Alaternin and Emodin with Hydroxyl Radical Inhibitory and/or Scavenging Activities and Hepatoprotective Activity on Tacrine-Induced Cytotoxicity in HepG2 Cells

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The antioxidative and hepatoprotective potentials of two anthraquinones, alaternin (2-hydroxy-emodin) and emodin, to scavenge and/or inhibit hydroxyl radicals generated by the Fenton reaction and to protect tacrine-induced cytotoxicity in human liver derived HepG2 cells were evaluated, respectively. The inhibitory activity on hydroxyl radical generated in a cell-free chemical system ($FeSO_4/H_2O_2$) was investigated by a fluorescence spectrophotometer using a highly fluorescent probe, 2',7'-dichlorofluorescein. The hydroxyl radical scavenging activity was determined by electron spin resonance spectroscopy using 5,5-dimethy-1-pyrroline-N-oxide as hydroxyl radicals trapping agents. Tacrine-induced HepG2 cell toxicity was determined by a 3-[4,5-dimethylthiazole-2yl]-2,5-diphenyltertrazolium bromide assay. Although the scavenging activity of alaternin on hydroxyl radical was similar to that of emodin in dose-dependent patterns, the inhibitory activity exhibited by the former on hydroxyl radical generation was stronger than that of the latter, with IC_{50} values of 3.05 ± 0.26 μM and 13.29 ± 3.20 μM, respectively. In addition, the two anthraquinones, alaternin and emodin showed their hepatoprotective activities on tacrine-induced cytotoxicity, and the EC_{50} values were 4.02 μM and 2.37 μM, respectively. Silymarin, an antihepatotoxic agent used as a positive control exhibited the EC_{50} value of 2.00 μM. These results demonstrated that both alaternin and emodin had the simultaneous antioxidant and hepatoprotective activities.

Key words: Alaternin, Emodin, Anthraquinone, Cassia tora L., Hydroxyl radical, Hepatoprotective activity, Tacrine, ESR spectroscopy, DCF

INTRODUCTION

Like other free radicals and reactive oxygen species (ROS), the short-lived hydroxyl radical (·OH, ~10^{-9} sec) is generated in the body. This highly reactive oxygen-centered radical is assumed to attack cellular molecules including hepatic tissue, because it is located less than a few nanometers from the site of their generation (Hipeli and Elstner, 1997). For experimental purposes, ·OH was produced by the reaction of transition metal ions including

iron and copper with H_2O_2, ultraviolet (UV) photolysis, and the electrocatalysis of H_2O_2, namely photo-Fenton and electro-Fenton reactions, respectively (Halliwell and Gutteridge, 1992, 1986; Ueda et al., 1996; Moraes et al., 2004; Oturan et al., 2000).

In the present work, we focused on the highly toxic ·OH to evaluate its antioxidative activities. There were two antioxidative experiments using ·OH, one was to inhibit the generation of ·OH, and the other was to scavenge ·OH. The inhibitory activities of alaternin (1,2,3,8-tetrahydroxy-6-methyl-9,10-anthraquinone) and emodin (1,3,8-trihydroxy-6-methyl-9,10-anthraquinone) on ·OH was measured with a highly fluorescent probe, 2',7'-dichloro-fluorescein (DCF) method, whereas the scavenging activities was monitored by an electron spin resonance

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(ESR)-spin trapping (Ueda *et al.*, 1996).

