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A Comparative Impact of Different Types of a Single Antioxidant Supplementation (β-Carotene, α-Tocopherols and Ascorbic Acid) on Lipid Profile in Hyperlipidemic Rats

[1]Salem Ali Salem, [1]Dalia Refaat Hassan and [2]Aida Rashad Mowafy

[1]Faculty of Specific Education, Fayoum University, Egypt
[2]Department of Nutritional Biochemistry, National Nutrition Institute, Egypt

Abstract: The present study was carried out to compare the effect of a single supplementation of different types of antioxidant nutrient (β-carotene, α-tocopherol or Ascorbic Acid) on some biochemical parameters and lipid peroxidation in hyperlipidemic rats. Thirty male Sprague-Dawley albino rats, were used in this study, the rats were divided into 5 groups, normal control group fed on standard diet, 4 groups were fed high lipid and cholesterol diet for 4 weeks then divided to Positive control group fed on high lipid and cholesterol diet only and the remaining 3 groups were fed the high lipid and cholesterol diets supplemented with single antioxidant β-carotene 300mg/kg diet, L- Ascorbic acid 1000mg/kg diet or RRR-α-tocopherols 800 mg/Kg diet, for 4 weeks. Serum total cholesterol (TC), HDL cholesterol (HDL-c), the LDL-Cholesterol (LDL-c), Serum triglyceride (TG) levels, blood glutathione (GSH) and livers malondialdhyde (MDA) were measured. Feeding high lipid and cholesterol diet resulted in a significant ($P \leq 0.01$) reduction of HDL-c and significant ($P \leq 0.01$) increment in TG, TC, LDL-c and LDL/HDL ratio, with a significant elevation of liver MDA and a significant reduction in blood GSH. Supplementation with Ascorbic acid produced a significant reduction in serum TG, TC, LDL-c levels ($P \leq 0.01$) and significant elevation of serum HDL-c levels ($P \leq 0.01$). β-carotene increased serum TG value ($P \leq 0.01$), reduce serum TC and LDL-c levels ($P \leq 0.01$) and elevate serum HDL-c levels ($P \leq 0.05$). Supplementation with α-tocopherols decreased serum HDL and TG levels ($P \leq 0.01$ and $P \leq 0.05$ respectively). Supplementation of β-carotene, Ascorbic acid or α-tocopherols resulted in a significant reduction of the liver MDA and elevation of Blood GSH. It could be concluded that, only Ascorbic acid supplemented group had the lowest atherogenic index, followed by β-Carotene supplemented group. Therefore, It may be worthwhile to investigate the beneficial supplementation with a combination of these two antioxidant nutrient (Ascorbic acid/β-carotene) to control hyperlipidemia.

Key words: Natural antioxidants · Lipid profile · MDA · GSH · Hyperlipidemic rats

INTRODUCTION

Antioxidant nutrients, including β-carotene, vitamin E and vitamin C, are thought to play a role in atherosclerosis. Mild to moderate deficiencies of these vitamins, although not severe enough to cause classic deficiency diseases, may be involved in the development of Cardiovascular Diseases CVD [1]. Therefore, it is thought that antioxidant supplementation may help reduce the incidence or progression of atherosclerotic CVD. Antioxidant used to prevent atherosclerotic heart disease is based on the hypothesis that lipid peroxidation or oxidative modification of low-density lipoprotein is the initiator of atherosclerosis [1]. Hypercholesterolemia and hypertriglyceridemia are independent risk factors that can accelerate the development of atherosclerosis and progression of atherosclerotic lesions [2].

One of the initial events in the development of atherosclerosis is the accumulation of cells containing excess lipids within the arterial wall. In addition, increased intracellular generation of Reactive oxygen species (ROS) plays an important role in chronic inflammatory responses to atherosclerosis [3]. Reactive oxygen species (ROS) may react with a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissue, thereby interfering with cell function. Propagating lipid peroxidation is a degenerative process that affects cell membranes and

Corresponding Author: Salem Ali Salem, Faculty of Specific Education, Fayoum University, Egypt.

other lipid-containing structures under conditions of oxidative stress [4]. Oxidative stress in hyperlipidemia is thought to be a factor in the development of atherosclerotic plaques [5]. A lot of oxygenated compounds, particularly aldehydes such as malondialdehyde (MDA) and conjugated dienes, are produced during the attack of free radicals on membrane lipoproteins and polyunsaturated fatty acids [6]. Therefore, cells and tissues have evolved both enzymatic and non-enzymatic antioxidant systems to combat the oxidative stress caused by ROS. However, such endogenous antioxidants are not sufficient under the conditions of extreme oxidative stress, thus making it necessary to rely on exogeneous antioxidants, such as β-carotene, vitamins E and C [4, 7]. Moreover, Yang *et al.* [8] suggested that, antioxidants have a therapeutic role in protecting from oxidative damage by ROS in the hyperlipidemia disease. Thus, in subjects with high risk for developing hyperlipidemia, supplementation with the right antioxidant might reduce the peroxidation rate, restore the body's antioxidant capacity and possibly prevent or delay development of this disease.

