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Original Article

Effect of Two Carotenoids (Lycopene and β-Carotene) Supplementation on Hyperlipidemia and Lipid Peroxidation in Experimental Albino Rats

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Abstract

Background: Carotenoids have been known to have important beneficial properties for human health and gained importance in recent years.
Objectives: The present study aimed to investigate the effect of supplementation with two carotenoids (Lycopene and β-Carotene) separately or in combination, on lipid peroxidation and some endogenous antioxidants in addition to their effect on serum levels of lipoproteins in male rats.
Methods: Thirty male Sprague-Dawley Albino rats, were randomly divided into 5 groups, normal control group fed on standard diet, 4 groups were divided to positive control group fed on high fat and cholesterol diet only, and the remaining 3 groups were fed on high fat and cholesterol diets supplemented with single antioxidant Lycopene 350mg/kg diet, β-carotene 350mg/kg diet, or mixture of Lycopene and β-carotene, for 4 weeks. The following parameters were measured, serum total cholesterol (TC), HDL, LDL, serum triglyceride (TG) levels, blood and liver glutathione (GSH), and livers malondialdhyde (MDA).
Results: Supplementation of High Fat Diet (HFD) with lycopene (HFD+ LYC), β-Carotene (HFD+ B-car) and their mixture (HFD+ (LYC+ B-car)) produced a significant reduction of Serum TC, LDL and TG levels, and a significant elevation of serum HDL levels. The supplementation of lycopene, β-carotene or their mixture resulted in a significant reduction of liver MDA, and a significant elevation of liver and blood glutathione (GSH), in comparison with the levels of HFD group.
Conclusion: These findings suggest that lycopene and ß-carotene supplementation, altered the pro-oxidation and anti-oxidation balance and suppressed oxidative stress by modulating endogenously the antioxidant system and cholesterol metabolism.
Key Words: *Rats- Lycopene - ß-carotene - LDL- HDL- MDA- Blood GSH, Liver GSH*

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INTRODUCTION

New life styles have driven consumers away from healthy dietary habits. In the last 40 or 50 years mankind has modified its diet to include a reduced amount of fruits and vegetables, while increasing its intake of processed foods with a high content of fats and simple carbohydrates. These changes have brought on consequences such as the development of dyslipidemia and oxidative stress. Oxidative stress resulting from excessive production of reactive oxygen species (ROS) have been implicated in playing a major role in the causation and progression of several chronic diseases. As a matter of fact, diets with antioxidant substances can reduce oxidative stress and play an important role in prevention of cardiovascular diseases.[(1)]

Carotenoids have been used commercially as natural food colorants and nutrient supplements. Rao and Rao[(2)], reported that, carotenoids have been known to have important beneficial properties for human health, and for a long period of time, β-Carotene was used as a standard model to study the relationship between oxidative stress and chronic diseases. Moreover, accumulating evidences indicated that, some of the carotenoids, their metabolites and/or related compounds are involved in different biologic functions in the human body and provide diverse health benefits.[(3)] More specifically, the health benefits of lycopene, the red pigment found in some plant

foods (tomatoes, watermelon, grapefruit, and other fruits) and of its oxidative metabolites have been the subject of major studies in the last decade.[3-5] Recently many studies suggested that, the combination of lycopene with other antioxidants, may be more potent in inhibiting lipid peroxidation, than lycopene per se, and that was explained on the basis that, different carotenoids show synergism as antioxidants in heterogeneous systems due to their different lipophilicity[4], and that lycopene absorption from dietary sources is influenced by several factors including the presence of lipids and other lipid-soluble compounds including other carotenoids.[5]

Hence the aim of this study was to investigate the effect of supplementation with two non-polar carotenoids (Lycopene and β-Carotene) separately or in combination, on lipid peroxidation and some endogenous antioxidants in addition to their effect on serum levels of lipoproteins in male rats fed with a high fat and cholesterol diet.

