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Possible effects of lycopene and silymarin on rat liver functions and oxidative stress markers

[Likopen ve silimarinin sıçan karaciğer işlevleri ve oksidatif stres göstergeleri üzerine olası etkileri]

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ABSTRACT

Objective: To evaluate effects of lycopene and silymarin on antioxidant enzymes, lipid peroxidation and possible liver toxicity in rats.

Methods: 15 female Wistar rats were divided into 3 groups: control group (Group I) received corn oil while Groups II and III were treated with 100 mg/kg oral dose of lycopene and silymarin for 7 days, respectively. The antioxidant enzyme activities of liver (superoxide dismutase and catalase) and malondialdehyde level were measured. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), cholesterol and urea levels were also analyzed. Besides, histopathological evaluations were performed in liver.

Results: Silymarin treatment resulted in significant decreases in catalase and cholesterol and increase in superoxide dismutase activities compared to control, while lycopene caused significant decrease in urea levels. Both of the antioxidants caused an increase in liver function enzymes AST and ALT compared to the control group. In lycopene treated group, ALP levels were also increased in comparison with control. There were significant differences in cholesterol, AST and ALP levels between silymarin and lycopene treatment groups. In histopathological examinations minimal changes were observed in liver tissues.

Conclusion: Lycopene and silymarin treatment may cause alterations in liver functions due to the dose and/or duration. Therefore, both of the lycopene and silymarin need to be investigated in detail for their possible beneficial and harmful effects.

Key Words: Histopathology, catalase, lycopene, lipid peroxidation, silymarin, superoxide dismutase, transaminases.

Conflict of Interest: The authors declare no conflict of interest.

ÖZET

Amaç: Likopen ve silimarinin antioksidan enzimler ve lipid peroksidasyon üzerine etkileri ile olası karaciğer toksisitesinin sıçanlarda araştırılması.

Metod: 15 dişi Wistar sıçan 3 guba ayrılmıştır: kontrol grubuna (Grup I) mısır yağı, Grup II ve Grup III'e sırasıyla 100 mg/kg likopen veya silimarin oral yolla 7 gün boyunca verilmiştir. Karaciğerdeki antioksidan enzim aktiviteleri (süperoksit dismutaz ve katalaz) ve malondialdehit seviyesi ölçülmüştür. Serumda ise alanine aminotransferaz (ALT), aspartate aminotransferaz (AST), alkalin fosfataz (ALP) aktiviteleri; kolesterol ve üre düzeyleri ölçülmüştür. Ayrıca, karaciğer histopatolojik açıdan değerlendirilmiştir.

Bulgular: Silimarin grubunda kontrol grubuna göre, katalaz ve kolesterolde istatistiksel olarak önemli bir azalma görülürken, süperoksit dismutaz aktivitesinde artış olmuştur. Likopen grubu kontrol grubuyla karşılaştırıldığında üre düzeyi önemli derecede azalmıştır. Karaciğer işlevini gösteren AST ve ALT enzimleri her iki grupta da kontrol grubuna kıyasla artmıştır. Likopen uygulama grubunda ALP aktivitesi de önemli dercede artmıştır. Ayrıca kolesterol düzeyleri, AST ve ALT aktivitesi silimarin ve likopen grupları arasında farklılık göstermiştir. Histopatolojik incelemelerde minimal değişiklikler gözlenmiştir.

Sonuç: Likopen ve silimarin kullanımı doz ve/veya süreye bağlı olarak karaciğer işlevleri üzerinde değişikliklere yol açabilir. Bu nedenle likopen ve silimarinin olası yararlı ve zararlı etkilerinin ayrıntılı olarak araştırılmasına gereksinim bulunmaktadır.

Anahtar Kelimeler: Histopatoloji, katalaz, likopen, lipit peroksidasyonu, silimarin, süperoksit-dismutaz, transaminazlar.

