Int Surg 2017;**102:**473–478
DOI: 10.9738/INTSURG-D-15-00075.1

Protective Effect of Caffeic Acid Phenethyl Ester on Antituberculosis Drug-Induced Hepatotoxicity in Rats

Cigdem Aliosmanoglu[1], Halil Erbiş[2], Ibrahim Aliosmanoglu[2], Mehmet Akif Türkoglu[2], Burak Veli Ulger[3], Ahmet Türkoglu[3], Hatice Yüksel[4]

[1]*State Hospital, Department of Pediatrics, Antalya, Turkey*

[2]*Department of General Surgery, Medical Faculty, Akdeniz University, Antalya, Turkey*

[3]*Department of General Surgery and* [4]*Department of Biochemistry, Medical Faculty, Dicle University, Diyarbakir, Turkey*

Isoniazid and rifampicin are drugs primarily used in antituberculosis treatment. Our aim in this study is to evaluate the effect of caffeic acid phenethyl ester's protective effect on liver function tests and to trace elements in hepatic damage caused by isoniazid and rifampicin on rats. Forty Wistar albino rats were divided into 4 groups. Group 1: Sham, Group 2: caffeic acid phenethyl ester application, Group 3: isoniazid and rifampicin given, Group 4: isoniazid + rifampicin and caffeic acid phenethyl ester application. After 30 days, the rats were sacrificed by taking blood from the heart. Alanine aminotransferase, aspartate aminotransferase, zinc, copper, total antioxidant capacity, total oxidative status, and oxidative stress index levels were evaluated. The rats to which isoniazid + rifampicin+ caffeic acid phenethyl ester were given had less oxidative stress and copper levels ($P < 0.001$, $P = 0.019$) but have higher zinc levels ($P = 0.001$) compared to the isoniazid + rifampicin group. Liver enzyme levels were also lower in rats that were given isoniazid + rifampicin + caffeic acid phenethyl ester ($P < 0.001$). The results of this study suggested that caffeic acid phenethyl ester influences the levels of trace elements (copper and zinc) that are important for the physiologic mechanisms of organisms, reducing liver damage.

Key words: Isoniazid – Rifampicin – Caffeic acid phenethyl ester – Hepatotoxicity

Corresponding author: Cigdem Aliosmanoglu, MD, State Hospital, Department of Pediatrics, 07058, Antalya, Turkey.
Tel.: +90 242 249 61 23; Fax: +90 242 2274444; E-mail: caliosmanoglu@gmail.com

Tuberculosis is a serious disease and is difficult to treat because of its resistance to drugs and long-term treatment period as well as its tendency to increase in diseases, such as HIV, along with patient compliance. Asymptomatic increases in transaminase are common during antituberculosis treatment, but hepatotoxicity can cause mortality when not noticed early and when therapy is not ceased in time. Isoniazid (INH) is a drug that is commonly used in the treatment of active and latent *Mycobacterium tuberculosis* infections. Rifampicin (RIF) is another primary component for the treatment and prophylaxis of tuberculosis. Both drugs are metabolized by the liver.[1] Isoniazid and rifampicin are potentially hepatotoxic drugs.[2] This hepatotoxicity mostly happens through oxidative stress.[3] Caffeic acid phenethyl ester (CAPE) is an active ingredient of honeybee propolis extract that has been used as a traditional medicine in the Far East.[4] The molecular weight and empirical formula of CAPE are 284.3 g/mol and $C_{17}H_{16}O_4$.[5] CAPE has no potentially harmful effects on normal cells.[6] It is a small, flavonoid-like, lipid soluble compound that has anti-inflammatory,[7] antiviral, anticarcinogenic, immunomodulatory,[8] and antioxidant activities.[9] It has been shown that CAPE suppresses lipid peroxidation, inhibits lipoxygenase activities and tumor promotion, lipid peroxidation, lipoxygenase activities, protein tyrosine kinase, and ornithine decarboxylase.[10] Korish *et al*[11] pointed out that CAPE creates hopeful results on correcting acute liver failure by affecting oxidative stress and disordered hemostasis. CAPE can prevent radiation induced liver cell apoptosis.[12] Zinc (Zn) is a trace element with anti-inflammatory and anti-apoptotic effects along with important antioxidant properties. In experimental animal models, although it was not fully clarified, it has been seen that zinc has hepatoprotective properties on acute and chronic liver damage.[13] Copper (Cu) is a trace element, acting as a cofactor in many enzymatic reactions.[14] Excessive copper build up in the liver has been reported in primary biliary cirrhosis, alcoholic cirrhosis, and other cholestatic syndromes.[15,16] All these studies show that extensive research is necessary for recognizing biochemical analysis and for healing liver damage, which can develop during antituberculosis drug treatment, primarily INH and RIF. Tuberculosis disease still remains a major health problem. When patients are having tuberculosis treatment they may be required emergency surgery. Therefore, our aim in this study is to investigate the protective effects of CAPE on liver function tests and trace elements on the hepatic damage caused by INH and RIF on rats.

