Original Article

Assessment of Lipid Lowering Effects of Vitamin E

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ABSTRACT

Objective: Aim of this study was to determine the role of antioxidant vitamin E in lowering the serum cholesterol and triglyceride levels.

Place and Duration: This study was conducted in the department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi. Duration of study was 42 days from1-03-2008 to 11-4-2008.

Materials and Methods: Male 20 rabbits were equally divided into 2 groups, group A was served as control and the group B was vitamin E treated. All the animals were maintained on cholesterol rich diet for a period of 30 days in order to induce hypercholesterolemia. After induction of hypercholesterolemia the group B was given vitamin E in a dose of 8.57mg/kg/day while the control group A was maintained on distilled water only for a period of 42 days. At the end of 42 days the blood samples were collected from marginal ear vein of rabbits and were analyzed to determine the levels of cholesterol and triglycerides.

Results: The results showed that there was decrease in the level of cholesterol but the level of triglyceride was reduced much significantly by administration of vitamin E. It has been reported earlier that vitamin E has potential to decrease levels of cholesterol and triglycerides.

Conclusion: It can be concluded that vitamin E use can reduce the level of cholesterol and triglyceride in hypercholesterolemia and may prove to be beneficial.

Key words: Alpha tocopherol or vitamin E, Hypercholesterolemia, Low density lipoprotein (LDL), Antioxidant, Oxidative stress.

INTRODUCTION

Hyperlipidemia is defined as an elevation of blood lipids particularly cholesterol and triglycerides (1).There is correlation between hyperlipidemia and lipid peroxidation so when there is increase in the lipid content more is the chance of oxidation as free radicals reacts more quickly with near by fats, charbohydrates and proteins (2). In hyperlipidemia there is increase in lipoproteins levels especially LDL which is also known as bad cholesterol. Increase lipoprotein can enhance the process of development of atherosclerosis (3). In vitro studies have shown that LDL cholesterol and, in particular, its oxidized derivative are injurious to the endothelium (4). Oxidized LDL has several biological consequences (5). It promotes vasoconstriction, promotes adhesion, stimulates cytokines such as interleukin-1 (IL-1), increases platelet aggregation, inhibits nitric oxide tissue factor secretion, and stimulates plasminogen activator inhibitor-1 synthesis. A growing evidence suggests implication of inflammatory reactions playing a major role in the development of atherosclerosis, thus, clearly supporting atherosclerosis as an inflammatory disease (6),(7). Major cellular participants in atherosclerosis include monocytes, macrophages, active vascular endothelium, T lymphocytes, platelets, and smooth muscle cells (8),(9),(10). In atherosclerosis there is up-regulation of pattern-recognition receptors for innate immunity, including scavenger receptors and toll-like receptors (11),(12). Scavenger receptors (e.g CD-36) internalize a broad range of molecules and particles bearing molecules with pathogen-like molecular patterns (11). Bacterial endotoxins, apoptotic cell fragments, and oxidized LDL particles are all taken up and destroyed through this pathway. If cholesterol derived from the uptake of oxidized LDL particles cannot be mobilized from the cell to a sufficient extent, it accumulates as cytosolic droplets. Ultimately, the cell is transformed into a foam cell, the prototypical cell in atherosclerosis.

Oxidative stress seems to play a key role in the pathogenesis of atherosclerosis (13). Free radicals, also known as reactive oxygen species, are chemical compounds that have one or more unpaired electrons, allowing them to react quickly and unpredictably with nearly any nearby protein, fat, carbohydrate, or nucleic acid in a chemical reaction called oxidation (2). Agents that protect low-density lipoprotein from oxidation have been shown in a range of in vitro and animal models to reduce the development and progression of atherosclerosis. These agents include antioxidant micronutrients such as vitamin E. They have gained

wide interest because of the potential for prevention of atherosclerotic vascular disease in humans (13).

Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. "Vitamin E" is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities (14).

Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity (14). Alpha-Tocopherol is the most prevalent of these, composing more than 90% of the tocopherols in animal tissues and displaying the most activity in vitro. While most of its functions have been thought to be related to antioxidation in the patient, new research has identified the hormonal effects of vitamin E. Homologues of alpha-tocopherol, for example, suppress arachidonic acid metabolism, and conjugates of vitamin E can affect cell signaling functions (15).

Vitamin E can modulate signal transduction and gene expression and thus affect many cellular reactions such as the proliferation of smooth muscle cells, the expression of cell adhesion and extra cellular matrix molecules, the production of superoxides by NADPH oxidase, the aggregation of platelets and the inflammatory response. Vitamin E may modulate the extracellular matrix environment by affecting the vascular smooth muscle cells differentiation and the expression of connective tissue proteins involved in vascular remodeling as well as the maintainence of vascular wall integreity (16).

