Food Sci. Biotechnol. Vol. 15, No. 4, pp. 612 ~ 616 (2006)

Food Science and Biotechnology

Water Extract of Ash Tree (*Fraxinus rhynchophylla*) Leaves Protects against Paracetamol-Induced Oxidative Damages in Mice

Jeong-Ryae Jeon

Department of Food and Nutrition, Obesity-Diabetes Advanced Research Center, College of Medicine, Yeungnam University, Daegu 705-717, Korea

Abstract The protective effect of water extract of ash tree leaves (ALE) against oxidative damages was investigated in paracetamol-induced BALB/c mice. Biochemical analysis of anti-oxidative enzymes, immunoblot analyses of hepatic cytochrome P450 2E1 (CYP2E1), and the gene expression of tumor necrosis factor (TNF-α) were examined to determine the extract's protective effect and its possible mechanisms. BALB/c mice were divided into three groups: normal, paracetamol-administered, and ALE-pretreated groups. A single dose of paracetamol led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA). This was associated with a significant reduction in the hepatic antioxidant system, e.g., glutathione (GSH). Paracetamol administration also significantly elevated the expression of CYP2E1, according to immunoblot analysis, and of TNF-α mRNA in liver. However, ALE pretreatment prior to the administration of paracetamol significantly decreased hepatic MDA levels. ALE restored hepatic glutathione and catalase levels and suppressed the expression of CYP2E1 and TNF-α observed in inflammatory tissues. Moreover, ALE restored mitochondrial ATP content depleted by the drug administration. These results show that the extract of ash tree leaves protects against paracetamol-induced oxidative damages by blocking oxidative stress and CYP2E1-mediated paracetamol bioactivation.

Keywords: paracetamol, ash tree leaves, cytochrome P450 2E1, TNF-α, ATP

Introduction

Living organisms are continuously exposed to oxidative damage from environmental factors such as ozone, nitrogen oxide, and xenobiotics, as well as by oxygen radicals produced endogenously via normal mechanisms (1). One proposed mechanism for the toxicity of various xenobiotics, such as paracetamol, carbon tetrachloride, adriamycin, and alcohol involves the free radical-mediated oxidation of biomolecules (2).

Paracetamol (*n*-acetyl-*p*-aminophenol), a widely used analgesic and antipyretic drug, is known to cause hepatotoxicity in experimental animals and humans at high doses (3-5). Following an oral therapeutic dose, a fraction of paracetamol is converted, via the cytochrome P-450 pathway, to a highly toxic metabolite, *n*-acetyl-*p*-benzo-quinone-imine (NAPQI) (6, 7); this metabolite is normally conjugated with glutathione (GSH) and then excreted in the urine. Overdoses of paracetamol deplete GSH stores, leading to accumulation of NAPQI (6), mitochondrial dysfunction (7, 8) and the development of acute hepatic necrosis (7, 8). Also, depletion of GSH enhances the expression of tumor necrosis factor alpha (TNF-α) (9). GSH significantly contributes to the intracellular antioxidant defense system, as it is a powerful consumer of superoxide, singlet oxygen, and hydroxyl radicals (5, 10). The breakdown of the GSH-dependent antioxidant defense system increases the intracellular flux of oxygen-free radicals, creating oxidative stress, and initiating apoptosis (5, 7, 9).

There is an increasing interest in medicinal plants, as they could potentially suppress oxidative damage to tissues by stimulating the natural defense system. Antioxidants of natural origin can serve as chemopreventive agents, ameliorating the toxicity caused by certain drugs and environmental chemicals or in disease states involving oxidative stress.

The stem bark of ash tree (*Fraxinus rhynchophylla*), a Chinese herbal drug, has traditionally been used to treat intense diarrhea and dysentery, especially dysentery with bloody stools, and to treat lung/heart syndrome with cough and dyspnea (11, 12). Although these plants have been used and studied for decades, little is known about the pharmacological effects or active components of its leaves. We have recently commenced developing medicinal uses for these plant leaves, and have found that these leaves have a protective effect against paracetamol-induced hepatotoxicity in a mouse model (3).

