The effects of caffeine on the renal antioxidant activity in rats

Ebru Ofluoğlu Demir*, Canan Yılmaz Demirtaş, Özge Tuğçe Paşaoğlu

Ratlarla böbrek antioksidan aktivitesi üzerine kafeinin etkileri

Objectives: In our study, the short-term effects of caffeine on the renal antioxidant activity in rats were investigated.

Methods: Caffeine was given orally at two different doses: 30 mg/kg and 100 mg/kg (a high non-toxic dose). The current study included 30 rats, which were divided into 3 groups: a control group and two caffeine-treated groups. Group 1 was given caffeine at 30 mg/kg and Group 2 was given caffeine at 100 mg/kg for 14 days. We measured advanced oxidation protein products (AOPP), malondialdehyde (MDA) and nitric oxide (NO) levels in the kidney tissue following caffeine administration. In addition, we also evaluated superoxide dismutase (SOD), and glutathione S transferase (GST) activities in the kidney tissue.

Results: Our results showed that caffeine administration decreased lipid peroxidation and advanced oxidation protein products in kidney. Especially, MDA levels in the kidney tissue of the caffeine-treated groups decreased significantly as a result of the dose. NO levels in the kidney tissue of the caffeine-treated groups were higher than those in the control group. GST activities in the kidney tissue of rats in the caffeine groups also increased significantly. In our study, we did not observe significant changes in renal SOD activities upon caffeine consumption.

Conclusion: These results show that short-term consumption of two different doses of caffeine may protect against oxidative stress in the kidney tissue of rats. This effect is related to the caffeine dosage. Determining the mechanisms and antioxidant effects of caffeine at suitable dose requires advanced animal and human studies.

Keywords: Caffeine, antioxidant, malondialdehyde, nitric oxide, oxidation protein products, kidney

*Corresponding author: Ebru Ofluoğlu Demir: Ahmet Erdoğan Vocational School of Health Services, Bülent Ecevit University, Zonguldak, Turkey, e-mail: ebruofluoglu@yahoo.com
Canan Yılmaz Demirtaş: Department of Medical Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey, e-mail: mdcanandemirtas@yahoo.com.tr
Özge Tuğçe Paşaoğlu: Department of Medical Laboratory, College of Health Care Services, Gazi University, Ankara, Turkey, e-mail: ozge.tugce@gmail.com
mizda kafein tüketimi üzerine börek SOD aktivitelerinde önemli değişiklikler gözlenmemiştir.

Sonuç: Bu sonuçlar, iki farklı doz kafeinin kısa süreli tüketiminin, börek dokusunda oksidatif stresse karşı koruma bileğini göstermektedir. Bu etkiler kafeinin dozu ile ilişkilidir. Kafeinin uygun dozdada, etki mekanizmalarının ve antioksidan etkilerinin belirlenmesi, ileri hayvan ve insan çalışmalari gerektirmektedir.

Anahtar Kelimeler: Kafein, antioksidan, nitrik oksit, oksidasyon protein ürünlerı, malondialdehit, börek

Introduction

Caffeine, a purine alkaloid (1, 3, 7-trimethylxanthine), is present in several popular drinks, such as coffee, tea and energy drinks [1,2]. Caffeine and other methylxanthines are also used as components of analgesics, appetite suppressants, myorelaxants, and stimulant drug formulations [3,4]. The plasma half-life of caffeine in a healthy person ranges from 2.5 to 4.5 h. The half-life would be shorter in rats and mice [5]. There is also a strong belief that people who consume high amounts of caffeine tend to carry a higher risk of developing bone problems, including osteoporosis, and problems in metal absorption and in the excretion and reabsorption processes of the intestines and kidneys [3,6]. Some of the effects of caffeine may favor the production of free radicals and lead to a subsequent increase of lipid peroxidation by increasing oxidative stress [7,8]. Some articles, however, claim that caffeine protects against cellular damage by producing beneficial antioxidant effects [9,10].

