Effects of Caffeine on Oxidant-Antioxidant Mechanisms in the Rat Liver

Kafeinin Rat Karaciğerinde Oksidan Antioksidan Mekanizmalara Etkisi

Canan Demirtaş[1], Ebru Ofluoğlu[2], Ahmed Hussein[1], Hatice Paşaoğlu[1]

[1]Department of Medical Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey
[2]Zonguldak Vocational School of Health Services, Zonguldak Karaelmas University, Zonguldak, Turkey

ABSTRACT

Objective: Caffeine (1, 3, 7-trimethylxanthine) is a purine alkaloid which exists in a variety of foods and drinks. Today, caffeine is a regularly consumed substance, found in coffee, tea, chocolate and cola. The main aim of our study was to compare the potential antioxidant effects of oral caffeine intake in rat the liver at two different doses over a short period of time.
Methods: We measured malondialdehyde (MDA) levels, which is a product of lipid peroxidation, in rat livers following caffeine administration. In addition, we evaluated superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S transferase (GST) activities as well as glutathione (GSH) levels in the liver. Thirty male Wister rats were used. Rats were equally divided into three groups. Group 1 was the control group, Group 2 received 30 mg/kg of caffeine and Group 3 received 100 mg/kg caffeine (non-toxic high dose) orally for 14 days (a short time period).
Results: Our results showed that the 30mg/kg and 100 mg/kg caffeine doses decreased lipid peroxidation in liver. Antioxidant enzyme activities in the rat liver, like SOD, catalase, GPx and GST, showed a statistically significant increase with caffeine intake. Liver glutathione levels, in comparison to the control group, showed a slight increase, but this was not statistically significant. Results from the Spearman analysis showed a strong negative correlation between MDA levels and GPx, GST and SOD activities. Tissue GST activity and tissue catalase activity showed a strong positive correlation.
Conclusion: Decreased lipid peroxidation and increased antioxidant enzyme activities demonstrate improved control of oxidative stress, suggesting that these doses of caffeine may have antioxidant activity.
(Gazi Med J 2012; 23: 13-8)

Key Words: Caffeine, malondialdehyde, antioxidant enzymes, liver

Received: 02.12.2011 **Accepted:** 19.02.2012

ÖZET

Amaç: Kafein (1, 3, 7 trimetilksantin) bir pürin alkoloit olarak birçok yiyecek ve içeceklerin içeriğinde bulunur. Kahve, çay, çikolata, kola ve bazı gazlı içecekler kafein ihtiva eder. Biz çalışmamızın temel hedefi olarak, kısa süreli oral kafein alımının rat karaciğerinde olası antioksidan etkilerini iki farklı dozda araştırmaya çalıştık.
Yöntemler: Kafein verilen ratların karaciğer dokularında lipit peroksidasyon ürünü olan MDA düzeylerini ölçtük. Bunun yanında kafeinin antioksidan özelliğini incelemek için, enzimatik ve non enzimatik antioksidan sistem üzerinde araştırmalar yaptık. Karaciğer dokularında SOD, katalaz, GPx, GST aktivitelerini ve GSH düzeylerini ölçtük. Çalışmamızda 30 adet (ortalama 250 gr ağırlığında) Wistar cinsi erkek rat kullanıldı. Ratlar üç eşit gruba ayrıldı. Grup 1: Kontrol grubuydu. Grup 2'ye 30 mg/kg, Grup 3'e 100 mg/kg (nontoksik yüksek doz) kafein 14 gün boyunca (kısa süreli) oral yol ile verildi.