METHODS

Study design: Experimental animal design was performed using a total of 30 male *Sprague-Dawley albino rats*, weighing 100±15 g, taken from animal house of Egyptian Organization for Biological Products and Vaccines. (VACSERA) Cairo, Egypt. The rats were randomly divided into 5 groups (6 rats each). The normal control group was fed on standard diet which had the following composition: corn starch 70.5%, corn oil 10%, salt mixture 4%, vitamin mixture 1%, DL methionin 0.3 gm and choline chloride 0.2%. The protein was added at 14% level at the expense of starch **[6]**. The other 4 groups were fed on high fat and cholesterol diet for 4 weeks and the diet was composed of standard diet in addition to 10% lard and 1% cholesterol at the expense of starch **[7]**. The positive control group was fed only on high fat and cholesterol diet (HFD), and the remaining 3 groups were fed on high fat and cholesterol diets supplemented with single antioxidant Lycopene 350mg/kg diet [8] (HFD+ LYC), β-carotene 350 mg/kg diet (HFD+ B-car), or mixture of Lycopene and β-carotene 350 and 350 mg /kg diet (HFD+ (LYC+B-car)) for 4 weeks. Lycopene and β-Carotene were obtained from Mepaco Company for herbs and drugs, Egypt. The rats were housed in stainless steel cages and received diet and water ad libitum during the experimental period and were maintained at an environmental temperature of 18-23 ^{o}C. All animals were treated according to ethical consideration for the care and use of animals in laboratory research.

Blood Samples: After four weeks of feeding, 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. All the procedures were performed in accordance with ethical consideration for the care and use of animals in laboratory research.

***Biochemical analysis*:** Serum total cholesterol concentrations (TC) were estimated using enzymatic kit.[9] High Density Lipoprotein Cholesterol (HDL) determined according to the method described by Burstein et al.[10] The LDL-Cholesterol (LDL) was estimated according to the method of Friedwald et al, **[11]**. Serum triglyceride (TG) levels were determined by the method of Fossati and Prencipe.[12] Total cholesterol was measured by the cholesterol oxidase assay, whereas HDL was measured by the same procedure after precipitating of LDL and VLDL. . The concentration of (VLDL) was estimated according to the following equation VLDL- c = Triglycerides/ 5 according to the method of Friedwald et al, **[11]**. Atherogenic Index was calculated by dividing the LDL/ HDL level. For the determination of blood glutathione (GSH), another 0.2 ml of heparinized blood was used **[13]**. Liver were isolated rapidly, and homogenized. The homogenate was used for determination of liver glutathione (GSH) and Malondialdhyde (MDA) according to the method of Beutler, et al, [13] and Uchiyama and Mihara [14], respectively.

***Statistical analysis*:** All data are expressed as (Means± S.E.). 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 16; SPSS Inc Chicago, USA).

Ethical Statement

The study was approved by the institutional review board of faculty of Specific Education, Fayoum University. The study procedure conformed to the international research guidelines, the ethical guidelines of the 1975 Declaration of Helsinki and Guidelines of the International Conference on Harmonization for Good Clinical Practice.

RESULTS

As shown in Table (1), and Figure (1), positive control group fed on high fat diet (HFD), showed a significant ($P \leq 0.01$) reduction of HDL, and significant ($P \leq 0.01$) increment of total cholesterol, LDL, TG, and LDL/HDL ratio (Atherogenic Index), when compared with normal control group (NC). The data in Table (1) and Figure (1) also illustrated that, the supplementation of HFD with lycopene (HFD+ LYC), resulted in a significant reduction of serum TC, LDL and TG levels ($P \leq 0.01$), and

significant elevation of serum HDL levels (P≤0.01). Supplementation of HFD with β-Carotene resulted in a significant reduction of serum TC, LDL and TG levels (P≤0.01), and significant elevation of serum HDL levels (P≤0.05). In comparison with the HFD group values, a significant reduction of serum TC, LDL and TG levels (P≤0.01), and a significant elevation of serum HDL levels (P≤0.01) were noticed in rats group fed on HFD supplemented with a mixture of lycopene and β-Carotene. Furthermore, Figure (2) showed that, the mixture of lycopene and β -carotene supplemented group had the lowest atherogenic index, followed by lycopene and β-Carotene supplemented groups when compared with HFD group. The results in Table (2) and Figures (3 and 4) showed that, HFD brings about notable modifications in the antioxidant defense mechanism against the process of lipid peroxidation. Detected high MDA concentration in the present study, together with the significant decreases in the levels of liver and blood glutathione (GSH) observed on HFD indicated that there was an increase in lipid peroxidation in animals fed on HFD when compared to the normal control group. It was clear from Figures (3 and 4) that, the supplementation of lycopene, β-carotene or their mixture resulted in a significant reduction (P≤0.01) of liver MDA, and a significant elevation (P≤0.01) of liver and blood GSH, in comparison with the levels of HFD group. The results of the present study revealed that oxidative stress was drastically reduced and antioxidant status was improved by supplementation of lycopene and its mixture with β-carotene. The treatment with lycopene, β-carotene and their mixture caused a marked inhibition in MDA production and significantly (P≤0.01) increased the liver and blood GSH levels, and further decreased oxidative stress by preventing glutathione oxidation.

