & III) were fed on cholesterol-enriched feed upto 12 weeks.

TABLE 2. CURATIVE EFFECT, LEVEL OF TRIGLYCERIDES, TOTAL-, HDL- AND LDL+ (VLDL)- BOUND CHOLESTEROL (mg/dl) IN THE PLASMA OF CHICKENS FED ON CHOLESTEROL-ENRICHED FEED (MEAN ± S.E.M.)

| Animal Group | Parameter | 8th week | 10th week | 12th week |
|---|---|---|---|---|
| *Group-I* (Atherosclerosis model) | Triglycerides | 108±9.4 | 180±6.9 | 184±6.9 |
| | Total cholesterol | 811±27.3 | 812±35.6 | 829±31.7 |
| | HDL-cholesterol | 122±7.5 | 118±7.7 | 103±7.0 |
| | LDL+(VLDL)-cholesterol | 691±23.8 | 692±25.7 | 730±31.2 |
| *Group-II* (Curative/therapeutic effect) | Triglycerides | 170±11.3 | 261±12.8 | 263±12.4 |
| | Total cholesterol | 955±38.2 | 963±29.2 | 1018±54.8 |
| | HDL-cholesterol | 83±4.9 | 117±6.3 | 96±4.4 |
| | LDL+(VLDL)-cholesterol | 875±32.5 | 847±41.7 | 919±38.8 |
| *Group-III* (Autoreversal effect) | Triglycerides | 130±4.8 | 196±11.8 | 245±15.3 |
| | Total cholesterol | 845±21.4 | 890±35.4 | 893±32.8 |
| | HDL-cholesterol | 111±9.7 | 121±8.3 | 105±11.5 |
| | LDL+(VLDL)-cholesterol | 734±28.4 | 772±24.2 | 786±32.4 |

All groups (I, II & III) were fed on cholesterol-enriched feed upto 12 weeks

TABLE 3. CURATIVE EFFECT, LEVEL OF TRIGLYCERIDES, TOTAL-, HDL-, AND LDL+(VLDL)-BOUND CHOLESTEROL (mg/dl) IN THE PLASMA OF CHICKENS RENDERED HYPERLIPIDEMIC AND THEN TREATED WITH GARLIC OR ALLOWED TO AUTOREVERSE BY PUTTING ON NORMAL FEED (MEAN ± S.E.M.)

| Animal Group | Parameter | 14th week | 16th week | 18th week |
|---|---|---|---|---|
| *Group-I* Atherosclerotic model (control) | Triglycerides | 142±8.6 | 120±7.1 | 108±5.4 |
| | Total cholesterol | 654±29.3 | 557±23.4 | 388±12.1 |
| *Treatment* cholesterol-enriched feed twice a week | HDL-cholesterol | 104±7.7 | 125±8.2 | 120±8.5 |
| | LDL+(VLDL)-cholesterol | 549±31.6 | 430±18.6 | 271±12.7 |
| *Group-II* (Curative/therapeutic effect) | Triglycerides | 122±5.9 | 118±6.9 | 110±9.0 |
| | Total cholesterol | 602±32.4 | 443±29.8 | 276±8.8 |
| *Treatment* Cholesterol-enriched feed twice a week + Garlic* | HDL-cholesterol | 120±9.7 | 118±9.5 | 121±12.4 |
| | LDL+(VLDL)-cholesterol | 483±23.8 | 328±13.8 | 157±17.5 |
| *Group-III* (Autoreversal effect) | Triglycerides | 116±4.8 | 122±8.6 | 120±6.7 |
| | Total cholesterol | 697±34.6 | 497±29.9 | 282±11.3 |
| *Treatment* Normal feed | HDL-cholesterol | 125±9.7 | 115±4.8 | 118±4.6 |
| | LDL+ (VLDL)-cholesterol | 575±19.8 | 381±18.3 | 164±9.3 |