22nd National Biochemistry Congress. 27-30 October 2010 / Eskişehir 4th National Molecular Medicine Congress. 27-30 June 2011 / İstanbul
Address for Correspondence / Yazışma Adresi: Dr. Canan Demirtaş, Department of Medical Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey Phone: +90 312 202 41 78 E-mail: mdcanandemirtas@yahoo.com.tr

doi:10.5152/gmj.2012.04

Bulgular: Çalışmamızın sonuçları, 14 gün düşük doz (30 mg/kg) ve toksik olmayan yüksek doz (100 mg/kg) kafein uygulamasının, karaciğerde lipit peroksidasyonununu azalttığını göstermektedir. Kafein alımıyla rat karaciğer dokusunda SOD, katalaz, GPx ve GST gibi antioksidan enzim aktivitelerinde ise istatistiksel olarak anlamlı artış saptanmıştır. Karaciğer dokusu glutatyon düzeyleri karşılaştırıldığında kontrol grubuna göre kafeinli gruplarda hafif artış tespit edilmiş, ancak gruplararasında istatistiksel olarak anlamlı fark bulunmamıştır. Spearman korelasyon analizi sonuçlarına göre doku MDA düzeyi azalırken, GPx, GST, SOD aktivitesi artmış ve güçlü negatif korelasyon görülmüştür. Doku GST aktivitesi ile doku katalaz aktivitesi arasında güçlü pozitif korelasyon bulunmuştur.
Sonuç: Kafeinin bu dozlarda; lipit peroksidasyonunu azaltması, antioksidan enzim aktivitelerini artırması ile oksidatif stresi iyileştirmesi, yapılan araştırmaların da ışığında antioksidan olabileceği görüşünü desteklemektedir. Kafeinin antioksidan olarak uygun dozunun belirlenmesinde, etki mekanizmalarının açığa kavuşturulmasında ileri hayvan ve insan çalışmalarının gerekli olduğunu düşünmekteyiz.
(Gazi Med J 2012; 23: 13-8)

Anahtar Sözcükler: Kafein, malondialdehit, antioksidan enzim, karaciğer

Geliş Tarihi: 02.12.2011 **Kabul Tarihi:** 19.02.2012

INTRODUCTION

Caffeine, a purine alkaloid (1, 3, 7-trimethylxanthine), is present in several popular drinks, such as coffee, tea and energy drinks. Methylxanthines are also used as components of analgesics, diuretics, myorelaxants and stimulant drug formulations (1-4). The subject of our study, caffeine, has caught the attention of many researchers because of its effects on hepatic metabolism and its antioxidant properties. Studies on the effect of caffeine on the liver have shown that it protects against damage caused by radiation and other agents, it decreases lipid peroxidation and protects membranes from damage caused by reactive oxygen species (5, 6). It is also thought that caffeine cleans up ROS (reactive oxygen species) and peroxide radicals (7). However, Moutaery et al. showed that high dose intraperitoneal caffeine (100-150 mg/kg) administration following experimental head trauma in rats dose dependently increased lipid peroxidation and caused oxidative stress in the cortex (8). A study by Karas et al. showed increased liver MDA levels in rats that had received allyl-alcohol for low grade hepatotoxicity and were given caffeine orally (150 mg/kg) (9).

The aim of our study was to assess the effects of oral caffeine intake on the rat liver at two different doses (30 mg/kg and 100 mg/kg) over a short period of time (14 days). We measured levels of MDA, a lipid peroxidation product, in rat livers. In addition, to investigate the antioxidant effects of caffeine, we studied the enzymatic and non-enzymatic antioxidant systems. We measured SOD, catalase, GPx and GST activities and GSH levels in liver tissue.

METHODS

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 was purchased and dissolved in distilled water. Freshly prepared caffeine solutions were administered orally at a volume of 1 mL/day for 14 days. The experimental protocols were conducted with the approval of the Animal Research Committee of Gazi University, Ankara, TURKEY (Code No: G.U.E.T-07.031).

Thirty male Wistar rats weighing about 250 g each were used in the experiment. The animals were randomly divided into three groups, each consisting of 10 members. The first group served as the control; these animals were given drinking water without caffeine. The second group of animals was given a caffeine dose of 30 mg/kg. The third group was given a 100 mg/kg dose of caffeine solution. The total daily dose was divided into two smaller doses, given in the morning and the afternoon. After 14 days, the rats were sacrificed, and the liver tissue was quickly removed and frozen at -80°C.