Materials and Methods

Animals

Forty female Wistar albino rats, each weighing 200 to 250 g were included in the study. The study was conducted in accordance with the rules of the National Institute of Health Guide for the Care and Use of Laboratory Animals, following approval from the Ethics Committee. Rats were housed under standard conditions in an air-conditioned room with 12-hour light and dark cycles and a constant temperature of 22 ± 2°C. The rats were housed in cages and were allowed free access to standard rat chow and water before the experiments. The animals were fasted overnight the day before surgery, but had access to water.

Experimental design

Forty Wistar albino rats were divided into 4 groups (n = 10):

Group 1 (Sham, S): Rats were fed with standard rat chow for 30 days.
Group 2 (CAPE): CAPE was applied daily at a dose of 10 mg/kg intraperitoneally for 30 days.
Group 3 (INH + RIF): INH (30 mg/kg/d) and RIF (30 mg/kg/d) were given via oral gavage for 30 days.
Group 4 (INH+RIF+CAPE): INH (30 mg/kg/day) and RIF (30 mg/kg/day) were given via oral gavage for 30 days and CAPE (10 mg/kg/day) was given intraperitoneally.

After 30 days, the rats were given 50 mg/kg ketamine hydrochloride (Ketalar, Parke Davis, Pfizer, Istanbul, Turkey) and 10 mg/kg Xylazine (Rompun, Bayer AG, Leverkusen, Germany) was injected intramuscularly to facilitate anesthesia. The rats were sacrificed by taking blood from the heart for biochemical analysis.

Biochemical analysis

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), Zn, and Cu were measured to evaluate the liver functions. To assess oxidative injury, the total antioxidant capacity (TAC), total oxidative status (TOS), and oxidative stress index (OSI) levels were determined.

Measurement of ALT, AST

ALT and AST were measured spectrophotometrically in serum using an Architect c16000 auto analyzer (Abbott Laboratories, Abbott Park, Illinois).

Measurement of Zn and Cu in serum

Zn and Cu were determined using Shimadzu 6401S atomic absorption/emission spectrometer. The acetylene flow rate and the burner height were adjusted in order to get the maximum absorbance signal with a slit of 0.5 nm at a wavelength of 213.9 nm for Zn and 324.8 nm for Cu. The radiation sources were hollow cathode lamps (Shimadzu, Japan). Operating conditions were those recommended by the manufacturer (Operation Manual—Atomic Absorption Spectrophotometer AA-6800, SHIMADZU, 2000).

Measurement of total oxidant status

The total oxidant status (TOS) of supernatant fractions was determined using a novel automated measurement method developed by Erel.[17] Oxidants present in the sample oxidize the ferrous ion o-dianisidine complex to ferric ion. The oxidation reaction is increased by glycerol molecules, which are commonly present in the reaction medium. The ferric ion gives a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of nmol H_2O_2 Equiv./mg protein.

Measurement of the total antioxidant capacity

The total antioxidant capacity (TAC) of supernatant fractions was determined using a novel automated measurement method developed by Erel.[17] In this method, hydroxyl radical, which is the most potent biologic radical, is produced. In the assay, ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals, such as the brown-colored dianisidinyl radical cations produced by the hydroxyl radicals are also potent radicals. Using this method, the antioxidative effect of the sample on the potent-free radical reactions initiated by the produced hydroxyl radical is measured. The assay has excellent precision values, which are lower than 3%. The results are expressed as nmol Trolox Equiv./mg protein.

