The atheroprotective effects of Vitamin E in hypercholesterolemic male rabbits

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Abstract

Background: Atherosclerosis remain as a major health problem occasioning early death in much of the world's people. The ancestry of this diseases related to genetic influences and dietary improperly. The main sources of dietary cholesterol are eggs, meat, and milk products, which induced hypercholesterolemia and atherosclerosis in some species of animals. Materials and Methods: Eighteen local domestic male rabbits will be randomly allocated into 3 groups, 6 in each Group: Group I (n = 6), control; Group II (n = 6), rabbits were receiving 1% cholesterol diet (induced untreated group); Group III (n = 6), 1% cholesterol-diet + Vitamin E (400 mg/kg daily orally). After zero time, four weeks and eight weeks of the study Blood samples were collected for lipid profile (triglycerides, total cholesterol, high density lip protein and serum IL-6, serum high sensitive C-Reactive Protein hs-CRP, serum MCP-1 and serum HMG-box1. Results: Data of this present study has shown that, high fat diet diet caused an increase in serum level of, TG, LDL-C, VLDL-C and TG  were increased and decrease serum level of HDL-C compared with the control group in the rabbits feed hypercholesterolemic diet (P < 0.05). Histologically all induced-untreated rabbit showed increase aortic intima-media thickness (P < 0.05). Vitamin E treated cause significant change on lipid profile (P < 0.05) compared with the induced untreated group in compared with induced untreated group (P < 0.05), Vitamin E showed significant the change in hs-CRP, IL-6, MCP-1 and HMG-box1.

Keywords: Vitamin E, atherosclerosis, inflammation, lipid profile
1. Introduction

Atherosclerosis is a chronic inflammatory and immune disease where lipids block the arteries (atheroma or plaques) [1]. In the United states atherosclerosis causes one third of the all the mortality [2]. It is characterized by the accumulation of fatty lesions along the walls of the aorta, which limits flow of blood [3]. Higher total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and triglycerides (TG) declines have been suggested to affect lipoprotein metabolism, including testosterone levels in men, both correlated with increased risk of atherosclerosis [4].

There are many risk factors for atherosclerosis which either:
1. Not adjustable such as age, origin race, gender and genetic factors.
2. Alteration such as Diet, high cholesterol, dyslipidemia, high blood pressure, diabetes, obesity and smoking [5,6].

High cholesterol (hypercholesterolemia) is the main risk factor for the development of atherosclerosis. The progression of atherosclerosis begins with an increase in the retention of low-fat lipoprotein (LDL) and very low density lipoprotein (VLDL) particles in the receptacle. Accordingly, the vessel walls liberate the inflammation and oxidation factors that alter these fatty proteins, such as by oxidation, leading to LDL oxidized (oxLDL). LDL can also be altered by the enzymes of hydrolyzing in the injury site [7,8]. Immune cells, especially monocytes, are recruited from the circulatory system when expressing for a chemical attractor such as tumor necrosis factor (TNF) and monoclonal protein (MCP-1) in atherosclerosis. Monoclonal cells are recruited in plaques. Altering fatty proteins and accumulating in this way leads to their development into foam cells. Foam cells increase the inflammatory response, leading to the recruitment of additional immune cells in atherosclerotic plaques. While a nucleus is formed and the atherosclerotic plaque develops, the capillary endothelium cells are destabilized. In an attempt to stabilize this cover, smooth muscle cells (SMC) multiply from the middle to the inside of the lining and become elastin and collagen. At the moment when the balance is supportive of destabilizing factors, the lid may explode, which may lead to a cardiovascular condition [9]. Many mediators of inflammation are secreted by cells in the vessel wall which affects the function of the buds. Also, secretion of mediators for
inflammation by damaged macrophages perform a significant role in the development of atherosclerotic plaque [10].

Vital signs of inflammation expressed through smooth muscle cells (SMCs) inside the plaque of atherosclerosis, such as TNF, interleukin, IL-6, MCP-1, IL-18, growth factors, CD-40 and insulin-1 growth factor (IGF-1) Platelet growth (PDGF), all produced by macrophages, has many effects on gene expression in surrounding cells and is involved in increased absorption of lipoproteins and recruitment of inflammatory cells in the plaque [11,12].

The effect of these biomarkers is different and includes the spread of intracellular matrix, vascularization, mutagenesis, and development of foam cells. Frequent inflammatory cycles cause the accumulation of macrophages, some of which can die at the site in this region, to produce the so-called necrotic nucleus, and stimulate the generation of smooth muscle cells and migration to plaque to form the fibrous cover of the advanced complex sclerotic lesion [13].

Fatty strands are the first to be visible to atherosclerosis, due to the accumulation of fat-packed foam cells in the inner layers of the artery. Over time, the fatty streak grows into a fibrous plaque, the main sign of arteriosclerosis. Eventually atherosclerosis may develop to contain larger amounts of fat. If they become unstable, plaque damage, or damage to the internal cells, it may lead to thrombosis in the artery [14].