The tissues were separately weighed and homogenised in 10 volumes of cold 50 mM Tris-HCL Buffer (pH 7.4) using an automatic homogeniser. Then, the homogenates were centrifuged at 15,000 rpm for 15 min at 4°C. Clear supernatants were used for the MDA, GST, catalase and GSH assays (10). Tissue protein levels were also measured at this step according to Lowry et al. (11).

The remaining supernatants were mixed with chloroform/ethanol mixture at a ratio of 1/1 (v/v) and centrifuged for 30 minutes at 4°C at 5000 g. Clear supernatants were used to measure SOD and GPx activities. Tissue protein levels were also measured at this step according to Lowry et al. (11).

Assay for Tissue MDA Concentrations

Tissue MDA assays were performed according to Ohkawa et al. (12). MDA is a product of lipid peroxidation that reacts with TBA under acidic conditions at 95°C, forming a pink coloured complex that absorbs at 532 nm. 1, 1, 3, 3-tetraethoxypropane was used as the standard. The results are expressed as nmol/g tissue.

Assay for GSH Concentrations

Liver GSH levels were detected in a 96-well microtitre plate using the Sigma Fluorometric Glutathione Assay Kit (Catalogue No: CS1020). The kit assay utilises a thiol probe (monochlorobimane) which can freely pass through the plasma membrane. The free, unbound probe shows very little fluorescence, but when bound to reduced glutathione in a reaction that is catalysed by glutathione S-transferase (GST), it forms a strongly fluorescent adduct. All samples were tested in duplicate. The results are expressed as nmol GSH/mg protein. Protein concentrations were assayed by Lowry's method (11).

Assay for SOD Activity

Liver SOD activity was assayed according to the method of Yi-Sun et al. (13). This method employs xanthine and xanthine oxidase to generate superoxide anions which react with nitroblue tetrazolium (NBT) and measured by the degree of inhibition of the reaction, such that one unit of enzyme provides 50% inhibition of NBT reduction. The results are expressed as IU/mg protein. Protein concentrations were assayed by Lowry's method (11).

Assay for GPx Activity

Liver GPx activity was assayed according to the method of Paglia et al. (14). GPx catalyses the oxidation of glutathione in the presence

of tert-butyl hydroperoxide (tBH). Oxidised glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH, while NADPH is oxidised to NADP. Reduction in the absorbance of NADPH at 340 nm is measured. By measuring the absorbance change per min and using the molar extinction coefficient of NADPH, GPx activity is calculated and expressed as IU/mg protein. Protein concentrations were assayed by Lowry's method (11).

Assay for GST Activity

Liver GST activity was assayed according to the method of Habig et al. (15). Briefly, 1 mM CDNB was added to a buffer containing 1 mM GSH and an aliquot of the sample to be tested. Upon the addition of CDNB, the change in absorbance at 340 nm was measured as a function of time. The extinction coefficient for this reaction is 9.6 mM^{-1} cm^{-1}. GST activity is calculated and expressed as nmol/min/mg protein. Protein concentrations were assayed by Lowry's method (11).

Assay for Catalase Activity

Liver catalase activity was assayed according to the method of Aebi et al. (16). Briefly, H_2O_2 was used as the substrate and the decrease of H_2O_2 concentration at 20°C in phosphate buffer was assayed by spectrophotometry at 240 nm. One unit of catalase activity is the amount of enzyme that degrades 1 µmol of H_2O_2/min; catalase activity is expressed as IU/mg protein. Protein concentrations were assayed by Lowry's method (11).

Statistical Analysis

All values were expressed as the mean±SD. Comparisons between groups were done using the Mann-Whitney U test. Probability values of less than 0.05 were accepted as significant. The correlation between groups assay of values in the tissues was evaluated using Spearman's correlation test. All analyses were done with the statistical package of software SPSS 11.5.