Oxidative stress index

The percent ratio of the TOS level to TAC level was accepted as oxidative stress index (OSI). OSI value was calculated according to the following formula:[18] OSI (Arbitrary Unit) = TOS (nmol H2O2 Equiv./mg protein)/TAC (nmol Trolox Equiv./mg protein).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows 11.5 (SPSS Inc, Chicago, Illinois). Data were expressed as mean ± SD (standard deviation) values for biochemical values. Groups were compared using the nonparametric Kruskal-Wallis test. Mann-Whitney *U* test was used for binary comparisons. A *P*-value of less than 0.05 was considered significant.

Results

All animals survived throughout the experimental procedures. The liver functions, serum Cu and Zn levels of all groups are shown in Table 1. Serum TAC, TOS, and OSI levels are shown in Table 2. Comparisons of the serum Cu and Zn levels between the groups are shown in Fig. 1. Serum ALT, AST, Cu, and TOS levels were found to be significantly higher in Group 3 compared to the other 3 groups ($P < 0.05$). When Group 1 and Group 2 were compared, serum Zn values were found to be higher in Group 2 ($P = 0.029$). There was no difference in the other parameters between the 2 groups ($P > 0.05$). When Group 1 and Group 4 were compared, differences between their TOS and OSI levels were detected ($P < 0.05$). Other parameters were the same ($P > 0.05$). There were significant differences between all parameters when Group 2 and Group 3 were compared ($P < 0.001$). When Group 2 and Group 4 were compared, Zn, TOS, and OSI levels were different ($P < 0.05$). All parameters were significantly different when Group 3 and Group 4 were compared ($P < 0.05$).

Discussion

Antituberculosis drug-induced hepatotoxicity causes substantial morbidity and mortality and decreases treatment effectiveness.[1] Adverse effects significantly contribute to nonadherence, eventually contributing to treatment failure, relapse, or the emergence of drug-resistance. Consequently, they diminish treatment effectiveness.[19,20] Numerous

Table 1 Serum ALT, AST levels (mg/dL) and Zn, Cu levels (μm/protein)

	S (Mean ± SD)	CAPE (Mean ± SD)	INH + RIF (Mean ± SD)	INH + RIF + CAPE (Mean ± SD)
ALT	57.9 ± 14.3	63.4 ± 15.8	189.8 ± 42.6[b,d]	73.7 ± 20.6[f]
AST	94.2 ± 20.9	79.4 ± 13.5	211.6 ± 63.5[b,d]	92.1 ± 30.2[f]
Zn	0.53 ± 0.18	0.73 ± 0.09[a]	0.38 ± 0.13[d]	0.57 ± 0.08[e,f]
Cu	0.88 ± 0.37	0.65 ± 0.27	189.80 ± 42.65[c,d]	73.70 ± 20.60[g]

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cu, copper; SD, standard deviation; Zn, zinc.

[a]Significantly different when compared with Sham group ($P < 0.05$).

[b]Significantly different when compared with Sham group ($P \leq 0.001$).

[c]Significantly different when compared with Sham group ($P < 0.01$).

[d]Significantly different when compared with CAPE group ($P < 0.001$).

[e]Significantly different when compared with CAPE group ($P < 0.01$).

[f]Significantly different when compared with INH + RIF group ($P < 0.001$).