The aim of our study was to evaluate the effects of two different doses of caffeine in short-term consumption on the renal antioxidant activity in rats. We hypothesized that there exists a relationship between caffeine intake and antioxidant activity in the kidney of rats, which suggests an alternative mechanism for caffeine’s effect on the renal antioxidant defense system.

For this purpose we have studied the influence of caffeine on the metabolism of antioxidant in the kidney by measuring glutathione S transferase (GST) and superoxide dismutase (SOD) activities and also malondialdehyde (MDA), nitric oxide (NO), and advanced oxidation protein products (AOPP) levels. These parameters are of particular importance for the maintenance of homeostasis especially oxidant/antioxidant status. MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Its level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients.

SOD, one of the most important antioxidative enzyme, catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. We also investigated the effect of caffeine on the enzymatic antioxidant systems by measuring SOD and GST activities in rat kidney tissue. GST takes on considerable importance as a mechanism for carcinogen detoxification and cellular protection. The biochemical protection mechanisms by GST involve both reduction of organic hydroperoxides, contributing to oxidative stress, and conjugation of electrophilic compounds with glutathione which facilitate their transportation from the cell.

NO is synthesized from L-arginine by nitric oxide synthase (NOS). It is a polyfunctional signalling molecule controlling the processes of vasodilatation, platelet aggregation, adhesion of leukocytes to the endothelium, and other processes. Diseases such as vascular dysfunctions are associated with the impaired production of NO, however, septic shock, profound vasodilatation, cardio-depression, and bacterial destruction are associated with NO overproduction [11]. AOPP were also suggested as one of the possible markers of oxidative damage, which originates under oxidative and carbonyl stress and increase inflammatory activity.

It has generally shown the effects of caffeine by creating pathological conditions such as hypertension, metabolic syndrome, diabetes or etc. and considering caffeine as a drug in both human and rat studies up to this time. In addition, there are few studies concerning the antioxidant activity of tissues by only applying caffeine on healthy rats except for our studies in the literature [12,13]. Therefore, we aimed to show potential antioxidant effects of caffeine on kidney tissue with some important oxidant/antioxidant parameters as mentioned above without creating any pathology and investigating kidney functions by considering caffeine as a nutrient which is consumed slightly by all of us in our daily lives instead of considering it as a drug in two other studies previously planned and studied by us. For this purpose we have planned to study in molecular level in the kidney.

Materials and Methods

This study was planned simultaneously with our other two studies and all tissues were examined in the same time period. (Paşaoğlu H et.al, Turk J Med Sci 2011; 41(4):665–71. and Demirtaş C et al. Gazi Med J 2012; 23:13–8.) The
experimental protocols were conducted with the approval of the Animal Research Committee at Gazi University, Ankara, Turkey (Code No: G.U.E.T-07031).

All chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, USA) and were of analytical grade or the highest grade available. Caffeine (Fluka) was purchased and dissolved in distilled water at concentrations of 30 mg/kg and 100 mg/kg body weight. Freshly prepared caffeine solutions were administered orally at a volume of 1 ml/day for 14 days. Thirty male Wistar rats weighing about 250 g were used in the experiment. The animals were randomly divided into three groups, each consisting of 10 members. The initial mean body weight of each group was similar.

The first group served as the control. Animals in this group were given drinking water without caffeine. The second group of animals (group I) was given a caffeine solution with a dose of 30 mg/kg body weight. The third group (group II) was given a dose of 100 mg/kg body weight. After 14 days, the rats were sacrificed, and the kidneys were quickly removed and frozen.

The kidney tissues were stored at -80°C until the processing stage. The frozen tissues were separately weighed and homogenised in 10 volumes of cold 0.01 M Tris–HCl buffer (pH 7.4) by means of an automatic homogeniser. Then, the homogenates were centrifuged at 25000 g for 15 min at 4°C. Clear upper supernatants were used for GST activity, NO, MDA, and AOPP assays [14]. The tissue protein levels were also measured at this step according to the protocol described by Lowry et al. [15] using bovine serum albumin (BSA) as standard.