There have been several studies dealing with the simultaneous antioxidative and hepatoprotective activities of natural products. In order to evaluate the protective activities regarding hepatotoxicity, several hepatotoxic model systems have been used. Cytotoxins such as carbon tetrachroride (CCl_4), acetaminophen, thioacetamide, tacrine, rubratoxin B (Nagashima *et al.*, 2001), and H_2O_2 (Yang *et al.*, 1999), along with lipopolysaccharide/D-galactosamine (Morita *et al.*, 2003) have been investigated. Several studies have shown that hepatotoxins may be involved in the metabolism of cytochrome P450. Furthermore, they are suspected of producing the reactive toxic metabolites (Wang *et al.*, 2004; Asha *et al.*, 2004; Bruck *et al.*, 1999). Tacrine (1,2,3,4-tetrahydro-9-aminoacridine hydrochloride) is an inhibitor of acetylcholinesterase, which plays a role in the mediation of nervous transmission and has been the focal point for the treatment of Alzheimer's disease, myasthenia gravis and glaucoma as well as the recovery of damage due to exposure to nerve agents, in the pharmaceutical industry (Doucet-Personeni *et al.*, 2001). Although, tacrine was the first drug approved in the United States for the treatment of the Alzheimer's disease, reversible hepatotoxicity, together with inclemental serum glutamate pyruvate transaminase activities, occurred in 30~50% of patients who took the medicine for a long time in therapeutic doses (Pan *et al.*, 2002; Watkins *et al.*, 1994). Furthermore, tacrine, like other hepatotoxins is thought to participate in the generation of ROS in HepG2 cells, a human liver cell line (Osseni *et al.*, 1999). Many related studies showed that sesquiterpenes, coumarins, diarylheptanoids and phenolic compounds exhibited hepatoprotective activities on tacrine-induced cytotoxicity of HepG2 cells (Song *et al.*, 2003, 2001; Oh *et al.*, 2002a; 2002b; Cho *et al.*, 2001).

Despite the important number of past investigations, the relationships between antioxidative and hepatoprotective capacities remained obscure. Consequently, many researchers have attempted to find the mechanisms that govern these two activities and the correlations between them.

The present study dealed with the ·OH scavenging/inhibitory activities and hepatoprotective activities of the two anthraquinones, emodin and alaternin. Moreover, the investigation try to elucidate the relationships between those activities.

MATERIALS AND METHODS

Chemicals

Analytic grade hydrogen peroxide (H_2O_2), diethyltri-aminopenta acetic acid (DTPA), spin trap 5,5-dimethy-1-pyrroline-*N*-oxide (DMPO), L-ascorbic acid, silymarin, 3-[4,5-dimethylthiazole-2yl]-2,5-diphenyltertrazolium bromide

(MTT) and tacrine were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). 2',7'-Dichlorofluorescin diacetate (DCFH-DA) and dimethyl sulfoxide (DMSO) were of high quality and purchased from Molecular Probes (Eugene, OR, USA) and Aldrich (Milwaukee, WI, USA), respectively.

Isolation of alaternin and emodin

Alaternin and emodin were isolated from *C. tora* L. as mentioned previously (Choi *et al.*, 1994) and its structure was identified by comparison with reported data (Choi *et al.*, 1994). The chemical structures of alaternin and emodin are shown in Fig. 1.

Inhibition of the ·OH generation by the DCF experiment

Two anthraquinones, alaternin and emodin, along with a positive control, L-ascorbic acid (1, 5, 10, 20 and 40 μM) were added to 1 mM H_2O_2, and 0.2 mM $FeSO_4$, and incubated at 37°C for 5 min. Then esterase-treated 2 μM DCFH-DA was added. DCFH-DA is a stable compound that easily diffuses into cells. It is chemically hydrolyzed not only at basic pH but also by intracellular or exogenous esterase to yield the reduced non-fluorescent compound of 2',7'-dichlorofluorescin (DCFH), which is trapped within cells. The ·OH obtained by Fenton reaction oxidized DCFH to a highly fluorescent dye, DCF. The fluorescence intensity of the oxidized DCF generated from reduced DCFH was monitored on a microplate fluorescence spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT, USA), with an excitation wavelength of 460 nm and the emission wavelength at 530 nm (Shao *et al.*, 2004; Komarov *et al.*, 2004; Rota *et al.*, 1999; Lebel and Bondy, 1990). The inhibitory activity due to ·OH expresses the 50% inhibition concentration (IC_{50}: μM) of ·OH generation in 1.0 mM H_2O_2 and 0.2 mM $FeSO_4$.

