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Full Length Research paper

Effects of *Amaranthus caudatus* l. extract and lovastatin on atherosclerosis in hypercholesterolemic rabbits

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Coronary artery disease (CAD), which is a multi-factorial disease frequently, causes morbidity and mortality in human beings. Scientists now believe that there is a good link between the inflammatory process and coronary atherosclerosis. A study was conducted to find out the anti- atherosclerosis effect of *Amaranthus caudatus* L. on rabbits, and was compared with lovastatin. Twenty five male New Zealand rabbits were randomly grouped into five and each were fed for 60 days with a standard diet, standard diet and cholesterol, standard diet and *A. caudatus* extract (150 mg/kg daily), standard diet and *A. caudatus* extract (150 mg/kg daily) with cholesterol, and standard diet with lovastatin (10 mg/kg) and cholesterol. At the 30th and 60th day of experiment, the biochemical factors were measured and the fatty streak formation was evaluated at the 60th day. Rabbits fed with high cholesterol diet and *A. caudatus* extract significantly decreased the cholesterol, LDL-C, triglyceride, oxidized LDL (Ox-LDL), apo-lipoprotein B (apoB), CRP, atherogenic index (AI) and HDL-C and apo-lipoprotein A (apoA) increased. Lesion severity, in extract recipient group, significantly decreased. *A. caudatus* extract decreased the most important risk factors (the serum lipoproteins, apoB and Ox-LDL) of cardiovascular diseases and inflammatory factors prevented atherosclerosis and was more effective than lovastatin.

Key words: Atherosclerosis, lipid, Ox-LDL, CRP, apolipoprotein, *Amaranthus caudatus,* amaranthaceae.

INTRODUCTION

Atherosclerosis is the most frequent cause of morbidity and mortality in the entire world (Ahaneku et al., 2001). Coronary artery disease (CAD) is a multi-factorial disease and about 250 different risk factors have been recognized (Parmley, 1997). Atherosclerosis is complex. Its exact cause is still unknown. It is thought that atherosclerosis is caused by a response to damage to the endothelium from high cholesterol, high blood pressure, and cigarette smoking. Apo-lipoprotein A (apo A) is the main protein of HDL-C and has a inverse relationship with CAD. Apolipoprotein B (apo B) is the main protein component of LDL-C and found to be an important factor in predicting on of CAD. Many scientists now believe that there is a link between the inflammatory process and coronary atherosclerosis. One of the markers of inflammation is CRP(C-reactive protein) that it is elevated when ischemic heart disease is present (Ross., 1999; Guldiken et al., 2005; Elias-Scale et al., 2007). Atherosclerosis is a soph-isticated and complex process which includes oxidation and inflammation (Stulnig et al., 1996; Nakamura et al. 2006). *Amaranthus caudatus* which is synonym with *Amaranthus paniculatus* L. was used in some studies. Grain and leaves of *Amaranthus* are utilized as food for human beings as well as for animal (Martirosyan, 2001)

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and their nutritional value have been extensively studied (Martirosyan, 2003).

Amaranthus leaves are an excellent source of protein (Kadoshnikov et al., 2005) and numerous studies on their grain oil content have been carried out in the last 2-3 decades (Pogojeva et al., 2006). However, research on *Amaranthus* as a vegetable has received significantly low attention than grains. In this study, effects of the aerial parts of *A. caudatus* on traditional and new risk factors of cardiovascular disease and fatty streak formation in hypercholesterolemic rabbits were assessed. Also its effect was compared with lovastatin which is one of the important and new medications in atherosclerosis prevention and control.

MATERIALS AND METHODS

Plant material

A. caudatus plants were provided from Isfahan Natural Resource Institute and authenticated by Dr. Lili Ghaemmaghami and the voucher specimen bearing number 13649 was deposited in Isfahan University Herbarium.

Preparation of extract

Aerial parts of the plant were dried at room temperature and it was extracted with 96% ethanol for 72 h and then filtered, and concentrated by vacuum distillation.

Total flavonoids content was measured at 424 nm, (Petry et al., 2001; Hartke., 1987) and anthocyanins at 535 nm (Francis, 1982) using spectrophotometeric method.

