

## Original Article

# The Effect of EDTA and Garlic Extract Combination on Plasma Lipids, Lipoproteins, and Fatty Streaks in Cholesterol Fed Male Rabbit Aorta

M.R.Sharifi MD\*, S. Javdan MS\*\*, M.Fesharaki MS\*\*

## ABSTRACT

**Background:** Garlic extract is used in treatment of hypercholesterolemia, although its efficacy isn't exactly clear. There is a little information about mechanism of garlic effect on plasma lipids. By intervention of garlic in atherogenesis process it has been shown that it has a protective effect against cardiovascular disease. On the other hand, it has been shown that EDTA (Ethylene Diamin Tetra Acetic Acid) improves blood flow and decreases the vascular atherosclerotic symptoms.

**Methods:** The objective of this study was to evaluate the effect of EDTA and garlic extract with together on plasma concentration of cholesterol, Triglyceride, LDL, and HDL, and formation of fatty streaks through the phenomena called "chelation therapy" in male Rabbit aorta. 32 male Rabbits were used and divided to 4 groups as group 1: cholesterol – rich diet (CRD) + EDTA + Garlic Extract (GE), group 2: CRD + EDTA, Group 3: CRD + GE, Group 4: CRD (control group). The animals were under food and drug diet for 5 weeks. Blood samples of Rabbits were taken before and after the test and then plasma cholesterol, triglyceride, LDL and HDL were measured. Finally, abdominal aorta was studied and pathologically evaluated.

**Results:** The experiments showed the significant decrease of mean cholesterol in case groups and mean triglyceride in the groups which have received EDTA + GE and EDTA. Our results showed that mean LDL and LDL/HDL Ratio in case groups have been decreased in comparison to control group. In addition, there was a significant increase in mean HDL in the group which has received EDTA + GE.

**Conclusion:** In this study it was shown that the using of fresh garlic and EDTA with together has a reductive effect on cholesterol, Triglyceride, and LDL concentration and also has an increasing effect on HDL concentration. But it seems that concomitant using of these tow substances with together strengthen the effect of each one, and it produces a great reduction of the blood lipids concentration.

**Key words:** atherosclerosis, garlic, EDTA.

Atherosclerosis is a complex and multifactorial disease which is characterized by factors such as: hypertension, diabetes mellitus, and especially familial hyperlipidemia. In this disease, some changes take place in plasma cholesterol concentration and then cholesterol and other lipids precipitation in arteries take place as well. They, per se lead to a narrow arterial lumen. Finally, this restricts the vital organ blood flow, and then this disturbance leads to produce various clinical syndromes such as: heart attacks and strokes<sup>1</sup>. The

increase in plasma concentration of cholesterol leads to increase in risk of vascular disease<sup>2</sup>. Diet therapy is the first step in treatment of hyperlipidemia. Some other studies also concluded that garlic is able to normalize plasma lipids<sup>1</sup>. Raw garlic or garlic extract has been used in various studies. It seems that Allicin is the principal bioactive matter in aqueous garlic extract. Some investigators have evaluated the effect of garlic on lipids metabolism in hypercholesterolemic rats. Their results showed that plasma cholesterol, triglyceride, and LDL

\* Associated Professor, Department of Physiology, Medical School, Isfahan University of Medical Sciences, Isfahan, Iran.

\*\* Animal Physiologist, Payam Nour University, Isfahan, Iran.

\*\*\* Physiologist, Medical school, Isfahan University of Medical Sciences, Hezar Jarib Avenue, Isfahan, Iran.

Correspondence to: Dr. Mohammad Reza Sharifi, Department of Physiology, Medical school, Isfahan University of Medical Sciences, Isfahan, Iran.

concentrations significantly decreased, but garlic had no effect on plasma HDL<sup>3</sup>. In a case-control study in which plasma cholesterol level in cases were greater than 200mg/dl, it was shown that the raw garlic can reduce plasma cholesterol down to 12%<sup>4</sup>. The other research showed that the garlic decreases the formation of fatty streaks and atherosclerosis in carotid artery<sup>5</sup>.