Scavenging activity due to the hydroxyl radical during the ESR experiment

The ·OH reacts rapidly with the nitrone spin trap DMPO; the resultant DMPO-OH adduct can be detected by use of ESR spectroscopy. The spectra were recorded 5 min and 10 min after samples (30 μL) were mixed in phosphate buffer (pH 7.2) with H_2O_2 (1 mM, 75 μL), $FeSO_4$ (0.2 mM,
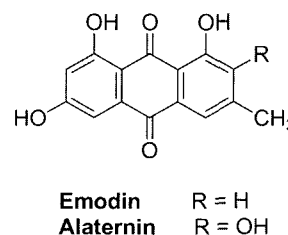

Emodin R = H
Alaternin R = OH

Fig. 1. Structures of emodin and alaternin

37.5 µL), DTPA (1 mM, 37.5 µL), and DMPO (0.3 M, 20 µL). The ESR experiments were carried out on a JEOL FE-3X spectrometer (JEOL Serving Advanced Technology, Tokyo, Japan) with an X-band microwave unit. The parameters of the ESR spectrometer were set at the following conditions: magnetic field, 336.5 mT; power, 1.00 mW; frequency, 9.4380 GHz; modulation amplitude, 0.16 mT; gain, 200; scan time, 0.5 min; scan width, 5.0 mT; time constant, 0.03 s; temperature 25°C. The relative ESR signal intensity was calculated as reported in the literature (Rimbach et al., 2004; Li et al., 2004; Yoshimura et al., 1999):

Relative ESR signal intensity of spin adducts = ΔH_S (sample)/ΔH_C (control).

ΔH_S (sample) = signal height of sample/signal height of no sample added (Mn^{2+}).

ΔH_C(control) = signal height of control (DMSO)/signal height of no sample added (Mn^{2+}).

Hepatoprotective activity on tacrine-induced toxicity by the MTT assay

Human liver derived HepG2 cells from American Type Culture Collection were maintained at 2×10^5 cells/well in a complete medium consisting of RPMI supplemented with 10% heat-inactivated FBS, penicillin G (100 IU/mL), streptomycin (100 µg/mL). They were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air (Yang et al., 1999). The Tacrine-mediated toxicity was evaluated with the MTT assay as previous described (Benoit et al., 1997; Galm et al., 2002). Cytotoxicity was assessed after the 3 h incubation period in the corresponding medium containing 1 mM of tacrine or not (control). Five concentrations (0.1, 1, 10, 50, and 100 µg/mL) were tested for each sample, and each experiment was triplicated. The results expressed the EC_{50} values as the percentage of viability vs. control. Silymarin was also tested as positive control. The data were statistically assessed by using a linear regression model.

RESULTS AND DISCUSSION

The DCFH generated from an esterase-related deacetylation of DCFH-DA was expected to oxidize to DCF as a result of the reaction of exogenous H_2O_2 with Fe^{2+} in a cell-free system (Shao et al., 2004; Komarov et al., 2004), and by oxidizing species including ROS and free radicals, and endogenous H_2O_2 and peroxidase in a biological system (Rota et al., 1999). In the system used, however, $FeSO_4$ and H_2O_2 reacted with two anthraquinones, 5 min after the addition of DCFH. Both alaternin and emodin exerted marked concentration-dependent inhibitory activities on ·OH generated by the Fenton reaction (Table

I & Fig. 2). In particular, the inhibition rate (%) of alaternin sharply increased to attain about 100% at a concentration of 5 µM (IC_{50} value of 3.05 ± 0.26 µM). Emodin also exhibited significant inhibitory activity with an IC_{50} value of 13.29 ± 3.20 µM. However, L-ascorbic acid, a positive control, acted as a weak inhibitor of ·OH for the generation of ·OH over 10 µM. This may be due to its prooxidant activity (Yen et al., 1997).

The inhibitory activities of the two anthraquinones on ·OH generation were more likely to be associated with the chelating of the metal ions, which may have reacted with H_2O_2 to generate ·OH in the Fenton reaction. Transition metal ions, such as iron or copper are known to be able to withdraw hydrogen atoms from membrane lipids and bring about lipid peroxidation. Metal ions may act as prooxidants during electron transfer releasing radicals (Stohs and Bagchi, 1995). Also, the ferric (Fe^{3+})/ferrous (Fe^{2+}) molar ratio plays a significant role in the initiation of lipid peroxidation. Metal irons are therefore not only involved in the generation of ·OH but also in the propagation of intracellular lipid peroxidation by catalyzing the decomposition of lipid peroxidation (Halliwell and Gutteridge, 1992). It has been suggested that the chelating potential of anthraquinones on Fe^{2+} is dependent on the number and positions of hydroxyl groups. Moreover, the o-dihydroxyl groups of anthraquinone are considered to improve