Table (1): Effects of feeding High Fat and Cholesterol Diet (HFD) and HFD supplemented with Lycopene, β-Carotene or their mixture for 4 weeks on rats' mean serum Total Cholesterol, HDL, LDL, and TG (Mean±S.E.)

	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	TG mg/dl
Normal control **(NC)**	122.33± 0.76	70.82± 0.15	37.22± 0.59	69.83± 0.71
Positive control **(HFD)**	205.83± 0.27 ^{a**}	55.75± 0.42 ^{a**}	124.02± 0.58 ^{a**}	128.26± 0.24 ^{a**}
Lycopene **(HFD+ LYC)**	140.60± 0.31 ^{b**}	60.03± 0.23 ^{b**}	58.67± 0.23 ^{b**}	109.03± 0.47 ^{b**}
B-Carotene **(HFD+ B-car)**	193.84± 0.89 ^{b**}	56.83± 0.16 ^{b*}	110.97± 0.93 ^{b**}	130.21± 0.57 ^{b**}
Lycopene + B-Carotene **(HFD+(LYC+ B-car))**	140.33± 0.38 ^{b**}	63.49± 0.44 ^{b**}	54.93± 0.47 ^{b**}	108.28± 0.48 ^{b**}

^{a*} Significant difference from normal control group (P≤0.05) , ^{a**} Significant difference from normal control group at (P≤0.01)
^{b*} Significant difference from positive control group at (P≤0.05), ^{b**} Significant difference from positive control group at (P≤0.01)

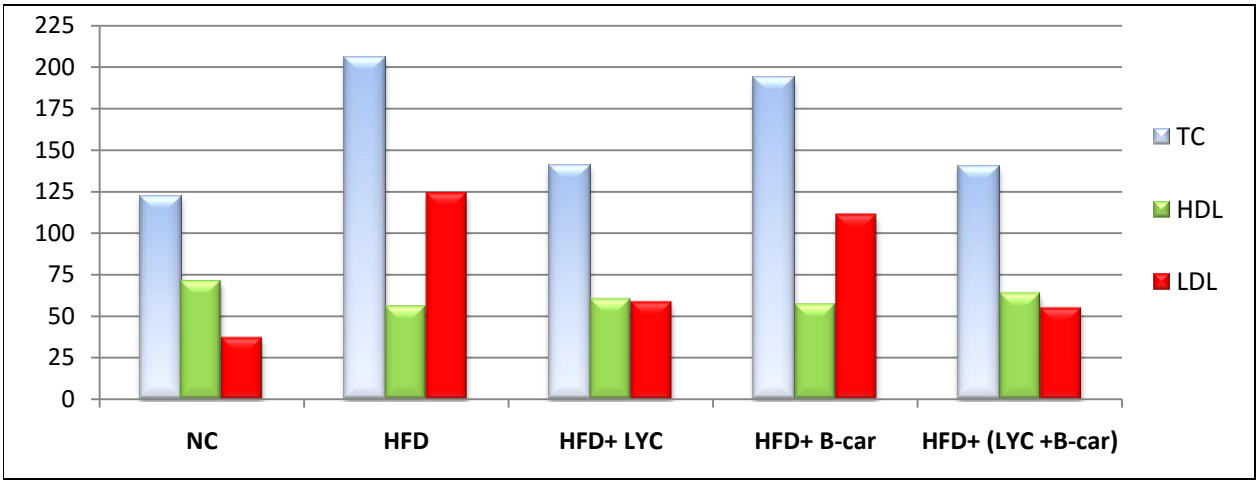

Figure (1): Mean Serum Total Cholesterol, HDL and LDL (mg/dl) of rats group fed on normal or high fat diet supplemented with lycopene, β-Carotene and their mixture (Mean±S.E.)