Assay for nitric oxide (NO) concentrations

NO assays were measured using the colorimetric assay of Cayman’s nitrate/nitrite Chemical kit (Cayman Chemical Company, Item No.780001, USA). NO levels obtained by this procedure represent the sum of nitrite and nitrate. All sample nitrate is converted to nitrite, by the enzyme nitrate reductase, followed by the colorimetric detection of nitrite as a azo compound of the Griess reaction that absorb visible light at 540 nm. Concentrations were calculated by comparison to nitrite standards. The results of the NO assays were expressed as μmol/g tissue.

Assay for malondialdehyde (MDA) concentrations

MDA assays were performed as described by Ohkawa et al. [16]. MDA, a product of lipid peroxidation, reacts with thiobarbituric acid (TBA) under acidic conditions at 95°C to form a pink-colored complex with an absorbance maximum at 532 nm. The results were expressed as nmol/g tissue.

Assay for superoxide dismutase (SOD) activity

The total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity was assayed according to the method of Sun et al. [17]. The principle of the method is based on the inhibition of nitroblue-tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The results were expressed in relation to the protein content of the samples as Units/mg protein.

Assay for glutathione S transferase (GST) activity

GST (EC 2.5.1.18) activity was assayed according to the method of Habig et al. [18]. This assay uses 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, which conjugates with the thiol group of the glutathione (GSH), causing an increase in absorbance. The enzyme activity was determined spectrophotometrically at 340 nm by measuring the formation of the conjugate of GSH and CDNB. The increase in absorbance per minute was estimated and the reaction rate at 340 nm was determined using the CDNB extinction coefficient (ε) of 9.6 mM⁻¹cm⁻¹. The results were expressed in relation to the protein content of the samples as nmol/min/mg protein.

Assay for advanced oxidation protein products (AOPP) concentrations

The products of the oxidative modification of proteins are known as AOPP. The tissue AOPP assays were performed as described by Uzun et al. [19]. AOPP concentrations were expressed in μmol/L of chloramine-T equivalents with an absorbance maximum at 340 nm. The results were calculated and expressed as μmol/mg protein.
Statistical analysis

Statistical analysis of the results was conducted using SPSS 20 software. Comparisons between the groups were made using Mann-Whitney U test with Bonferroni Correction test. Probability values less than 0.0167 were accepted as significant. The correlation between groups assay of values in the tissues was evaluated using Spearman's correlation test.

Results

The results are given in Table 1. Renal GST activities increased significantly with caffeine administration (p<0.0167, Table 1), but there was no statistically significant difference between the GST activities of the caffeine groups (p>0.0167, Table 1).

SOD activities were also investigated in rat tissue samples. There was no statistically significant difference in the SOD activities between the two caffeine groups and control (p>0.0167, Table 1).

Renal MDA concentrations, a measure of the level of lipid peroxidation, decreased in the caffeine-treated rats with respect to rats in control, and there was a statistically significant difference between the MDA levels of the caffeine groups (p<0.0167, Table 1).

AOPP levels in kidney tissue from rats in the 100 mg/kg caffeine-treated also decreased significantly (p<0.0167, Table 1), but there was no statistically significant difference between the AOPP levels of the caffeine groups (p>0.0167, Table 1).

Renal NO levels increased in the group of animals treated with 100 mg/kg caffeine with respect to control (p<0.0167, Table 1) but there was no statistically significant difference between the NO levels of the two caffeine groups (p>0.0167, Table 1).

Results from the Spearman correlation analysis showed strong negative correlation between GST activities and MDA levels, and also between NO and MDA levels. On the other hand, it showed a positive correlation between MDA levels and AOPP levels, and also between NO levels and GST activities.