The present study was designed to compare between the effect of a single supplementation of different types of antioxidant nutrient (β-carotene, α-tocopherol or Ascorbic Acid) on lipid profile and lipid peroxidation in hyperlipidemic rats.

MATERIALS AND METHODS

Animals and Diets: Thirty male Sprague-Dawley albino rats, weighting 100-150g were used in this study. The rats were housed in stainless steel cages and received standard diet and water *ad libitum* during the first week and were maintained at an environmental temperature of 18-23°C. After that the rats were randomly divided into 5 groups of 6 rats each, as normal control fed on standard casein diet [9, 10, 11]. The other 4 groups were fed high lipid and cholesterol diet for 4 weeks [12, 13]. Positive control group was fed on high lipid and cholesterol diet and the remaining 3 groups were fed the experimental diets for 4 weeks, only groups β-carotene, α-tocopherol and Ascorbic Acid supplementation were fed on high lipid and cholesterol diet Supplemented with one of the antioxidants β-carotene 300 mg/kg diet [14], L- Ascorbic acid 1000mg/kg diet or RRR-α-tocopherols 800 mg/kg diet [15]. RRR-α-tocopherols "vitamin E" and L- Ascorbic acid "vitamin C" and β-Carotene was obtained from Mepaco Company for herbs and drugs, Egypt.

Blood Samples: After four weeks of feeding period the rats were anesthetized and hepatic portal vein blood samples were withdrawn with EDTA. The serum from each blood sample was recovered by centrifugation at 2500 rpm.

Biochemical Analysis: Serum total cholesterol concentrations (TC) were estimated using bioMérieux enzymatic kit [16]. HDL cholesterol (HDL-c) determined using bioMérieux kit according to the method of Burstein *et al.* [17]. The LDL-Cholesterol (LDL-c) was estimated according to Friedwald *et al.* [18]. Serum triglyceride (TG) levels were determined by the method of Fossati and Prencipe [19]. For the determination of blood glutathione (GSH), another 0.2 ml of heparinized blood was used [20]. Livers were isolated rapidly and homogenized. And the homogenate was used for determination of Malondialdhyde (MDA) according to the method of Uchiyama and Mihara [21].

Statistical Analysis: All data are expressed as (means±S.E.) for animals in each group. The statistical significance of mean differences between groups was tested by one way analysis of variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at $P \leq 0.05$ and $P \leq 0.01$. All the data analysis was performed using SPSS software (Version 10; SPSS Inc Chicago, USA).

RESULTS AND DISSCUSION

The effects of feeding of high lipid and cholesterol diet on lipid profile were shown in Table 1, feeding high lipid and cholesterol diet resulted in a significant ($P \leq 0.01$) reduction of HDL-c and significant ($P \leq 0.01$) increment of TG, TC, LDL-c and LDL/HDL ratio (atherogenic index).

The results were in agreement with the findings of Shils *et al.* [22] who demonstrated that, a diet containing lard was significantly more atherogenic, as the consumption of saturated fats produced an elevation in circulating total and LDL Cholesterol levels. From Table 1, it could be noticed that, feeding rats on a high lipid and cholesterol diet induced a significant elevation of liver MDA and a significant reduction in blood GSH when compared with the normal control values. This could be due to the fact that, feeding the high fat diet result in a large amounts of fat intake and further increment of Poly Saturated Fatty Acids (PUFA) in serum

Table 1: Effects of feeding high lipid and cholesterol diet for 4 weeks on rats' Serum TG, TC, HDL-c, LDL-c, VLDL-c, LDL/ HDL Ratio, Blood GSH and Liver MDA (Mean ± S.E.)

