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The Effect of Turkish Coffee on Performance, Biochemical, and Physiological Parameters in Rabbits

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Abstract: Coffee is one of the most widely consumed hot beverages in the world. Melanoidins, which are formed with brown color during coffee roasting, are reported to be a strong antioxidant. Discussions related to the more and less consumption of coffee still continue. This study investigated the effect of a low and high amount of coffee consumption on antioxidant parameters in this context. A total of 15 female New Zealand rabbits weighing 1500-2500 g were used in the study. The control group was fed as *ad-libitum* control with basal feed. 1% and 5% Turkish coffee were added to the feed of the 1% TC and 5% TC groups, respectively. Blood was collected once a week throughout the study. All animals were euthanized in accordance with ethical rules at the end of the study. Samples (plasma and tissue) were taken for the analyses. Glutathione (GSH) and malondialdehyde (MDA) analyses were performed on all samples. Plasma GSH values were observed to increase in the groups given TC according to the analyses. Plasma MDA values decreased in the TC groups. An increase was observed in liver and heart tissue in the TC group upon the evaluation of tissue GSH parameters. MDA values were found to decrease in lung, heart, and kidney tissues in TC groups. Additional effects were observed in plasma and tissues in the 5% TC group upon the evaluation of 1% TC and 5% TC groups. No statistical differences were found in performance parameters. It has been revealed as a result that TC is a strong antioxidant, and its effect increases in high consumption.
Keywords: *GSH, MDA, Turkish coffee.*

Türk Kahvesinin Tavşanlarda Performans, Biyokimyasal ve Fizyolojik Parametreler Üzerine Etkisi

Özet: Kahve dünyada en yoğun tüketilen sıcak içeceklerdendir. Kahve kavrulması sırasında başlayan kahverengi rengi ile meydana gelen melanoidin güçlü antioksidan olarak bildirilmektedir. Kahvenin az ve çok kullanımı ile ilgili tartışmalar devam etmektedir. Bu bağlamda bu çalışmada az ve yoğun alınan kahvenin antioksidan parametreler üzerine etkisini araştırıldı. Çalışma kapsamında 1500-2500 g ağırlığında toplamda 15 adet dişi Yeni Zelanda tavşanı kullanıldı. Kontrol grubuna normal yem ile *ad-libitum* olarak beslendi. %1 TK ve %5 TK grubunda ise rasyonlarına sırasıyla %1 ve %5 Türk kahvesi ilave edildi. Çalışma boyunca haftada bir kan alındı. Çalışma sonunda etik kurallara uygun bir şekilde tüm hayvanların yaşamına son verildi. Analizler için gerekli numuneler (Plazma ve Doku) alındı. Alınan tüm numunelerden glutatyon (GSH) ve malondialdehit (MDA) analizi yapıldı. Yapılan analizlere göre plazma GSH değerleri TK verilen gruplarda artış gözlendi. Plazma MDA değerlerinde ise TK gruplarında azalma belirlendi. Doku GSH parametreleri değerlendirildiğinde ise karaciğer ve kalp dokusunda TK grubunda artış gözlendi. Doku MDA değerlerinde ise akciğer, kalp ve böbrek dokusunda TK gruplarında azalma belirlendi. %1 TK ve %5 TK değerlendirildiğinde plazma ve dokularda %5 TK'de daha fazla etki gözlendi. Performans parametrelerinde ise istatistiksel bir fark belirlenmedi. Sonuç olarak, TK'nın güçlü bir antioksidan olduğu ve yoğun kullanımda etkisinin arttığı ortaya çıkmaktadır.
Anahtar Kelimeler: *GSH, MDA, Türk Kahvesi.*

Introduction

Coffee and tea are among the most widely consumed beverages. However, studies on tea are more common than coffee in *in vivo* and *in vitro* studies. Many heat treatment procedures are applied to coffee from the beginning of production. Melanoidins, which are brown polymers formed by Maillard reaction during roasting of coffee, the beginning of these processes, constitute 25% of the dry substances of coffee beverages. Likewise, melanoidins were to increase in heat treatment during brewing (Borrelli et al., 2002). Melanoidins formed in the last stage of the Maillard reaction were to have certain functional properties such as antioxidant, antimicrobial, and antihypertensive activities (Rufian-Henares and Morales, 2007). In particular, the mechanism of antioxidant action relies on their ability to capture positively charged electrophilic species, purify oxygen radicals, or perform metal chelation to form inactive complexes

(Delgado-Andrade et al., 2005; Pastoriza and Rufián-Henares, 2014).