In this study, two major possibilities were examined to evaluate the antioxidant activity of ash tree leaf extract against paracetamol-induced oxidative damages: [1] whether leaves protect against paracetamol-induced oxidative damages by enhancing cellular defense mechanisms, [2] whether the plant leaves decrease the bio-activation of paracetamol by suppressing P450 enzymes. Thus, to evaluate the antioxidant effects of the plant leaves, the present study monitored parameters such as GSH, lipid peroxidation, cytochrome P450 2E1, TNF-α, hepatorenal antioxidant enzymes, and ATP content.

Materials and Methods

Preparation of ash tree leaf extract Ash tree leaves were collected from Forestry of Daegu City, Korea in May 2004, and a voucher specimen was deposited in the College of Medicine, Yeungnam University (Ash leaf

*Corresponding author: Tel: 82-53-620-4335; Fax: 82-53-651-3651
E-mail: jeonjr@yumail.ac.kr
Received June 2, 2006; accepted July 18, 2006

101). The extraction of the leaves was performed according to Jeon and Park's method (13), and freeze-dried ash tree leaf extract (ALE) was stored at 4°C until use.

Animal care and induction of hepatotoxicity by paracetamol Specific pathogen-free male BALB/c mice (6-8 weeks of age) were purchased from Hyochang Science (Umsung, Korea) and used in this experiment. After an adaptation period of one week, the mice were randomly divided into three groups: normal group (Normal), paracetamol-treated group (Paracetamol), and ALE pre-treated paracetamol group (ALE+paracetamol). All of the animals were fed standard mouse chow (Sam Yang Food Co., Wonju, Korea) and had free access to water. Experimental oxidative damage was induced by intraperitoneal injection of paracetamol at a dosage of 600 mg/kg body weight. The ALE and paracetamol (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in warm phosphate buffered saline. The ALE was administered orally at 300 mg/kg once daily for 7 consecutive days. On the seventh day, three hours after treatment with ALE, mice were injected intraperitoneally with paracetamol solution. Animals in the normal group were given only vehicle. Paracetamol-induced oxidative damages were determined 24 hr after paracetamol-induction, and mice were fasted 15 hr before sacrificing. Animals were anesthetized with pentothal sodium (40 mg/kg). The care of animals followed NIH guidelines for the care and the use of laboratory animals.

Biochemical assay Blood was collected from the abdominal aorta of each mouse with a heparinized syringe. The blood was centrifuged at 5,000×*g* for 5 min at 4°C to separate the plasma, and hepatic enzyme activities were analyzed by commercial kits (Asan Pharm Co., Seoul, Korea). Liver tissues were quickly excised, and portions of the liver sections were homogenized in cold 0.25 M sucrose buffer / 0.2 M EDTA (in four volumes of ice-cold 20 mM Tris-HCl buffer, pH 7.4, containing 0.15 M KCl using a Potter-Elvehjem homogenizer with a Teflon pestle); the remaining portions of the livers were stored at -80°C for RNA analysis. Mitochondrial and cytosolic fractions were prepared from liver homogenates by differential centrifugation. Protein concentrations were determined by the Bradford assay (14). The glutathione level was assayed in the liver homogenate by the method of Beutler (15) with Ellman's reagent. Lipid peroxidation (LPO) in the homogenate was ascertained by the formation of malondialdehyde (MDA) and measured by the thio-barbituric acid-reactive method (16). Catalase (CAT) activity was determined according to the method of Bergmyer (17). Superoxide dismutase (SOD) activity was determined by the method of Martin and Bendich (18). Inhibition of spontaneous hematoxylin oxidation was determined as a measure of SOD activity. For calculations, a standard curve of SOD activity was generated and used.