RESULTS

Statistically significant correlations ($p<0.05$) were found between:

(1) MDA and GPx activities in liver tissue ($p=0.001$, $r=-0.561$)
(2) MDA and GST activities in liver tissue ($p=0.05$, $r=-0.361$)
(3) MDA and SOD activities in liver tissue ($p=0.03$, $r=-0.396$)
(4) GST and catalase activities in liver tissue ($p=0.005$, $r=0.502$)

DISCUSSION

Caffeine (1, 3, 7-trimethylxanthine), a form of purine alkaloid, exists in variety of foods and drinks (1). Today, caffeine is a regularly consumed substance. It is known that coffee, tea, chocolate, cola and some soft drinks contain caffeine which excites the central nervous system (2-4). A low dose ethanol and caffeine mixture (10 mg/kg) is called caffeinol, which has been shown to protect the cortical parts of the brain from damage caused by distal focal ischaemia at rats. It has been reported that alcohol used alone worsens stroke, but used with caffeine, it protects neurons (17, 18).

Chronic use of low dose (10 mg/kg) caffeine at rats with ischaemic brain damage has been shown to decrease global ischaemia. The neuroprotective effect of chronic caffeine use is thought to be mediated by its both inhibiting and adaptive effects on the adenosine receptor pathway. Acute low dose teophilin in rats decreases brain damage after cerebral hypoxia-ischaemia. Caffeine and its metabolites have been shown to have various effects in the treatment of ischaemia, depending on dose, administration and the model of ischaemia (19).

Experiments with rats have shown that caffeine protects against damage caused by radiation and other agents; 2 mM caffeine taken before or after radiation exposure almost completely inhibits apoptosis induced by radiation. It has been reported that even at millimolar concentrations; caffeine decreases lipid peroxidation in rat liver microsomes and protects membranes against damage caused by reactive oxygen radicals as an antioxidant (5, 6).

Oral caffeine admission in mice has been reported to regulate tumour suppressor genes (including p53). In addition, the effect of caffeine has been found to both decrease and increase mutations. Caffeine and its catabolic products, theobromine and xanthine, show pro-oxidant activity by decreasing the transformation of Cu(II) to Cu(I) at high copper concentrations and are thought to act as oxygen radicals. Furthermore, caffeine has been reported to act as an anti-oxidant and inhibit hydroxyl radical formation and DNA breaks (20). Caffeine can clean up hydroxyl radicals and singlet oxygen, inhibit lipid peroxidation and decrease LDL concentration and protein carbonyl levels produced by protein oxidation. Due to these effects caffeine, is considered to be cardioprotective (21, 22).

When acute doses of caffeine (5-15 mg/kg) were added to the drinking water of mice, gamma ray-induced chromosomal damage decreased rapidly. This implies that caffeine cleans up ROS and peroxyl radicals. It has been reported that drinking two cups of coffee per day decreases radiation-induced toxicity in some cancer patients. Furthermore, the protective effects of caffeine on chemical and UV-B-induced carcinogenesis have been noted (7). It has been demonstrated that caffeine administration at a dose of 20 mg/kg per day for 30 days increased hepatic catalase and SOD activities and decreased MDA levels (23). The effects of caffeine on hepatic metabolism, particular its antioxidant activity, have promoted caffeine as an area of research. The aim of our study was to evaluate the antioxidant effects of caffeine on the liver.

We measured levels of hepatic MDA, a lipid peroxidation product, in rats following caffeine administration. In addition, we investigated the effect of caffeine on the enzymatic and non-enzymatic antioxidant systems by measuring SOD, catalase, GPx and GST activities as well as GSH levels in rat liver tissue.

The liver is the main organ for xenobiotic detoxification. For this reason, the liver is the first organ to be affected when caffeine consumed. It is also known that free radicals are created by some physiological events. We measured the effects of caffeine at two different doses (30 mg/kg and 100 mg/kg) over a short time period (14 days) on free radicals in the rat liver. Lipid peroxidation is one of the important mechanisms of cell injury related to free radicals. MDA, which is one of these lipid peroxidation products, is a very toxic substance that has many effects on ion exchange at the cell membrane, which impairs ion permeability and enzyme activity (24).

