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Does the high sulfur content in apricots affect oxidative stress? Running title: Effect of sulfur amount on oxidative stress

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Abstract

Apricots are one of the most important fruits that can be produced worldwide. Most of the importer countries are sensitive about sulfur which is used in the protection of dried apricots. The objective of this study was to compare the effects of feeding with high and low sulfur content apricots on oxidative stress parameters of the liver. In total, 42 Wistar albino rats were fed for 12 weeks with the use of dried apricots containing different amounts of sulfur and sun-dried apricots. There was no significant difference in IL-1β, 8-OHdG and TOS levels between the control group and the group fed with sun dried apricots. There was no statistically significant difference between the groups fed with low and high sulfur-containing apricots.Non-necrotic changes were observed in 3000 ppm and 4000ppm groups in microscopic examination. No significant effect of feeding with sulfur treated apricots was found in the liver tissues of rats.

Keywords: Histology, liver, oxidative stress, Prunus armeniaca, rat, sulfur compounds

Introduction

Apricots are one of the most important fruits that can be commercially produced in many regions worldwide [1]. Between 2.5 and 2.8 million tons of fresh apricots production is made from about 70 million trees over the world each year. In Turkey, 400-600.000 ton fresh apricots production is made from about 16 million apricot trees. Each year 250-300.000 tons of this amount is produced in Malatya province. A revenue between 150-200 million US dollars is obtained from the export of 50-120.000 ton dried apricots to about 100 countries annually. Apricots have become an irreplaceable product with the economic contribution it provides to Malatya province.

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A large part of the produced apricots is dried by the sulfurization process. The sensitivity of the importer countries about sulfur which is used in the protection of dried apricots is increasing day by day. The amount of sulfur in dry apricots is limited to 2000 ppm in many European countries and in our country, and this amount is likely to be further decreased. Although experiments conducted on voluntary persons have shown that a sulfur amount higher than 13-14 mg/kg caused throat and gastric burning, headache, and even toxic symptoms such as vomiting [2], many studies have been conducted with laboratory findings but found controversial results. No evidence could be found about sulfurization contents causing danger for majority of society. Some persons give no reaction to a sulfur amount of 50 mg/kg body weight, while far less amount may cause symptoms in other persons such as headache and vomiting [3].

Recent studies showed that increased free oxygen radicals and lipid peroxidation play an important role in the pathogenesis of many diseases [4]. Oxidative stress has been associated with many diseases including cardiologic diseases such as myocardial

infarction, neurological diseases, asthma, diabetes mellitus, rheumatic disease including rheumatoid arthritis, cancer and aging [5]. Free radicals are defined as short-lived, unstable, low-molecular-weight highly potent molecules that have one or more unpaired electrons. Free radicals have different chemical structures such as hydroxyl, superoxide, nitric oxide, and lipid peroxide radicals. The most important free radicals in biological systems are the radicals consisting of oxygen. These radicals are generally named as Reactive Oxygen Species (ROS) [6]. Some defense systems have been developed in the body in order to prevent or decelerate the damage caused by ROS. These defense systems are known as antioxidant defense mechanisms. The condition known as oxidative stress occurs as a result of the severe imbalance between the formation of free radicals and antioxidant defense systems. This imbalance usually leads to tissue damage [7].

Macrophages are the most important source of interleukin-1 (IL-1), and the main mechanism in which they function is the formation of immunologic and inflammatory response. Besides macrophages, endothelial cells, smooth muscle cells and fibroblasts also secrete IL-1 [8]. IL-1 produces the immunologic response by providing development, adhesion and functional activation of T and B lymphocytes from different aspects, and maturation of thymic T and B cell precursors, and by increasing the synthesis of lymphokine and lymphokine receptors. In addition, IL-1 provides stimulation of arachidonic acid metabolism, synthesis of the acute phase proteins in hepatocytes, and an increase of IL-2 [9].

The main reason for the guanine base being the base that most easily experiences oxidation is that it has the lowest ionization potential among the other bases. 8-hydroxy-2'- deoxyguanosine (8-OHdG) is formed as a result of the reaction between hydroxyl radical and double bond at the C-8 position. 8-OHdG is the most commonly found oxidative DNA damage repair product in humans, and this highly stable product is used in the determination of oxidative DNA damage [10]. In addition, G: C and T:A base change mutations that may lead to severe outcomes may occur with the oxidation of the guanine base [11].