Cytochrome P450 2E1 immunoblot assay A SDS-PAGE analysis was performed according to the method of Laemmli (19) using the Bio-Rad Mini-protean apparatus (Bio-Rad Lab, Hercules, CA, USA). In brief, microsomal proteins were separated by 10% SDS-PAGE, and electrophoretically transferred to Bio-Rad nitrocellulose membrane. The membrane was then immunoblotted with rabbit anti-human/rat cytochrome P450 enzyme (CYP2E1) polyclonal antibody (Chemicon Inc., Tamecula, CA, USA) or polyclonal rabbit anti-β-actin. Blotting-grade horse radish peroxidase-conjugated goat anti-rabbit IgG (H&L, Bio-Rad Lab.) and goat polyclonal anti-rabbit IgG (H&L, Bio-Rad Lab.) were used as the secondary antibodies. The CYP2E1 and β-actin bands were visualized by development with the ECL-Plus detection reagent (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA), and the intensities of the bands were measured using an image documentation system (Image Master; Amersham Pharmacia Biotech. Inc.).

Total RNA Extraction and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis Total RNA was isolated from liver tissue using TRI Reagent (Sigma Chemical Co.). The RNA concentration was quantitated by measuring the absorbance at 260 and 280 nm. The RT reaction was performed using a RT-PCR kit (Qiagen, Hilden, Germany) according to the protocol provided by the manufacturer. One μg of total RNA was reverse transcribed into cDNA using Omniscript RT, Sensiscript RT, and primers. The sense primer sequence for TNF-α was TGGCCCAGACCCTCACACTC and the antisense primer sequence for TNF-α was CTCCTGGTA TGAAATGGCAAATC. β-Actin was used as an internal standard (sense: TCTACAATGAGCTGCGTGTG; antisense: GGTCAGGATCTTCATGAGGT). The amplification was initiated at 50°C for 30 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at the appropriate primer-pair annealing temperature for 1 min and extension at 72°C for 1 min, then a final extension step of 10 min at 72°C. The RT-PCR products were electrophoresed on 1-1.5% agarose gels and visualized by ethidium bromide staining. The intensities of the bands were measured with the Image Master documentation system (Amersham Pharmacia Biotech. Inc.).

Measurement of ATP content The Enliten™ ATP Assay system with bioluminescence detection kit (Promega, Madison, WI, USA) was used to measure ATP content. In this assay, luciferase from *Photinus pyralis* catalyzes the conversion of D-luciferin in the presence of ATP and oxygen to oxyluciferin, P_i, AMP, carbon dioxide, and light. As described above, liver tissue was homogenized in the presence of 500 μL pre-cooled $HClO_4$ (10%). The homogenate was centrifuged at 4,500×*g* for 10 min at 4°C. The supernatant (500 μL) was neutralized with 200 μL of 2.5 M KOH, and the precipitate was removed by centrifugation at 4,500×*g* for 5 min. An aliquot from the supernatant stored at -80°C until assay. The aliquot was then diluted 5-40 fold with 0.1 M Tris-EDTA (pH 7.75), and 100 μL of this diluted extract were used for the ATP assay. For the ATP analysis, 10 μL of sample extract or standard solution were placed in a 1.5-mL tube, followed by the addition of 50 μL of the ATP assay mix. The luminescence signal was measured in a luminometer set with a lag time of 1 sec and an integration time of 5 sec, as described by the supplier. Light photons were measured by a luminometer (TD 20/20; Turner BioSystems, Sunnyvale,

CA, USA) and compared to an ATP standard curve to calculate ATP concentration. ATP content is expressed as mol per mg protein for tissue samples, mol per 5 mg tissue for section punches (20).

Statistical analysis of data All results were expressed as mean ± SEM. For multiple comparisons, one-way analysis of variance (ANOVA) was used. When ANOVA showed significant differences, post-hoc analysis was performed with the Newman-Keuls multiple range test by SPSS.

Results and Dissicusion

GSH and MDA levels A single administration of paracetamol depleted hepatic GSH content by 61% compared to the control group. However, pretreatment of mice with ALE caused a 2-fold elevation in hepatic GSH levels over that of paracetamol alone (Table 1). Paracetamol also caused a marked increase (2.2-fold) in the formation of hepatic MDA, as assayed to examine paracetamol-induced lipid peroxidation (Table 1). Pretreatment with ALE attenuated the elevated MDA formation by 70% of paracetamol level ($p<0.05$).

