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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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The Effect of α-tocopherol on Cholesterol and Lipoproteins Levels in Rats

A.A. Al-Juary, *K.H.M. Al-Awwad and F.A. Al-Torahi
Department of Nutrition and Food Science, Applied Science Private University, Amman, Jordan

Abstract: The effect of the α-tocopherol on the reduction of plasma cholesterol, HDL, and LDL have been investigated in rats. No significant differences were observed on the rate of body weight gain, food intake, and food efficiency of rats fed with diet supplemented with α-tocopherol. There were significant decreases in serum cholesterol, and LDL+VLDL of animals fed α-tocopherol supplementation compared to control. In contrast the HDL cholesterol showed no significant decrease in animal groups fed α-tocopherol compared to control group. The lowest bile acid levels were in feces of control group, and the highest levels were for the groups fed α-tocopherol supplementation.

Key words: α-tocopherol, cholesterol, LDL, HDL-Cholesterol, rats

Introduction

Vitamin E "Tocopherol" is a significant antioxidant in the biological system. It suppresses per oxidation of membrane lipids and also acts as an essential part in protecting plasma lipoproteins against oxidative modification. Epidemiological studies propose that a high dietary intake of vitamin E reduces the risk of coronary heart disease. Lipid oxidation also appears to be involved in the initiation and promotion of carcinogenesis, a process which may be prevented by antioxidants.

It has been known for years that vitamin E status of rats and rabbits can reduce their plasma cholesterol and total fat concentration (Sebely, 2003). Ronald *et al.* (1999) reported that α-tocopherol supplementation inhibits hydroxy methyl glutaryl-CoA reductase (HMG-COA) and decreases the plasma LDL and cholesterol.

The aim of the present study was therefore to find out whether α-tocopherol could reduce total plasma cholesterol, HDL, and LDL-cholesterol in rats. Four concentrations of α-tocopherol, 50, 250, 500, and 1000 mg were tested for this role.

Materials and Methods

Animals: Male rats, weighing between 150 and 160 g were obtained from national center for pharmaceutical research, Ministry of Health, Iraq. The rats were distributed into five groups and were housed in stainless steel cages with wire mesh floor. Animals had free access to standard casein diet (Table 1) (Morita *et al.*, 1997) and water for one week. Group one of animals was only fed with the casein diet without vitamin E and served as control group. The other groups (2, 3, 4, and 5) were fed on casein diet with 50, 250, 500 and 1000 mg α-tocopherol acetate/Kg diet respectively throughout the experimental period which lasted for 4 weeks after a one week adaptation period with the casein diet. During the study period, food intake and weight gain were recorded.

Table 1: Composition of diet

Component	g/Kg diet
Casein	250
Starch	535
Sucrose	100
Conrail	70
Mineral mixture (1)	35
Vitamin mixture (2)	10

Collection of blood samples: Blood was collected from each rate under chloroform anesthesia via heart puncture and transferred into sample tubes containing EDTA as an anticoagulant (Raederstorff *et al.*, 2002). The serum from each blood sample was recovered by centrifugation at 2500 rpm.

Biochemical analysis: Serum total cholesterol and HDL-cholesterol concentrations were estimated using linear laboratories (Spain) kit. The LDL-Cholesterol and very low density lipoprotein in the serum were obtained by subtracting the value for HDL-Cholesterol from total cholesterol (Keaney *et al.*, 1994). Serum triglyceride levels were determined by the method of Franey and Elias (1968). Total cholesterol in some organs and feces were measured according to Franey and Elias (1968), cholic acid and deoxycholic acid were determined using Thin-Layer Chromatography (TLC).

Statistical analysis: All data are expressed as means for animals in each diet group. The statistical significance of mean differences between dietary groups was tested by the analysis of variance. If significant differences were found, the Duncan's test was used, P=0.01 was considered significant. All the analyses were performed with statistic SAS.