Statistically significant correlations were found between:

1. GST activities and MDA levels in kidney tissue (p=0.003, r=-0.526)
2. MDA and NO levels in kidney tissue (p=0.039, r=-0.379)
3. GST activities and NO levels in kidney tissue (p=0.038, r=0.380)
4. MDA and AOPP levels in kidney tissue (p=0.03, r=0.397)

Discussion

Caffeine, a purine alkaloid (1,3,7-trimethylxanthine), is a stimulant of the central nervous system. Ingestion of moderate doses of caffeine, which is 50–300 mg for a person...
of 60–70 kg and is equivalent to 1–3 cups of coffee, usually reduces drowsiness and fatigue and results in a more rapid and clearer flow of thought, as well as feelings of well-being and alertness. On the contrary, when ingested in higher doses (300–800 mg), caffeine induces nervousness, insomnia, and panic attacks. Moreover, caffeine produces tonic-clonic seizures in both rats and mice when given in excessive doses [5,20,21].

It has been reported that caffeine acts on various tissues, in particular the central nervous system and skeletal and cardiac muscle. The mechanism by which caffeine improves performance is not clear, but several possibilities have been proposed, such as antagonizing adenosine receptors and increasing catecholamine release [22,23].

Previous studies indicated that chronic caffeine consumption has adverse renal effects in nephropathy associated with high blood pressure and insulin resistance in obese, diabetic ZSF1 rats. The studies reported that caffeine improves insulin sensitivity but increases plasma cholesterol levels and impairs renal function in obesity with the metabolic syndrome and hypertension. Their results implied that the health consequences of chronic caffeine consumption may depend heavily on underlying pathophysiology process. [24,25].

As in the above studies and as mentioned in the information section, it has generally shown the effects of caffeine by creating pathological conditions and considering caffeine as a drug in the studies. We aimed to show potential antioxidant effects of caffeine on healthy kidney tissue with some important oxidant and antioxidant parameters without creating any pathology and investigating kidney functions by considering caffeine as a natural dietary supplement which is consumed slightly by all of us in our daily lives. Because there are few studies concerning this topic [12,13], we were not able to compare with the same sort of study. Therefore we could compare rats formed in pathological or etc. conditions with healthy rats in this study.

In our study, we have demonstrated that 30 mg caffeine dosage didn’t have any effects on MDA levels, on the other hand 100 mg caffeine dosage decreased MDA levels, and also there was a statistically significant difference between the MDA levels of these two caffeine groups. These results suggest that the dose of 100 mg caffeine is much more effective than 30 mg caffeine dosage.

In some previous studies investigating the relationship between caffeine and lipid peroxidation, it was shown that MDA levels were reduced considerably through caffeine treatment, whereas some studies found an increase in the levels of MDA. For example, Al Moutaery et al. [26] showed that among the rats that had experimental head trauma, the administration of a high dose of caffeine (100–150 mg/kg) intraperitoneally increased lipid peroxidation in the cortex and caused oxidative stress. Another example, Karas et al. [27] stated that the administration of caffeine (150 mg/kg, orally) increased MDA levels in the livers of rats that had been treated with allyl alcohol to reduce the hepatotoxicity of the high dose of caffeine. On the other hand, Yukawa et al. [28] demonstrated that consuming 150 ml of coffee (3 times a day) for 7 days reduced total serum cholesterol and MDA levels. Mukhopadhyay et al. [29] stated that caffeine administration to rats (20 mg/kg, 30 days) caused augmentation of the activities of hepatic catalase (CAT) and SOD and reduction of MDA levels.

After reviewing these studies, we chose two different doses of caffeine (30 and 100 mg/kg) to be administered for 14 days in order to investigate possible alternative results. Our results showed that short-term caffeine consumption decreases MDA levels in the renal tissues of 100 mg caffeine dosage used in this study. According to this result, the level of MDA is especially sensitive to dosage and also 100 mg caffeine dosage could be used as an antioxidant in decreasing MDA levels.