Our study showed that when Group 1 (control) and Group 2 (30 mg/kg caffeine) were compared, Group 2 had lower MDA levels but this was not statistically significant ($p=0.427$). The comparison of Group 1 with Group 3 (100 mg/kg caffeine) was statistically significant ($p=0.003$). There was also a statistically significant difference between Group 2 and Group 3 ($p=0.003$) (Table1). Nikolic et al. (25) reported that caffeine affected L-arginine metabolism in brain. They

Table 1. MDA, GSH levels and SOD, GPx, GST and Catalase Activities in the rat liver

	MDA (nmol/g tissue)	GSH (nmol GSH/mg protein)	SOD (IU/mg protein)	GPx (IU/mg protein)	GST nmol/min/mg protein	Catalase (IU/mg protein)
Group 1	115.78±15.86	0.111±0.026	7.82±1.36	4.82±2.63	48.58±23	277.33±22
Group 2	103.57±24.65	0.137±0.044	11.20±2.06[a]	4.69±1.18	61.47±17	280.01±36
Group 3	67.48±8.10[a,b]	0.129±0.043	10.85±2.61[a]	8.04±1.74[a,b]	86.37±18[a,b]	342.49±52[a,b]

The results are presented in Table 1. Results are expressed as mean±SD
[a]:p<0.05 as compared to control (Group 1), [b]:p<0.05 as compared to Group 1 and Group 2 and 3

added caffeine to rats' drinking water at 2 mg/mL for three days, which was then increased to 4 mg/mL for seven days. It was noted that caffeine decreased arginase activity and MDA levels in the brain.

Some studies based on caffeine and lipid peroxidation have shown a significant decrease in MDA levels in serum, liver, heart, brain and kidney, but on the other hand, some studies have shown an increase in MDA levels in the brain and liver (5-9, 21, 23). Lee et al. demonstrated that caffeine cleans up hydroxyl radicals created by the Fenton reaction (Fe^{+2}/ H_2O_2). In addition, they reported that millimolar concentrations of caffeine decreased hepatic microsomal lipid peroxidation by inhibiting, and they also noted that caffeine was more potent than ascorbic acid at the same millimolar concentrations. The same researchers found decreased chromosomal damage induced by gamma rays when caffeine (5-15 mg/kg) was added to the drinking water of rats (7). In a publication by Devasagayam et al., even millimolar concentrations of caffeine decreased rat hepatic microsomal lipid peroxidation and protected cell membranes from damage caused by reactive oxygen species (5). Yukawa et al. reported that consuming 150 mL of (Arabic) coffee for seven days, three times per day, decreased total serum cholesterol, LDL cholesterol and MDA levels, but did not change Lp(a), HDL cholesterol and triglyceride levels in human subjects (21). In a study by Karas et al., oral caffeine administration (150 mg/kg, oral) increased hepatic MDA levels in rats which had allyl-alcohol induced low-grade hepatotoxicity (9).

Our results on hepatic SOD activity showed that there was statistically significant increase in the 30 mg/kg caffeine dose group (Group 2) compared to the control group (Group 1) (p=0.001), and there was also statistically significant increase in the 100 mg/kg dose group (Group 3) compared to the control group (p=0.001). There was no statistical significance between Group 2 and Group 3 (p=0.326) (Table 1). When hepatic tissue catalase activity between Group 1 and Group 2 was compared, there was no statistical significance (p=5.45), but there was statistically significant significance between Group 1 and Group 3 (p=0.007). There was also a statistically significant difference between group 2 and 3 (p=0.005) (Table 1). Mukhopadhyay et al. published a paper on increased hepatic catalase and SOD activity, along with decreased MDA levels, after administration of 20 mg/kg of caffeine to rats for 30 days (23). Birkner et al. administered NaF and caffeine (for 50 days, 3 mg/kg/day) into rats' drinking water and showed no statistically significant difference between the control group and the NaF group in terms of SOD levels, but reported a decrease in catalase activity and an increase in GPx activity (26). Rossowska et al. demonstrated no statistically significant difference in liver and heart tissue total SOD, GPx or catalase activities between the control group and two groups of rats which had consumed 20 mg/kg caffeine for 22 and 30 days (27). We did not find a statistically significant significance in hepatic GPx activity between Group 1 and Group 2 (p=0.940). On the other hand, we found a statistically significant increase in GPx activity Group 3 when compared to the control group (p=0.010); the difference between Groups 2 and 3 was also statistically significant (p=0.001) (Table 1).