Grouping and feeding of the rabbits

Twenty five male New Zealand rabbits were provided from Razi Institute, Karaj, Iran and randomly divided into five groups and each was fed with standard diet, standard diet and cholesterol (1%), standard diet and *A. caudatus* extract (150 mg/kg daily), standard diet and *A. caudatus* extract (150 mg/kg daily) and cholesterol, and standard diet with lovastatin (10 mg/kg daily) and cholesterol.

The *A. caudatus* extract, cholesterol and lovastatin were fed through feeding tube for 60 days. Isfahan Cardiovascular Research Center Ethics Committee which is a member of office for human research protections, US department of health and human services, approved the present study.

Measurement of biochemical factors

The rabbits were put in fasting conditions for 12 h and blood samples were taken from the marginal auricular vein prior to the beginning of diets, on the 30^{th} and 60^{th} day of feeding. Total cholesterol (Chol.), triglyceride (TG), LDL-C (Low density lipoprotein cholesterol), HDL-C (High density lipoprotein cholesterol), apoA and apoB were measured in an autoanalyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo) using special kits (DiaSys, Germany). CRP(C-reactive protein) was measured by rabbit CRP ELISA (Rapidbio, USA). Ox-LDL measured by rabbit Ox-LDL ELISA (Rapidbio, USA). All of these biochemical factors were measured at the beginning, 30^{th} day and 60^{th} day of the experiment except for Ox-LDL, which was measured only at 60^{th} day. Atherosclerosis index (AI) was calculated using the following formula: AI= LDL-C/HDL-C (Mertz, 1980).

Histopathological analysis of aorta

On the 60^{th} day, all the animals were killed by pentobarbital (5%) injection. Aortic tissue parts were stained by Hematoxyline-Eosin method. Chekanov scale was used for grading of atherosclerotic plaques and the results were determined on a scale of 1-4 in relation to the thickness of media layer (Chekanov, 2003).

Statistical analysis

Differences between groups were statistically analyzed by one-way ANOVA, and between the means of groups were separated by least significant difference (LSD) test. All data were presented as mean ± SD. Values of $P<0.05$ were considered as significant.

RESULTS

Each 100 g powder of *A. caudatus* results in 3.8 ± 0.029 g extract powder. The amount of total flavonoids based on hyperoside and anthocyanins in 100 g of *A. caudatus* extract is 0.379 ± 0.02 g and 24.1 ± 1.29 mg, respectively.

High cholesterol diet caused a significant increase in Chol, TG, LDL-C, HDL-C, AI, apoB and CRP in comparison with standard group and also standard diet with A. caudatus extract measured at the 30^{th} and 60^{th} day of experiment (Figure 1).

For Rabbits fed with standard diet and *A. caudatus* extract, there was no significant changes in biochemical factors except for HDL-C level, which was significantly increased in this group at the 60^{th} day of experiment (Figure 1)

Rabbits fed with high cholesterol diet with *A. caudatus* extract TG, CRP, apoB, apoA and AI was significantly decreased at the 30^{th} day as compared with high cholesterol diet and Chol, and LDL-C was significantly decreased at the end of study (60^{th} day). There was no significant difference between the lovastatin and *A. caudatus* extract in hypercholesterolemic rabbit on biochemical factors except for apoB where the effect of lovastatin was not significant.

Also Ox-LDL level in high cholesterol diet plus *A. caudatus* extract or lovastatin in comparison with high cholesterol group was reduced significantly at the end of study (60^{th} day) (Figure 1).

Histological study showed that, standard group had completely normal arteries without any lesion in intima or media. In high cholesterol group, atheroma plaques were formed with macrophages filled with fat which created foamy cells and also smooth muscle cells. Plaque thickness was also increased to more than half of media thickness, equal to degree 3 of Chekanov scale. In standard diet plus *A. caudatus*, no pathologic lesions were seen.