*4 gm paste/bird/day

TABLE 4.CURATIVE EFFECT, LEVEL OF TRIGLYCERIDES, TOTAL-, HDL-, AND LDL+(VLDL)-BOUND CHOLESTEROL (mg/dl) IN THE PLASMA OF CHICKENS RENDERED HYPERLIPIDEMIC AND THEN TREATED WITH GARLIC OR ALLOWED TO AUTOREVERSE BY PUTTING ON NORMAL FEED (MEAN ± S.E.M.)

| Animal Group | Parameter | 20th week | 22nd week | 24th week |
|---|---|---|---|---|
| *Group-I* Atherosclerotic model (control) | Triglycerides | 96±4.5 | 82±3.4 | 85±4.1 |
| | Total cholesterol | 221±7.3 | 240±6.9 | 188±8.9 |
| *Treatment* Cholesterol-enriched feed twice a week | HDL-cholesterol | 112±6.4 | 113±4.3 | 94±5.6 |
| | LDL+(VLDL)-cholesterol | 112±12.3 | 128±5.1 | 95±10.3 |
| *Group-II* (Curative/therapeutic effect) | Triglycerides | 106±9.5 | 76±3.8 | 72±4.3 |
| | Total cholesterol | 162±8.7 | 132±7.6 | 130±6.8 |
| *Treatment* cholesterol enriched feed twice a week + Garlic* | HDL-cholesterol | 108±6.3 | 106±4.8 | 100±5.9 |
| | LDL+(VLDL)-cholesterol | 56±4.1 | 26±4.2 | 30±3.5 |
| *Group-III* (Autoreversal effect) | Triglycerides | 125±8.3 | 103±5.7 | 98±4.8 |
| | Total cholesterol | 215±12.9 | 163±8.3 | 142±10.3 |
| *Treatment* Normal feed | HDL-cholesterol | 98±5.4 | 91±5.6 | 88±4.9 |
| | LDL+(VLDL)-cholesterol | 116±10.3 | 70±7.7 | 57±10.1 |

*4 gm/bird/day

II and III). However, the percent decrease in these values was lower in the control (Group-I) and the autoreversal (Group-III) animals as compared with the garlic-treated group (Table 5). Thus there was a 7% decrease in triglycerides, 49.9% in total cholesterol and 58.8% in the LDL(+VLDL)-bound cholesterol in the control (Group-I) animals. In the case of autoreversal (Group-III) animals, the decrease was 22.4% in triglycerides, 58.7% in the case of total cholesterol and 67.8% in the

TABLE 5. CURATIVE EFFECT, LEVEL OF TRIGLYCERIDES, TOTAL-, HDL-, AND LDL+(VLDL)-BOUND CHOLESTEROL; IN THE CHICKENS RENDERED HYPERLIPIDEMIC BY FEEDING CHOLESTEROL-ENRICHED DIET AND THEN TREATING WITH GARLIC OR ALLOWED TO REVERT TO NORMAL FEED (MEAN±S.E.M.) OF OVERALL VALUES.

| Animal Group | Group-I Control | | Group-II Garlic treated | | Group-III Autoreversal | |
|---|---|---|---|---|---|---|
| | Before treatment | After treatment | Before treatment | After treatment | Before treatment | After treatment |
| Triglycerides (mg/dl) | 114±22.2 | 106±9.3 | 175±33.9 | 101±8.8 | 147±24.6 | 114±4.5 |
| Total cholesterol (mg/dl) | 749±37.5 | 375±79.2 | 914±61.3 | 291±79.1 | 807±32.4 | 333±89.7 |
| HDL-cholesterol (mg/dl) | 109±3.8 | 111±4.5 | 96±4.6 | 112±5.8 | 102±5.4 | 106±6.3 |
| LDL+(VLDL)-cholesterol (mg/dl) | 640±36.4 | 264±77.2 | 819±60.9 | 180±76.6 | 705±27.3 | 227±84.6 |

Group I, II and III were fed on cholesterol-enriched feed for 12 weeks. After that Group I and II received cholesterol-enriched feed twice weekly and for the rest of 5 days were given normal feed. Group III was reverted to normal feed after the initial period of 12 weeks.