Despite all this information, no study was found in the literature comparing the effects of apricots treated with sulfur and sun-dried apricots or effects of increasing sulfur amounts. Within this context, the objective of this study was to compare the effects of long-term feeding with high and low sulfur-containing apricots on oxidative stress parameters of the liver tissues.

Materials and Methods

Experimental Animals

A total of 42 male Wistar albino rats were used in this study. They were supplied from the Inonu University, Experimental Animals Production, and Research Center. The study followed the principles of the İnönü University Experimental Animals Ethics Committee. Rats of 18 weeks weighed 205±13 g and were kept in standard housing cages until the day of the experiment. Drinking water was changed and standard cages were cleaned daily during the experiment. The rats were harbored in well-ventilated rooms with 24-27°C room temperature and a 12-hours dark/light cycle. Rats in each group were distributed as two rats in each cage to provide a homogeneous pellet feed.

Experimental groups were designed as follows

1. Control Group (n=7): Rats in these groups were fed with commercial pellets.
2. Sun-Dried Apricots Group (n=7): Pellets prepared with 10% sun-dried apricots were given ad libitum.
3. 1000 ppm Sulfured Apricots Group (n=7): Pellets prepared with apricots containing 1000 ppm sulfur were given ad libitum.
4. 2000 ppm Sulfured Apricots Group (n=7): Pellet prepared with apricots containing 2000 ppm sulfur was given ad libitum.
5. 3000 ppm Sulfured Apricots Group (n=7): Pellets prepared with apricots containing 3000 ppm sulfur were given ad libitum.
6. 4000 ppm Sulfured Apricots Group (n=7): Pellet prepared with apricots containing 4000 ppm sulfur was given ad libitum.

Determination of Sulfur Amount of Apricots

The sulfur amount in the apricots was determined according to the Turkish Standard no:8131. The apricots were classified according to the sulfur content, and ground, and stored at +4°C in portions.

Preparation of Pellets Containing Sun-Dried Apricots and Sulfur-Containing Apricots

During the experiments, sun-dried and sulfur-containing apricots were mixed with pellets at 10%. To minimize the decrease of sulfur ratio due to waiting for the prepared pellets, the pellets were prepared as 3kg at every turn. For this purpose, 300 g of the apricots that were previously ground and kept at +4°C were weighed and a powder pellet was added until a 3kg pellet was obtained (approximately 2700g). Some water was added to the mixture, which was then worked until it became thoroughly homogenized and then made pellet again utilizing a pellet machine.

Obtaining Liver Tissues from the Rats

At the end of the 12-week-long feeding period, the rats were sacrificed under anesthesia by drawing blood from the heart. The livers of the rats were removed from the abdominal cavity and divided into two parts. One of these parts was kept in formaldehyde, while the other part was kept at -80°C by wrapping it with an aluminum foil.

Tissue Homogenization

To prevent activity loss of tissues taken from the deep freezer, they were treated on the ice during the homogenization process. The tissues were divided into small pieces weighing about 200 mg. The weighted tissues were put into tubes that were inserted into the ice-filled transporters and numbered. 1mL of 50 mM Tris-HCl buffer (pH:7.4) was added to each of the tubes with tissues and homogenized at 16000 revolutions per minute for 2 minutes. Then the solution was added further 1mL 50 mM Tris-HCl buffer (pH:7.4) and homogenized for 1 minute. This process was applied separately for each tube. Following the homogenization process,

all tubes were centrifuged at 4000 rpm and +4°C for 10 minutes. The formed supernatants were portioned into Eppendorf tubes and used for the measurement of biochemical parameters.

Biochemical Measurements

TAS and TOS activities of the liver tissue were analyzed by using Total Antioxidant Status and Total Oxidant Status kits (Rel Assay Diagnostics, Turkey) respectively. The IL-1β and 8-OHdG levels were measured using commercial double-antibody sandwich ELISA kits (SunRed, China). Biotek ELx800 device was used for photometric reading of plates.

Histological Examination

For histological examination, liver tissue samples were fixed in 10% formaldehyde at room temperature for 48 hours. Following the fixation, liver tissue samples were subjected to ethanol series of increased degrees (50%-%99), dehydration, and xylene series and cleared. Then following melted paraffin infiltration at 62°C, the samples were embedded into the paraffin blocks. Sections of 6 µm were cut from the paraffin blocks using a microtome and taken on the slides. Liver sections were stained with hematoxylin-eosin (H&E) and examined with Nikon Optiphot-2 light microscope, Nikon DS-Fi2 camera, and Nikon DS-L3 image analysis system (Nikon Corporation, Tokyo, Japan).