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Antioxidants which form a diverse group of compounds with different properties operate by inhibiting oxidant formation, intercepting oxidants and repairing oxidant-induced injuries [1]. Antioxidants are quite important to human life, and lycopene and silymarin are two of the most popular. Epidemiological studies have indicated that food products which contain lycopene, the most potent antioxidant compound among carotenoids, have chemopreventive effects against cancers and other diseases [2]. Human cell studies showed that lycopene can reduce oxidative DNA damage, stimulate the immune system and facilitate intercellular communication [3]. Many reports on the health benefits of lycopene are attributed to its ability to protect cells against oxidative damage [2-6]. Besides, it has also been observed that silymarin possesses antioxidant, antiinflammatory, antifibrotic and antiproliferative properties. Silymarin is a flavonolignan mixture composed of silybinin, silydianin and silychristin, isolated from the seeds of milk thistle *(Silybum marianum)*. Hepatoprotective potential of silymarin has been reported due to its cellular regeneration and cytoprotection activities [7-9]. Silymarin showed liver regenerating property in many cases by direct interactions with cell membrane. The inhibition of lipid peroxidation in erythrocytes, hepatocytes and human mesangial cells *in vitro* has been accepted as one of the major protective mechanisms [7].

Reactive oxygen species (ROS) are normally produced in both unstressed and stressed cells [10]. Various ROS may be produced from biochemical and essential metabolic processes or from external sources such as exposure to a variety of agents present in the environment [11]. ROS and reactive nitrogen species are well known to play a dual role as both deleterious and beneficial, since they can be either harmful or beneficial to living system [12]. The term "oxidative stress" was formerly defined as a disturbance in the pro-oxidant-antioxidant balance leading to potential cellular damage. Most cells can tolerate a mild degree of oxidative stress, because they have sufficient antioxidant defense capacity and repair systems [11]. The interaction of ROS with cellular macromolecules such as protein, lipid, DNA may contribute to the aetiology of many chronic diseases of high prevalence. Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) are considered as cellular antioxidant defence mechanisms [12-16]. SOD enzyme functions at the first step to detoxify superoxide anion radical and GPx and CAT complete the process [10,17,18]. GPx is found throughout the cell, whereas CAT is often restricted to peroxisomes. In humans, the highest levels of CAT are found in liver, kidney, and erythrocytes, where it decomposes the majority of hydrogen peroxide (H_2O_2). Many natural substances reduce oxidative stress by radical scavenging, metal ion chelating and inhibiting the activity of radical generating enzymes. It also has been well defined that chemopreventive reagents for instance phenolic antioxidants, dithiolethiones, isothiocyanates selectively induce the activation of phase II detoxifying and antioxidant enzymes through the Keap1–Nrf2 pathway [18].

Lipid peroxidation refers to the oxidative deterioration of lipids containing any number of carbon-carbon double bonds, such as unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Radical scavengers can directly react and quench peroxide radicals to terminate the chain reaction. Lipid peroxidation has been extensively used as a research model for identifying natural antioxidants as well as the studies of their mechanisms of action. Studies on antioxidants such as vitamins, polyphenols, flavones and ginsenosides against free radical-induced lipid peroxidation have been undertaken in several systems [18].

Cholesterol has been exploited with great advantage to detect any oxidation process in cell membranes. In contrast with unsaturated fatty acids, cholesterol exists as a single molecular species, its oxidation products are thus much less complicated to isolate and characterize. The identification of cholesterol oxidation products may be used as a mechanistic proof in various oxidant systems [19].

Herbal antioxidants have become a popular area of research due to their therapeutic potential against diseases and efficacy to counteract toxicity induced by chemicals [17]. Therefore, the present study was undertaken to evaluate the effects of two antioxidants lycopene and silymarin on the balance of oxidative stress-antioxidant status in rats by measuring the activities of SOD and CAT, and lipid peroxidation levels. Furthermore, the basic biomarkers of hepatotoxicity such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), levels of cholesterol and urea were also investigated. The findings were also supported with liver histopathological examination.

Materials and Methods

Chemicals

Lycopene was purchased from Roche as Redivivo™ while silymarin was obtained from Sigma (Darmstadt, Germany). They were prepared in corn oil (Ülker, Turkey). All other chemicals used in the study were analytical grade and obtained from Sigma Chemical Co.

Animals

This study was performed with the official permission of the *"Ethical Committee for the Protection of Animals in Research"* of the Hacettepe University. Fifteen female Wistar rats with body weights (bw) of 170–210 g were obtained from the Unit of Laboratory Animals. The rats were housed as 5 in a cage, at 22-24 ºC and 50±10% humidity with a 12:12-h light-dark cycle and they had access to food and water ad libitum. The animals were divided into 3 groups and all of the treatments were applied for

Table 1. Alteration of gravimetric parameters in study groups

Parameters	Group I	Group II	Group III
Initial body weight (g)	192±5	180±4	194±5
Final body weight (g)	193±9	169±6	200±3
Relative liver weight	0.045±0.001	0.035±0.002[a]	0.041±0.001[a]

Values are mean ± SEM. Statistically significant difference: [a]p<0.05 vs controls (Group I).