Evans showed that injection of 300mg/day of EDTA reduced plasma cholesterol in hypercholesterolemic rabbits<sup>6</sup>. In a 12-week study on 42 persons, results have shown that plasma cholesterol and LDL reduced 6% in those who had received 900mg/dl of garlic, whereas in the other group whose people had received placebo, their plasma cholesterol has decreased only 1%<sup>7</sup>. It seems that garlic usage as dry garlic preparation (600 mg/day), fresh garlic, or high Allicin yielding garlic (10-20g/day) appears to reduce total plasma cholesterol significantly over a 1-3 month period<sup>1</sup>. There are some clinical reports that suggest that chelation therapy has beneficial effects in treatment of atherosclerosis<sup>8</sup>. Removing metal ions such as iron from blood by EDTA can reduce free radicals and lipids peroxidation production.

Furthermore, EDTA binds to calcium ion and leads to reduction of blood calcium through this way. EDTA leads to atherosclerotic plaque damage through blood calcium reduction, because calcium reduction is compensated by different sources such as calcium which is present in these plaques. Excretion of calcium from the plaques leads to their cholesterol liberation<sup>9</sup>. The goal of this study was to evaluate the concomitant use of EDTA and garlic extract on plasma cholesterol, triglyceride, LDL, and HDL levels and accumulation of fatty streaks in male aortic wall.

## Materials and Methods

In this study, 32 white and male rabbits, weighted  $1.18 \pm 0.1$  kg, were used which were prepared by Pastor Institute in Iran. Blood samples (5ml) were taken from the heart of the rabbits to determine plasma cholesterol, triglyceride, and LDL, and HDL concentration after taking care of them for a week in animal house. Then they were divided into 4 groups randomly as following:

Group1: Cholesterol-rich diet (CRD) + EDTA (100mg/case) + garlic extract (GE) (400mg/case),  
Group2: CRD + EDTA,  
Group3: CRD + GE,  
Group4: CRD (control group).

Cholesterol rich diet (CRD) that contains 1% of cholesterol was prepared by dissolving 1gr of cholesterol powder in 4ml of olive oil and adding it to 1kg animal food<sup>10</sup>. Dose of garlic extract was 400mg/day<sup>11</sup>. Experiments carried out during 5 weeks and animals feeding were interrupted for 12 hours, then they anesthetized by injecting 1ml urethane 20% intraperitoneally. 5 ml of blood was taken from each of the rabbits for determination of cholesterol, triglyceride, LDL, and HDL concentration by enzyme method; finally animals were sacrificed through the bleeding. Abdominal aorta was excised off for pathologic studies after opening their abdomen. The results reported as mean  $\pm$  SE. All groups were compared as intra groups by t-paired test from the statistical view, and ANOVA test was used for determination of significant differences among the groups. If P-value was less than 0.05, it was evaluated significant.

## Results

Results are shown in table 1 and figure 1. Evaluation of cholesterol before and after the test in 4 groups shows that cholesterol concentration has decreased significantly in three groups Who have received EDTA and GE with together or each one alone in comparison to control group (group has taken EDTA + GE: 29.4 times, group has taken EDTA: 26.4 times, group has taken GE: 27.5 times and control group: 40 times).