In our research, the NO levels in renal tissue from rats in the 100 mg/kg dose caffeine group increased significantly when compared with the control group. This result means that 30 mg caffeine dosage didn’t have any effects on NO levels, on the other hand 100 mg caffeine dosage increased NO levels, and this increase was sensitive to dosage. Previous research into the relationship between caffeine intake and NO levels has indicated that NO increases significantly after intake of caffeine [30–32]. Lee et al. [30] stated that adding caffeine (40 mg/kg, 1 day) to the drinking water given to rats reduced α and β Na+/K+-ATPase (sodium-potassium pump) activities and increased NOx (nitrite + nitrate) levels by raising the endothelial NOS expression in the kidneys. Another example, Hashiguchi et al. [31] investigated the effect of caffeine on NO production in the brain. In their research, they found that NO levels rose in the brain when caffeine was added to the rat’s drinking water (0.6 mg/ml, 1–3 weeks). Alasehirli et al. [32] also found that NO production increased when increasing doses of intraperitoneally-injected caffeine (25–50–100 mg/kg, 21 days) were given to pregnant rats. As mentioned above in studies, the results has shown that the effects of caffeine on NO levels may be explained by dose of caffeine, orally or injectable administration, especially duration of treatment, tissue type, conditions or etc. Our findings also serve as evidence for possible antioxidant effects of 100 mg caffeine on NO levels.

In some previous studies investigating the relationship between caffeine and antioxidant enzymes activities, it was shown that some antioxidant enzyme activities were...
increased considerably through caffeine treatment. We [12] demonstrated previously that short-term consumption of two different doses of caffeine (30 mg/kg and 100 mg/kg, orally, 14 days) may protect against oxidative stress in the liver tissue of rats. We indicated that hepatic SOD, CAT, glutathione peroxidase (GPx), and also GST activities increased significantly with caffeine administration. On the other hand, Rossowska and Nakamoto [33] demonstrated that there was no statistically significant difference in the hepatic and cardiac SOD, GPx or CAT activities between the two caffeine groups (20 mg/kg caffeine for 22 and 30 days) and control group. Abd el-Rahman and Sherif [35] stated that caffeine (80 ml/kg, 21 days, intraperitoneal injection) and aspirin administrations to rats resulted in significant improvement in renal and hepatic functions associated with reduction in oxidative stress caused by gamma irradiation. In their research, they reported that MDA levels decreased, SOD and CAT activities increased after caffeine administration (one hour prior to irradiation) in both tissues of the irradiated rats. Birkner et al. [36] reported that adding caffeine (3 mg/kg, 50 days) and sodium fluoride (NaF) to the drinking water given to rats reduced catalase activities and increased GPx activities in the kidneys. In their research, they also showed that renal SOD activities were not affected by caffeine.

The results of our study demonstrated compliance with the results of the above studies. In the current study, the kidney tissue GST activities increased significantly after caffeine administration, but there was no statistically significant difference between the GST activities in the kidney tissue of the two caffeine groups. This means in two doses of caffeine (either 30 mg or 100 mg) showed the same effect on GST activities. On the other hand, we found no statistically significant difference between the SOD activities of rats in the control group and rats in the group which was given caffeine.

This result has shown that any dosage of caffeine didn’t have positive or negative antioxidant effects on SOD levels. Contrary to this result, two dosage of caffeine had antioxidant effects on GST levels.

All of these effects of caffeine may be explained by dose of caffeine, orally or injectable administration or etc. Our results may be indicative of the negative effect of caffeine on radical production and its positive effect on antioxidant enzyme activities.

In the literature, there is only one study investigating the association between caffeine intake and AOPP levels [13]. Paşaoğlu et al. [13] investigated the effect of caffeine on oxidative stress in liver and heart tissues of rats. We previously showed in this study that MDA and AOPP levels in the liver, and also in the heart were reduced considerably after treatment with caffeine. According to our research, these markers of oxidative damage in the liver were affected by caffeine dosage.

In current study, we also measured AOPP levels in the kidney tissues. AOPP levels were statistically reduced in the 100 mg/kg dose caffeine-treated rats compared to rats in control group. This result means that 30 mg caffeine dosage didn’t have any effects on AOPP levels, on the other hand 100 mg caffeine dosage decreased AOPP levels.