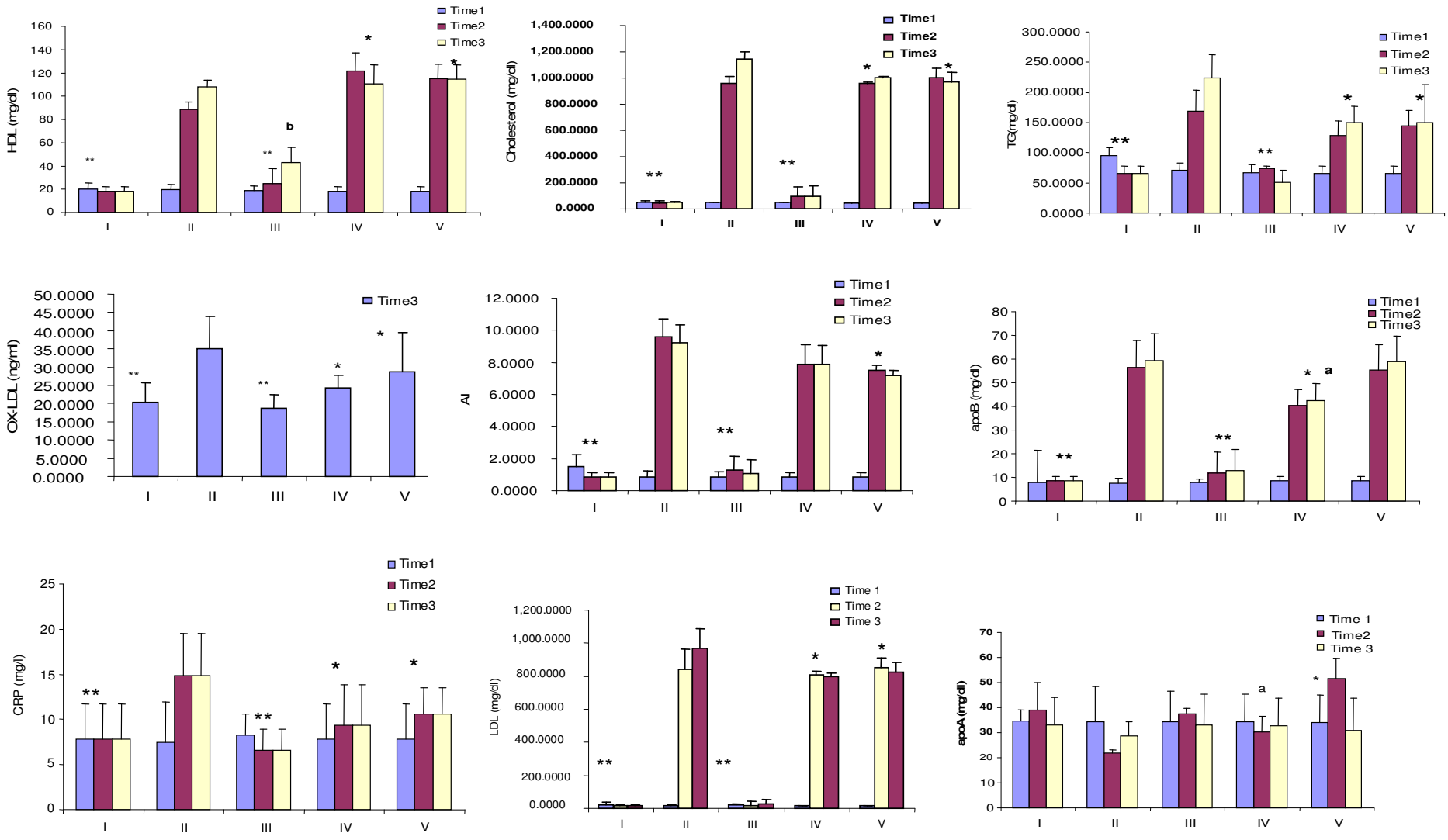

Figure 1. Comparing the biochemical factors in each experimental groups prior to the beginning of diets (Time 1), at the 30th day (Time 2), and 60th day (Time 3) of the experiment., I; normal group, II; high cholesterol group, III; normal diet with *Amaranthus Caudatus* extract, IV; high cholesterol diet with *Amaranthus Caudatus* extract, V; high cholesterol diet with lovastatin, TG; triglyceride, LDL-C; Low Density Lipoprotein, apo B; apolipoprotein B, CRP, Ox-LDL; oxidized LDL, HDL-C High Density Lipoprotein;, apo A apolipoprotein A;, AI (atherogenic index)=LDL-C/HDL-C. *P<0.05; significant difference between high cholesterol treated groups (IV or V) in comparison to high cholesterol group. **P<0.05; significant difference between normal treated groups (I or III) in comparison to high cholesterol group. a(P<0.05); significant difference between high cholesterol with *Amaranthus Caudatus* extract groups in comparison to high cholesterol group. b(P<0.05); significant difference between normal group and normal diet with *Amaranthus Caudatus* extract. Data are shown as mean ± standard deviation.