Parameters	Normal Control	Positive Control
TG. mg/dl	92.4±1.420	159.8±0.48**
TC. mg/dl	85.42±1.52	175.94±0.53**
HDL-c mg/ dl	37.36±0.30	47.55±0.83**
LDL-c mg/ dl	29.13±1.19	106.42±1.16**
VLDL-c mg/ dl	18.9±0.280	21.96±0.10**
LDL/ HDL Ratio	0.77±0.01	2.23±0.03**
Blood GSH mg/ dl	22.60±0.51	16.47±0.32**
MDA nmol /g liver	38.46±0.67	101.05±0.78**

**Highly Significant at P≤0.01

Table 2: Effect of dietary supplementation with β-Carotene, α-Tocopherol and Ascorbic Acid each individually on lipid profile of hyperlipidemic rats (Mean±S.E.)

			Serum Lipoproteins			
Group	Triglycerides mg/dl	T. Cholesterol mg/dl	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl	LDL/ HDL
Positive Control	159.8±0.48	175.94±0.53	47.55±0.83	106.42±1.16	21.96±0.10	2.23±0.03
β-Carotene	163.37±1.14**	169.05±1.79**	49.01±0.32*	97.65±1.76**	22.67±0.23**	1.99±0.02**
α -tocopherol	156.48±2.40*	174.72±1.65	45.64±1.26**	107.54±1.94	21.29±0.48*	2.36±0.04**
Ascorbic Acid	115.28±0.87**	135.43±1.25**	76.53±1.46**	40.76±0.95**	16.374±0.17**	0.53±0.01**

* Significant difference from positive control group at P≤0.05

**Significant difference from positive control group at P≤0.01

or liver. Large amounts of PUFA contents being more susceptible to lipid peroxidation, as the liver Thiobarbituric Acid Reactive Substances (TBARS) was higher in animals fed high fat than animals fed low fat diet [23]. Recent study, Yang *et al.* [8] stated that, increased levels of MDA in the higher lipid and hyperlipidemic groups could be attributed to increased ROS production and/or deficiency of antioxidant defense system, as the activities of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-px) in higher lipid subjects were decreased. Thus, the insufficient detoxification of reactive oxygen species by antioxidant enzymes may lead to an occurrence of imbalance between antioxidant and oxidant systems. Moreover, a high cholesterol diet compromises the endogenous antioxidant defense mechanisms as indicated by the reduction of Glutathione Reductase (GR), which could be possible reason for the depletion of GSH [24].

It was obvious from Table 2 and Fig.1 that, supplementation with Ascorbic acid produced a significant reduction of Serum TG, TC, LDL-c levels (P≤0.01) and significant elevation of serum HDL-c levels (P≤0.01), when compared to the hyperlipidemic control values. Compared with the Positive control values, β-carotene significantly increased serum TG value (P≤0.01), significantly reduce serum TC and LDL-c levels (P≤0.01) and significantly elevate serum HDL-c levels (P≤0.05). In contrast α-tocopherols supplementation significantly decreased serum HDL and TG levels (P≤0.01 and P≤0.05 respectively), when compared to Positive control values. From Fig. 2 it could be concluded that, only Ascorbic acid supplemented group had the lowest atherogenic index, followed by β-Carotene supplemented group. In contrast the α-tocopherols supplemented group had higher atherogenic index compared to the positive control group. The present findings came in agreement with that of Alan *et al.* [25] who stated that, an increase in the level of dietary β –carotene resulted in progressive decreases in fasting serum total and LDL cholesterol. β-carotene supplementation did not significantly reduce serum VLDL cholesterol concentrations. Compared with rats fed the basal diet, rats fed diets containing 250 or 500 mg β -carotene/kg had significantly lower serum LDL cholesterol. The ratio of HDL cholesterol to total cholesterol was slightly increased with β -carotene supplementations. These results suggest that, β -carotene may play a role in altering the rate of cholesterol metabolism in the liver or the efficiency of cholesterol absorption in the intestine [25].

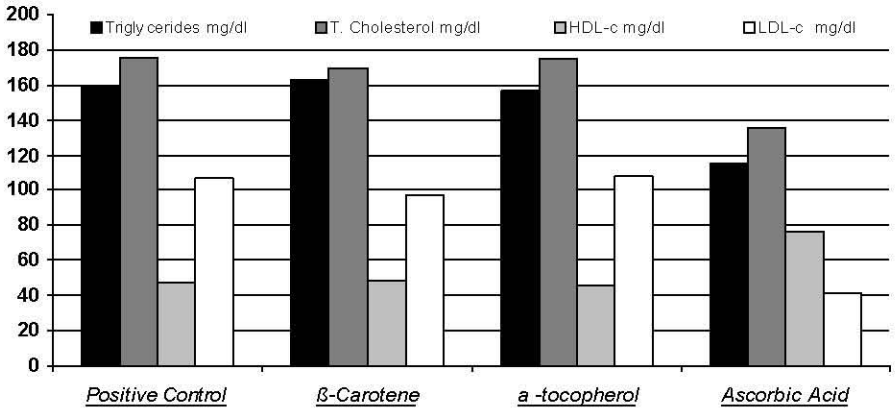

Fig. 1: Effect of dietary supplementation with β-Carotene, α-tocopherol and Ascorbic Acid on lipid profile of hyperlipidemic rats.