According to the results of the Spearman correlation analysis, the kidney tissue MDA levels decreased together with kidney tissue AOPP levels. Consequently, the kidney tissue GST activities and NO levels increased. The results also showed a strong negative correlation between GST activities and MDA levels, and also between NO and MDA levels.

These results serve as evidence for possible antioxidant effects of 100 mg caffeine dosage as shown by decreased oxidation protein products (AOPP) and also lipid peroxidation (MDA).

In this situation and conclusion, It should be noted that, as in the case of many other compounds and supplements, the effects of caffeine largely depend on the dose. Caffeine treatment may cause either favorable or disadvantageous biochemical changes, as well as even resulting in dysfunctions of specific tissues and metabolic disorders depending on its dose. The caffeine doses applied in our study were similar to those applied to human successfully and safely. It can show the antioxidant effect of caffeine especially at 100 mg dosage applied in this study. Caffeine could be taken into consideration as a natural dietary supplement. We applied this study in short term period (14 days), it could be investigated whether there is an antioxidant effect of caffeine at 100 mg dosage in long-term period for further studies.

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

References

[1] MacDonald J. The potential adverse health effects of energy drinks. Am Fam Physician 2013; 87(5):321.
[2] An SM, Park JS, Kim SH. Effect of energy drink dose on exercise capacity, heart rate recovery and heart rate variability after high-intensity exercise. J Exerc Nutrition Biochem 2014; 18(1):31–9.
[3] Kolayli S, Ocak M, Kucuk M, Abbasoglu R. Does caffeine bind to metal ions? Food Chem 2004; 84(3):383–8.
[4] Son HY, Nishikawa A, Kanki K, Okazaki K, Kitamura Y, Lee KY et al. Synergistic interaction between excess caffeine and...
deficient iodine on the promotion of thyroid carcinogenesis in rats pretreated with N-bis(2-hydroxypropyl)nitrosamine. Cancer Sci 2003; 94(4):334–7.

[5] Tanda G, Goldberg SR. Alteration of the behavioral effects of nicotine by chronic caffeine exposure. Pharmacol Biochem Behav 2000; 66(1):47–64.

[6] Heckman MA, Weil J, Gonzalez de Mejia E. Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. J Food Sci 2010; 75(3):77–87.

[7] Vistisen K, Poulsen HE, Loft S. Foreign compound metabolism capacity in man measured from metabolites of dietary caffeine. Carcinogenesis 1992; 13(9):1561–8.

[8] Dianzani MU, Muzio G, Brocca ME, Canuto RA. Lipid peroxidation in fatty liver induced by caffeine in rats. Int J Tissue React 1991; 13(2):79–85.

[9] Krisko A, Kveder M, Pifat G. Effect of caffeine on oxidation susceptibility of human plasma low density lipoproteins. Clin Chim Acta 2005; 355(2-3):47–53.

[10] Demirtaş C, Ofluoğlu E, Hussein A, Paşaoğlu H. Effects of Caffeine on Oxidant-Antioxidant Mechanisms in the Rat Liver. Gazi Med J 2012; 23(1-2):125–8.

[11] De Cruz SJ, Kenyon NJ, Sandrock CE. Bench-to-bedside review: the role of nitric oxide in sepsis. Expert Rev Respir Med 2009; 3(5):511–21.

[12] Paşaoğlu H, Ofluoğlu E, Demirtaş C, Hussein A, Paşaoğlu OT. The effect of caffeine on oxidative stress in liver and heart tissues of rats. Turk J Med Sci 2011; 41(4):665–71

[13] Ilhan A, Akyol O, Gurel A, Armutcu F, Iraz M, Oztas E. Protective effects of caffeic acid phenethyl ester against experimental allergic encephalomyelitis-induced oxidative stress in rats. Free Radic Biol Med 2004; 37(3):386–94.

[14] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193(1):265–75.