TABLE 6.CREATIVE EFFECT, PERCENT CHANGES FOUND IN THE LEVEL OF TRIGLYCERIDES, TOTAL-, HDL-, AND LDL+(VLDL)-BOUND CHOLESTEROL IN VARIOUS GROUPS (I, II & III), AFTER TREATMENT.

| Parameter | Group-I (Control) | Group-II (Garlic-treated) | Group-III (Autoreversal) |
|---|---|---|---|
| | % Increase* or Decrease** | | |
| Triglycerides (mg/dl) | 7.0** | 42.3** | 22.4** |
| Total-cholesterol (mg/dl) | 49.9** | 68.2** | 58.7** |
| HDL-cholesterol (mg/dl) | 1.8* | 16.7* | 3.9* |
| LDL+(VLDL)-cholesterol (mg/dl) | 58.8** | 78.0** | 67.8** |

Group I, II and III were fed on cholesterol-enriched feed for 12 weeks and then Group I and II received cholesterol- enriched feed twice weekly and for the rest of 5 days were given normal feed. Group II was also given garlic paste (4 gm/bird/day) for 12 weeks. Group III was reverted to normal feed after the initial period of 12 weeks.

case of LDL (+VLDL)-bound cholesterol which is comparatively higher than found in the control group. A maximum decrease in the values of the above parameters was found in the case of garlic-treated group which showed a decrease of 42.3% in triglycerides, 68.2% in the case of total cholesterol and 78.0% in the case of LDL (+VLDL)-cholesterol. There was also a slight increase in the HDL-cholesterol levels (Table 6) but this change was not statistically significant.

The results of the autopsy studies showing the gradation of aortic lesions are given in Table 7. The intensity of aortic damage was significantly higher in the Group-I animals which constitute the atherosclerotic model (control), than was found in the other two groups II and III, curative and autoreversal group respectively. The intensity of damage was higher in autoreversal group as compared to the damage found in the curative group. Thus out of the 6 animals belonging to the curative group, 4 animals showed aortic lesions of stage-I and two showed lesions of stage-II. Among the atherosclerotic model group, one animal showed aortic lesions of stage-I and 5 animals showed aortic lesions of stage-II.

Figures 1-3 show the antiaggregatory effects of the aqueous extracts of garlic at different concentrations and after challanging the human PRP with adrenaline, adenosine diphosphate (ADP) and sodium arachidonate. A maximum inhibition was observed at 3.75 mg concentration of the garlic extract against all the three aggregating agents (Table 8).

Studies on the curative effect of garlic were undertaken to see if garlic feeding can reverse or lessen the damage caused by experimental induction of atherosclerosis through cholesterol feeding. All the three groups received cholesterol-enriched feed for 12 weeks and then reverted to normal feed. However, the animals of group-I and II continued to receive cholesterol-enriched feed twice a week till the conclusion of the studies to simulate the conditions prevalent in human atherosclerotic disease wherein hypercholesterolemia is usually present [3]. We observed an overall decrease in the level of triglycerides, total-, and LDL (+VLDL)-bound cholesterol in all the groups (I, II and III). Comparing on quantitative basis however, we found that percentage decrease in the parameters mentioned above was maximum in the garlic-treated group. In the autoreversal group, the levels were slightly improved than was observed in the case of atherosclerotic model group but they were not significantly different (Table 6).

The assessment of the aortic damage provided a similar picture. Damage being least in the garlic-treated group and comparatively lesser in the autoreversal group (Table 7).