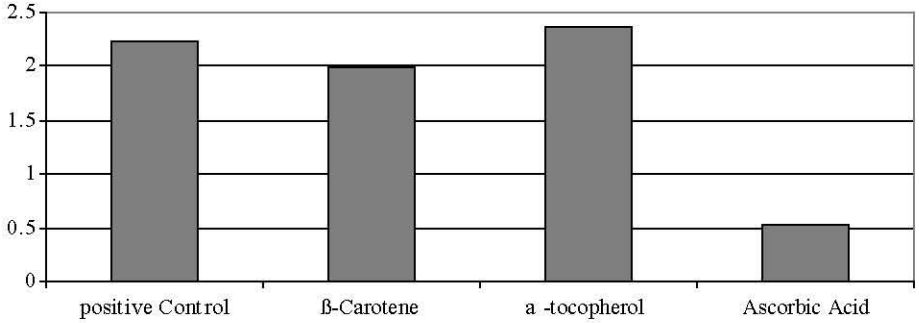

Fig. 2: Effect of dietary supplementation with β-Carotene, α –Tocopherol and Ascorbic Acid on LDL/ HDL ratio in hyperlipidemic rats.

Supplementation with Ascorbic acid produced a significant ($P \leq 0.01$) reduction of serum TG, TC, LDL-c levels, this could be due to the fact that, Ascorbic Acid enhanced the conversion of cholesterol to bile acids (most important pathway of cholesterol catabolism), simultaneous with increased fecal and liver bile acids through activation of cholesterol 7-α-hydroxylase, the limiting step in cholesterol transformation to bile acids [26]. The results of the present study were in agreement with the finding of Fomihiko *et al.* [27] who indicated that, the activity of hepatic cholesterol 7-α-hydroxylase, the rate-limiting step in cholesterol biotransformation to bile acids, seemed to be lower in a rat mutant unable to synthesize Ascorbic acid (OD rats), than in OD rats fed a diet supplemented with 300 mg of ascorbic acid/kg. Ascorbic acid deficiency caused a significant decrease in fecal excretion of bile acids in OD rats fed a diet with cholesterol. This could be explained on the basis that, the primary effect of Ascorbic Acid deficiency is the depression of hepatic activity of cholesterol 7-α-hydroxylase, followed by the reduction of bile acid synthesis and a lower bile acid turnover rate and a prolonged bile acid half-life and consequently a lower rate of bile acid excretion and a reduction in the size of the bile acid pool in Ascorbic acid deficiency.

The results concerning α-tocopherols were in harmony with that of Kesaniemi and Grundy [28]. Which showed that vitamin E caused no decrease in plasma total cholesterol, very low-density and low-density lipoprotein cholesterol and triglyceride concentrations and no increase in high-density lipoprotein cholesterol level. The present data was in agreement with a previous study, in which vitamin E therapies in postmenopausal women increased the ratio of LDL to HDL cholesterol [29]. Furthermore, Reaven *et al.* [28] reported that, supplementation of vitamin E alone in mildly hyperlipidemic volunteers for five months, resulted in a slightly higher total cholesterol levels, but the LDL cholesterol and HDL cholesterol levels were similar to the placebo period [30]. Alpha-Tocopherol does not seem to have any consistent effect on plasma lipids and lipoproteins levels.

