Article

Toxicology and Industrial Health
2016, Vol. 32(1) 15–21

DOI: 10.1177/0748233713485890
tih.sagepub.com

The protective effects of caffeic acid phenethyl ester against toluene-induced nephrotoxicity in rats

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Abstract

Caffeic acid phenethyl ester (CAPE) has antioxidant and anti-inflammatory properties. The aim of this study is to examine the negative effects of toluene on kidney tissues and functions and to investigate the protective effects of CAPE against toluene-induced nephrotoxicity in rats. A total of 21 male Wistar rats were divided into three groups of equal number in each. The rats in group I were the controls. Toluene was intraperitoneally injected into the rats in group II with a dose of 500 mg/kg. Rats in group III received CAPE daily while exposed to toluene. After 14 days of experimental period, all rats were killed by decapitation. Enzymatic activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) and malondialdehyde (MDA) levels were studied in the rat kidneys. Blood urea nitrogen (BUN) and serum creatinine levels were measured for renal function. The CAT and SOD enzyme activities and serum creatinine levels were significantly increased in rats treated with toluene when compared with the controls. But GSH-Px activity, MDA, and BUN levels showed statistically nonsignificant changes. However, increased CAT and SOD enzyme activities and decreased serum creatinine levels were detected in the rats that received CAPE while exposed to toluene. The GSH-Px activity and MDA and BUN levels in the same group did not show statistically significant changes. The results of our study demonstrated that toluene damages kidney tissue and is a nephrotoxic substance. CAPE was able to prevent the renal damage as antioxidant, antitoxic, and nephroprotective agent.

Keywords

Oxidative stress, toluene, antioxidant, renal, occupational health

Introduction

Toluene is an aromatic hydrocarbon and an organic solvent that is harmful to the human body. It is widely used in industries where adhesives, erasers, plastics, leather, dyes, dye thinners, and printing pastes are manufactured. Toluene exposure is quite common among individuals who are working in the abovementioned industries (ATSDR, 2007; Thiel and Chahould, 1997). Furthermore, toluene has an addictive potential. Therefore, substance abuse can be a matter of concern (ATSDR, 2007; Coskun et al, 2006; Mendoza-Cantu et al., 2006; Tomei et al., 1999; Tsatsakis et al., 1997). Toluene has lipophilic properties and can rapidly diffuse into the tissues of certain organs such as the brain, liver, and kidneys. Therefore, even short-term exposure may cause the emergence of

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side effects. Depending on its concentrations in blood, toluene may cause severe clinical disorders including coma (ATSDR, 2007).

Caffeic acid phenethyl ester (CAPE) is an active component of propolis, a substance produced by honeybees. CAPE has antiproliferative, antioxidant, anti-inflammatory, and immune-modulator effects (Ogetürk et al., 2005; Kus et al., 2004; Borrelli et al., 2002; Hepsen et al., 1997; Natarajan et al., 1996; Mirzeova and Calder, 1996). In many studies, it was determined that CAPE was capable of increasing the enzyme activities of some antioxidants and preventing oxidative damage in tissues (Ogetürk et al., 2005; Kus et al., 2004). However, even though the antioxidant effect of CAPE is demonstrated in many studies, there are no studies related to its effect on biochemical and histological alterations that may occur in kidney tissues as a result of toluene exposure. Therefore, we examined the protective effect of CAPE against toxic effects that may occur in kidney tissues as a consequence of toluene administration in this study.

Materials and methods

Animals and treatments

Adult male Wistar rats (200–250 g) were obtained from Experimental Research Centre of Gaziantep University (Gaziantep, Turkey). The animal studies were carried out according to the guidelines of the European Community Council for experimental animal care. Animals were housed individually on a 12-h light:12-h dark cycle (lights on at 08.00 h), at a temperature of $21 \pm 1^{\circ}$C and 50% humidity. Food (standard pellet diet) and tap water were supplied *ad libitum*. The animals were equally divided into three groups. Rats in group I ($n = 7$) served as controls, which were given a vehicle (0.1 ml/10 g/day corn oil, intraperitoneally). Toluene was intraperitoneally injected into the rats in group II ($n = 7$) with a dose of 500 mg/kg/day (0.1 g/ml, in corn oil) (Chien et al., 2005). Rats in group III received CAPE (10 μmol/kg) daily, while being exposed to toluene (500 mg/kg/day, intraperitoneally). After 14 days of experimental period, all rats were killed by decapitation. Blood samples were collected for the determination of serum creatinine and blood urea nitrogen (BUN) levels. The kidneys of the rats were removed. Some of the kidney tissue specimens were washed twice with cold saline solution, placed into glass bottles, labeled, and stored frozen (-30°C) for the eventual determination of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and malondialdehyde (MDA) production. The other kidney tissue specimens were used for light microscopic evaluations.