Results

The body weight gain, food intake and food efficiency of the rats at the end of five weeks are presented in Table

Table 2: Body weight, Feed intakes and feed efficiency of rats in experimental groups

Diet groups	Initial weight (g)	Final weight (g)	Body weight gain (g)	Feed intake (g)	% Feed efficiency
Group 1	148.60	208.20	59.60	361.20	92.98
Group 2	149.50	222.90	77.8	389.30	93.87
Group 3	153.00	233.10	86.62	381.80	94.26
Group 4	145.10	234.7	82.56	397.60	94.76
Group 5	144.20	208.60	61.70	362.30	93.40

Table 3: Serum lipid profile of rats in control and diet groups often one, two, and four weeks

Diet groups	After one week			After two weeks			After four weeks		
	Total Cholesterol (TC)	HDL	LDL + VLDL	Total Cholesterol (TC)	HDL	LDL + VLDL	Total Cholesterol (TC)	HDL	LDL + VLDL
Group 1	63.58	26.44	37.04	72.60	30.68	42.08	109.10	42.16	67.60
Group 2	65.26	29.30	35.50	76.76	29.82	46.90	89.90	40.28	49.60
Group 3	65.46	29.60	35.86	74.02	31.90	42.10	82.98	37.00	45.90
Group 4	61.52	30.82	30.80	73.90	32.90	40.96	87.30	40.26	47.04
Group 5	63.46	23.94	39.50	74.40	28.16	44.26	86.12	39.78	46.34

2. Dietary treatment with α-tocopherol had no significant effect on body weight, food consumption and feed efficiency of rats.

The serum lipid profile of the rats after four weeks of feeding with various diets is shown in Table 3. Animals fed on casein diet (control group) demonstrated plasma total cholesterol, HDL cholesterol, and LDL + VLDL cholesterol of 109.10, 42.16, and 67.60 mg/dl, respectively. Animals receiving diets containing 50, 250, 500, and 1000 mg α-tocopherol for four weeks demonstrated a significant decrease ($p \leq 0.01$) of plasma total cholesterol, and LDL + VLDL compared to control animals. In contrast, the HDL cholesterol showed no significant decrease in animal groups fed α-tocopherol compared to control group. Dietary treatment with α-tocopherol had some significant effect ($p \leq 0.01$) on the fat and cholesterol levels of liver, spleen and heart of animals (Table 4).

Table 5 revealed the presence of significant differences ($p \leq 0.01$) in the level of feces lipids, cholesterol and some bile acids, while the lowest increase in lipids levels was found in feces of the control group, the highest level was in the group fed on the diet supplemented with 500 mg α-tocopherol. The levels of cholesterol in the feces were lowest in control group and the highest was in the group fed on the diet supplemented with 50 mg α-tocopherol. Finally, the bile acid concentrations in feces of control group were low compared with the group fed on the diet supplemented with 250 mg α-tocopherol.

Discussion

Cholesterol and LDL are important lipids associated with cardiovascular diseases. The harmful effect of hyperlipidemia underscores the need for research on this management.