Antioxidant enzyme activities Catalase (CAT) activity was significantly lower (by 45%) in the paracetamol-treated mice than in the control mice (Table 2). Pretreatment with ALE attenuated the decrease in the CAT activity by 17%. Superoxide dismutase (SOD) activity was not significantly decreased by paracetamol administration alone, but the activity was increased by ALE pretreatment once daily for 7 days prior to the administration of paracetamol; however, no significant differences were observed between the paracetamol group and the ALE+ paracetamol group (Table 2).

Table 1. Effect of ash tree leaf extract on glutathione and lipid peroxidation following paracetamol-induced oxidative damages in mice

Group[1)]	GSH	LPO
	μmol/g of liver tissue	(MDA, nmol/g of liver tissue)
Normal	3.18±0.06[2)]	12.61±0.40
Paracetamol	1.23±0.63*	27.29±0.90*
ALE+paracetamol	2.49±0.17**	19.29±0.80**

[1)]Normal: Normal+PBS, Paracetamol: paracetamol+PBS, ALE+paracetamol: ALE-pretreatment group (ALE administered once daily for 7 days prior to paracetamol administration.). Oxidative damages were determined 24 hr after paracetamol-induction.
[2)]Mean±SE (n=7), *$p<0.05$ vs. Normal, **$p<0.05$ vs. Paracetamol.

Table 2. Effect of ash tree leaf extract on antioxidant enzyme activities following paracetamol-induced oxidative damages in mice

Group[1)]	Catalase	SOD
	reduced H_2O_2 nmol/min/mg protein	unit/mg protein
Normal	127.16±2.61[2)]	17.77±0.30
Paracetamol	70.47±1.50*	16.94±0.39
ALE+paracetamol	82.31±0.84**	17.31±0.16

[1)]Normal: normal+PBS, Paracetamol: paracetamol+PBS, ALE+paracetamol: ALE-pretreatment group (ALE administered once daily for 7 days prior to paracetamol administration). Oxidative damages were determined 24 hr after paracetamol-induction.
[2)]Means±SE (n=7), *$p<0.05$ vs. Normal, **$p<0.05$ vs. paracetamol.

Immunoblot analysis of CYP2E1 Immunoblot analysis of hepatic microsomal CYP2E1, which mediates bioactivation of paracetamol to NAPQI, was used to examine the hepatoprotective effect of ALE associated with CYP2E1 inhibition. The expression of hepatic microsomal CYP2E1 significantly increased following a single administration of paracetamol at a dose of 600 mg/kg (Fig. 1). However, CYP2E1 expression was suppressed by ALE pretreatment for 7 days before paracetamol administration.

TNF-α mRNA expression in liver homogenate TNF-α is a cytokine that is rapidly produced in response to tissue injury and directly induces liver cytotoxicity. To investigate the protective effect of ALE on paracetamol intoxication, TNF-α mRNA expression in liver homogenate was analyzed. As shown in Fig. 2, paracetamol significantly increased hepatic TNF-α mRNA expression, but ALE pretreatment attenuated the expression.

Mitochondrial ATP content Depletion of mitochondrial ATP, which is likely the most critical event for paracetamol toxicity, was observed in the paracetamol-injected mice and was also partially prevented in the mice pretreated with ALE for 7 days (Fig. 3).

Many recent studies indicate that, in most plants, leaf extracts have the most potent antioxidant agents, thereby raising interest in these extracts world wide. The stem bark of ash tree was commonly used to treat intense diarrhea and dysentery in China. However, unlike the bark of the plant, there is little known about the physiological