Biochemical analysis

The tissues were weighed and homogenized in four volumes of ice-cold Tris–hydrochloric acid buffer (50 mM, pH 7.4) containing 0.50 ml/l Triton X-100 in a homogenizer (IKA Ultra-Turrax T 25 Basic, IKA Werke GMBH & CO., KG, Staufen, Germany) for 2 min at 13,000 r/min. All procedures were performed at $+4^{\circ}$C. Tissue homogenates were centrifuged at 5000*g* for 60 min to remove debris, and the clear supernatant fluids were separated and kept at -40°C until the enzyme activity measurements were performed (about a week later).

Determination of SOD activity. Total (copper–zinc and manganese) SOD (EC 1.15.1.1) activity was determined based on the method of Sun et al. (1988). The principle of the method is based on the inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1 ml of an ethanol–chloroform mixture (5:3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as units per gram of protein.

Determination of GSH-Px activity. GSH-Px (EC 1.6.4.2) activity was measured by the method of Paglia and Valentine (1967). The enzyme reaction in the tube containing nicotinamide adenine dinucleotide phosphate, reduced glutathione (GSH), sodium azide, and GSH reductase was initiated by the addition of hydrogen peroxide (H_2O_2), and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was expressed as units per gram of protein.

Determination of CAT activity. CAT (EC 1.11.1.6) activity was measured according to the method of Aebi (1974). The principle of the assay is based on the determination of the rate constant k (dimension: per second) of H_2O_2 decomposition. By measuring the absorbance changes per minute, the rate constant of the enzyme was determined. Activities were expressed as rate constant (k) per gram of protein.

Determination of MDA level. The tissue MDA level was determined using the method of Esterbauer and

Table 1. The activities of SOD, CAT and GSH-Px enzymes and levels of MDA in renal tissue in control, toluene, and toluene + CAPE.[a]

Group	MDA (nmol/g tissue)	SOD (U/mg protein)	CAT (k/g protein)	GSH-Px (U/g protein)
I: Control	4.65 ± 0.51	0.059 ± 0.004	1.71 ± 0.09	2.04 ± 0.12
II: Toluene	4.58 ± 0.39	0.041 ± 0.001	1.10 ± 0.07	2.17 ± 0.06
III: Toluene + CAPE	4.60 ± 0.33	0.053 ± 0.002	1.48 ± 0.11	1.93 ± 0.08
$p <$ (I vs. II)	NS	0.001	0.001	NS
$p <$ (I vs. III)	NS	NS	NS	NS
$p <$ (II vs. III)	NS	0.01	0.01	NS

CAPE: caffeic acid phenethyl ester; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; CAT: catalase; MDA: malondialdehyde.
[a]Results are presented as mean ± SEM.

Cheeseman (1990), based on its reaction with thiobarbituric acid (TBA) at 90–100°C. In the TBA test reaction, MDA and TBA react to produce a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2–3 and at 90°C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was centrifuged and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm. Results were expressed as nanomoles per gram of wet tissue with reference to a standard curve prepared from measurements made with a standard solution (1,1,3,3-tetramethoxypropane).

Determination of serum creatinine and BUN. The BUN and serum creatinine levels were determined with an autoanalyzer (Synchron LX 20, Ireland) using commercial Beckman Coulter diagnostic kits (Beckman Coulter Inc., CA, USA).

Histological examination

For light microscopic examinations, kidney samples were fixed in 10% neutral-buffered formalin. After dehydrating with graded alcohol series, tissues were embedded in paraffin. Several 5-µm-thick transverse sections were obtained from kidney tissue blocks and stained with hematoxylin and eosin for histological evaluation. Sections were examined and photographed with an Olympus DP20 camera attached–Olympus CX41 photomicroscope (Olympus Europa Holding GMBH, Hamburg, Germany) for characteristic histological changes.