The studies on antithrombotic effects of garlic showed a marked inhibitory effect on platelet aggregation In vitro when tested with the aqueous extract of garlic (Table 8, Fig. 1-3) indicating that garlic can reduce the tendency to thrombus formation. It is, therefore, confirmed that administration of garlic can provide a significant protection against hyperlipidemia and thrombus formation and thus can be of some benefit in the cardiovascular anomalies.

One of us [21] studied the effect of garlic paste and solvent extracts in normal fed chickens and found significant decreases in the serum lipids and LDL-cholesterol levels. Present studies confirm these findings in chickens with cholesterol-induced atherosclerosis.

The mechanism of hypolipidemic action of garlic is still not fully understood. There appear to be three possible ways in which this could occur. Firstly, it has been observed by a number of workers that garlic administration in experimental animals leads to increase excretion of bile acids and neutral steroids in faeces [22,23] which are the end products of cholesterol metabolism. Secondly, hypocholesterolemic agents are reported to decrease the plasma LDL- and VLDL-bound cholesterol fractions and, in certain cases, a concomitant increase in HDL-cholesterol levels [24,25]. It has also been demonstrated that HDL inhibits the LDL uptake by the arterial

TABLE 7. CURATIVE EFFECT, GRADATION OF AORTIC LESIONS IN THE CONTROL AND TREATED ANIMALS.

| Animal Group | Total number of animals | Nature of lesions | | |
|---|---|---|---|---|
| | | Normal | Stage-I | Stage-II |
| *Group-I* (Atherosclerosis model) | 6 | 0 | 1 | 5 |
| *Group-II* (Therapeutic effect) | 6 | 0 | 4 | 2 |
| *Group-III* (Autoreversal) | 6 | 0 | 2 | 4 |

TABLE 8. INHIBITION OF PLATELET AGGREGATION BY AQUEOUS EXTRACT OF GARLIC.

| Aggregating agent used | Concentration of the garlic extract (mg) | Percent Inhibition |
|---|---|---|
| Epinephrine | 1.25 | 31.8 |
| (200 μM) | 1.87 | 54.5 |
| | 2.50 | 85.6 |
| | 3.75 | 92.4 |
| Adenosine | 1.25 | - |
| Diphosphate (ADP) | 1.50 | 26.0 |
| (2.2 μM) | 2.50 | 74.7 |
| | 3.75 | 100 |
| Sodium | 1.25 | 25.9 |
| arachidonate | - | - |
| (1.7 μM) | 2.50 | 100 |
| | 3.75 | 100 |

wall and facilitates transport of cholesterol from peripheral tissue to liver for catabolisation [26,27]. Since garlic partly corrects the imbalance in the lipoprotein profile, it is possible that garlic may exert its hypolipidemic effect through this

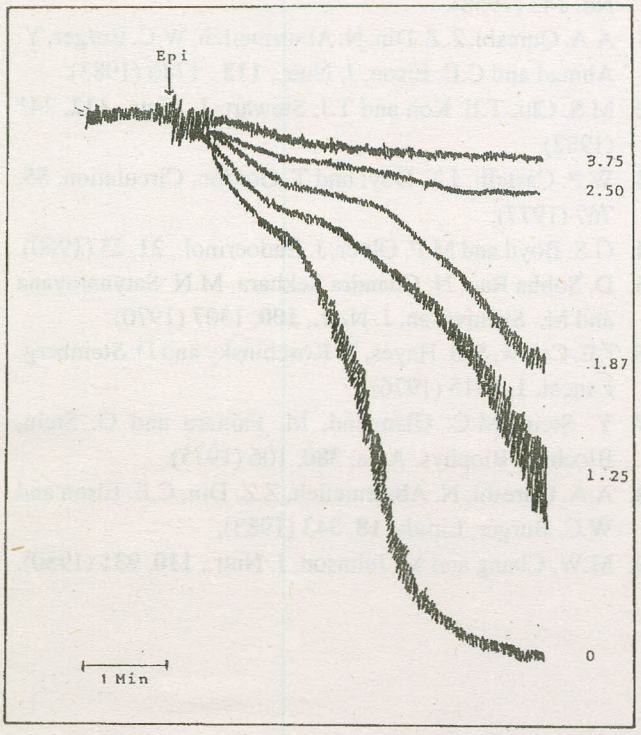


Fig. 1. Effect of aqueous garlic extract on platelet aggregation induced by Epinephrine.