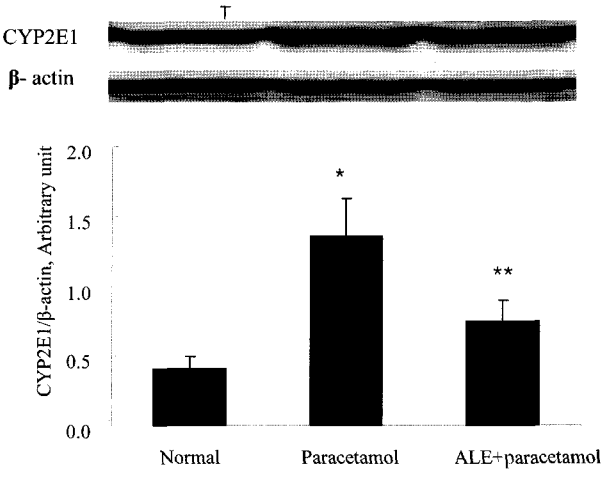

Fig. 1. Immunoblot analysis of cytochrome P450 2E1. Normal, normal+PBS; Paracetamol, paracetamol+PBS; ALE+paracetamol, ALE-pretreatment group (ALE administered once daily for 7 days prior to paracetamol administration). *$p<0.05$ vs. Normal, **$p<0.05$ vs. Paracetamol.

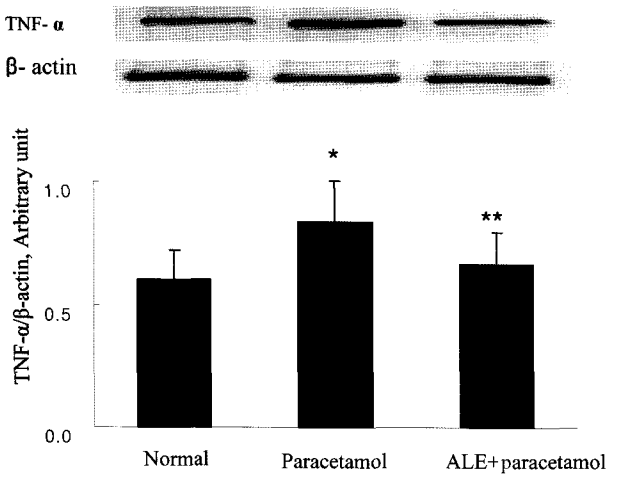

Fig. 2. Effect of ash tree leaf extract on TNF-α mRNA expression of paracetamol-induced mice. Normal, normal+PBS; Paracetamol, paracetamol+PBS; ALE+ paracetamol, ALE-pretreatment group (ALE administered once daily for 7 days prior to paracetamol administration). $^{*}p<0.05$ vs. Normal, $^{**}p<0.05$ vs. Paracetamol.

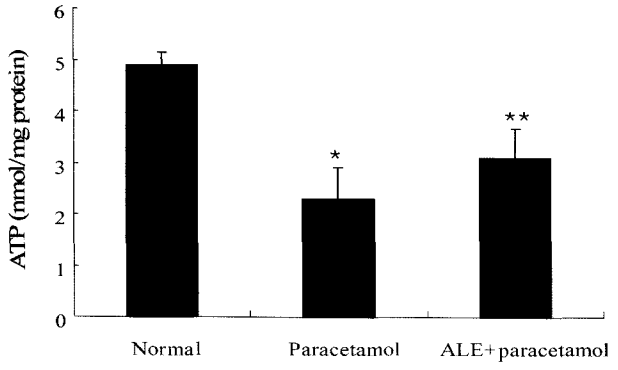

Fig. 3. Mitochondrial ATP content. Normal, normal+PBS; Paracetamol, paracetamol+PBS; ALE+paracetamol, ALE-pretreat-ment group (ALE administered once daily for 7 days prior to paracetamol administration). $^{*}p<0.05$ vs. Normal, $^{**}p<0.05$ vs. Paracetamol.

properties of the plant leaves. Accordingly, the present study was performed to investigate the protective effect, as well as possible mechanisms, of the leaf extract against paracetamol-induced oxidative stress.

Glutathione (GSH) is known to protect liver cells against oxidative stress through non-enzymatic and enzymatic reactions (5, 6, 8). As may be expected, a single paracetamol administration caused remarkable depletion of cellular GSH in mice. This may be related to its direct conjugation with the paracetamol metabolite, NAPQI, and/or to paracetamol-induced lipid peroxidation, as evidenced by elevated MDA levels in liver homogenate (8). In our study, a substantial increase in hepatic lipid peroxidation by paracetamol, and its suppression to nearly normal levels by ALE pretreatment, supports the view that the plant leaf prevents paracetamol-induced depletion of GSH.