[g]Significantly different when compared with INH + RIF group ($P < 0.05$).

studies have demonstrated that oxidative damage is an important mechanism of antituberculosis drug-induced hepatotoxicity.[21–23] Isoniazid can cause mild to moderate elevation of plasma liver enzyme activity in 10% to 20% of patients and severe hepatotoxicity in approximately 0.5% to 2%[24] of patients. It has been reported that the incidence of hepatotoxicity is approximately 2.6% with isoniazid–rifampin co-administration, but only 1.6% with isoniazid treatment alone and 1.1% with rifampin alone. This suggests that there is higher incidence of severe hepatotoxicity in patients cotreated with these 2 drugs.[25] CAPE is a natural flavonoid-like compound that has protective effects against drug-induced hepatotoxicity.[26] Ashwag *et al* detected that CAPE decreases liver damage by increasing antioxidant capacity in tamoxifen-induced hepatotoxicity.[27] Kart *et al* found in a study that CAPE has a protective property in cisplatin-induced hepatotoxicity and that this was related to its antioxidant property.[28] In our study, when the control group and the INH + RIF group were compared, it was found that in the INH + RIF group, rats had a lower TAC value ($P = 0.001$) and higher OSI and TOS values ($P < 0.001$). Furthermore, when the 2 groups were compared, we saw that the AST and ALT values were higher in the INH + RIF group of rats ($P < 0.001$). These results show that INH and RIF, which have been commonly used for a long time in tuberculosis treatment, can increase liver damage by elevating oxidative stress. In several experimental animal models, it has been shown that changes in the Cu and Zn levels in the liver tissue affect liver damage.[29,30] In addition, it has been shown that increasing Cu and decreasing Zn levels are related to oxidative stress.[31] In our study, we saw that when the group that were only given CAPE and the group given INH + RIF were compared, the serum Zn levels of the CAPE group rats were elevated ($P < 0.001$) and the Cu levels were decreased ($P < 0.001$). By comparing these 2 groups, we determined that the TAC levels were higher ($P < 0.001$) and the TOS

Table 2 Oxidative and antioxidative parameters in groups

	S (Mean ± SD)	CAPE (Mean ± SD)	INH + RIF (Mean ± SD)	INH+RIF + CAPE (Mean ± SD)
TAC (nmol Trolox Eqv./mg protein	1.99 ± 0.56	2.13 ± 0.24	1.21 ± 0.33[a,c]	1.98 ± 0.29[e]
TOS (nmol H_2O_2 Egv./mg protein	33.09 ± 9.01	31.29 ± 6.42	205.90 ± 97.32[a,c]	45.59 ± 10.19[b,d,e]
OSI (arbitrary unite)	17.38 ± 5.20	14.89 ± 3.59	178.26 ± 110.62[a,c]	23.16 ± 4.52[b,c,e]

OSI, oxidative stress index; SD, standard deviation; TAC, total antioxidant capacity; TOS, total oxidative status.

[a]Significantly different when compared with Sham group ($P \leq 0.001$).

[b]Significantly different when compared with Sham group ($P < 0.05$).

[c]Significantly different when compared with CAPE group ($P \leq 0.001$).

[d]Significantly different when compared with CAPE group ($P < 0.01$).

[e]Significantly different when compared with INH + RIF group ($P < 0.001$).

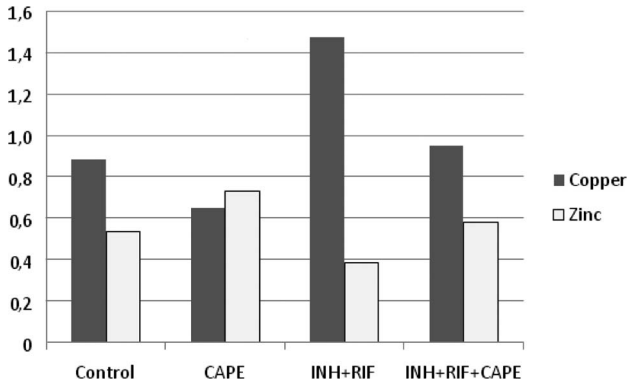

Fig. 1 Comparison of serum Zn and Cu levels between groups.

and OSI levels were lower ($P < 0.001$) in the CAPE group. Furthermore, the AST and ALT levels were significantly higher in the INF + RIF group ($P < 0.001$). These results show that CAPE can affect liver damage. Albukhari *et al*[27] determined in a study that antioxidant enzyme levels were higher and oxidative status was lower in CAPE + tamoxifen rats when compared to tamoxifen only rats; therefore, liver enzyme levels were lower. Kart *et al*[28] compared rats that were given cisplatin with rats that were given CAPE + cisplatin and found that in the CAPE + cisplatin group, liver enzymes were lower. Lee et al[32] compared rats that were given t-BHP to CAPE + t-BHP and saw that the second group had less oxidative stress. Our study also showed elevated Zn levels ($P = 0.001$) and decreased Cu levels ($P = 0.019$) in the INH + RIF + CAPE group when compared to the INH + RIF group. Again, when we compared these 2 groups, we determined that TAC increased in the INH + RIF + CAPE group and the TOS and OSI decreased ($P < 0.001$). Furthermore, in the INH + RIF + CAPE group, the AST and ALT values were lower ($P < 0.001$).