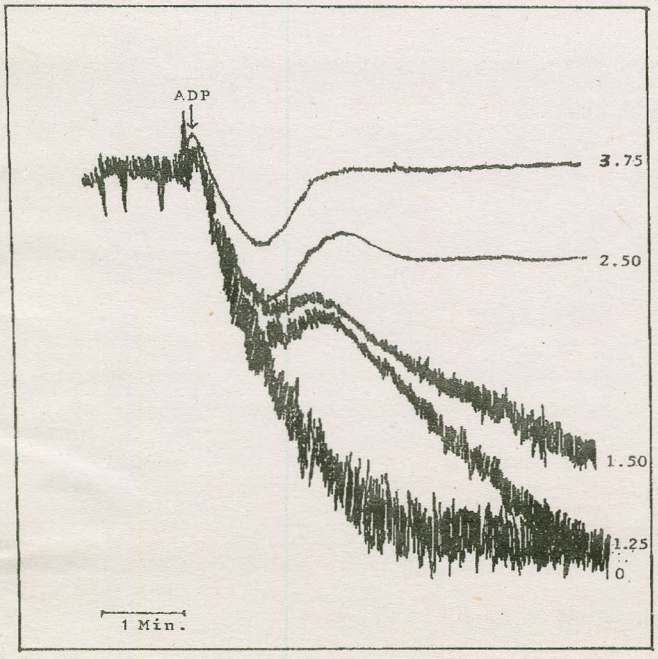


Fig. 2. Effect of aqueous garlic extract on platelet aggregation induced by ADP.

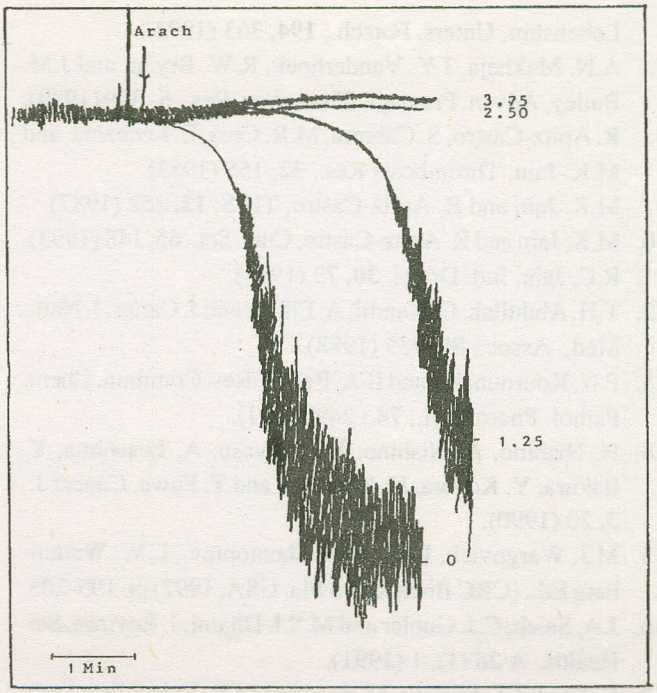


Fig. 3. Effect of aqueous garlic extract on platelet aggregation induced by Arachidonic acid.

pathway. Thirdly, a decrease in the hepatic synthesis of cholesterol and fatty acids has also been observed in garlic fed animals [28,29]. It is, therefore, likely that one or more of these mechanisms may be operative in garlic-induced hypolipidemia. Further studies using radio-labelled cholesterol are necessary to elucidate the actual mechanism.

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