Evaluation of triglyceride before and after the test shows that triglyceride concentration has increased in 4 groups after the test (group has taken EDTA + GE: 1.8 times, group has taken EDTA: 3.8 times, group has taken GE: 2.1 times, and control group: 2.7 times), but triglyceride concentration has significantly elevated in control group in comparison to the groups that have received EDTA + GE with together or each one alone. Evaluation of plasma LDL concentration in 4 groups before and after the test shows that LDL concentration has elevated after the test (group has taken EDTA + GE:

33 times, group has taken EDTA: 33 times, group has taken GE: 41 times, and control group: 66 times), but its concentration has significantly reduced in the groups that have received EDTA alone and EDTA + GE (in comparison to control group). Evaluation of plasma HDL concentration in 4 groups before and after the test shows that HDL concentration has elevated after the test (group has taken EDTA + GE: 9.1 times, group has taken EDTA: 11.1 times, group has taken GE: 11.9 times, and control group: 10.4 times), but its concentration in the groups that have received EDTA + GE with together and GE alone also was more than control group. LDL/HDL ratio has decreased in 4 groups after the test (group has

taken EDTA + GE: 2.6 times, group has taken EDTA: 1.4 times, group has taken GE: 2.9 times, and control group: 4.1 times), but its reduction was significant in the groups that have received EDTA + GE with together and EDTA or GE alone in comparison to control group. Evaluation of pathologic lesions shows that the rate of the lesions in the group that has received EDTA + GE has significantly reduced in comparison to control group, whereas there isn't significant difference between control group and group that has received GE alone. Pathologic results are shown in figure 1.

**Table 1.** Comparison of mean plasma cholesterol (Chol), triglyceride (TG), HDL, LDL, and HDL in 4 experimental groups before and after experiment (mg/dl).

Parameter	Group	Before treatment	After treatment
Cholesterol	1	42.4 ± 8.32	1235.6 ± 44
	2	52.75 ± 4.96	1375.8 ± 90.28
	3	61.8 ± 6.61	1678 ± 39.62
	4	64 ± 6.3	2584.16 ± 56.9
Triglyceride	1	43 ± 7.7	79.75 ± 9.75
	2	37 ± 4.01	142.8 ± 25.26
	3	38.4 ± 6.51	83.25 ± 12.26
	4	57.5 ± 3.26	157.5 ± 32
LDL	1	31.86 ± 8.41	1034.6 ± 62.15
	2	34 ± 2.15	1142 ± 51.17
	3	34.5 ± 5.6	1417.75 ± 72.2
	4	32.75 ± 7.65	2179.5 ± 27.04
HDL	1	18.75 ± 2.25	172.4 ± 11.11
	2	18.4 ± 2.15	204.8 ± 7.99
	3	20.5 ± .50	245.75 ± 8.59
	4	23 ± 2.09	240.83 ± 10.48
LDL / HDL	1	2.3 ± 1	6.08 ± 0.54
	2	3.74 ± 1.01	5.33 ± 0.23
	3	1.97 ± 0.58	5.57 ± 0.42
	4	2.48 ± 0.57	10.39 ± 1.07

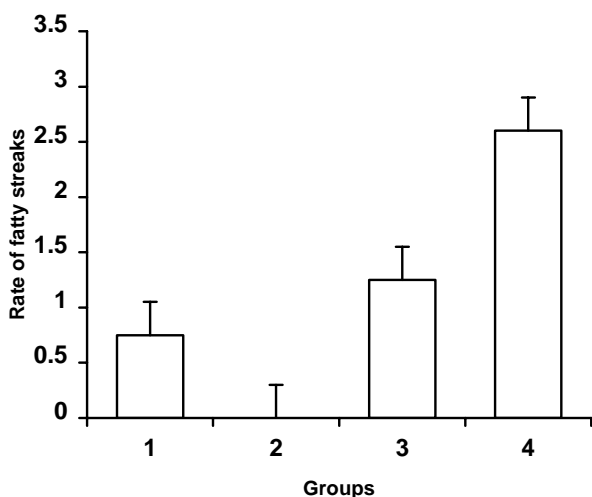
Group 1: CRD + EDTA + GE

Group 2: CRD + EDTA

Group 3: CRD + GE

Group 4: CRD (Control group)

Before and after values were significantly different for all parameters in all groups.