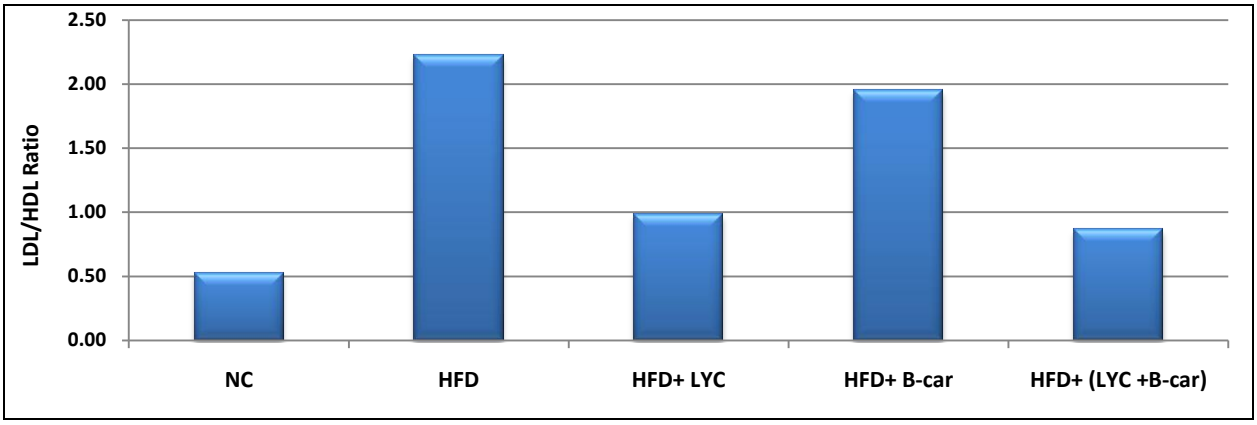

Figure (2): Mean LDL/HDL Ratio of rats groups fed on normal or high fat diet supplemented with lycopene, β- Carotene and their mixture (Mean±S.E.)

Table (2): Mean Liver MDA, Liver GSH and Blood GSH of rats groups fed on normal or high fat diet supplemented with lycopene, β-Carotene and their mixture (Mean±S.E.)

	Malondialdhyde (MDA) nmol /g liver	Liver Glutathione (GSH) mg/g liver	Blood Glutathione (GSH) mg/dl
Normal control **(NC)**	25.73± 0.48	2.56± 0.08	16.75± 0.19
positive control **(HFD)**	82.45± 0.93 ^{a**}	1.84± 0.13 ^{a**}	11.50± 0.15 ^{a**}
Lycopene **(HFD+ LYC)**	10.58± 0.06 ^{b**}	2.22± 0.03 ^{b**}	14.55± 0.22 ^{b**}
B-Carotene **(HFD+ B-car)**	13.53± 0.18 ^{b**}	2.32± 0.02 ^{b**}	14.67± 0.13 ^{b**}
Lycopene + B-Carotene **(HFD+(LYC+ B-car))**	10.79± 0.11 ^{b**}	2.39± 0.03 ^{b**}	15.49± 0.33 ^{b**}

^{a*} Significant difference from normal control group (P≤0.05) , ^{a**} Significant difference from normal control group at (P≤0.01)
^{b*} Significant difference from positive control group at (P≤0.05), ^{b**} Significant difference from positive control group at (P≤0.01)