Vitamin E (α-tocopherol) is the primary lipid-soluble component in the antioxidant system of cell defense, it is mainly obtained from the diet. the antioxidant activity of Vitamin E plays important roles within the body [15]. Vitamin E is a good chain-breaking antioxidant that prevents the development of reactive oxygen species molecules when lipids are oxidative and free radical reactions spread [16].

through decreasing overall oxidative stress, annihilation of LDL oxidation, removal of scavenger receptors and adhesion molecules, reduction of pro-inflammatory cytokines, inflammation and macrophage activation and decreased thromboxane output Vitamin E plays its anti-atherosclerotic function [17].
2. Materials and Methods

Eighteen local domestic male rabbits will be randomly allocated into 3 groups, 6 in each Group: The first group I, control; The second group II, rabbits were receiving 1% cholesterol diet; The third Group III, Receiving Vitamin E(400 mg/kg daily orally) and 1% cholesterol-diet. After zero time, four weeks and eight weeks of the study Blood samples were collected for measurement of lipid profile (triglycerides, total cholesterol, high density lip protein and serum IL-6, serum high sensitive C-Reactive Protein hs-CRP, serum MCP-1 and serum HMG-box1.

Blood sampling

From the central ear artery about 5 ml of blood was collected from each rabbit following an overnight fasting. The blood sampling was made at zero time, 4weeks and 8 weeks of the induction period. The samples of blood were permitted to clot at 37 C and starting centrifuged at 6000 rpm for 12 minute; The serum were isolated, and analyzed for measurement of serum TC, TG, HDL- C, LDL-C, VLDL-C, IL-6, C-Reactive protein, MCP-1 and HMGbox1.

3. Results

Data of this present study has shown that, high fat diet diet caused an increase in serum level of, TG, LDL-C, VLDL-C and TG were increased and decrease serum level of HDL-C compared with the control group in the rabbits feed hyper cholesterolemic diet (P < 0.05). Histologically all induced-untreated rabbit showed increase aortic intima-media thickness (P < 0.05). Vitamin E treated cause significant change on lipid profile (P < 0.05) compared with the induced untreated group. in compared with induced untreated group (P < 0.05), Vitamin E showed significant the change in hs-CRP, IL-6, MCP-1 and HMG-box1.

**Table 1.** effect of hight cholesterol diet and Vitamin E on lipid profile for three experimental groups.
# Table 2: effect of high cholesterol diet and Vitamin E on (hs-CRP, IL-6, MCP-1 and HMGbox1).

|                          | TC mg/dl | HDL mg/dl | TG mg/dl |
|--------------------------|----------|-----------|----------|
| control group            |          |           |          |
| 0 time                   | 49.5±2.1 | 21.0±1.7  | 48.8±3.0 |
| 4 weeks                  | 50.8±3.4 | 20.1±1.5  | 47.0±3.8 |
| 28 days                  | 44.3±0.8 | 21.5±0.8  | 46.5±2.4 |
| Induced untreated group  |          |           |          |
| Zero time                | 57.3±1.8 | 20.2±1.4  | 46.3±3.7 |
| 4 weeks                  | 123.7±17.8* | 13.9±0.5* | 625.0±12.1* |
| 28 days                  | 172.0±6.7† | 12.0±0.2† | 859.0±73.8† |
| Vitamin E treated group  |          |           |          |
| 400 mg/kg                |          |           |          |
| 0 time                   | 51.5±3.3 | 20.8±0.8  | 49.2±2.7 |
| 4 weeks                  | 143.7±11.6* | 10.8±0.2* | 704.8±24.7* |
| 28 days                  | 85.2±11.2† | 16.4±0.4† | 335.2±31.2† |

* consider significant as a comprised to zero time, † consider significant as a comprised to 4weeks.

**Table 2:** effect of high cholesterol diet and Vitamin E on (hs-CRP, IL-6, MCP-1 and HMGbox1).

|                          | IL-6 pg/l | hs-CRP mg/l | MCP-1 | HMGbox1 |
|--------------------------|-----------|-------------|-------|---------|
| control group            |           |             |       |         |
| Zero time                | 1.3±0.1   | 3.0±0.2     | 0.7±0.1 | 0.7±0.03 |
| 4 weeks                  | 1.3±0.1   | 3.4±0.1     | 1.0±0.1 | 0.8±0.1 |
| 28 days                  | 1.2±0.2   | 3.3±0.2     | 0.7±0.1 | 0.7±0.2 |
| Induced untreated group  |           |             |       |         |
| 0 time                   | 1.5±0.3   | 3.0±0.2     | 1.0±0.2 | 0.8±0.1 |
| 28 days                  | 4.7±0.3*  | 5.5±0.2*    | 4.2±0.3* | 2.9±0.04* |
| 28 days                  | 6.6±0.3†  | 7.5±0.2†    | 5.6±0.2† | 4.1±0.09† |
| Vitamin E treated group  |           |             |       |         |
| 400 mg/kg                |           |             |       |         |
| 0 time                   | 1.4±0.3   | 3.1±0.2     | 0.9±0.2 | 0.7±0.3 |
| 4 weeks                  | 5.3±0.4*  | 6.3±0.4*    | 4.5±0.2* | 3.3±0.2* |
| 28 days                  | 0.4†±2.9  | 5.4±0.4†    | 2.6±0.2† | 1.6±0.1† |