Statistical analysis

Data were analyzed using a commercially available statistics software package (SPSS for Windows v. 12.0, Chicago, Illinois, USA). Distribution of the groups was analyzed with one sample Kolmogrov–Smirnov test. All groups showed normal distribution; therefore, parametric statistical methods were used to analyze the data. One-way analysis of variance test was performed and *post hoc* multiple comparisons were made using least-square differences. Results are presented as mean ± SEM; $p < 0.05$ was regarded as statistically significant.

Results

Biochemical results

Reduction in CAT and SOD enzyme activities was found to be statistically significant in toluene-administered rats when compared with rats in the control group ($p < 0.001$). However, despite alterations in GSH-Px enzyme activity, MDA levels were found to be statistically insignificant. Both CAT and SOD enzyme activities increased in rats when toluene was administered along with CAPE ($p < 0.01$). Once again, in the same group, variations in GSH-Px activity and MDA levels were considered statistically insignificant (Table 1).

Serum creatinine levels were significantly increased in rats treated with toluene when compared with the control group ($p < 0.001$). Additionally, the decreased serum creatinine levels were detected in the rats administered with CAPE acid while exposed to toluene ($p < 0.01$). However, BUN levels were found to be statistically insignificant when compared between groups (Table 2).

Light microscopic results

Histological assessment displayed normal glomerular and tubular structures of the kidney tissue in the control group (Figure 1). However, glomerular and tubular structures of the kidney tissue in the toluene group

Table 2. The serum creatinine and BUN levels in control, toluene, and toluene + CAPE groups.[a]

Group	Creatinine (mg/dl)	BUN (mg/dl)
I: Control	0.36 ± 0.01	24.8 ± 0.83
II: Toluene	0.48 ± 0.38	23 ± 1.68
III: Toluene + CAPE	0.37 ± 0.15	25.1 ± 1.57
p < (I vs. II)	0.001	NS
p < (I vs. III)	NS	NS
p < (II vs. III)	0.01	NS

BUN: blood urea nitrogen; CAPE: caffeic acid phenethyl ester.
[a]Results are presented as mean ± SEM.

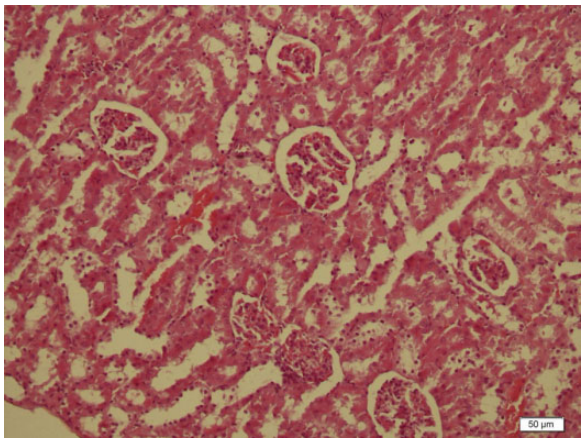

Figure 1. Normal glomerular and tubular structure in the control group (hematoxylin and eosin, ×200).

were found to be damaged when compared with the control group (Figures 2 and 3). It was determined that shrinkage in the glomerular tufts, differentiation in the capsule, and increase in the connective tissue in the interstitial area all occurred. Glomerular tufts and the renal tubular structure of the toluene + CAPE group were found to be almost the same as the control group (Figures 4 and 5).

Discussion

In the current study, we investigated the negative effects of toluene on kidney tissues and functions. Accordingly, serum creatinine levels and BUN values were measured. SOD, GSH-Px, and CAT enzyme activities, along with MDA levels, were measured to evaluate oxidative stress. Additionally, histopathological alterations were also assessed. Furthermore, in addition to abovementioned effects, the antioxidant and nephroprotective effects of CAPE were investigated against the toxic effect caused by toluene.