Conclusion

The results of this study showed that INH + RIF, important drugs in antituberculosis treatment, increased oxidative damage and elevated liver enzymes. When CAPE was added to the treatment, Zn levels increased and Cu levels decreased. Furthermore, oxidative stress also decreased. As a result of all this, we detected a significant correction in liver enzymes. As a conclusion, we determined that CAPE decreases liver damage. This situation may be associated with changes in the levels of Zn and Cu. Additional studies are needed to clarify this situation.

Acknowledgments

The authors declared that there were no financial disclosures and no conflicts of interest.

References

1. Tostmann A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. *J Gastroenterol Hepatol* 2008;**23**(2):192–202
2. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. *Lancet* 2003;**362**(9387):887–899
3. Sodhi CP, Rana SV, Mehta SK, Vaiphei K, Attari S, Mehta S. Study of oxidative-stress in isoniazid-rifampicin induced hepatic injury in young rats. *Drug Chem Toxicol* 1997;**20**(3): 255–269
4. Grunberger D, Banerjee R, Eisinger K, Oltz EM, Efros L, Caldwell M *et al*. Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. *Experientia* 1988;**44**(3):230–232
5. Akyol S, Ugurcu V, Altuntas A, Hasgul R, Cakmak O, Akyol O. Caffeic acid phenethyl ester as a protective agent against nephrotoxicity and/or oxidative kidney damage: a detailed systematic review. *ScientificWorldJournal* 2014;**2014**:561971
6. Altuntaş A, Yılmaz HR, Altuntaş A, Uz E, Demir M, Gökçimen A *et al*. Caffeic acid phenethyl ester protects against amphotericin B induced nephrotoxicity in rat model. *Biomed Res Int* 2014;**2014**:702981
7. Chen YJ, Shiao MS, Wang SY. The antioxidant caffeic acid phenethyl ester induces apoptosis associated with selective scavenging of hydrogen peroxide in human leukemic HL-60 cells. *Anticancer Drugs* 2001;**12**(2):143–149
8. Park EH, Kahng JH. Suppressive effects of propolis in rat adjuvant arthritis. *Arch Pharm Res* 1999;**22**(6):554–558
9. Rao CV, Desai D, Kaul B, Amin S, Reddy BS. Effect of caffeic acid esters on carcinogen-induced mutagenicity and human colon adenocarcinoma cell growth. *Chem Biol Interact* 1992; **84**(3):277–290
10. Sırmalı M, Solak O, Tezel C, Sırmalı R, Ginis Z, Atik D *et al*. Comparative analysis of the protective effects of caffeic acid phenethyl ester (CAPE) on pulmonary contusion lung oxidative stress and serum copper and zinc levels in experimental rat model. *Biol Trace Elem Res* 2013;**151**(1):50–58
11. Korish AA. Effect of caffeic acid phenethyl ester on the hemostatic alterations associated with toxic-induced acute liver failure. *Blood Coagul Fibrinolysis* 2010;**21**(2):158–163
12. Chu J, Zhang X, Jin L, Chen J, Du B, Pang Q. Protective effects of caffeic acid phenethyl ester against acute radiation-induced hepatic injury in rats. *Environ Toxicol Pharmacol* 2015;**39**(2):683–689