* consider significant as a comprised to zero time, † consider significant as a comprised to 4weeks.
Table 3: Aortic intimal thickness (px) after 8 weeks. All data are presented as mean ± SEM.

| Group                      | Aortic intimal media thickness (μm) |
|----------------------------|-------------------------------------|
| control group              | 36.5±2.8                            |
| Dietary induced untreated group | 454.6±38.3*                      |
| Vitamin E treated          | 135.0±11.0†                         |

* Consider significant as compared to control group, † Consider significant as a comprised to induced-untreated group

Figure 1: Photomicrograph showing no development of aortic intimal media thickness (red arrow) in the aorta (normal control group). The segment was contaminated with eosin and hematoxylin (x10).
Figure 2: Photomicrograph showing development of aortic intimal media thickness (red arrow) in the aorta (induced untreated group). The segment was contaminated with eosin and hematoxylin (x10).

Figure 3: Photomicrograph showing decrease of aortic intimal media thickness (red arrow) aorta (Vitamin E treated group). The segment was contaminated with eosin and hematoxylin (x10).
4. Discussion

Atherosclerosis is described by decreased high density lipoprotein (HDL) and increased serum total cholesterol, low density lipoprotein (LDL), deposition of fibrous and lipids in large and medium size arteries. Higher of cholesterol with oxidized LDL and free radical stress has been reported to be directly embroiled in the start of atherosclerosis by Shaheen et al [18]. In this study, it is shown that the feeding of a cholesterol enriched diet to rabbits for 8 weeks resulted in marked hypercholesterolemia in lipid profile (total cholesterol, triglyceride, low density lipoprotein, and very low density lipoprotein increased significantly. While, high density lipoprotein level decreased significantly. Thus observation in conform with earlier studies by (Mudhafar et al., 2018) [19] and Majeed et al [20]. Serum of TC, TG, HDL, LDL-C, and VLDL in Vitamin E treated rabbits significant changes in comparison with induced untreated rabbits. These results were confirm by (Adedapo, Adepoju and Olusanya, 2019) [21]. This results demonstrated that the Vitamin E showed a significant lipid as a consequence of its antioxidant and anti-hyperlipid effects, Vitamin E lowering the capability of LDL oxidation, reducing the cholesterol concentration.

In addition, reduce of triglyceride concentration may result from the antioxidant vitamin E effect of decreasing glucocorticoid excretion., Inhibiting stimulation tissue lipase and lipoprotein thereby lowering Tissue Lipid mobilization [18], This decrease may also be attributed to prevent fatty acid synthesis and other metabolic enzymes like glucose-6-phosphate dehydrogenase and fatty acid synthetized.

Treatment with Vitamin E significantly decrease hs-CRP level These findings agreement with Libinaki et al [22], Who found that Vitamin E reduce CRP, IL-6 and IL-8 and reducing lesion development in a hypercholesterolaemic rabbit model, this result could be attributed to the free radical activity salvaging by antioxidants and possible clearance by the immune system [21]. In the current study, Treatment with vitamin E causes important reduction of IL-6 level these result are in line with (Lira et al., 2011)[23] who showed that Vitamin E supplementation have an anti-inflammatory impact through decreasing Interleukin-6. In the current study, Vitamin E causes a
significant decrease in level of MCP-1 these result may be due to that vitamin E prevent
the development of MCP-1 by stimulated endothelium, This, in effect, prevents the
recruitment of monocytes to the stimulation site. It leads to inhibiting the development of
fatty lesions in the early stages of atherosclerosis and may impede the progression of
established lesions. Wu et al [24].

Vitamin E decreased aortic intimal media thickness This positive effect could be due its
anti-oxidation effect Tang et al [25]. Another potential mechanism for anti-ischemic
improvement by vitamin E has also been suggested by Freedman et al [26] who showed
that the antioxidant properties of vitamin E prevent platelet formation by reducing
smooth muscle cell proliferation and platelet aggregation. In addition,
Vitamin E contributes to many components of the circulatory system, for example,
smooth muscle cells ,Endothelial cells and platelets, which make it possible to alter many
inflammatory processes contributed in atherogenesis [27,24].

Finally, data from this study have showed that Vitamin E was increase HDL and decrees
of serum lipid profile (TC, TG, LDL and VLDL) and decrees inflammatory biomarker
IL-6, hs-CRP, MCP-1 and HMG-box1. Furthermore, Vitamin E decreased aortic intimal
media thickness [28-31].

Conclusion

The results suggest the treated with Vitamin E can be exploited for prevention of lipid
profile and inflammatory biomarkers in hypercholesterolemic rabbits.

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