Antioxidants are important compounds that help us stay healthy by reducing oxidative stress caused by the overproduction of reactive oxygen (ROS) or nitrogen species (RNS). The terms "ROS" and "RNS" refer to various free radicals such as superoxide, hydroxyl, peroxyl, nitric oxide, nitrogen dioxide radicals, and non-radical reagents in general such as hydrogen peroxide (H_2O_2) and peroxynitrite. These free radicals are produced in our organism under normal physiological conditions but exacerbate under pathological conditions and play an important role in pathological processes and regulatory activities. Antioxidants can scavenge free radicals in the organism, inhibit pro-oxidative enzymes, and chelate metal ions (Martinez-Gomez et al., 2020). Melanoidins were reported to provide protection against DNA damage caused by ROS (Langner and Rzeski, 2014).

Measuring levels of DNA/RNA damage, lipid peroxidation, and protein oxidation/nitration instead of direct measurement of reactive oxygen species indirectly reveals oxidative stress. A common method of determining the degree of lipid peroxidation is to measure the level of malondialdehyde (MDA), a by-product of the lipid peroxidation process. In addition, the measurement of changes in reduced glutathione (GSH) concentration, an endogenous antioxidant, was also used as an indicator of the severity of lipid peroxidation (Smith et al., 2005).

The antioxidant effect on some tissues and plasma was determined by adding coffee to the feed of New Zealand rabbits in our study.

Material and Methods

Animals and Trial Design: The study was started after the approval of the Local Ethics Committee for Animal Experiments of Kafkas University (KAÜ-HADYEK/2020-006). The rabbits were supplied by the Atatürk University Experimental Animal Breeding Unit. A total of 15 female New Zealand rabbits weighing 1500-2500 g were used in the study. The rabbits were divided into 3 groups with 5 animals in each group. Animals were individually housed in cages specially designed for their species and fed as *ad-libitum* with basal feed (%16 CP and 2500 kcal/kg ME). The temperature of the room where the animals were housed was set to 25°C and the illumination was set to 12 hours of light and 12 hours of darkness. Body weight gain was determined by weighing the initial and final body weights of the rabbits. Feed consumption was recorded in all groups. The feed conversion rate was calculated by dividing the feed consumption by the body weight gain.

Experimental groups are as follows;

Group I (Control): The control group fed as *ad-libitum* control with basal feed without any application,

Group II (1% Turkish Coffee): The group in which 1% Turkish Coffee (TC) was added to their feed for 21 days,

Group III (5% Turkish Coffee): The group in which 5% Turkish Coffee (TC) was added to their feed for 21 days.

Sampling and Analysis: Blood was collected from the ear veins of the animals once a week into anticoagulant tubes for 3 weeks. Tissue samples required for analysis were taken after all animals were euthanized under anesthesia on the 21st day in accordance with ethical rules at the end of the study. The tissue samples were homogenized with phosphate buffer (pH 7.4) and centrifuged at 3000 RPM for 5 minutes and their homogenates were separated. Blood samples were centrifuged at 3000 RPM for 5 minutes and their plasma was separated. Tissue homogenates and plasmas were stored at -20°C until the day of the analysis. GSH analysis was determined according to the method of Beutler et al. (1963) whereas the method of Yoshioka et al. (1979) was used in MDA analysis.

Statistical Analysis: One-way analysis of variance (ANOVA) method was used to determine the differences between the initial body weights, final body weights, body weight gain, feed consumption, feed conversion ratio, and MDA and GSH values between the groups. GLM Repeated Measures test was used to analyze the measurement results of MDA and GSH parameters of the groups on the 0th, 7th, 14th, and 21st days of the study (Graphpad Prism 8/San Diego, CA). The results were interpreted using Tukey's HSD test. The results were given as mean±standard deviation (SD).