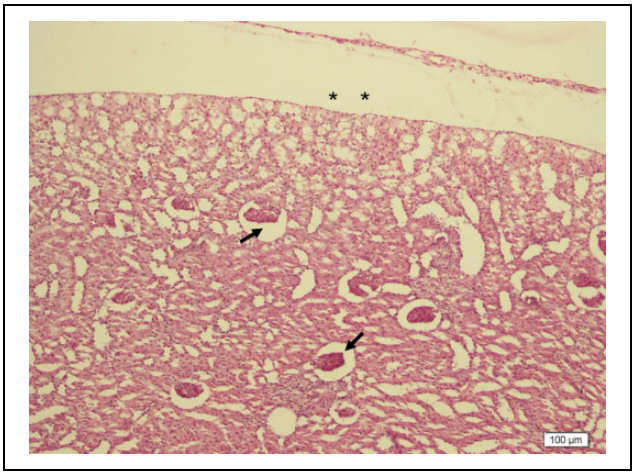

Figure 2. Shrinkage in glomerular tufts (à) and differentiation of the capsule (*) in the toluene group (hematoxylin and eosin, ×100).

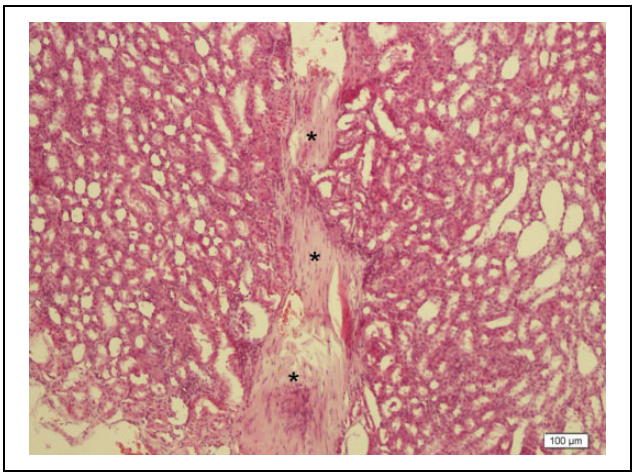

Figure 3. Increase in connective tissue at the interstitial area (*) in the toluene group (hematoxylin and eosin, ×100).

There are several studies on substances that cause renal toxicity (Tutanc et al, 2012; Zararsiz et al., 2007; Ogetürk et al., 2005). One of them is toluene. Toluene has a toxic effect on many organs such as the brain, liver, and kidneys. It has been reported that toluene exposure leads to serious conditions such as metabolic acidosis, hypokalemia, hematuria, proteinuria, distal tubular renal acidosis, formation of renal stones, and pyuria as a result of nephrotoxicity (ATSDR, 2007; Streicher et al., 1981; Kroege et al., 1980). It is obvious that toluene can deteriorate the renal glomerular and tubular structures and cause the emergence of the mentioned clinical disorders. The histological assessment of the current study demonstrated that the glomerular and tubular structures of the renal tissues in the control group were normal.

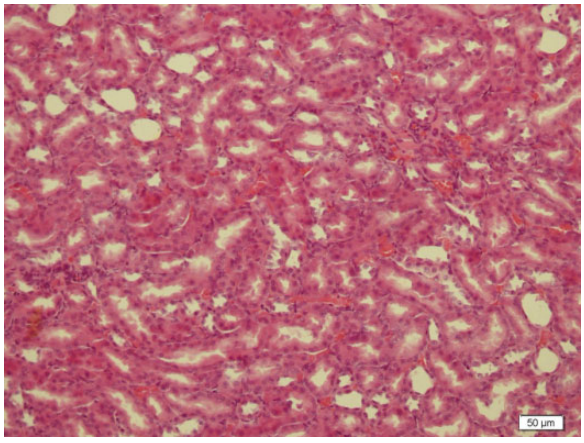

Figure 4. General tubular structure in the toluene + CAPE group similar to the control group (hematoxylin and eosin, × 200). CAPE: caffeic acid phenethyl ester.