In high cholesterol plus *A. caudatus*, severity of lesions were significantly reduced in comparison to high cholesterol group and was limited to a small number of macrophages in intima layer. Plaque degree was 1 and plaque thickness was less than a half of media thickness. In lovastatin group severity of plaques was equal to degree 1 of Chekanov scale and atherogenic lesion severity was less in comparison to cholesterol recipient. However, in comparison to treated group with cholesterol, lesion severity seems to be higher (Figure 2).

The mean of thickness and length of lesion in high cholesterol plus *A. caudatus* decreased significantly in comparison to high cholesterol group and lovastatin (Figure 3).

DISCUSSION

This study showed that *A. caudatus* extract and also lovastatin significantly decreased Chol, LDL-C, TG, Ox-LDL, CRP, AI and increased apoA and HDL-C in hyper-cholesterolemic rabbits. Further, *Amaranthus* extract decreased the apoB significantly more than lovastatin. Also its effect on HDL-C level was as same as of lovastatin. Recent studies have shown that apo B is one of the most important risk predictors for cardiovascular disease (Rasouli et al., 2006). It seems that the extract by hypolipidemic, anti-oxidant and anti-inflammatory properties, prevent atherosclerosis. In the present study, the rabbits fed with high cholesterol diet plus *A. caudatus* extract showed significantly lower levels of CRP in comparison with the rabbits fed with high cholesterol diet, and the rabbits fed with high cholesterol diet plus lovastatin. Study further revealed this extract decreased atherosclerotic lesions in hypercholesterolemic rabbits significantly more than lovastatin. Histological study demonstrated that the length and thickness of atherosclerotic lesions in the rabbits fed with high cholesterol diet plus *A. caudatus* extract decreased as compared to high cholesterol diet group (and group fed with high cholesterol diet plus lovastatin). Andrea et al. (2002), shows positive effect of *A. caudatus* extract to decrease of cholesterol level, LDL-C, VLDL-C and TG (Andrea et al., 2002). Qureshi et al. (1991), shows that*A. caudatus* extracts contains tocotrienol and tocopherol (Qureshi et al., 1991). Recently, it has shown that these two substances regulate cholesterol metabolism (Yu et al., 2006). Qureshi et al. (1996) observed that consumption of 200 mg per day tocotrienol for 8 weeks reduces cholesterol and LDL-C level 15 and 8%, respectively (Qureshi et al., 1996). Danz et al. (1992) observed that beneficial effect of *A. caudatus* on lipid levels in rats is dependent on its soluble and insoluble fibers (Danz et al., 1992).

A. caudatus contains flavonoids, anthocyanins, carotenoids, vitamin C, folate, and folic acid, high amount of methionine, lysine, and unsaturated fatty acids. These antioxidants scavenge the free radicals and thereby reduce their damage. Beta carotene has strong antioxidant effect for damage of free radicals and it has beneficial effect on lipid oxidation (Qureshi et al., 1991). Ascorbic acid preserves membrane against peroxidation by increasing tocopherol activity (Qureshi et al., 1996). Also ascorbic acid decreases tocopherol activity and consequently causes activation of free radicals scavenging by tocopherol (Yu et al., 2006). Flavonoids protect alpha tocopherol and probably other antioxidants against oxidation (Yu et al., 2006). The results of recent studies suggest that flavonoids decrease plasma lipids and atherosclerosis and it is denoted that anti-hypercholesterolemic effect of flavonoids is related to decrease of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) and decrease in apo B secretion in hepatocytes (Thakur et al., 2001; Borradaile et al., 2002). Anthocyanins have anti inflammatory and free radical scavenging activity (Pergola et al. 2006). A great number of studies have shown that, anthocyanins prevents endothelial damages and act as an inhibitor of endothelial cell death (Borradaile et al., 2002; Pergola et al., 2006). Anthocyanins protect endothelial cell by inhibition peroxynitrites that it leads to oxidative damages (Pergola et al., 2006).