Results

Plasma GSH values of the control (13.34±0.43, 13.68±0.38, 13.56±0.80, 13.60±0.56, respectively), 1% TC (13.61±0.29, 14.23±0.20, 14.57±0.54, 14.98±0.43, respectively), and 5% TC (13.49±0.80, 14.53±0.31, 14.67±0.55, 15.79±0.25, respectively) groups on the 0th, 7th, 14th, and 21st days are presented in Figure-1A. A significant increase was found on the 21st day in the 1% TC ($p<0.01$) and 5% TC ($p<0.0001$) groups compared to the control group upon the evaluation of the plasma GSH parameter. A significant increase was found on the 21st day ($p<0.05$) in the 1% TC group compared to the 0th day. A significant increase was found on the

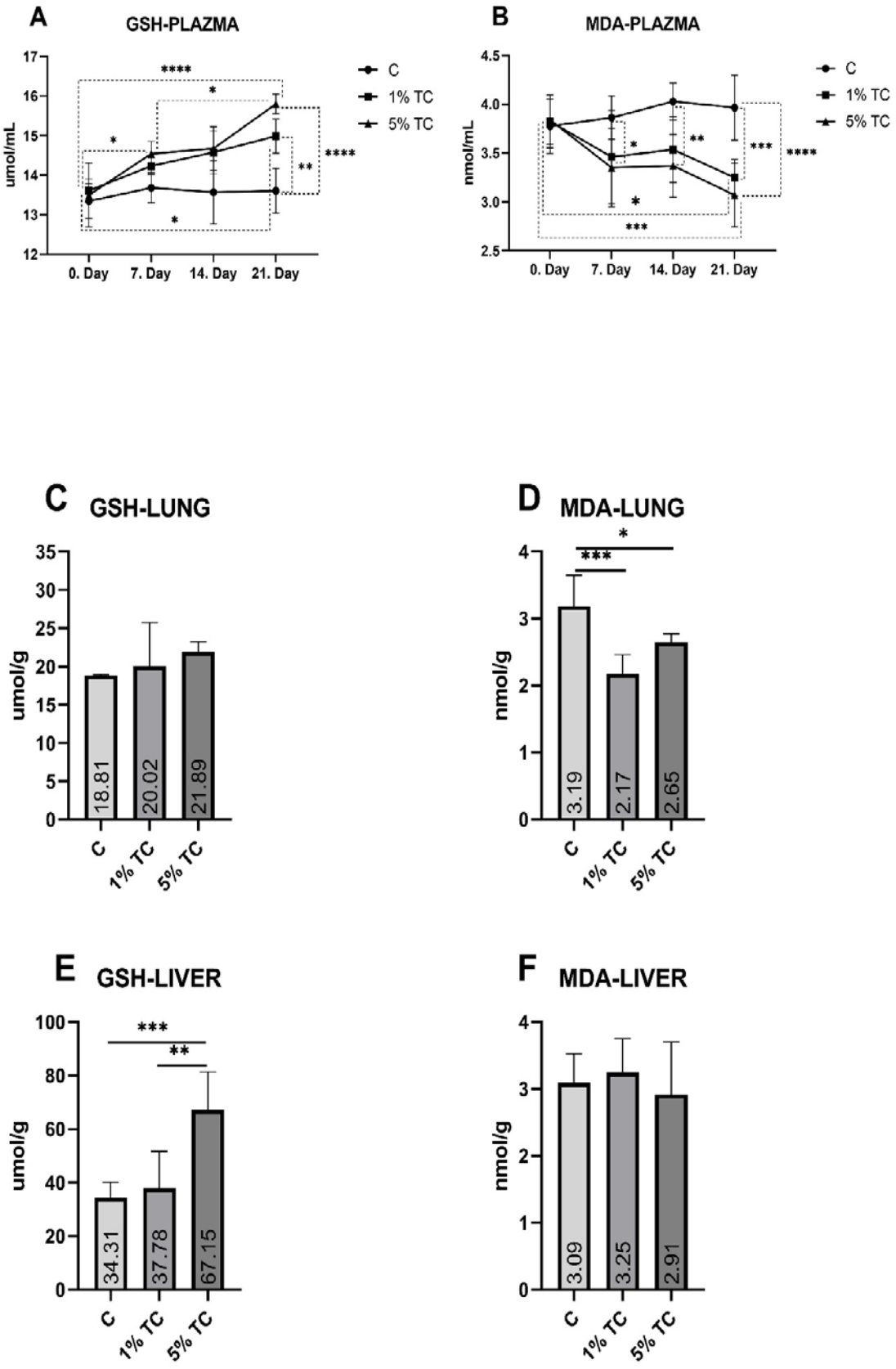

Figure 1. Means and Std. deviation of the three groups for biochemical parameters. *p<0,05, **p<0,01, ***p<0,001, ****p<0,0001.