Table 2. Lipid peroxidation, urea and cholesterol levels of the study groups

Parameters	Group I	Group II	Group III
Liver MDA (nmol/g tissue)	0.22±0.03	0.16±0.03	0.15±0.03
Urea (mg/dl)	66±4	47±4[a]	69±3
Cholesterol (mg/dl)	61±4	57±3	47±2[a,b]

Values are mean ± SEM. Statistically significant difference: [a]p<0.05 vs controls (Group I); [b]p<0.05 vs lycopene-treated rats (Group II).

7 days. Group I was used as a control group and treated with corn oil while Groups II and III were treated with 100 mg/kg (bw) dose of lycopene and silymarin, respectively. Changes in the body weights of each animal were observed once a day during the assay period.

Determination of hepatic functions

At the end of the experimental period, the rats were sacrificed under anaesthesia with Ketalar® (Pfizer, Turkey) and Alfazyne® (Alfasan International, Holland). Blood samples were collected and centrifuged for 15 min at 3000 rpm (Hanil Science Industrials Co.). Obtained plasma samples were stored at -20 ºC until the analysis of urea, total cholesterol and the enzyme activities, AST, ALT and ALP. Determination of AST, ALT and ALP activities and cholesterol and urea levels were done by using biochemical kits (Audit Diagnostics, Ireland) by Shimadzu CL-770 Clinical Spectrophotometer (Japan).

Determination of specific SOD and CAT activities

Each liver was immediately removed, absolute organ weights were recorded and relative organ weights were calculated. Liver tissues were stored at -80 ºC until the analysis. In order to detect SOD and CAT activities, the liver homogenates were prepared from the stored tissues. Following centrifugation steps, catalase activity [20] and superoxide dismutase activity [21] were measured. In order to calculate the specific enzyme activity, protein content of the samples was determined [22].

Determination of the lipid peroxidation products

The liver homogenates were used for determination of malondialdehyde (MDA) levels by using the method based on thiobarbituric acid (TBA) reactivity [23] with tetraethoxypropane as a standard [24].

Histopathological evaluation

Each liver tissue sample was fixed in 10% formalin solution, embedded into paraffin, 5 µm thick tissue sections were stained with routine haematoxylin and eosin (H&E) and examined under Olympus BX51 system light microscope for histopathology.

Statistical analysis

Statistical analysis was performed using SPSS software. Statistical significance was assigned at the $p \leq 0.05$ levels. The homogeneity of variance and normal distribution between groups were evaluated using general linear model procedure and Kolmogorov-Smirnov nonparametric test. Serum parameters were analyzed using one-way ANOVA. To identify the sources of significant main effects, post hoc comparisons (Games-Howell, Tukey) were used. Body and relative organ weights were examined using one-way ANOVA and Games-Howell post hoc test.

Results

As basic toxic response indicators, whether death and body weight changes were investigated in all study groups. There was no mortality observed in any of the groups during the experimental period. Changes in the body weight gains and relative liver weights of the groups were given in Table 1. There were not any significant differences in body weight gains among the groups. The significant alteration was observed in liver weights; both of the lycopene and silymarin caused decreases in relative liver weights compared to the control group (both, $p<0.05$). As shown in Figure 1, lycopene did not cause any significant change in liver CAT enzyme activity ($p>0.05$) while silymarin treatment led to a significant decrease in CAT activity compared to the control ($p<0.05$). On the other hand, no difference was observed between silymarin and lycopene treated groups ($p>0.05$). Results of the liver SOD specific activities were shown in Figure 2. Both of the lycopene and silymarin treatments resulted in elevations of the SOD activity. However, only