**Figure 1.** The rate of fatty streaks in all groups after the test.

Group 1: CRD + EDTA + GE

Group 2: CRD + EDTA

Group 3: CRD + GE

Group 4: CRD (Control group)

## Discussion

Atherosclerosis alone accounts for more than half of deaths in wide world, and accounts for almost one million fatality in the United States in each year<sup>12</sup>. Studies have indicated that active compound in raw garlic is a sulfur compound called Allicin, which is produced when raw garlic is chewed<sup>13</sup>. Allicin has antiatherosclerotic effect on vascular wall; this compound inhibits liver lipogenic and cholesterogenic enzyme activity such as fatty acid synthetase and HMG-coA reductase. However, recent in vitro studies show that there is a soluble organophosphor compound called S-allyl disulfide (SAC), present in garlic oil. This substance is a potent cholesterol synthesis inhibitor<sup>1</sup>. The studies demonstrate that chelation therapy with EDTA reduces cholesterol concentration in hypercholesterolemic rabbits<sup>9</sup>, but its mechanism isn't clear, whereas on the basis of Rosenman's findings, EDTA hasn't any effect on liver cholesterol metabolism in rat<sup>14</sup>.

In previous studies it was shown that the rate of incorporation of glycerol to fatty acids reduced in hepatocyte cell culture of whome was treated with garlic extract. Thus, the triglyceride lowering effect of garlic extract maybe somehow due to inhibition of

fatty acid synthesis<sup>15</sup>. In one another study in hypercholesterolemic rats induced by high cholesterol diet, administration of garlic has reduced cholesterol and LDL concentration significantly, but hasn't any effect on plasma HDL<sup>1</sup>. In the present study the results showed that GE and EDTA combination reduced plasma cholesterol significantly in comparison to those groups which have received EDTA or GE alone. In addition, in this study, elevation of triglyceride concentration demonstrated a significant difference between the groups that have received EDTA and GE with together and GE alone in comparison to control group. However, triglyceride concentration in group which has received EDTA alone hasn't significant difference with control group. The meaning of this difference is that GE acts as a potent triglyceride reducer. In this study, evaluations revealed that there is a relative elevation in plasma HDL in group which has received GE alone in comparison to control group. This effect is probably due to fresh garlic form. In spite of EDTA property in blood lipid lowering experimentally, there isn't any logical explanation for this act. But however, the role of this substance in chelating of metal ions which can catalyze some oxidative reactions is approved<sup>16</sup>. On the basis of previous study, Allicin which is in fresh garlic reduces fatty acid synthetase activity. Presence of %1-4 Allicin in daily food can decrease plasma LDL concentration<sup>11</sup>. Plasma LDL evaluation in present study shows that its concentration in three case groups have decreased. But the differences in LDL concentration between groups which have received EDTA and GE with together and EDTA alone in comparison to control group are significant. The differences between group which has received EDTA and GE with together in comparison to control group is too much. In assessment of previous studies and present study, it seems that concomitant use of EDTA and GE is responsible for LDL concentration reduction.

Abramovis has studied the effect of fresh garlic on fatty streaks formation in hypercholesterolemic rat aorta. The microscopic and statistical analysis of this study has shown that formation of these lesions decreased down to %70 in rats which has received Allicin<sup>17</sup>. Evaluation of pathologic lesions in present study shows that formation of the lesions in three groups of test reduced in comparison to control group, but this reduction in groups which have

received EDTA and GE with together and EDTA alone was significant. In group which has received EDTA alone there isn't any fatty lesion. It seems that EDTA has inhibited free radical formation in blood by removing metal ions, and by this way, it has influenced formation of atherosclerotic plaques.

Finally, it is concluded that concomitant use of EDTA and GE with together strengthens the effect

of each one, and it produces a great reduction in blood lipids and lipoproteins concentration. Although EDTA has beneficial effects, but with chelating of metal ions such as calcium and iron may leads to reduction of blood calcium and iron and then hypocalcemia and anemia.

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