SOD is the main cleanser of superoxide radicals. It is also known that H_2O_2 is created by this reaction. While H_2O_2 is not accepted as a radical, it is the main source of hydroxyl radicals in the human body. GPx and catalase are two important enzymes for H_2O_2 metabolism. Our study indicated an increase in SOD, GPx and catalase in both treatment groups compared to the control group. Liver tissue GST activity was not statistically different between Group 1 and Group 2 (p=0.151). When Group 1 and Group 3 were compared, there was a statistically significant difference (p=0.002). There was also a statistically significant difference between Groups 2 and 3 (p=0.013) (Table 1). Comparison of the caffeine groups with the control group showed no significant difference, although there was a slight increase (Table 1). Abraham et al. administered caffeine by gavage to three groups of rats (2.25 mg/kg, 4.5 mg/kg and 9 mg/kg) and, in comparison to the control group, showed no significant differences in liver GST activity. The same study also showed no differences in hepatic GST activity and sulphydryl (-SH) levels when caffeine was added to the drinking water (two weeks, 70 mg/100 mL) (28).

Glutathione has a low molecular weight and is an important tripeptide which contains thiol group. It has multiple functions in DNA and protein synthesis, regulation of enzyme activities, intra and extracellular transport and is also an antioxidant with a role in cell protection. It is known that reduced glutathione causes a high redox state in the cell by its thiol group which protects the cell from oxidative damage (29-31). Noschang et al. studied the effect of chronic caffeine administration on parameters related to oxidative stress in different brain regions of stressed and non-stressed rats. Lipid peroxide levels and the total radical-trapping potential were assessed, as well as antioxidant enzyme activities, superoxide dismutase, glutathione peroxidase and catalase in the hippocampus, striatum and cerebral cortex. Their results showed interactions between stress and caffeine, especially in the cerebral cortex, since caffeine increased the activity of some antioxidant enzymes, but not in stressed animals. They concluded that chronic administration of caffeine led, in some cases, to increased activity of antioxidant enzymes. However, these effects were not observed in the stressed animals (32). Ofluoglu et al. investigated the short-term effects of caffeine on L-arginine metabolism in the rat brain. They reported that MDA levels decreased significantly in both the serum and brain tissue of animals, while on the other hand, brain tissue and serum nitric oxide (NO) levels increased significantly after caffeine administration (33). Varma et al. reported that caffeine was found to be effective in preventing oxidative stress to the lens induced by iron under ambient conditions. The protective effect was attributed to its ability to scavenge ROS, particularly the hydroxyl radical. Lenses incubated in medium containing Fe_8Br_8

undergo oxidative stress, as evidenced by the inhibition of Na+/-K+/ ATPase-driven rubidium transport and the loss of tissue glutathione and ATP. These effects were prevented in the presence of caffeine. Caffeine has been found to inhibit the latter processes as evidenced by the measurement of tissue ATP levels. In the presence of caffeine, the level was well-maintained, as was the case with the maintenance of the levels of GSH and the rubidium transport activity. Caffeine has been found to be effective in protecting the lens against damage induced by ROS generated by the redox cycling of trace metals, such as iron (34). Pasaoglu et al. investigated the effect of caffeine on the levels of MDA, nitric oxide (NO) and advanced oxidation protein products (AOPP) in the liver and heart tissues of rats. Their results showed that MDA and AOPP levels in the liver tissue of the caffeine-treated groups decreased significantly, and MDA and AOPP levels in the heart tissue also decreased, but this effect was not significantly affected by the dose. In addition, they reported that NO levels in the liver tissue of the caffeine-treated groups were higher than those in the control group; in heart tissue, however, NO levels were not significantly affected by caffeine. Their results showed that short-term consumption of two different doses of caffeine may potentially protect against oxidative stress in the liver; this effect was related to the dose of caffeine in the liver tissue (35).