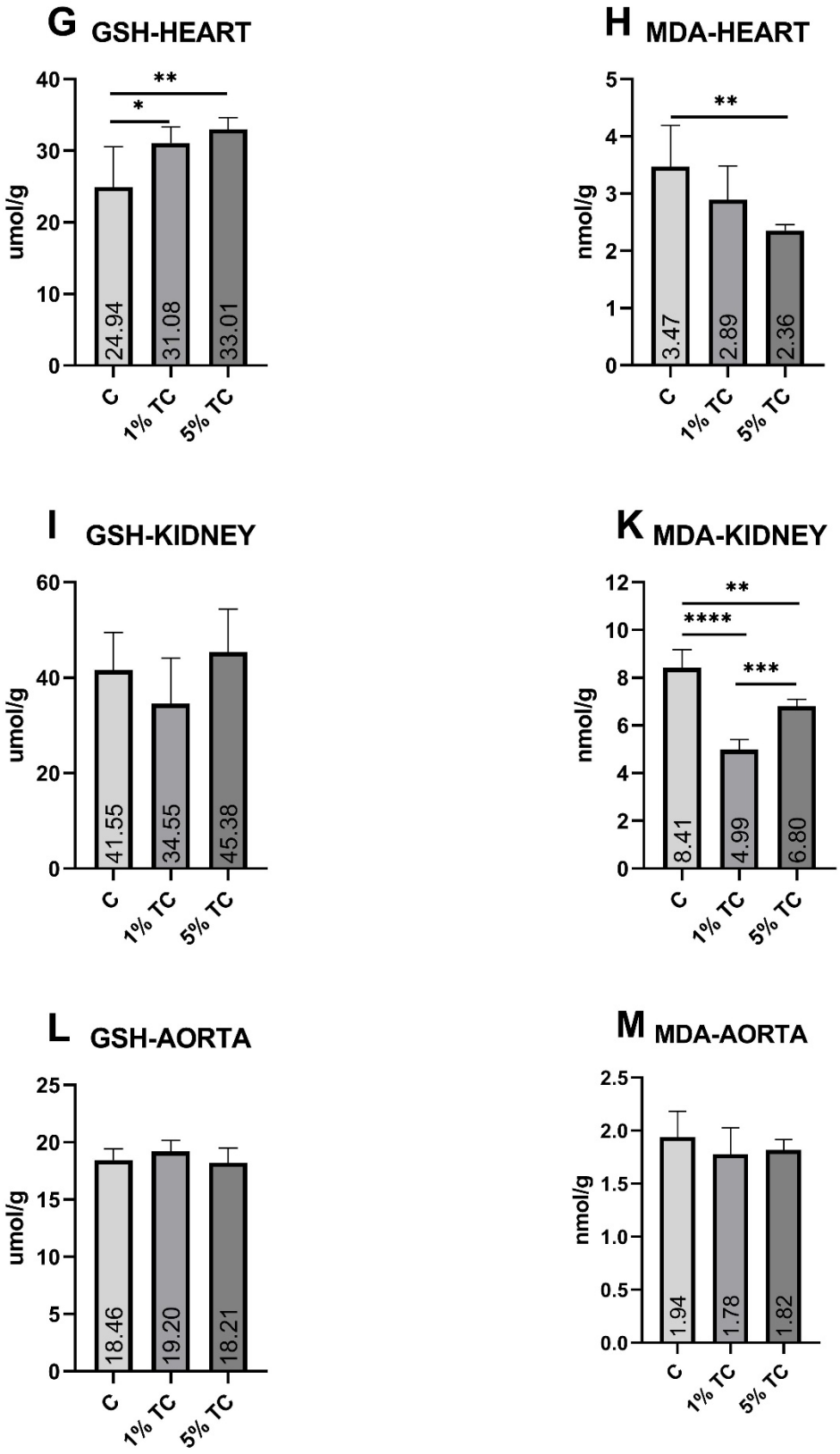

Figure 2. Means and Std. deviation of the three groups for biochemical parameters. *p<0,05, **p<0,01, ***p<0,001, ****p<0,0001.