This study demonstrates that the incorporation of α-tocopherol did not alter the body weight, the food intake and the feed efficiency of the rats at the end of five weeks (Table 2). This indicates that the addition of α-tocopherol to the diet did not significantly alter the palatability of the diet, its nutrient content, and the caloric values. These results corroborate with those made by Kub *et al.* (1997), Farwer *et al.* (1994), Al-Sebahi (2000), and Peluzio *et al.* (2001). Plasma VLDL + LDL- cholesterol represent mobilization of fats from the liver to adipose tissue. LDL carry 60%-70% of the total cholesterol in the serum (Edijala *et al.*, 2005) and also VLDL is the main carrier of triglycerides. A statistically significant relationship was demonstrated between α-tocopherol intake and the changes seen in the total and LDL + VLDL cholesterol, without a significant change in HDL cholesterol levels (Table 3), thus increasing the HDL/LDL ratio. High HDL/LDL ratios are associated with a decreased risk of vascular diseases (Raederstorff *et al.*, 2002, and Wilson, 1999) and, therefore, a dietary supplement of α-tocopherol might be advantageous in reducing the total and LDL + VLDL-cholesterol. This study confirms the hypocholesterolemic effect of α-tocopherol observed in rats (Liu and Boylan, 1994) and other animal models (Khoja and Marzouki, 1994; Chien *et al.*, 1972; Jack *et al.* 1977; Al-Sebahi, 2000; Raederstorff *et al.*, 2002 and Hidiroglou *et al.* 2004). One may speculate that the effects induced by dietary supplements of α-tocopherol acetate in rats are due to an inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity by α-tocopherol, the rate-limiting enzyme in cholesterol biosynthesis (Oriani *et al.*, 1997; Guorong *et al.*, 2000; and Sebely *et al.*, 2003) and on the cellular lathosterol concentration, an index of cholesterol synthesis (Sebely *et al.*, 2003). It was observed that the maximal effect of α-tocopherol levels was at 50 mg/Kg diet. Knor and Chieng (1996) also tested a higher dose of 1000mg/ Kg of diet but found no further improvement when compared to the lower doses. This study provides further evidence that α-tocopherol lowered the total and

Table 4: Total fat and cholesterol in some organs of rats in the experimental groups at the end of four weeks

Diet groups	Total Fat			Cholesterol		
	Liver (mg/g)	Spleen (mg/g)	Heart (mg/g)	Liver (mg/g)	Spleen (mg/g)	Heart(mg/g)
Group 1	52.6	27.10	38.00	2.54	1.40	1.56
Group 2	42.8	28.40	30.70	1.73	1.66	1.34
Group 3	40.97	31.20	32.03	1.81	1.88	1.23
Group 4	40.45	25.40	34.50	1.93	1.38	1.31
Group 5	43.7	24.40	31.30	1.99	1.58	1.38

Table 5: Total fat, cholesterol and bile acids in rats feces in experimental groups at the end of four weeks

Diet Groups	Total fat (mg/g)	Total Cholesterol (mg/g)	Bile Acids (mg/g)
Group 1	102.4	5.14	1.39
Group 2	131.03	8.32	1.66
Group 3	154.3	7.14	2.36
Group 4	159.5	7.90	2.31
Group 5	136.3	7.18	1.99

LDL-cholesterol plasma levels in rats. Upon absorption, α-tocopherol is transported to the liver and mobilized from liver stores via an α-tocopherol transfer protein and transported in the circulatory system to tissue cells, where lipoprotein lipase hydrolyzes the blood triglycerides (Moreira and Mahan, 2002). Our results suggest that both fats and cholesterol in liver and heart decreased significantly ($p \leq 0.01$). In animals fed on diet supplemented with α-tocopherol (Table 4), deposition of α-tocopherol in the liver, adipose tissue, and in the lipoprotein membranes of other body cell seems to occur in response to the supplemental dietary level of α-tocopherol and in response to the cell's ability to hydrolyze the blood triglyceride (Moreira and Mahan, 2002). Jensen *et al.* (1990) demonstrated that the serum and liver responded more rapidly to supplemental α-tocopherol or carrot diet (Nicolle *et al.*, 2003). The effect of α-tocopherol feeding on fecal bile acids is shown in table 5. Fecal bile acids and cholesterol levels were increased by α-tocopherol supplementation. Bile acids synthesis and subsequent excretion in the feces represent a significant mechanism for elimination of excess cholesterol.

In conclusion, dietary treatment with α-tocopherol decreased total cholesterol, LDL+VLDL cholesterol levels in dose dependent manner in rats. Moreover, in this study there seems to be a relationship between the fecal bile acids and cholesterol levels and the α-tocopherol supplementation.

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