Hypolipidemic effect of *Amaranthus hypochondriacus* in animal and antidiabetic and antioxidative effect of *Amaranthus esculantus* in streptozotocin-induced diabetic rats concurrent with improvement of lipid profile is well established (Berger et al., 2003; Kim et al., 2006). The inclusion of amaranth oil in the diet had a beneficial effect upon the clinical presentation of coronary heart disease and hypertensive patients. Amaranth oil decreased the amount of Chol, TG, LDL-C and VLDL-C significantly and contributes to an increase in the concentration of polyunsaturated fatty acids, particularly, long-chain omega-3 fatty acids in patients suffering from hypertension and coronary heart disease (Martirosyan et al., 2007; Pogojeva et al., 2006). The results of this study therefore suggest that the addition of *A. caudatus* to hyperlipidemic diets may prevent progression of atherosclerosis. Therefore, *Amaranthus* and its products could be a valuable additive for cereals to prevent atherosclerosis, and it is especially recommended for allergic hypercholesterolemic patients.

Conclusion

Atherosclerosis can begin in the late teens, but it usually takes decades to cause symptoms. Some people experience rapidly progressing atherosclerosis during their thirties, others during their fifties or sixties. Atherosclerosis is complex. Its exact cause is still unknown. It is thought that atherosclerosis is caused by a response to damage to the endothelium from high cholesterol, high blood pressure, and cigarette smoking.

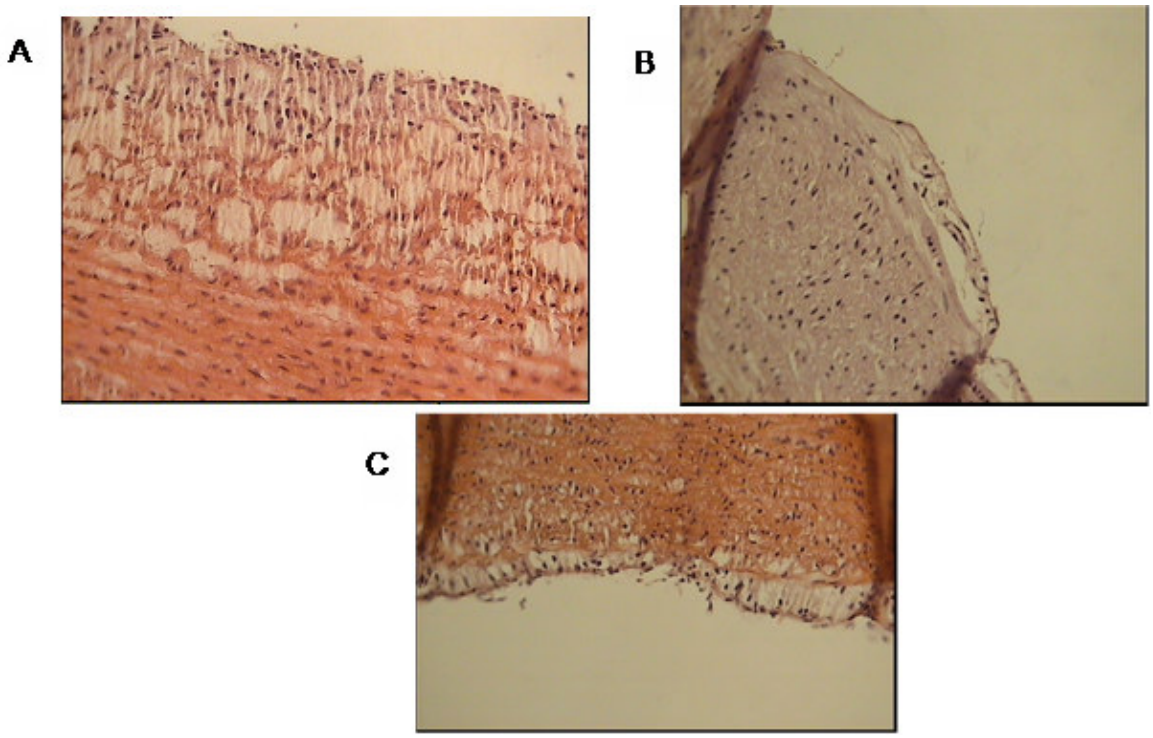

Figure 2. Cross sections of the aorta: (a) group fed with high cholesterol diet, magnification ×40, (b) group fed with high cholesterol diet+ *Amaranthus Caudatus* extract, magnification ×40 (c) group fed with high cholesterol diet and lovastatin, magnification ×40.