7th day (p<0.05) and the 21st day (p<0.0001) in the 5% TC group compared to the 0th day. A statistical increase was also observed on the 21st day (15.79±0.25) compared to the 7th day (p<0.05).

Plasma MDA values of the control (3.77±0.28, 3.86±0.22, 4.02±0.19, 3.96±0.33, respectively), 1% TC (3.82±0.27, 3.46±0.47, 3.53±0.34, 3.24±0.18, respectively), and 5% TC (3.84±0.24, 3.35±0.40, 3.37±0.32, 3.07±0.32, respectively) groups on the 0th, 7th, 14th, and 21st days are presented in Figure-1B. A statistically significant decrease was found on the 7th, 14th, and 21st days (p<0.05, p <0.01, p<0.0001, respectively) in the 5% TC group and on the 21st day (p<0.001) in the 1% TC group compared to 7th, 14th, and 21st days upon the evaluation of plasma MDA values. A significant decrease was observed on the 21st day in the 1% TC (p<0.05) and 5% TC (p<0.001) groups compared to the 0th day in the change of the groups by days.

GSH values in tissues are presented in Figure 1-C, 1-E, and Figure 2-G, 2-I, 2-L. A statistically significant increase was observed in both 1% TC (31.08±2.28, p<0.05) and 5% TC (33.01±1.62, p<0.01) groups compared to the control group (24.94±5.61) in heart tissue. A significant increase was observed in the 5% TC (67.15±14.24) group compared to the control (34.31±5.86) group (p<0.001) in the liver tissue. A significant difference was also found between the 1% TC (37.78±13.96) group and the 5% TC (67.15±14.24) group in liver tissue (p<0.01). No statistical difference was found in kidney, lung, and aortic tissue (p>0.05).

MDA values in tissues are presented in Figures 1-D, 1-F, and Figures 2-H, 2-K, 2-M. A statistically significant decrease was found in lung tissue in the 1% TC (2.17±0.28, p<0.001) and 5% TC (2.64±0.12, p<0.05) groups compared to the control (3.18±0.45) group upon the examination of tissue MDA data. Similarly, a significant decrease was observed in 1% TC (4.98±0.41, p<0.0001) and 5% (6.80±0.29, p<0.01) TC groups compared to the control group (8.41±0.76) in kidney tissue. In addition, a significant difference was found between the 1% TC (4.98±0.41) and 5% TC (6.80±0.29) groups in kidney tissue (p<0.001). A statistical decrease was observed only in the 5% TC (2.35±0.10) group compared to the control (3.47±0.72) group (p<0.01) in the heart tissue. No statistical differences were found in kidney and aortic tissues (p>0.05).

The initial body weights, final body weights, body weight gain, feed consumption, and feed conversion rate of the animals are presented in Table 1. There was no statistical difference according to the results (p>0.05).

Table 1: Effect of TK on performance parameters of rabbits.

	Control	1% TC	5% TC	P
Initial Body Weight	2105.00±52.29	2091.00±43.17	2066.00±55.88	0.861
Final Body Weight	2248.00±66.72	2265.00±35.78	2218.00±69.17	0.852
Body Weight Gain (0-21 days)	142.20±22.19	174.50±21.39	151.70±35.18	0.691
Feed Consumption (0-21 days)	24.89±0.88	26.67±1.17	25.40±0.63	0.414
Feed Conversion Rate (0-21 days)	3.96±0.15	3.99±0.11	3.89±0.13	0.256

Discussion and Conclusion

Organisms try to detoxify these molecules, and oxidative stress arises because of the imbalance between the ability to detoxify these free radical products produced as a result of free radical production. Thus, it causes cellular damage. Free radicals are generally known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are reactive substances because they have one or more separating electrons (Sepidarkish et al., 2020). On the other hand, antioxidants are low or high molecular weight substances that try to prevent or repair cellular damage by destroying the harmful properties of free radicals (Poprac et al., 2017; Valko et al., 2007).