Statistical Analyses

The normal distribution of the data obtained was tested using the Shapiro-Wilk test. ANOVA test was used in the comparisons between the groups. All statistical analyses were performed with IBM SPSS Statistics version 23 package software. $p<0.05$ values were considered statistically significant.

Results

Results of the biochemical measurement in liver tissue were given in Table 1. There was no significant difference in IL-1β, 8-OHdG, and TOS levels between the control group and the group fed with sun-dried apricots. TAS levels were significantly higher in the group fed with sun-dried apricots compared to the control group, and OSI results were significantly lower in the group fed with sun-dried apricots compared to all groups. Although the IL-1β, 8- OHdG, TOS, and TAS measurements of all groups fed with sulfuric apricots were higher than the control group, there was no statistically significant difference between the groups fed with low and high sulfur-containing apricots. When the OSI results were evaluated, it was determined that there was no statistical difference between the groups except for the group fed with sun-dried apricots.

Table 1. Result of the biochemical measurement of liver tissue

Groups	n	IL-1β (pg/L)	8-OHdG (ng/mL)	TOS(µmol H_2O_2/L)	TAS(mmol Trolox/L)	OSI(Arbitrary Unit)
Control	7	842.49±68.85^{c}	1.53±0.21^{b}	12.34±0.28^{b}	1.56±0.34^{c}	8.19±1.71^{a}
Sun-Dried	7	881.09±79.80^{c}	1.32±0.15^{b}	13.25±1.96^{b}	3.44±0.30^{a}	3.89±0.82^{b}
1000ppm	7	1245.06±99.76^{a,b}	2.27±0.33^{a}	19.05±2.51^{a}	2.72±0.22^{b}	7.02±1.04^{a}
2000ppm	7	1109.08±168.97^{b}	2.29±0.58^{a}	19.20±2.05^{a}	2.60±0.46^{b}	7.56±1.51^{a}
3000ppm	7	1318.19±108.93^{a}	2.35±0.52^{a}	19.43±2.61^{a}	2.74±0.34^{b}	7.24±1.61^{a}
4000ppm	7	1089.23±109.65^{b}	2.47±0.28^{a}	21.69±6.88^{a}	2.54±0.30^{b}	8.55±2.73^{a}

Liver sections were evaluated in normal histologic structure in microscopic evaluations of the sections in the control, Sun-Dried, 1000 ppm, and 2000 ppm groups. Liver portal triad connective tissue with artery, vein and bile ductules, hepatocytes, sinusoids, and central vein were also normal (Figure 1; A, B, C, D). However, minimal congestion was observed in the examined section of all four groups. Histological examination of the liver sections of 3000 ppm revealed sporadic central vein damage, nuclear chromatolysis in the hepatocytes around the central vein, and although low, hepatocyte damage. Minimal sinusoidal dilatation was observed in central hepatic lobules. Minimal inflammatory cell infiltration and vascular congestion were found in periportal connective tissue areas. In addition, there was minimal cytoplasmic eosinophilia and increased nuclear heterochromatin in the hepatocytes (Figure 1; E). Histologic evaluation of the liver sections of the 4000 ppm group revealed central vein damage, sporadically diffuse nuclear chromatolysis, and heterochromatic pyknotic nucleus in the hepatocytes. Sinusoidal dilatation was found in some sections with being more prominent in the center of liver lobules. There were inflammatory cell infiltration and vascular congestion in the periportal connective tissue areas (Figure 1; F).