13. Stamoulis I, Kouraklis G, Theocharis S. Zinc and the liver: an active interaction. *Dig Dis Sci* 2007;**52**(7):1595–1612
14. Kovacic P, Somanathan R. Unifying mechanism for eye toxicity: electron transfer, reactive oxygen species, antioxidant benefits, cell signaling and cell membranes. *Cell Membr Free Radic Res* 2008;**2**(1):56–69
15. Zalewski PD, Truong-Tran AQ, Grosser D, Jayaram L, Murgia C, Ruffin RE. Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets. A review. *Pharmacol Ther* 2005;**105**(2):127–149
16. Aliosmanoglu I, Kapan M, Gul M, Arikanoglu Z, Onder A, Taskesen F *et al*. Effects of erythropoietin on serum and liver tissue levels of copper and zinc in rats with obstructive jaundice. *J Med Biochem* 2012;**32**(1):47–51
17. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005;**38**(12):1103–1111
18. Bolukbas C, Bolukbas FF, Horoz M, Aslan M, Celik H, Erel O. Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. *BMC Infect Dis* 2005;**5**:95
19. Kaona FA, Tuba M, Siziya S, Sikaona L. An assessment of factors contributing to treatment adherence and knowledge of TB transmission among patients on TB treatment. *BMC Public Health* 2004;**4**:68
20. Wares DF, Sing S, Acharya AK, Dangi R. Non-adherence to tuberculosis treatment in the eastern Tarai of Nepal. *Int J Tuberc Lung Dis* 2003;**7**(4):327–335
21. Bhadauria S, Singh G, Sinha N, Srivastava S. Isoniazid induces oxidative stress, mitochondrial dysfunction and apoptosis in Hep G2 cells. *Cell Mol Biol* 2007;**53**(1):102–114
22. Chowdhury A, Santra A, Bhattacharjee K, Ghatak S, Saha DR, Dhali GK. Mitochondrial oxidative stress and permeability transition in Isoniazid and Rifampicin induced liver injury in mice. *J Hepatol* 2006;**45**(1):117–126
23. Attri S, Rana SV, Vaiphei K, Sodhi CP, Katyal R, Goel RC *et al*. Isoniazid- and rifampicin-induced oxidative hepatic injury–protection by N-acetylcysteine. *Hum Exp Toxicol* 2000;**19**(9): 517–522
24. Nolan CM, Goldberg SV, Buskin SE. Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. *JAMA* 1999;**281**(11):1014–1018
25. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. *Chest* 1991;**99**(2): 465–471
26. Lee KJ, Choi JH, Khanal T, Hwang YP, Chung YC, Jeong HG. Protective effect of caffeic acid phenethyl ester against carbon tetrachloride-induced hepatotoxicity in mice. *Toxicology* 2008; **248**(1):18–24
27. Albukhari AA, Gashlan HM, El-Beshbishy HA, Nagy AA, Abdel-Naim AB. Caffeic acid phenethyl ester protects against tamoxifen-induced hepatotoxicity in rats. *Food Chem Toxicol* 2009;**47**(7):1689–1695
28. Kart A, Cigremis Y, Karaman M, Ozen H. Caffeic acid phenethyl ester (CAPE) ameliorates cisplatin-induced hepatotoxicity in rabbit. *Exp Toxicol Pathol* 2010;**62**(1):45–52
29. Bayliss EA, Hambidge KM, Sokol RJ, Stewart B, Lilly JR. Hepatic concentrations of zinc, copper and manganese in infants with extrahepatic biliary atresia. *J Trace Elements Med Biol* 1995;**9**(1):40–43
30. Suzuki K, Oyama R, Hayashi E, Arakawa Y. Liver diseases and essential trace elements. *Nippon Rinsho* 1996;**54**(1):85–92
31. Sirmali M, Uz E, Sirmali R, Kilbaş A, Yilmaz HR, Altuntaş I *et al*. Protective effects of erdosteine and vitamins C and E combination on ischemia–reperfusion-induced lung oxidative stress and plasma copper and zinc levels in a rat hind limb model. *Biol Trace Elem Res* 2007;**118**(1):43–52
32. Lee KJ, Choi JH, Hwang YP, Chung YC, Jeong HG. Protective effect of caffeic acid phenethyl ester on tert-butyl hydroperoxide-induced oxidative hepatotoxicity and DNA damage. *Food Chem Toxicol* 2008;**46**(7):2445–2450