Our suggestion as to why different studies on caffeine provide different results is the variety of models used, with different doses, for different durations of treatment, different methods of administration and tissue specificity of the antioxidant system. It is known that some physiological reactions create radicals. Oxygen derivatives increase hydrogen peroxide levels, which is the source of superoxide and hydroxyl radicals. This increases the MDA level, which is a marker of lipid peroxidation. Our study suggests that a significant decrease in MDA levels and an increase in SOD, GPx, GST and catalase enzyme levels in the caffeine administered groups against control groups are indicative of the negative effect of caffeine on radical production and its positive effect on antioxidant enzymes.

Our results in this short time interval (14 days) at both the low dose (30 mg/kg) and the non-toxic high dose (100 mg/kg) of oral caffeine administration showed a decrease in liver MDA levels. It was also noted that there was an increase in SOD, catalase, GPx and GST enzyme activities with caffeine intake in rat liver. When liver glutathione levels were compared to the control group, there was a slight increase associated with caffeine intake, but this increase was not statistically significant (Table 1).

These results support the idea of caffeine as an antioxidant with effects on lipid peroxidation, antioxidant enzyme activities and oxidative stress. The route of administration, dose, duration and various research models with antioxidant systems dependent on tissue type, in our opinion, may be reasons for having different results in various studies. Determining the optimal dose of caffeine for its antioxidant effect and understanding the mechanisms of its effects on that system through animal and human studies may enhance our understanding of caffeine.

Acknowledgements

This study was supported by the Department of Scientific Research Projects at Gazi University (Project number: 01/2007-86).

Conflict of Interest

No conflict of interest was declared by the authors.

REFERENCES

1. Kolaylı S, Ocak M, Kucuk M, Abbasoglu R. Does caffeine bind to metal 1. Kolaylı S, Ocak M, Kucuk M, Abbasoglu R. Does caffeine bind to metal ions? Food Chem 2004; 84: 383-8.
2. 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: 334-7. [CrossRef]
3. Al-Deeb S, Al-Moutaery K, Arshaduddin M, Biary N, Tariq M. Effect of acute caffeine on severity of harmaline induced tremor in rats. Neurosci Lett 2002; 325: 216-8. [CrossRef]
4. Concas A, Porcu P, Sogliano C, Serra M, Purdy PH, Biggio G. Caffeine-induced increases in the brain and plasma concentrations of neuroactive steroids in the rat. Pharmacol Biochem Behav 2000; 66: 39-45. [CrossRef]
5. Devasagayam TP, Kamat JP, Mohan H, Kesavan PC. Caffeine as an antioxidant; inhibition of lipid peroxidation induced by ROS. Biochim et Biophys Acta 1996; 1282: 63-70. [CrossRef]
6. George KC, Hebbar SA, Kale SP, Kesavan PC. Caffeine protects mice against whole-body lethal dose of γ -irradiation. J Radiol Prot 1999; 19: 171-6. [CrossRef]
7. Lee C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. Clin Chim Acta 2000; 295: 141-54. [CrossRef]
8. 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: 704-11. [CrossRef]
9. Karas M, Chakrabarti SK. Influence of caffeine on allyl alcohol-induced hepatotoxicity in rats. In vivo study. J Environ Pathol Toxicol Oncol 2001; 20: 141-54.
10. İlhan 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: 386-94. [CrossRef]
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with the folin phenol reagent. J Biol Chem 1951; 193: 265-75.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95, 351-8. [CrossRef]
13. Yi-Sun S, Oberly LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497-500.
14. Paglia DE, Valentina WN. Studies on quantitative and qualitative characterization of erytrocte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-69.
15. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases: The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-9.
16. Aebi H. Catalase, In: H.U.Bergmeyer, (Ed): Methods of Enzymatic Analysis, Academic Press, New York and London 1974; pp: 673-7.
17. Belayev L, Khoutorova L, Zhang Y, Belayev A, Zhao W, Busto R, et al. Caffeinol confers cortical but not subcortical neuroprotection after transient focal cerebral ischemia in rats. Brain Res 2004; 1008: 278-83. [CrossRef]
18. Aronowski J, Strong R, Shirzadi A, Grotta JC. Ethanol plus caffeine (caffeinol) for treatment of ischemic stroke. Stroke 2003; 34: 1246. [CrossRef]
19. 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: 515-22. [CrossRef]
20. Azam S, Hadi N, Khan NU, Hadi SM. Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. Med Sci Monit 2003; 9: 330-5.
21. Yukawa GS, Mune M, Otani1 H, Tone Y, Liang M, Iwahashi H, et al. Effects of Coffee Consumption on Oxidative Susceptibility of Low-Density Lipoproteins and Serum Lipid Levels in Humans. Biochemistry (Moscow) 2004; 69: 70-4. [CrossRef]
22. Kamat JP, Boloor KK, Devasagayam TPA, Jayashree B, Kesavan PC. Differential modification by caffeine of oxygen-dependent and independent effects of γ-irradiation on rat liver mitochondria. Int J Radiat Biol 2000; 76: 1281-8.