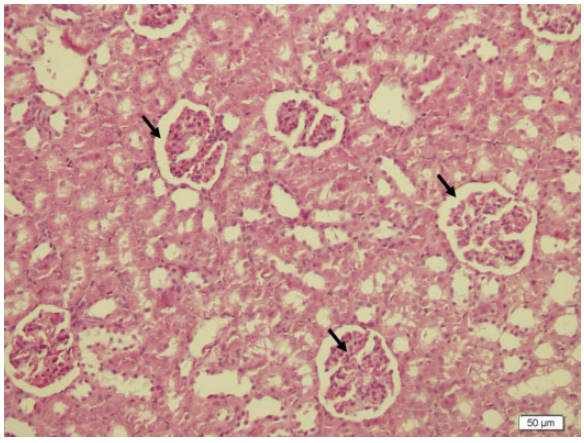

Figure 5. Glomerular tufts (à) in the toluene + CAPE group similar to the control group (hematoxylin and eosin, × 200). CAPE: caffeic acid phenethyl ester.

However, the normal structures of the mentioned tissues were corrupted and there were signs of shrinkage in the glomerular tufts and differentiations in the capsule. Additionally, an increase in the connective tissue at the interstitial area was observed. These findings reveal the nephrotoxic effects of toluene. In our study, when toluene and CAPE-administered groups were considered, we determined that the corrupted glomerular and tubular structures in the toluene group were quite close to the corrupted glomerular and tubular structures in the control group. Consequently, we may assume that CAPE displayed a nephroprotective effect. In a study carried out by Parlakpinar et al. (2005), it was stated that the deterioration was observed in the glomerular and tubular structures as a result of renal toxicity, while CAPE was able to recover the mentioned damage. Such findings show a similarity to the findings of our current study. In another study, it was reported that CAPE was capable of removing histopathological deterioration that occurs in the kidneys as a result of the toxic effect of toluene (Ogetürk et al., 2005).

The increase in free radicals or the decrease in antioxidant enzymes can cause oxidative damage in tissues. Enzymes such as SOD, CAT, and GSH-Px are recognized as antioxidant enzymes. Another indicator of oxidative damage is the MDA level (Coskun et al., 2006; Ozyurt et al., 2006; Elsayed and Bendich, 2001; Karla et al., 1991). In many studies, toluene is found to lead to oxidative stress in multiple tissues. A study carried out by Coskun et al. (2006) on peripheral nerves indicated a decrease in tissue SOD and GSH-Px levels in the toluene group when compared with the control group, while there were no increases in tissue CAT levels. In another study, aromatic hydrocarbons such as toluene were found to reduce the ratio of liver SOD and CAT enzyme levels in the toluene group when compared with the levels of the control group (Otitoloju and Olagoke, 2011). In our study, CAT and SOD enzyme activities significantly reduced in toluene-applied rats when compared with the rats in the control group. However, it was also determined that the changes in the GSH-Px enzyme activity and the MDA levels were statistically insignificant. Our findings demonstrate a similarity with the findings mentioned above. Consequently, toluene is expressed to cause oxidative stress in the renal tissue. However, it is possible to state that the oxidative stress did not occur due to lipid peroxidation, but occurred as a result of a decrease in the level of antioxidant enzymes, which led to a deterioration of the balance in the antioxidant defensive system.

The antioxidant properties of CAPE are displayed in multiple studies. In a study on renal toxicity, it was reported that SOD, CAT, and GSH-Px activities increased in the CAPE group when compared with the activities in the toxic group (Parlakpinar et al., 2005). In another study conducted on lithium toxicity and the kidneys, it was also reported that the activities of the mentioned enzymes showed an increase in the CAPE group when compared with the activities in the lithium group (Oktem et al., 2005). In a recent study, it was determined that SOD, CAT, and GSH-Px enzyme levels in the CAPE group showed a significant increase when compared with the ischemia group (Shi et al., 2010). In an experimental study on acute

renal failure, it was stated that CAPE was able to only increase the SOD level among the SOD, CAT, and GSH-Px enzyme activity levels (Aydogdu et al., 2004). In the study carried out by Gokce et al. (2009), it has been reported that CAPE alone may directly increase CAT activity and prevents cyclosporine A-induced nephrotoxicity in rats. In the current study, an increase was determined in the SOD and CAT enzyme activity levels in rats that received CAPE and toluene together when compared with the rats in the toluene group. However, no change was determined in the GSH-Px enzyme activity level and the MDA levels. Based on these findings, we can assume that CAPE is able to prevent oxidative damage in renal tissue that is induced by toluene.