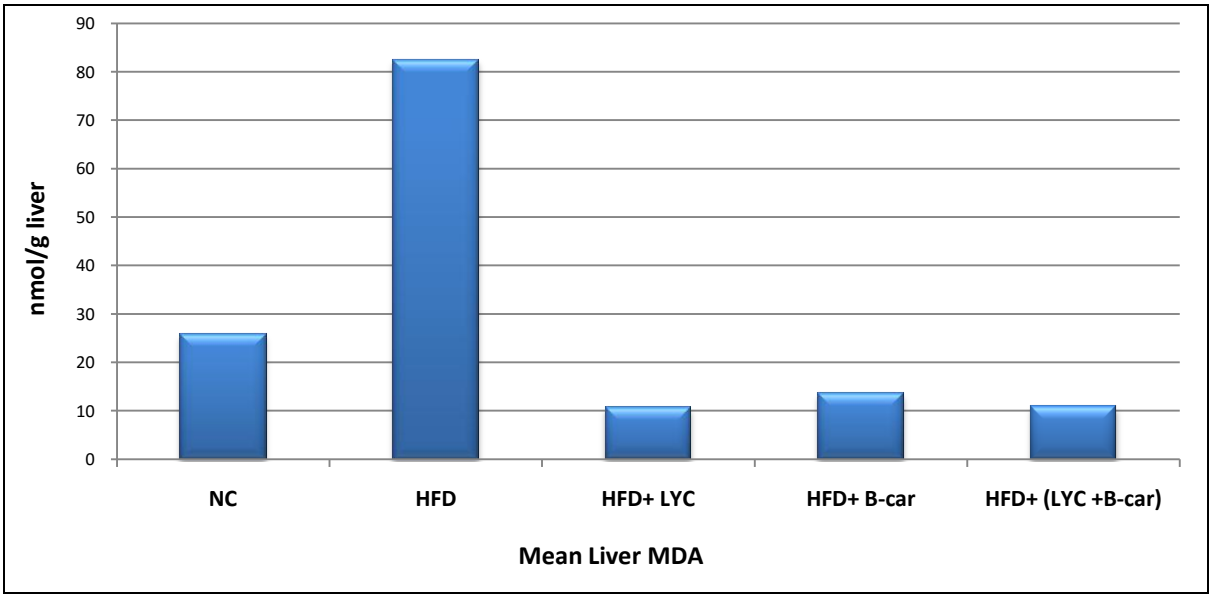

Figure (3): Mean Liver MDA (nmol/g liver) of rats groups fed on normal or high fat diet supplemented with lycopene, β-Carotene and their mixture (Mean±S.E.)

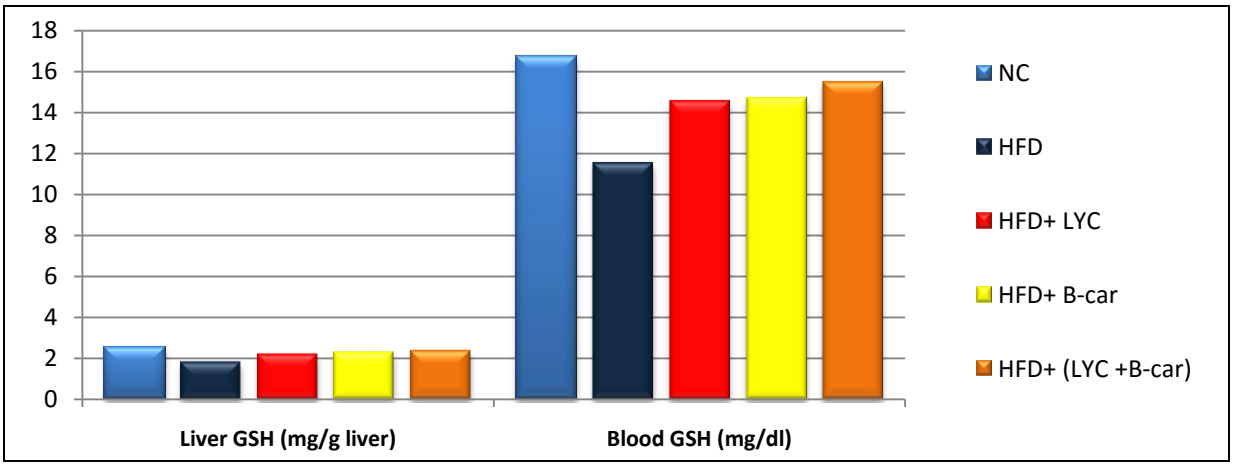

Figure (4): Mean Liver GSH (mg/g liver), and Blood GSH (mg/dl) of rats groups fed on normal or high fat diet supplemented with lycopene, β-Carotene and their mixture (Mean±S.E.)

DISCUSSION

It is well known that a diet containing lard is significantly more atherogenic, as the consumption of saturated fats produces an elevation in circulating total and LDL cholesterol levels.[15] Our study findings (Table 1 and Figures 1 and 2) showed that the consumption of HFD resulted in a significant reduction of HDL, and significant increment of total Cholesterol, LDL, TG, and LDL/HDL ratio (Atherogenic Index), when compared with normal control group (NC). These results were in agreement with the finding of Shih et al, [16] who reported that, when rats were fed on a high-cholesterol (1%) and high-fat diet, plasma and hepatic cholesterol concentrations elevated significantly, and with the finding of Nouri and Abbasabad [17] which concluded that adding cholesterol to rats diet increased the concentration of total cholesterol, LDL and TG but decreased the HDL level. Furthermore, these results were observed in other animal model (New Zealand White rabbits) as a diet rich in cholesterol led to changes in their lipid profiles including a rise in their triacylglycerols, total and LDL cholesterol and a reduction in HDL cholesterol. In addition, oxidative stress markers were also elevated when cholesterol was administered in the diet.[18]