The objective of the present study is to determine the role of antioxidant vitamin E in lowering hyperlipidemia particularly cholesterol and triglyceride levels.

MATERIALS AND METHODS

The present study was carried out on 20 locally bred rabbits weighing from 900 –1500 gram purchased from local market rabbit supplier. All animals were equally divided into 2 groups each comprising of 10 animals, 1 group was served as control and the second group was vitamin E treated.

The animals were caged in pair in iron cages. Before administration of drugs the rabbits were fed on 5 ml cholesterol rich diet twice daily orally for a period of one month in order to induce hypercholesterolemia. Along with the cholesterol rich diet animals were also given chow and tap water. All the animals were maintained under constant environment condition 21 ± 1°C and humidity (50-60%).

After day 30^{th}, the blood samples were drawn from animals in order to ensure that hypercholesterolemia has been induced. After inducing the hypercholestreolemia the effect of vitamin E has been studied.

The antioxidant dose of vitamin E i.e 400 IU/day= 600mg/day restores endothelial function in hyperlipidemia (17).

70 kg adult = 400 IU = 600 mg of vitamin E

1 kg = 8.57 mg of vitamin E

Hence the dose of vitamin E is 8.57mg/kg/day

The control group animals were given distilled water only. The dose of the drugs was given to the animals daily for a period of 42 days. During the course of treatments the animals were maintained on same cholesterol rich diet.

Before starting the dosing, the blood samples were collected from marginal ear vein of each control rabbit. The blood samples of the animals were then collected on day 42 to study the effect of drug.

After separation the of serum, cholesterol and triglyceride levels were analyzed within 3 hours of sample collection on Humalyzer 3000 (Semi-automatic chemistry analyzer Model # 16700 by Human Germany) using standard kits supplied by Human.

Statistical analysis

Comparison of difference of mean between control and vitamin E treated group was made by using student's t-test. A *p* value less than 0.05 was considered statistically significant and *p* value less than 0.005 was considered highly significant.

RESULTS

Table 1 showing the effect of vitamin E on cholesterol levels of rabbits. In control group the serum cholesterol level after inducing hypercholesterolemia was 100.66 ± 12.07 mg/dl which was reduced to 71.62 ± 6.37 with $p < 0.05$.

Table 2 showing the effect of vitamin E on triglyceride levels of rabbits. In control group the serum triglyceride level after inducing hypercholesterolemia was 161.69 ± 10.57 mg/dl which was reduced to 23.72 ± 3.59 with $p < 0.005$.

Table – 1: Effect of drugs on cholesterol level of rabbits

Drugs	Cholesterol mg/dl
	42 Days
Control	100.66±12.07
Vitamin. E	71.62±6.37

Values are mean ± S.E (n=10)

P<0.05* = Significant

P<0.01** = Moderately Significant

P<0.005*** = Highly Significant

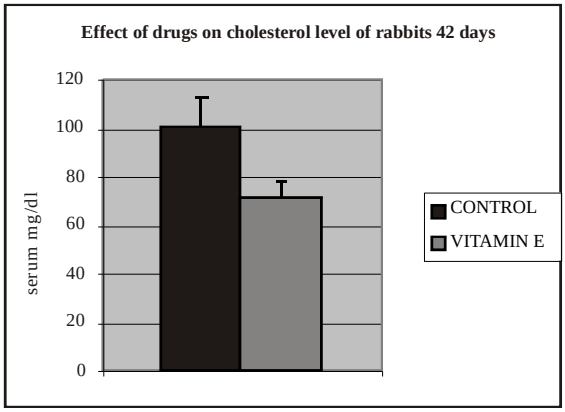


Table – 2: Effect of drugs on triglyceride level of rabbits

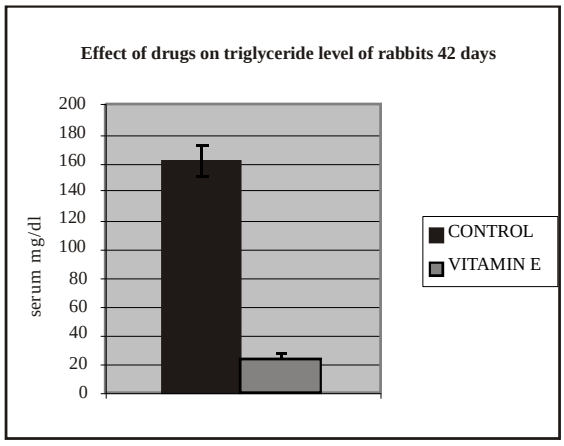
Drugs	Triglyceride mg/dl
	42 Days
Control	161.69±10.57
Vitamin. E	23.72±3.59

Values are mean ± S.E (n=10)

P<0.05* = Significant

P<0.01** = Moderately Significant

P<0.005*** = Highly Significant



DISCUSSION

A significant decrease in the level of cholesterol and triglyceride was observed in vitamin E treated group after day 42. Vitamin E and other antioxidants protect different structures against oxidative damage and lipid peroxidation, often measured as the production of malondialdehyde in plasma and tissues (18).