Table I. Hydroxyl radical inhibitory activity of anthraquinones

	IC_{50} (µM)[a]
Alaternin	3.05 ± 0.26
Emodin	13.29 ± 3.20
L-Ascorbic acid[b]	ND[c]

[a]·OH is the 50% inhibition concentration of hydroxyl radical generation in 1.0 mM H_2O_2 and 0.2 mM $FeSO_4$ (IC_{50} :mM), [b]L-Ascorbic acid is a positive control in this system, [c]ND represents no inhibitory effect at a range of tested concentrations.
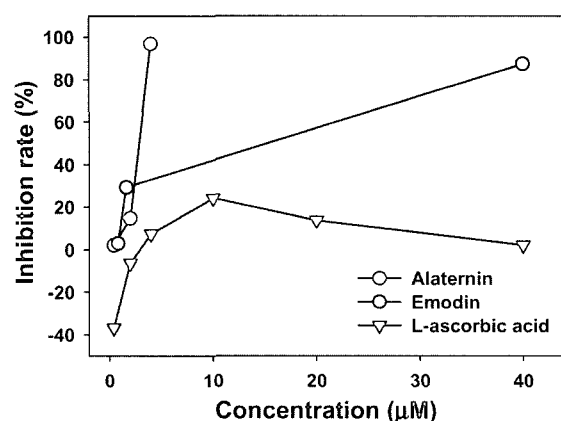

Fig. 2. Concentration-dependent inhibitory activity of two anthraquinones and L-ascorbic acid on hydroxyl radical generation

chelating power (Yen *et al.*, 2000). This may be the reason why alaternin exhibited an activity four times stronger than emodin in the inhibition of ·OH generation.

The ·OH scavenging activity of emodin and alaternin was investigated utilizing DMPO, a spin trap product with the ·OH in the Fenton reaction. The typical 1:2:2:1 signals of the DMPO-OH adduct could be measured by ESR spectroscopy. Since the DMPO connects directly with the ·OH, the intensity of the spin adduct reflects the amount of ·OH generated by the Fenton reaction. The ESR experiment was carried out in phosphate buffer with using DMPO as a spin trap. It was reported that the trapping of ·OH with DMPO was exclusively due to hydrogen peroxide, not in the photo-Fenton reaction but during the ferrous ion-Fenton reaction, as previously described (Lloyd *et al.*, 1997). At the end of the 10 min Fenton reaction after the addition of the hydroxyl peroxide solution, signal changes resulted from the decomposition of DMPO-OH adducts (Li *et al.*, 2004). Also, ferrous ions and phosphate ions were found to quench the DMPO-OH adducts rapidly, and reducing the signal intensity of the ESR spin trap. To avoid misinterpretation from the Fe^{2+}, DTPA as an electron donor was found to enhance the signal intensity of DMPO-OH adducts by forming a complex with the Fe^{3+} and/or Fe^{2+}. DMSO as a ·OH scavenger prevented the diminishing of intensity signals of the DMPO-OH adducts in the ESR spin trapping experiments (Mothilal *et al.*, 2004; Li *et al.*, 2004).

In this ESR spin trapping method with DMSO and DTPA, it was expected that the stronger the scavenging activity of the ·OH of the two anthraquinones, alaternin and emodin, the lesser the signal intensity of DMPO-OH adducts. As shown in Fig. 3, the spin intensities, which were measured 5 min and 10 min after the addition of the
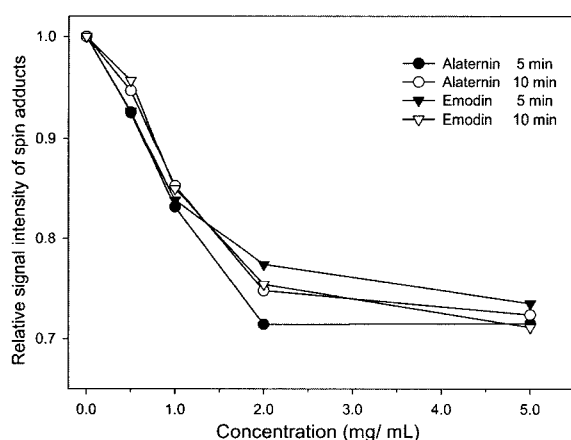