Supplementation of HFD with lycopene (HFD+ LYC) resulted in a significant reduction of serum TC, LDL and TG levels ($P \leq 0.01$), and significant elevation of serum HDL levels ($P \leq 0.01$). These results were in agreement with those of Hassan and Edrees,[19] Verghese et al, [20] Basuny et al, [21] and Palozza et al.[22] It was reported that, rats fed on high fat diet with grade lycopene, showed significant decreases in serum cholesterol, indicating that lycopene can lower the concentration of serum total cholesterol.[21] In addition Palozza et al, [22] stated that, in animal models of atherosclerosis, lycopene decreased plasma total cholesterol, LDL cholesterol and increased high-density lipoprotein cholesterol. Lycopene seems to possess direct hypocholesterolemic properties; it is quite likely that lycopene acts with other carotenoids to exert its biological activity. These effects could be attributed to the role of lycopene in protecting LDL or phospholipid in LDL from oxidation in addition to its role in inhibition of cholesterogenesis by inhibiting 3-hydroxy-3-methyl-glutaryl-CoA (HMG-Co-A) reductase activity, and to upregulate LDL receptor activity in macrophages, or may be due to its powerful antioxidant effect that has been shown to neutralize free radicals, resulting in protection against chronic diseases especially coronary heart disease.[19] Furthermore, Verghese et al, [20] suggested that lycopene may reduce cholesterol biosynthesis through the inhibition of hepatic HMG-Co-A reductase and Acyl-CoA: cholesterol O-acyltransferase (ACAT), resulting in lower hepatic cholesterol level by a decreased absorption of dietary cholesterol contributing to a simultaneous increase in fecal cholesterol excretion in lycopene fed rabbits. However, administration of lycopene in a high cholesterol diet significantly decreased the activities of both hepatic HMG-CoA reductase and ACAT.

Supplementation of HFD with β-Carotene resulted in a significant reduction of serum TC, LDL and TG levels, and significant elevation of serum HDL levels. These results (Table 1 and Figures 1and 2) were in line with those in the study of Shih et al, [16] which showed that, when rats were fed on a high-

cholesterol (1%) and high-fat diet, plasma and hepatic cholesterol concentrations elevated significantly. The study also showed that β-carotene supplementation improved the higher plasma and hepatic cholesterol concentrations induced by cholesterol suggesting that β-carotene supplementation had modulating effects on cholesterol metabolism. In comparison with the HFD group values, a significant reduction of serum TC, LDL and TG levels ($P \leq 0.01$), and significant elevation of serum HDL levels ($P \leq 0.01$) were noticed in rats group fed on HFD supplemented with a mixture of lycopene and β-Carotene. This could be explained on the basis that, when the two antioxidants are ingested together, they work in cooperation, as lycopene may reduce cholesterol biosynthesis [20], whereas β-Carotene has modulating effects on cholesterol metabolism.[16]

Moreover, different carotenoids show synergism as antioxidants in heterogeneous systems due to their different lipophilicity, and the mechanism behind seems to depend on the spatial distribution of carotenoids.[4] Furthermore, the combination of the two carotenoids may have synergistic therapeutic effect due to the fact that, β-carotene being highly lipophilic and non-polar, is transported in the circulation in association with various classes of lipoproteins. It could likely be incorporated into the hydrophobic core of various lipoprotein particles such as chylomicrons and their remnants VLDL, IDL and LDL.[23] On the other hand, the many conjugated double bonds of lycopene make it a potentially powerful antioxidant, a characteristic believed to be responsible for its beneficial effects. In addition, these hydrocarbon carotenoids, including ß-carotene and lycopene, are transported primarily in LDL, which puts them in prime position to protect LDL from oxidation.[24]