[15] Concas A, Porcu P, Sogliano C, Serra M, Purdy RH, Biggio G. Caffeine-induced increases in the brain and plasma concentrations of neuroactive steroids in the rat. Pharmacol Biochem Behav 2000; 66(1):39–45.

[16] Strong R, Grotta JC, Aronowski J. Combination of low dose ethanol and caffeine protects brain from damage produced by focal ischemia in rats. Neuropharmacology 2000; 39(3):515–22.

[17] Davis JM, Zhao Z, Stock HS, Mehl KA, Buggy J, Hand GA. Central nervous system effects of caffeine and adenosine on fatigue. Am J Physiol Regul Integr Comp Physiol 2003; 284(2):399–404.

[18] Greer F, Friars D, Graham TE. Comparison of caffeine and theophylline ingestion: exercise metabolism and endurance. J Appl Physiol (1985) 2000; 89(5):1837–44.

[19] Yokota S, Nakajima M, Inoue K, Utsunomiya Y, et al. Effects of caffeine on antioxidant enzymes of Ehrlich ascites tumor-bearing mice. Indian J Exp Biol 2003; 41(4):283–9.

[20] Yue X, Mune M, Liang XM, Iwahashi H, et al. Effects of coffee consumption on oxidative susceptibility of low-density lipoproteins and serum lipid levels in humans. Biochemistry (Mosc) 2004; 69(3):70–4.

[21] Mukhopadhyay S, Mondal A, Poddar MK. Chronic administration of caffeine: effect on the activities of hepatic antioxidant enzymes of Ehrlich ascites tumor-bearing mice. Indian J Exp Biol 2003; 41(4):283–9.

[22] Lee J, Ha JH, Kim S, Oh Y, Kim SW. Caffeine decreases the expression of Na+/K+–ATPase and the type 3 Na+/H+ exchanger in rat kidney. Clin Exp Pharmacol Physiol 2002; 29(7):559–63.

[23] Hashiguchi W, Nagatomo I, Akasaki Y, Uchida M, Tominaga M, Takigawa M. Influences of caffeine on nitric oxide production and zonisamide concentration in the brain of seizure-susceptible EL mice. Psychiatry Clin Neurosci 2001; 55(4):319–24.

[24] Alasehirli B, Cekmen M, Nacak M, Balat A. Effects of caffeine on placental total nitrate concentration: A 21-day, vehicle-controlled study in rats. Curr Ther Res Clin Exp 2005; 66(2):130–7.

[25] Rosowska MJ, Nakamoto T. Effects of chronic caffeine feeding and zonisamide concentration in the brain of seizure-susceptible EL mice. Psychiatry Clin Neurosci 2001; 55(4):319–24.

[26] Al Moutaery K, Al Deeb S, Ahmad Khan H, Tariq M. Caffeine impairs short-term neurological outcome after concussive head injury in rats. Neurosurgery 2003; 53(3):704–12.

[27] Karas M, Chakrabarti SK. Influence of caffeine on allergy-induced hepatotoxicity in rats. In vivo study. J Environ Pathol Toxicol Oncol 2001; 20(2):141–54.

[28] Tofovic SP, Kost CK Jr, Jackson EK, Bastacky SJ. Long-term caffeine consumption exacerbates renal failure in obese, diabetic, ZSF1 (fa/fa) rats. Kidney Int 2002; 61(4):1433–44.

[29] Tofovic SP, Kusaka H, Jackson EK, Bastacky SJ. Renal and metabolic effects of caffeine in obese (fa/fa), diabetic, hypertensive ZSF1 rats. Ren Fail 2001; 23(2):159–73.

[30] Al Moutaery K, Al Deeb S, Ahmad Khan H, Tariq M. Caffeine impairs short-term neurological outcome after concussive head injury in rats. Neurosurgery 2003; 53(3):704–12.

[31] Karas M, Chakrabarti SK. Influence of caffeine on allergy-induced hepatotoxicity in rats. In vivo study. J Environ Pathol Toxicol Oncol 2001; 20(2):141–54.