Alpha tocopherol is the most important lipid-soluble antioxidant that protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. Alpha tocopherol has properties of a peroxyl radical scavenger. The importance of this function is to maintain the integrity of long-chain polyunsaturated fatty acids in the membranes of cells and thus maintain their bioactivity (19).

As antioxidant, vitamin E acts in cell membranes which prevents the propagation of free radical reactions. Non-radical oxidation products are formed by the reaction between alpha-tocopheryl radical and other free radicals, which are conjugated to glucuronic acid and excreted through the bile or urine (20).

In addition to its activities as an antioxidant, vitamin E is involved in immune function and, as shown primarily by in vitro studies of cells, cell signaling, regulation of gene expression, and other metabolic processes (20). Alpha-tocopherol inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets, and monocytes. Protein kinase C inhibition by α-tocopherol is in part attributable to its attenuating effect on the generation of membrane-derived diacylglycerol, a lipid that facilitates protein kinase C translocation, thus increasing its activity.Vitamin E enrichment of endothelial cells downregulates the expression of intercellular cell adhesion molecule (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), thereby decreasing the adhesion of blood cell components to the endothelium. Vitamin E also upregulates the expression of cytosolic phospholipase A_2 and cyclooxygenase-1. The enhanced expression of these two rate-limiting enzymes in the arachidonic acid cascade explains the observation that vitamin E, in a dose-dependent fashion, enhanced the release of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation in humans (21).As we have discussed earlier that there is implication of inflammatory processes in atherosclerosis so all the above actions of vitamin E contributes in improving immune function, lowering inflammatory processes and hence eventually atherosclerosis.

In one study, the role of Vitamin E on CD36 expression in an in vivo model was investigated. Atherosclerosis was induced by 2% cholesterol containing Vitamin E poor diet. Three groups of six rabbits each were studied. The first group (control) was fed on Vitamin E poor diet. The second group was fed with Vitamin E poor diet containing 2% cholesterol and the rabbits in the third group were fed with Vitamin E poor diet containing 2% cholesterol and received injections of 50 mg/kg of Vitamin E I/M. After 4 weeks, aortas were removed and analyzed by light microscopy for atherosclerotic lesions. Aortic samples were analyzed for CD36 mRNA expression. The aortas of cholesterol-fed rabbits showed typical atherosclerotic lesions, detected by macroscopic and microscopic examination, and exhibited an increase in CD36 mRNA expression. Vitamin E fully prevented cholesterol induced atherosclerotic lesions and the induction of CD36

mRNA expression. The effects observed at the level of CD36 scavenger receptor expression in vivo suggest an involvement of reduced foam cell formation in the protective effect of Vitamin E against atherosclerosis (22). The above described all actions of vitamin E together contributes in lowering the cholesterol level in hypercholesterolemic rabbits.

The level of triglyceride was reduced highly significant than cholesterol levels. The effect of vitamin E may be due to the inhibition of fatty acid peroxidation with less formation of malondialdehyde and a larger amount of n-3 fatty acids in their sites of action in the liver, resulting in a greater decrease in the synthesis of triglyceride and fibrinogen (23). Vitamin E in the body not only protects unsaturated fatty acids from oxidation charges (24) but is also thought to have controlling influence on linoleyl and arachidonyl residues with in the membrane phospholipids, which cannot be explained by the antioxidant function of vitamin E (25). Besides its antioxidant effect vitamin E is thought to stabilize various membranes. It has also been reported that triglyceride levels might be more sensitive than cholesterol levels to the action of vitamin E (23). Lipoprotein lipase (a triglyceride hydrolase) is located on the walls of blood capillaries, anchored to the endothelium by negatively charged proteoglycan chains of heparin sulfate (26). We know that vitamin E has a role in improving vascular wall integrity therefore we can say that by improving the smooth muscle endothelium wall vitamin E improves the activity of lipoprotein lipase and hence with the passage of time i.e on 42nd day it was observed that vitamin E lowers the triglyceride level.

CONCLUSION

Vitamin E as an antioxidant can affect the cholesterol and triglyceride levels however further studies are recommended.

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