The finding of the present study (Table 2 and Figures 3and 4) revealed that, HFD brings about notable modifications in the antioxidant defense mechanism against the process of lipid peroxidation. That was in agreement with the study of Kaviarasan et al. [25] It was illustrated by recent studies that, in the last stage of the peroxidation process, peroxides are decomposed to aldehydes like malondialdhyde (MDA). Furthermore, Hu et al, [26] reported that, MDA is the major marker of endogenous lipid peroxidation. Krishnamoorthy et al, [27] stated that, reduced glutathione (GSH) level is one of the best markers to assess the oxidative imbalance within the cell and it is inversely proportional to the level of oxidized glutathione (GS-SG). In addition the HDL level of HFD group significantly decreased, and according to Hansel et al, [28] this low level of HDL is a key feature for oxidative stress status, as HDL exerts its anti-atherogenic and antioxidative effects when present in sufficient amounts. Detected high MDA concentration in the present study indicated that, there was an increase in lipid peroxidation in animals fed on HFD when compared to the normal control group. Moreover, GSH plays critical role in the detoxification process against reactive species, and the significant decrease in the levels of liver and blood glutathione observed on HFD feeding might be due to impaired GSH biosynthesis.[1]

Table (2) and Figures (3 and 4) showed that the supplementation of lycopene, β-carotene or their mixture resulted in a significant reduction ($P \leq 0.01$) of liver MDA, and a significant elevation ($P \leq 0.01$) of liver and blood GSH, in comparison with the levels of HFD group. These results were in line with the finding of Birben, et al,[29] who reported that, carotenoids have been shown to interact with virtually all the radicals present in biological systems and are consequently significant antioxidants. In addition, Vardi et al, [30] reported that, β-carotene is to increase antioxidant enzymes in tissues, or prevent their decrease. In rats, pretreatment with β-carotene may protect the hepatocyte membrane integrity by preventing the peroxidation of fatty acids by free radicals. Unlike some other carotenoids, lycopene does not have pro-vitamin A properties, and because of the unsaturated nature of lycopene it is considered to be a potent antioxidant and a singlet oxygen quencher. Furthermore, the antioxidant activity of lycopene is mainly dependent on its scavenging properties of $O_2^{\bullet}-$ and $HO^{\bullet}$. Simultaneous supplementation of lycopene decreased oxidative stress by preventing ROS production and glutathione oxidation.[27]

The results of the present study revealed that, oxidative stress was drastically reduced and antioxidant status was improved by supplementation of lycopene. As an antioxidant, lycopene has a singlet oxygen quenching ability twice as high as that of β-carotene. It protects critical cellular biomolecules including lipid, proteins and DNA and creates first line of defense against free radicals, thus there is improvement in the levels of all antioxidants in lycopene supplemented groups. In addition, Hu et al, [26] found that low dose of lycopene administration significantly decreased MDA level in all tissues. Lycopene powder has the ability to reduce the level of malonaldehyde and increase the superoxide dismutase, catalase and glutathione peroxidase level when they were gavaged to rats up to 1068 mg/kg body weight daily. Therefore they concluded that lycopene enhances the antioxidation system in vivo and inhibits the generation of free radicals, and the lycopene treatment caused a marked inhibition in MDA production. This decrease indicated that lipid peroxidation of rats' liver tissue was reduced. The protective effect of lycopene is most likely due to its ability to scavenge the free radicals.

The combination of lycopene with other natural antioxidants, as in tomatoes, may be more potent in inhibiting lipid peroxidation, than lycopene per se. The antiatherogenic effects of lycopene are generally believed to be due to its antioxidant properties. El-Raey, et al, [24] illustrated that lycopene had the strongest singlet oxygen-quenching capacity of several carotenoids, with α-carotene, ß-carotene. The hydrocarbon carotenoids, including ß-carotene and lycopene, are transported primarily in LDL, which puts them in prime position to protect LDL from

oxidation. Moreover the modes of action for antioxidants depended on their position in the cell. Carotenes such as lycopene lie parallel with the membrane surface, whereas β-carotene could likely be incorporated into the hydrophobic core of various lipoprotein particles such as chylomicrons and their remnants VLDL, IDL and LDL [(23)]. Thus, the combinations of lycopene and other antioxidants such as β-carotene exhibited higher scavenging activity than their individual antioxidant activity.

CONCLUSION AND RECOMMENDATIONS

lycopene and ß-carotene supplementation had synergistic modulating effects on lipid peroxidation and antioxidant levels in rats fed on a high-cholesterol, high-fat diet. These findings suggest that mixture of lycopene and ß-carotene supplementation altered the pro-oxidation and anti-oxidation balance and suppressed cholesterol-induced oxidative stress by modulating endogenously the antioxidant system and cholesterol metabolism. And it is recommended that ample amounts of these natural antioxidants should be consumed daily in order to prevent CHD.

Conflict of interest: There are no conflicts of interest. The author certifies that he has no affiliations with or involvement in any organization or entity with financial or non financial interest.

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