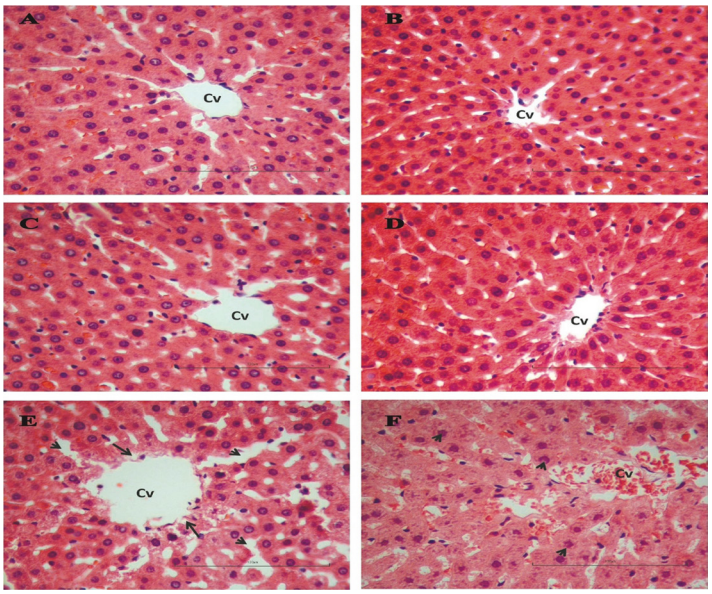

Figure 1. Liver central vein (Cv) images (40x magnification). A. Control group, B. Sun dried group, C. 1000 ppm group, D. 2000 ppm group, E. 3000 ppm group central vein damage (arrows) and dilated sinusoids (arrowheads), F. Congestion in the central vein and chromatolysis in hepatocyte nuclei in 4000 ppm group (arrowheads)

Discussion

In our study, we examined liver oxidative stress parameters and DNA damage markers in rats fed with apricots containing different amounts of sulfur and sun-dried apricots. The results obtained indicated that the oral intake of sulfured apricots does not significantly affect oxidative stress and DNA damage.

Articles related to sulfured apricots in the literature are mostly about the inhalation of SO_2 gas regarding worker safety. According to these studies, workers in the sulfuration process expose to 106-722 ppm SO_2 gas, and exhibit symptoms such as throat burning, eye, and nose itching, and cough one hour after the exposure [12]. In a study by N. Koksal et al., serum levels of TNF-α, IL1-β, IL-6, and IL-8 were increased in workers who were exposed to SO_2 gas for one hour. However, it was reported that the increased TNF-α and IL1-β were released from the macrophages, and high levels were expected since these were early released cytokines in acute lung damage [13]. Gokirmak et al. found decreased serum levels of GPx, CAT, and SOD, and increased MDA in workers who were exposed to SO_2 gas [14]. Uren et al. treated human peripheral lymphocytes with SO_2 gas at 0.1, 0.5, and 1 ppm concentrations and reported that SO_2 gas caused sister chromatid exchange, increasing mitotic index and replication index, and showing a cytotoxic effect [15]. The main reason for the different results between the mentioned studies and our study is the different ways of the sulfur entrance the body. Usually, sulfur as an air pollutant is applied to animals by inhalation [16]. Since our study aimed to investigate the effects of the sulfur amount in apricots, it is the most appropriate way to give apricots mixed with pellets to rats by the oral route [17-18].

It is known that treating apricots with sulfur leads to physical and chemical changes in the fruits. It has been reported that sulfurization decreases the amounts of total polyphenol, malic acid, citric acid, and β-carotene [19]. T. Kan and S. Z. Bostan observed decreases in the number of flavonoids such as catechin, epicatechin, and rutin at high levels of sulfur [20,21]. Given the decrease in these flavonoids and total polyphenols, the apricots used in our study might be subjected to the same condition. The lack of difference between the groups in many parameters might have resulted from the degradation of the compounds that play a major role in the antioxidant activities of the apricots.

Despite biochemical parameters that did not show significant differences among the experimental animals fed with apricots containing different amounts of sulfur, the results of the histologic examination were different. In the microscopic findings, liver damage was increased with the amount of sulfur in the apricots. However, this increase was not statistically significant. Nevertheless, some points should be noted. First, the most important change that may occur in the liver is necrosis [22], and necrosis was not found in any of our experimental groups. All changes were inflammatory changes that can be spontaneously resolved. Within this context, we can say that a high amount of sulfur has mild effects on the liver tissue, and did not cause permanent damage to the tissue.

Remarkably, histological changes did not reflect on biochemical parameters. As is known, biochemical parameters in the cells can instantly change and the cells can show adaptation to the existing condition after acute increases. In addition, unlike instant changes in biological parameters, morphologic changes take a longer time. Oxidative stress factors increase with age as well as with different stress factors such as hunger, infections, and space restriction. It should be noted that rodents were kept in cages for as long as 12 weeks in this study. The increase in oxidative stress levels may have occurred due to this restriction.

Conclusion

Our results indicated that the amount of sulfur in dried apricots does not significantly affect oxidative stress and DNA damage in the liver. However, further studies are needed to investigate the specific effects of sulfur amounts on different organs.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

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Ethical approval

Ethics committee approval of this study was given by İnönü University Experimental Animal Ethics Committee (Date: 08.01.2016).

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