silymarin treated group showed significance compared to the control ($p<0.05$). There was no difference in SOD activity between lycopene and silymarin treatment groups ($p>0.05$). Levels of MDA, cholesterol and urea were presented in Table 2. Lycopene and silymarin diminished MDA levels in comparison with the control, but there was no statistical significance between the groups (both, $p>0.05$). Lycopene caused a significant decrease in urea levels while silymarin led to reduce in cholesterol levels (both, $p<0.05$). There was a significant difference in cholesterol levels between silymarin and lycopene treatment groups ($p<0.05$). As shown in Figure 3, lycopene and silymarin significantly increased AST levels compared to control (both, $p<0.05$). However, there was not any difference in ALT levels in treated groups with lycopene or silymarin ($p>0.05$). As presented in Figure 4, both of lycopene and silymarin increased ALT levels compared to control (both, $p<0.05$). Treatment of silymarin caused a significant increase in the ALT level compared to lycopene treatment ($p<0.05$). In treated group with lycopene, ALP levels were significantly increased in comparison with control ($p<0.05$) while silymarin did not change ALP levels in comparison with control ($p>0.05$) (Figure 5). Moreover, there was a considerable difference in ALP levels between antioxidant treated groups ($p<0.05$). In histological examination, there were minimal changes such as congestion in the parenchyma and hyperaemia in vessel in the liver of only one of five rats in both lycopene and silymarin groups. The minor alterations were presented in Figure 6.

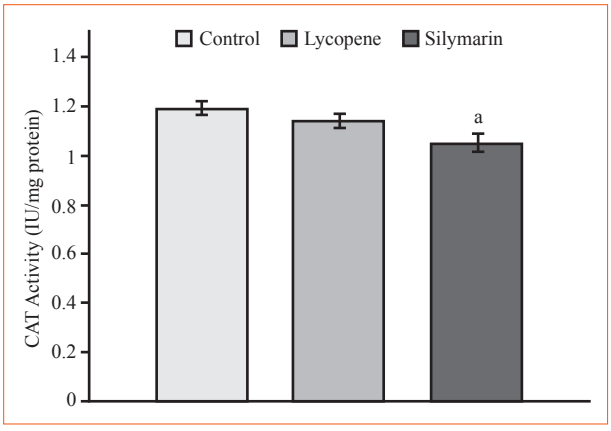

Figure 1. Effect of lycopene and silymarin on specific catalase (CAT) activity in rat liver. Values are mean ± SEM of 5 animals. Statistically significant difference: [a]$p<0.05$ vs control rats.

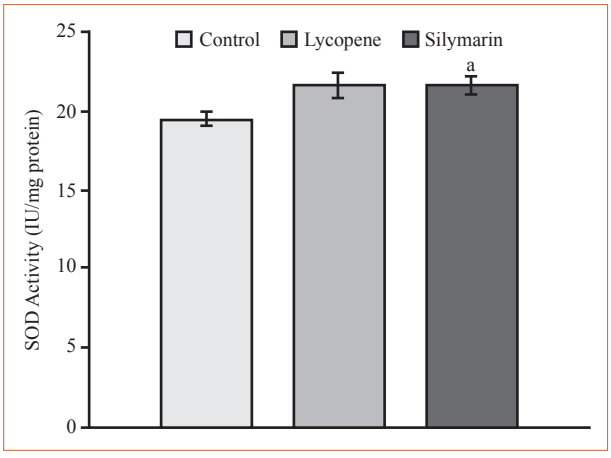

Figure 2. Effect of lycopene and silymarin on specific superoxide dismutase (SOD) activity in rat liver. Values are mean ± SEM of 5 animals. Statistically significant difference: [a]$p<0.05$ vs control rats.

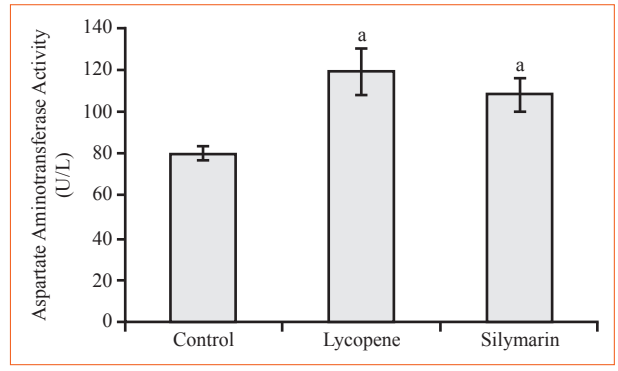

Figure 3. Effect of lycopene and silymarin on AST activity. Values are mean ± SEM of 5 animals. Statistically significant difference: a $p<0.05$ vs control rats.