Free radicals are reported to be at low or moderate concentrations within cells and play a vital role in the cellular defense system by invading pathogenic agents (Sepidarkish et al., 2020). The most commonly used test to measure the effects of hydroxyl radical damage in cells is the thiobarbituric acid test for the presence of MDA. Furthermore, measuring changes in reduced glutathione (GSH) concentration, an endogenous antioxidant, has also been reported to be more effective in determining the severity of lipid peroxidation (West, 2000).

Melanoidins, known as powerful antioxidants, are available in many heat-treated products, especially beer, coffee, and tea. They exhibit antioxidative action by eliminating ROS and RNS, which cause oxidative damage (Cammerer et al., 2012). Our study has determined the antioxidative

effect of coffee given to rabbits. It is thought that this effect may be caused by melanoidins in coffee.

An inverse relationship was reported between coffee consumption and cirrhosis (Muriel and Arauz, 2010). Liver damage was also reported to be inversely proportional to coffee consumption in Italy and Japan (Kono et al., 1994; Muriel and Arauz, 2010; Ruhl and Everhart, 2005). Lv et al. (2010) evaluated the effect of caffeine against alcohol-related liver diseases and determined MDA and GSH values. They observed a decrease in MDA level and an increase in GSH level in the caffeinated group in relation to liver damage according to their study. Vitaglione et al. (2010) reported the antioxidant effect of melanoidins taken with coffee consumption on the liver in their study. An increase in GSH level was found in the group which was given coffee compared to the control group, similar to our study. Studies have shown that coffee physiologically increases the antioxidant capacity in liver damage. Studies on coffee and caffeine similarly reported that antioxidant parameters increased and oxidant parameters decreased (Amer et al., 2017; Nogaim et al., 2020; Shaposhnikov et al., 2018).

Stress is the main cause of cardiovascular diseases. Ischemic heart disease and stroke, especially associated with atherosclerosis, have been the leading cause of morbidity or mortality worldwide for decades. Oxidative stress has been found to be associated with some risk factors for atherosclerosis such as hypertension, diabetes mellitus, hyperlipidemia, obesity, and smoking. In addition, increased ROS formation in the arterial wall through oxidase activation of nicotinamide adenine dinucleotide phosphate (NADPH) via Type 1 angiotensin II receptors causes increased oxidative stress (Cheng et al., 2017). Some researchers have reported that antioxidants in foods may inhibit the atherosclerosis process (Torres et al., 2015). Our study did not determine a statistical effect on the aorta related to coffee consumption whereas antioxidant GSH level in cardiac tissue increased and oxidant MDA level decreased. However, studies have reported that consumption of coffee prevents cardiovascular diseases, which supports our study (Kleemola et al., 2000; LaCroix et al., 1986; Wu et al., 2009). Another study reported that oxidative damage occurred by increasing the formation of ROS due to increased iron load in the liver (Güvendi et al., 2020). It is thought in such cases that coffee may have an antioxidant effect.

The exact mechanism by which coffee consumption reduces the risk of renal failure is not fully known. However, there are reports that various components of coffee (such as melanoidins, caffeine) can protect glomerular endothelial cells from oxidative stress (Wijarnpreecha et al., 2017). We found in our study that the levels of MDA in the kidney tissue decreased in the groups which were given coffee, reducing the oxidant effect.

The mechanism of the antioxidative activity of melanoidins is not fully understood. However, it is believed to be based on the elimination of oxygen radicals and chelation of metals to form inactive complexes. Antioxidant activity has been demonstrated in both cell and animal experiments. Studies have reported that digested coffee melanoidins protect human hepatocytes, HepG2, under oxidative stress. Coffee melanoidins in humans have been reported to increase plasma antioxidant capacity by up to 7% in a short time after consumption and protect human lymphocytes against ROS-induced DNA damage (Rufián-Henares and Pastoriza, 2016). Our study has determined an increase in plasma antioxidant level but a decrease in the oxidant level.

It is seen as a result that the antioxidant capacity in blood plasma and tissues is increased with an antioxidant such as caffeine and melanoidins taken with coffee consumption. The increase in antioxidant capacity with intensive consumption shows that it is directly proportional to the increase in coffee use when 1% TC and 5% TC in our groups are compared.

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