The depletion of cellular GSH leaves the cell particularly vulnerable to oxidative insults following the administration of paracetamol. Some endogenous protec-tive factors, such as CAT and SOD, play important roles in the elimination of ROS derived from the redox process of xenobiotics in liver tissues (18). In our study, paracetamol administration caused a decrease in the activity of antioxidant enzymes; however, this decrease was not significant for SOD. Free radicals generated by paracetamol may inhibit antioxidant enzymes, which is consistent with the theory that oxidative stress is a mechanism for paracetamol toxicity. Pretreatment with ALE restored the activity of the CAT in paracetamol-induced mice, thus, it enhanced, in part, the antioxidant defense system impaired by paracetamol. Many studies have reported the antioxidant activity of medicinal plants, such as the effects of Moutan cortex (21) and *Ambrosia maritima* (22), against paracetamol-induced toxicity in rats.

Another mechanism by which paracetamol produces liver injury involves its biotransformation to a more toxic chemical, NAPQI, by cytochrome P450 enzymes. It is evident from experiments using CYP2E1 knockout mice (7) and a variety of CYP2E1 inhibitors (23) that CYP2E1 is the most important cytochrome P450 enzyme in the bioactivation of paracetamol to NAPQI. Our results are in agreement with this theory; a single dose of paracetamol increased the expression of hepatic microsomal CYP2E1 protein. In the presence of ash tree leaves, the transformation of paracetamol to its reactive metabolite could be inhibited. This inhibition would offer protection against paracetamol-induced hepatotoxicity by inducing an increase in the intracellular concentration of nonmetabolized paracetamol, thus preventing GSH consumption required by NAPQI (21).

Both clinical and experimental studies have shown that any noxious event is perceived by tissue macrophages and monocytes, which in turn secrete cytokines and proinflammatory mediators such as TNF-α, complement factors, and also the anti-inflammatory cytokine, interleukin-10 (9, 24). TNF-α is a unique among cytokines in that it can also directly induce cytotoxicity and has been implicated in apoptosis (9). As evidenced in the present study, the administration of paracetamol resulted in increased expression of hepatic TNF-α, indicating a role for this cytokine in this toxicity. On the other hand, ALE depressed the TNF-α response. Thus, it seems likely that the alleviation of paracetamol-induced oxidative tissue damage by ALE involves the suppression of a variety of pro-inflammatory mediators produced by leukocytes and macrophages (25).

In this study, ATP content was measured to estimate mitochondrial function. Because various mitochondria dysfunctions have been observed with paracetamol toxicity, including inhibition of respiration, a decrease in hepatic ATP levels, a decrease in membrane potential, and a loss of mitochondrial Ca^{2+} (21), mitochondria are predicted to be target organelles for oxygen radicals (5). Indeed, GSH deficiency leads to mitochondrial damage in hepatic tissue (5, 7, 20). In the present study, hepatic ATP was significantly reduced 24 hr after paracetamol administration. This reduction of ATP was restored by ALE. These results indicate that ALE restores the function of mitochondria, which, when exposed to paracetamol, partially mediate the induced oxidative stress.

In conclusion, the protective effect of ash tree leaves against paracetamol-induced oxidative damages in mice

appears to be related to the inhibition of lipid peroxidative processes and to the prevention of GSH depletion. In addition, ash tree leaves restore ATP content and may inhibit hepatic TNF-mRNA expression and CYP2E1 protein expression in paracetamol-induced cell damage, thereby alleviating hepatic injury by reducing the production of the active metabolite, *n*-acetyl-*p*-benzoquinone-imine.