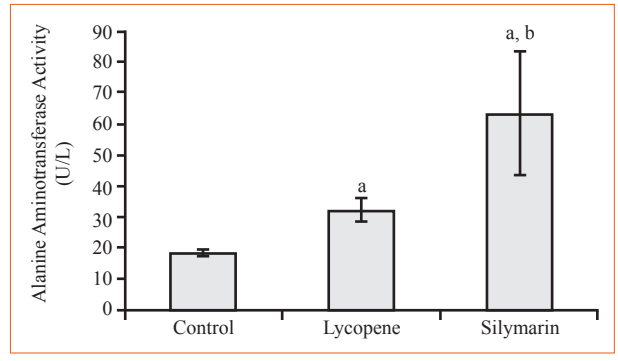

Figure 4. Effect of lycopene and silymarin on ALT activity. Values are mean ± SEM of 5 animals. Statistically significant difference: [a]$p<0.05$ vs control rats; [b,] $p<0.05$ vs lycopene-treated rats.

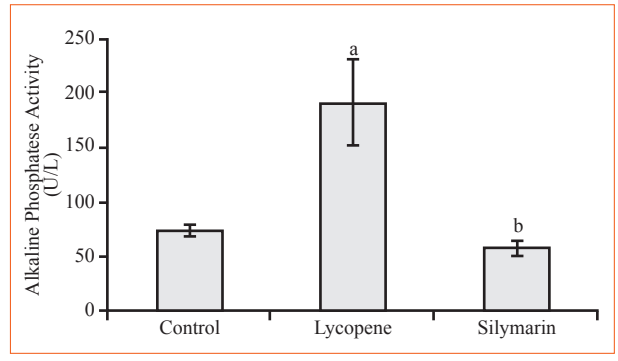

Figure 5. Effect of lycopene and silymarin on ALP activity. Values are mean ± SEM of 5 animals. Statistically significant difference: [a]$p<0.05$ vs control rats; [b]$p<0.05$ vs lycopene-treated rats.

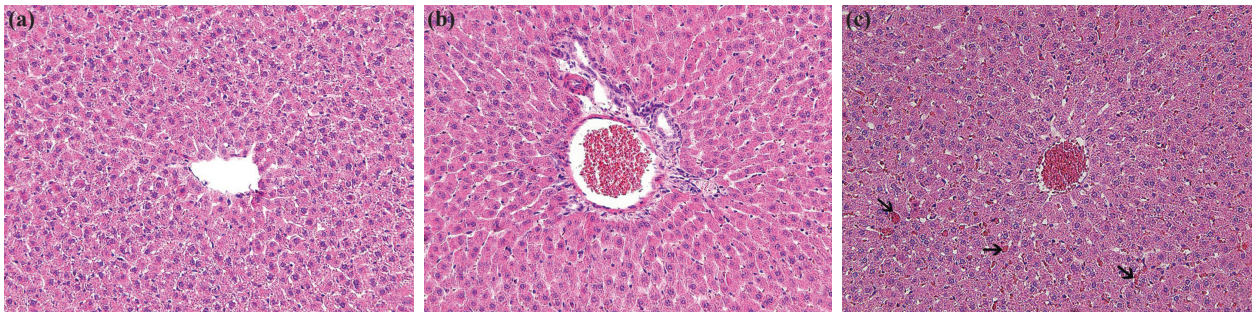

Figure 6. Photomicrographs of liver tissues of rats in control and lycopene and silymarin treatment groups stained with H&E. **(a)** Control group; **(b)** Lycopene group, hyperaemia in vessel; **(c)** Silymarin group, congestion in the parenchyma (arrow) and hyperaemia in vessel (100x).

Discussion

In past two decades, consumptions of natural antioxidants such as dietary supplements have not only been promoted by manufacturers in order to protect the organism and maintain the health against deleterious effects, but also they have been introduced as natural compounds without any toxicity even at high doses [19]. Supplementation with excessive quantities of antioxidants can shift the oxidant-antioxidant balance toward the oxidant side. The hazard of this nutritional manipulation is not still clear, but the ability of ROS to affect signal transduction and to modify metabolic processes suggests that antioxidants may be an important factor modulating how an organism will respond to oxidative stress and injury [11]. Antioxidants may indirectly decrease the formation of free radicals by inhibiting the activities or expressions of free radical generating enzymes or by enhancing the activities and expressions of other antioxidant enzymes [19].