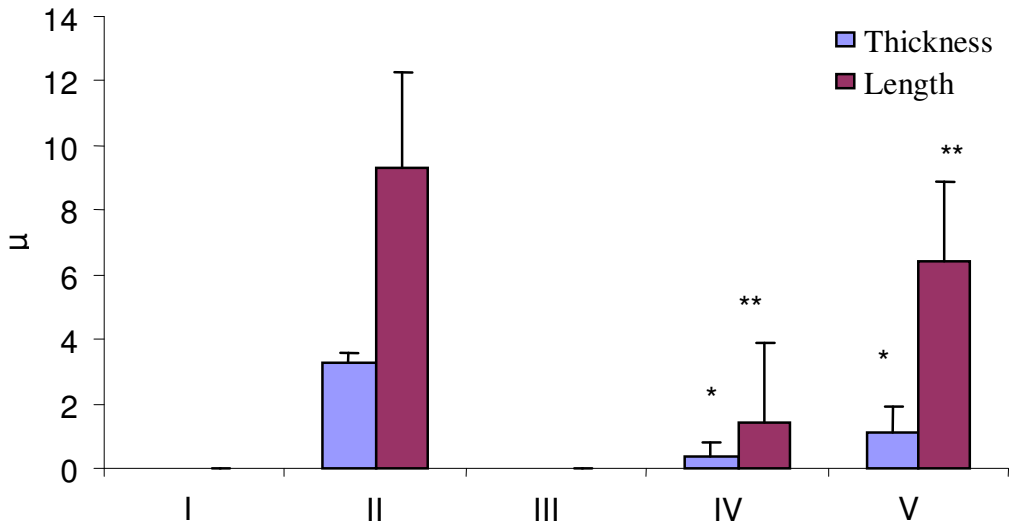

Figure 3. Plaque thickness and Length in studied groups *P < 0.05; significant difference of the thickness of plaque between high cholesterol treated groups (IV or V) in comparison to high cholesterol group ** P < 0.05 significant difference of the length of plaque between high cholesterol treated groups (IV or V) in comparison to high cholesterol group.

A person who has all three of these risk factors is eight times more likely to develop atherosclerosis than is a person who has none. Physical inactivity, diabetes, and obesity are also risk factors for atherosclerosis. High levels of the amino acid homocysteine and abnormal levels of protein-coated fats called lipoproteins also raise the risk of coronary artery disease. These substances are the targets of much current research. The role of

triglycerides, another fat that circulates in the blood, in forming atherosclerotic plaques is unclear. High levels of triglycerides are often associated with diabetes, obesity, and low levels of high-density lipoproteins (HDL cholesterol). The more HDL ("good") cholesterol, in the blood, the less likely is coronary artery disease. These risk factors are all modifiable. Non-modifiable risk factors are heredity, sex, and age.

Risk factors that can be changed are:

1. Cigarette/tobacco smoke: Smoking increases both the chance of developing atherosclerosis and the chance of dying from coronary heart disease. Second hand smoke may also increase risk.
2. High blood cholesterol: Cholesterol, a soft, waxy substance, comes from foods such as meat, eggs, and other animal products and is produced in the liver. Age, sex, heredity, and diet affect cholesterol. Total blood cholesterol is considered high at levels above 240 mg/dl and borderline at 200-239 mg/dl. High-risk levels of low density lipoprotein (LDL cholesterol) begin at 130-159 mg/dl.
3. High triglycerides: Most fat in food and in the body takes the form of triglycerides. Blood triglyceride levels above 400 mg/dl have been linked to coronary artery disease in some people. Triglycerides, however, are not nearly as harmful as LDL cholesterol.
4. High blood pressure: Blood pressure of 140 over 90 or higher makes the heart work harder, and over time, both weakens the heart and harms the arteries.
5. Physical inactivity: Lack of exercise increases the risk of atherosclerosis.
6. Diabetes mellitus: The risk of developing atherosclerosis is seriously increased for diabetics and can be lowered by keeping diabetes under control. Most diabetics die from heart attacks caused by atherosclerosis.
7. Obesity: Excess weight increases the strain on the heart and increases the risk of developing atherosclerosis even if no other risk factors are present.

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