References

1. Williams AT, Burk RF. Carbon tetrachloride hepatotoxicity: An example of free radical-mediated injury. Semin. Liver Dis. 10: 279-284 (1990)
2. Amdur MO, Doull J, Klassen CD. Toxicology. 4th ed. Pergamon Press Inc., New York, NY, USA p.338 (1991)
3. Jeon JR, Choi JH. Effect of ash tree leaf extract on acetaminophen-induced hepatotoxicity in mice. Food Sci. Biotechnol. 15: in press (2006)
4. Jaeschke H, Cover C, Bajt ML. Role of caspases in acetaminophen-induced liver injury. Life Sci. 78: 1670-1676 (2006)
5. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J. Pharmacol. Exp. Ther. 187: 211-217 (1973)
6. Dahlin DC, Miwa GT, Lu AY, Nelson SD. N-acetyl-p-benzoquinoneimine: a cytochrome P-450-mediated oxidation product of acetaminophen. P. Natl. Acad. Sci. USA 81: 1327-1331 (1984)
7. Lee SS, Buters JT, Pineau T, Fernandez-Salguero P, Gonzalez FJ. Fernandez-Salguero, P. Role of CYP2E1 in the hepatotoxicity of acetaminophen. J. Biol. Chem. 271: 12063-12067 (1996)
8. Gibson JD, Pumford NR, Samokyszyn VM, Hinson JA. Mechanism of acetaminophen-induced hepatotoxicity: covalent binding versus oxidative stress. Chem. Res. Toxicol. 9: 580-585 (1996)
9. Agarwal S, Piersco NP. Poly ADP-ribosylation of a 90-kDa protein is involved in TNF-alpha-mediated cytotoxicity. J. Immunol. 15: 473-481 (1994)
10. Miesel R, Zuber M. Copper-dependent antioxidant defenses in inflammatory and autoimmune rheumatic diseases. Inflammation 17: 283-294 (1993)
11. Hu SG, Li L, He XW. Solid-phase extraction of esculetin from the ash bark of Chinese traditional medicine by using molecularly imprinted polymers. J. Chromatogr. A 1062: 31-37 (2005)
12. Tsai JC, Tsai S, Chang WC. Effect of ethanol extracts of three Chinese medicinal plants with anti-diarrheal properties on ion transport of the rat intestinal epithelia. J. Pharm. Sci. 94: 60-66 (2004)
13. Jeon JR, Park JR. Effects of *Chrysanthemum boreale* M. water extract on serum liver enzyme activities and Kupffer cells of carbon tetrachloride-induced rats. Food Sci. Biotechnol. 14: 290-296 (2005)
14. Bradford, MM. A rapid and sensitive method for the determination of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254 (1976)
15. Beutler EA. Glutathione in red blood cell metabolism. Manual of biochemical methods. Grune & Stratton. New York, NY, USA. pp. 112-114 (1975)
16. Ohakawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358 (1979)
17. Bergmyer HU. Method of enzymatic analysis. Academic Press, Inc. New York, NY, USA, pp. 865-872 (1974)
18. Machlin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB J. 1: 441-445 (1987)
19. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685 (1970)
20. Masubuchi Y, Suda C, Horie T. Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. J. Hepatol. 42: 110-116 (2005)
21. Shon YH, Nam KS. Protective effect of Moutan cortex extract on acetaminophen-induced hepatotoxicity in mice. J. Ethnopharmacol. 90: 415-419 (2004)
22. Ahmed MB, Khater MR. Evaluation of the protective potential of *Ambrosia maritima* extract on acetaminophen -induced liver damage. J. Ethnopharmacol. 75: 169–174 (2001)
23. Kim ND, Kwak MK, Kim SG. Inhibition of cytochrome P450 2E1 expression by 2-(allylthio) pyrazine, a potential chemoprotective agent: hepatoprotective effects. Biochem. Pharmacol. 53: 261-269 (1997)
24. Dambach DM, Durham SK, Laskin JD, Laskin DL. Distinct roles of NF-kappa B p50 in the regulation of acetaminophen-induced inflammatory mediator production and hepatotoxicity. Toxicol. Appl. Pharm. 211: 157-165 (2006)
25. Bohlinger I, Leist M, Barsig J, Uhlig S, Tiegs G, Wendel A. Interleukin-1 and nitric oxide protect against tumor necrosis factor alpha-induced liver injury through distinct pathways. Hepatology 22: 1829-1837 (1995)