Renal functions can be followed by monitoring serum creatinine and BUN levels. However, clinical serum creatinine levels are more important than BUN levels at the early stages of renal disease (Erdem et al., 2000). In the current study, a significant increase was observed in serum creatinine levels in the rats included in the toluene group when compared with the rats included in the control group. This finding indicates that toluene causes deterioration in renal functions. When CAPE was administered talong with toluene, it was observed that CAPE caused a decrease in serum creatinine levels in the group of rats when compared with the rats in the toluene group. Thus, we can assume that CAPE was able to improve renal functions. However, no changes were determined when BUN levels of both the groups were compared. In a study carried out by Parlakpinar et al., serum creatinine and BUN levels showed a tendency to increase due to nephrotoxicity, but CAPE was able to reduce the mentioned levels. Based upon this finding, it was stated that CAPE demonstrated antioxidant properties against the types of reactive oxygen that impair the glomerular filtration rate (GFR) (Parlakpinar et al., 2005). GFR is inversely proportional to serum creatinine and is widely used in the measurement of renal functions.

The results obtained from biochemical and histological assessment demonstrated damage to the kidney tissue, and nephrotoxicity was found to be occurred as a consequence of toluene administration. CAPE was able to prevent the mentioned damage. At the end of this study, we conclude that CAPE could be a useful antioxidant, antitoxic, cytoprotective, and nephroprotective agent against tissue damage that may occur in individuals who are exposed to toluene as a result of toluene addiction or occupational obligations. Additionally, toluene addiction and occupational toluene exposure is a social problem. Any attempts made to remove addiction and prevent exposure may be helpful to solve this problem. CAPE can be used as an add-on therapy to the main treatment. However, it would be wise to investigate the effect of CAPE on the life quality and lifespan of individuals in future studies.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Aebi H (1974) *Catalase*. New York and London: Academic Press, pp. 673–677.

Agency for Toxic Substances and Disease Registry (ATSDR) (2007) *CSEM Toluene Toxicity: Environmental Medicine Case Study*. Atlanta, GA: ATSDR.

Aydogdu N, Atmaca G, Yalcin O, Batcioglu K, and Kaymak K (2004) Effects of caffeic acid phenethyl ester on glycerol-induced acute renal failure in rats. *Clinical and Experimental Pharmacology and Physiology* 31(9): 575–579.

Borrelli F, Maffia P, Pinto L, Ianaro A, Russo A, Capasso F, et al. (2002) Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. *Fitoterapia* 73(1): 53–63.

Chien TH, Chan MH, Tang YC, and Chen HH (2005) Toluene exposure during the brain growth spurt reduces behavioral responses to noncompetitive *N*-methyl-D-aspartate receptor antagonists in adult rats. *Psychopharmacology* 182(4): 468–474.

Coskun O, Yüncü M, Kanter M, and Büyükbas S (2006) Ebselen protects against oxidative and morphological effects of high concentration chronic toluene exposure on rat sciatıc nerves. *European Journal of General Medicine* 3: 64–72.

Elsayed NM, Bendich A (2001) Dietary antioxidants: potential effects on oxidative products in cigarette smoke. *Nutrition Research* 21(3): 551–567.

Erdem A, Gündoğan NU, Usubütün A, Kilinç K, Erdem SR, Kara A, et al. (2000) The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. *Nephrology Dialysis Transplantation* 15(8): 1175–1182.

Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods in Enzymology* 186: 407–421.

Gokce A, Oktar S, Yönden Z, Aydin M, Ilhan S, Ozkan OV, et al. (2009) Protective effect of caffeic acid phenethyl ester on cyclosporine A-induced nephrotoxicity in rats. *Renal Failure* 31(9):843–847.

Hepsen IF, Bayramlar H, Gultek A, Ozen S, Tilgen F, and Evereklioglu C (1997) Caffeic acid phenethyl ester to inhibit posterior capsule opacification in rabbits. *Journal of Cataract and Refractive Surgery* 23(10): 1572–1576.

Karla J, Chaudhary AK, and Prasad K (1991) Increased production of oxygen free radicals in cigarette smokers. *International Journal of Experimental Pathology* 72(1): 1–7.