23. 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: 283-9.
24. Moslen MT. Reactive oxygen species in normal physiology, cell injury and phagocytosis. Adv Exp Med Biol 1994; 366: 17-27. [CrossRef]
25. Nikolic J, Bjelakovic G, Stojanovic I. Effect of caffeine on metabolism of L-arginine in the brain. Mol Cell Biochem 2003; 244: 125-8. [CrossRef]
26. Birkner E, Grucka-Mamczar E, Zwirska-Korczala K, Zalejska-Fiolka J, Stawiarska-Pieta B, Kasperczyk S, et al. Influence of sodium fluoride and caffeine on the kidney function and free-radical processes in that organ in adult rats. Biol Trace Elem Res 2006; 109: 35-48. [CrossRef]
27. Rossowska MJ, Nakamoto T. Effects of chronic caffeine feeding on the activities of oxygen free radical defense enzymes in the growing rat heart and liver. Experientia 1994; 50: 465-8. [CrossRef]
28. Abraham SK, Singh SP. Anti-genotoxicity and glutathione S-transferase activity in mice pretreated with caffeinated and decaffeinated coffee. Food Chem Toxicol 1999; 37: 733-9. [CrossRef]
29. Meister A. New aspects of glutathione biochemistry and transport selective alteration of glutathione metabolism. Nutrition Rev 1984; 12: 397-410.
30. Gözükara EM. Biyokimya İ.Ü. Yayınları Malatya; 1989.
31. Meister A. Selective moification of glutathione metabolism. Science 1983; 220: 472-7. [CrossRef]
32. Noschang CG, Krolow R, Pettenuzzo LF, Avila MC, Fachin A, Arcego D, et al. Interactions between chronic stress and chronic consumption of caffeine on the enzymatic antioxidant system. Neurochem Res 2009; 34: 1568-74. [CrossRef]
33. Ofluoglu E, Pasaoglu H, Pasaoglu A. The Effects of Caffeine on L-Arginine Metabolism in the Brain of Rats. Neurochem Res 2009; 34: 395-9. [CrossRef]
34. Varma SD, Hegde KR. Prevention of Oxidative Damage to Lens by Caffeine. J Ocul Pharmacol Ther 2010; 26: 73-7. [CrossRef]
35. Paşaoğlu H, Ofluoğlu E, Demirtaş C, Husseın A, Paşaoğlu OT. The effect of caffeine on oxidative stress in liver and heart tissues of rats. Turk J Med Sci 2011; 41: 665-71.