Table 3: Effect of dietary supplementation with β-Carotene, α –Tocopherol and Ascorbic Acid each individually on Blood GSH and liver MDA of hyperlipidemic rats (Mean ± S.E)

Items	Positive Control	β-Carotene	α -tocopherol	Ascorbic Acid
Blood GSH mg/dl	16.47±0.32	20.38±0.18**	18.72±0.25**	20.71±0.5**
MDA nmol/g liver	101.05±0.78	16.04±0.24**	12.34±0.42**	12.07±0.11**

* Significant difference from positive control group at P≤0.05

**Significant difference from positive control group at P≤0.01

Data presented in Table 3 showed that, the supplementation of single antioxidants β-carotene, Ascorbic acid or α-tocopherols resulted in a significant reduction (P≤0.01) of liver MDA and a significant elevation (P≤0.01) of Blood GSH, when compared to the Positive control levels. High fat diet brings about remarkable modifications in the antioxidant defense mechanism against the process of lipid peroxidation that was in agreement with the recent study of Kaviarasan *et al.* [31]. Inability to maintain serum ascorbic acid and the consequent reduction in antioxidant capacity may result in an increased flux of harmful ROS [32]. In the last stage of the peroxidation process, peroxides are decomposed to aldehydes like MDA [33]. Furthermore, an Ascorbic acid deficiency enhances lipid peroxidation in plasma LDL and liver, Kimura *et al.* [34] postulate that, even with no increase in the serum lipid concentration, injury of the endothelium in the blood vessels occurred in ascorbic acid-deficiency as lipid peroxide production increased, suggesting an important relationship between ascorbic acid deficiency, lipid peroxides and atherosclerosis. It was found that, the dietary addition of 300 mg ascorbic acid/kg diet would be sufficient to maintain the normal concentration of lipid peroxide in plasma LDL and liver found in Ascorbic acid deficient rats.

Vitamin C serves directly as an antioxidant by scavenging aqueous peroxyl radicals and indirectly by regenerating reduced vitamin E [35]. Recent study by Fakher *et al.* [36] indicated that, the increase in free radicals causes overproduction of MDA; furthermore, the decreasing of free radicals production or inhibiting their oxidative damage could result in decreasing lipid peroxidation and MDA level. Supplementation of single antioxidants β-carotene, Ascorbic acid or α-tocopherols resulted in a significant elevation (P<0.01) in blood GSH compared to the hyperlipidemic group. These results could be explained by the findings that, low blood GSH peroxidase activity that was associated with Multiple Sclerosis (MS) activity could be increased by the antioxidant administration. Similarly, antioxidant treatment with vitamin C and E restored GSH levels as well as the GSH peroxidase activity in erythrocytes of MS patients, due to their free radical scavenging, metal chelation and radical chain reaction-breaking properties, nonenzymatic antioxidants, vitamin C, vitamin E, thiol-based antioxidants and flavonoids are important in prevention of oxidative stress [37]. Supplementation of α-tocopherol was shown to reduce plasma MDA levels significantly [38], this could be due to the fact that, oral supplementation with α-tocopherol increased the level of SOD and GPx. These enzymes scavenge free radicals and prevent oxidative damage [39].

There is some evidence that tocopherols have a specific function in cell membranes; the phytyl side chain of RRR-α-tocopherol can interact closely with the methylene-interrupted cis-double bonds of arachidonic acid and other long-chain polyunsaturated fatty acids in membranes, both stabilizing membrane structure and also protecting the fatty acids from oxidative damage [40]. Some studies have found that carotenoids increase the oxidative stability of LDL [41]. That could be explained on the fact that, most carotenoids are stored in adipose tissue. In plasma, the more hydrophobic carotenoids (β-carotene) are deep within chylomicrons or very low-density lipoproteins, as antioxidants, trapping singlet oxygen generated by lipid peroxidation of membranes [40]. As an antioxidant, ascorbate is an efficient scavenger, or reducing antioxidant, capable of donating its electrons to ROS and eliminating them. Because the ascorbyl radical is relatively stable, it makes ascorbate a powerful, important antioxidant. This radical can lose its electron and be transformed to dehydroascorbic acid or regenerated to the reduced form by obtaining an electron from another reducing agent, such as GSH or NADH, via the mediation of an enzyme like NADH-semidehydroascorbate reductase [33]. Furthermore, vitamin C prevents the pro-oxidant activity of vitamin E by decreasing the activity of α-tocopheroxyl radical to α-tocopherols, thereby acting as a co-antioxidant and further contributing to increased total antioxidant status and reduced oxidative stress [42].

It could be concluded that, only Ascorbic acid supplementation caused lowest atherogenic index, followed by β-Carotene supplemented group. Therefore, It may be worthwhile to investigate the beneficial supplementation with a combination of these two antioxidant nutrient (Ascorbic acid and β-carotene) to control hyperlipidemia. Antioxidant should be administered to the body continuously, in high concentrations and targeted to the biological site susceptible to oxidative damage. Cautions must be taken as excessive antioxidants supplementation may have potential side effects as upper toxic dose can easily be reached.

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