Kroege RM, Moore RJ, Lehman TH, Giesy JD, and Skeeters CE (1980) Recurrent urinary calculi associated with toluene sniffing. *Journal of Urology* 123(1): 89–91.

Kus I, Colakoglu N, Pekmez H, Seckin D, Ogetürk M, and Sarsilmaz M (2004) Protective effects of caffeic acid phenethyl ester (CAPE) on carbon tetrachloride-induced hepatotoxicity in rats. *Acta Histochemica* 106(4): 289–297.

Mendoza-Cantu A, Castorena-Torres F, Bermudez de Leon M, Cisneros B, Lopez-Carrillo L, Rojas-Garcia AE, et al. (2006) Occupational toluene exposure induces cytochrome P450 2E1 mRNA expression in peripheral lymphocytes. *Environmental Health Perspectives* 114(4): 494–499.

Mirzeova OK, Calder PC (1996) The effects of propolis and its components on eicosanoid production during the inflammatory response. *Prostaglandins Leukotrienes and Essential Fatty Acids* 55(6): 441–449.

Natarajan K, Singh S, Burke TR Jr, Grunberger D, and Aggarval BB (1996) Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NFkappaB. *Proceedings of the National Academy of Sciences of the United States of America* 93(17): 9090–9095.

Ogetürk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, and Sarsilmaz M (2005) Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. *Journal of Ethnopharmacology* 97: 273–280.

Oktem F, Ozguner F, Sulak O, Olgar S, Akturk O, Yilmaz RH, et al. (2005) Lithium-induced renal toxicity in rats: Protection by a novel antioxidant caffeic acid phenethyl ester. *Molecular and Cellular Biochemistry* 277(1–2): 109–115.

Otitoloju A, Olagoke O (2011) Lipid peroxidation and antioxidant defense enzymes in *Clarias gariepinus* as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons. *Environmental Monitoring and Assessment* 182 (1–4): 205–213.

Ozyurt H, Pekmez H, Parlaktaş BS, Kuş İ, Özyurt B, and Sarsılmaz M (2006) Oxidative stress in testicular tissues of rats exposed to cigarette smoke and protective effects of caffeic acid phenethyl ester. *Asian Journal of Andrology* 8(2): 189–193.

Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. *Journal of Laboratory Clinical Medicine* 70(1): 158–169.

Parlakpinar H, Tasdemir S, Polat A, Bay-Karabulut A, Vardi N, Ucar M, et al. (2005) Protective role of caffeic acid phenethyl ester (cape) on gentamicin-induced acute renal toxicity in rats. *Toxicology* 207(2):169–177.

Shi Y, Wu X, Gong Y, Qiu Y, Zhang H, Huang Z, et al. (2010) Protective effects of caffeic acid phenethyl ester on retinal ischemia/reperfusion injury in rats. *Current Eye Research* 35(10): 930–937.

Streicher HZ, Gabow PA, Moss AH, Kono D, and Kaehny WD (1981) Syndromes of toluene sniffing in adults. *Annals of Internal Medicine* 94(6): 758–762.

Sun Y, Oberley LW, and Li Y (1988) A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry* 34(3): 497–500.

Thiel R, Chahould I (1997) Postnatal development and behaviour of Wistar rats after prenatal toluene exposure. *Archives of Toxicology* 71(4): 258–265.

Tomei F, Giuntoli P, Biagi M, Baccolo TP, Tomao E, and Rosati MV (1999) Liver damage among shoe repairers. *American Journal of Industrial Medicine* 36(5): 541–547.

Tsatsakis AM, Dolapsakis G, Troulakis G, Christodoulou R, Relakis K, Trikilis N, et al. (1997) Fatal and non-fatal outcome by accidental intoxication with paint thinner. *Journal of Clinical Forensic Medicine* 4(3): 133–137.

Tutanc M, Arica V, Yılmaz N, Nacar A, Zararsiz I, Basarslan F, et al. (2012) Effects of erdosteine on cyclosporin-A-induced nephrotoxicity. *Human and Experimental Toxicology* 31(6): 565–573.

Zararsiz I, Sarsilmaz M, Tas U, Kus I, Meydan S, and Ozan E (2007) Protective effect of melatonin against formaldehyde-induced kidney damage in rats. *Toxicology and Industrial Health* 23(10): 573–579.