There are several reports on protective effects of lycopene and silymarin against various disorders. On the other hand, reports are rare and confusing about the side effects of their daily consumption in healthy individuals. This study was designed to elucidate liver function profile and antioxidant/protective effects of both lycopene and silymarin in rats. Lycopene and silymarin as recently most popular antioxidant compounds were tested at 100 mg/kg whether they have any effect on hepatic functions and oxidative/antioxidative status. Although the bioavailability of lycopene was very low (1–3% absorbed) in animals, it was found to be concentrated in various body tissues, such as the liver, adrenals, and adipose tissue [2]. Lycopene molecule has been shown [25] to be absorbed with having similar tissue distribution in rats and humans, therefore rats were chosen as an appropriate animal model for assessing the potential effects of lycopene in humans. We preferred to use lycopene at a higher dose than the acceptable daily intake (ADI) which was calculated from the no-observed-adverse-effect level (NOAEL, 3000mg/kg(bw)/day for synthetic lycopene) [26]. In spite of the fact that former studies have reported that silymarin is well tolerated and non-toxic, and there is no known median lethal dose [27], results of the present study showed that silymarin and lycopene at 100 mg/kg for 7 days caused some alterations in oxidative/antioxidative processes and hepatic functions.

Antioxidant ability of the most substances is not completely clear. Many studies have been focused on the structure modification of antioxidants to improve the antioxidant property and to enhance SOD activity; for instance, administration of a modified ocotillol type saponin, ginsenoside Rg3 in dogs caused a decrease in lipid peroxidation and an increase in SOD activity; but the underlying mechanisms for these effects are not clear [28]. Chemical models such as metalcurcumin which can be used to study the SOD activity have been established [29]. Cell line models have been used to study the effect of antioxidants such as anthocyanins, resveratrol, and curcumin on the activation of the internal antioxidant enzymes. It has been found that natural anthocyanins act as chemopreventive phytochemicals and could stimulate the intracellular antioxidant system to resist oxidant-induced injury. Resveratrol, however, either has no effect on, or reduces the activities of GPx, CAT, and SOD, while it dramatically and progressively induces mitochondrial SOD expression and activity [18]. Our results indicated alterations in SOD and CAT activities; both lycopene and silymarin had direct induction or inhibition effect on the antioxidant system. For this reason treatments of lycopene and silymarin resulted in increase in liver SOD activities, while decrease in CAT activity. Apparently their supplementations led to a decrease in lipid peroxidation in liver. Şentürk et al. has reported that silymarin at dose of 100 mg/kg caused an increase in kidney MDA levels and CAT activities of rats applied renal ischemia-reperfusion [30]. Most probably, decrease in MDA levels at 25-30% by lycopene and silymarin may be originated from elevation in liver SOD activities and/or they act as a radical scavenger. It is known that lipid peroxidation processes involve radical formations including initiator radicals and lipid peroxyl radicals. The antioxidant may directly react with initiator radicals or lipid peroxides, and it may also inhibit the formation of active radicals. These mechanisms of action of any antioxidant are critical and warrant for further investigations with radical scavenging assays and ion chelating tests [18].

Decreases in urea with lycopene or in cholesterol with silymarin were found in the present study. It is noted that lycopene shares similar initial synthetic pathway with cholesterol, which is synthesized in animal but not in plant cells. It was reported that dietary supplementation of carotenoids may act as moderate hypocholesterolemic agents, secondary to their inhibitory effect on the rate-limiting enzyme in cholesterol synthesis [4]. Surprisingly, lycopene and silymarin applications caused significant changes in AST, ALT and ALP activities. It might be thought that the reason of lower relative liver weights in treated groups compared to controls was compensation against the increase in AST, ALT and ALP levels. Precisely, to express anything about this is difficult at the moment and this point remains unclear. Regarding the histopathological examination, we observed minor changes in the liver. However, there were elevations in AST and ALT levels in the treatment groups, no marked changes were determined in liver according to histopathological examinations. Elevations in these enzymes seem to be transient liver dysfunction due to treatments with lycopene and silymarin. Apart from mild congestion in parenchyma, the liver had a nearly normal appearance. However, the data showing the increases in ALT and AST levels that are main biomarkers for liver function [31] were not accompanied by other indications of hepatotoxicity, such as liver histopathology. Considering possible long term intake, it is clear that these compounds need to be investigated extensively for both of their beneficial and untoward effects.

In conclusion, as it is well known that each natural or synthetic xenobiotic can show useful/harmful effects due to its dose and interval. Therefore, further studies are needed in order to confirm our results at their various doses and treatment duration. Moreover, it should be considered that natural compounds can never be used at uncontrolled amounts and long time. It is important to note that antioxidant compounds should be carefully consumed for the maintenance of health.

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Conflict of Interest

There are no conflicts of interest among the authors.

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