Fig. 3. Effect of anthraquinones on DMPO-OH adduct signal intensity in the Fenton reaction. ESR conditions and the calculation of relative signal intensity of spin-adducts were as mentioned in the text (Methods and Materials). The detection of spin adducts was carried out twice at 5 min and 10 min after the mixing of all reagents.

hydroxyl peroxide solution, happened to have no significant difference, except at the concentration of 2.0 mg/mL. Alaternin and emodin caused a concentration-dependent decrease in the amount of the DMPO-OH adduct, meaning that the two are more capable to scavenge free radicals, such as ·OH. This can be explained by the fact that emodin and alaternin had almost similar radical scavenging activity during the ESR experiment.

Previous studies have evaluated the antioxidative activities of emodin and alaternin obtained from *C. tora* as well as their ability to scavenge stable radicals (DPPH, authentic peroxynitrites) inhibit the generation of peroxynitrite, total ROS, 3-morpholinosydnonimine (SIN-1) induced peroxynitrites and peroxy radicals formed by lipid peroxidation (Choi *et al.*, 1993; 1994; 2000; Yen *et al.*, 1998; Huang *et al.*, 1995; Ng *et al.*, 2003; Demierezer *et al.*, 2001). As shown in Fig. 1, the chemical structures of the two anthraquinones, alaternin and emodin are different by the number of functional groups, designated to 1,2,3,8-tetrahydroxy-6-methyl-9,10-anthraquinone and 1,3,8-trihydroxy-6-methyl-9,10-anthraquinone, respectively. Concerning the antioxidant activity, an important factor is that alaternin possesses an *ortho*-dihydroxyl group at the positions C-1 and C-2. Actually, several investigations have shown that alaternin acts as scavenger and/or inhibitor of free radicals and ROS, including DPPH, the superoxide anion radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), the peroxy radical (ROO·), peroxynitrite, and nitric oxide (NO·). Unlike alaternin, emodin exerted only chain-breaking actions on the lipid peroxidation system using the thiocyanate method (Choi *et al.*, 1994; 2000). In our present work, both alaternin and emodin were expected to chelate the Fe^{2+} transition metal ions and scavenge the ·OH.

Also, anthraquinones such as emodin have been reported to exhibit inhibitory activity on NADH oxidase and succinate oxidase (Chen *et al.*, 1988) as well as xanthine oxidase (Noro *et al.*, 1987). Furthermore, they have shown marked inhibitory activities on the enzymatic superoxide anion radical generation with the phenazine methosuldate (PMS)-β-nicotinamide adenine dinucleotide (NADH) system. However, they inhibitory activities were moderate on ·OH generation from both lipid peroxidation and the Fenton reaction (Ng *et al.*, 2003; Yen *et al.*, 2000).

Investigations on hepatoprotective activities have focused on two pathways; the evaluation of activities not only by the detoxification of reactive and toxic hepatotoxins such as CCl_4, acetaminophen, thioacetamide, tacrine, and rubratoxin B and H_2O_2 but also by the antioxidant defense system of the body, including superoxide dismutase, catalase and glutathione peroxidase. The other pathway consists at judging from the activity and level of serum marker enzymes such as glutamate pyruvate transaminase and glutamate oxaloacetate transaminase, which are

Table II. Hepatoprotective activities on tacrine-induced cytotoxicity in human liver-derived HepG2 cells

	EC50	
	(μg/mL)[a]	(μM)[a]
Alaternin	11.50	4.02
Emodin	6.40	2.37
Silymarin[b]	9.65	2.00

[a] ·OH is the 50% scavenging concentration of hydroxyl radical in HepG2 cells (EC50 : μM and μg/mL), [b] Silymarin, antihepatotoxic agent is a positive control in this system.

released when the liver is impaired (Wang et al., 2004). It has been observed that the scavenging and/or inhibitory activities on free radicals, including trichloromethyl free radicals ($CCl_3·$) and trichloromethyl peroxy radicals ($CCl_3OO·$) by means of cytochrome P450 systems-related CCl_4 metabolism, are likely to be related to the hepatoprotective activity on the cellular membrane and organelles by the prevention of oxidation (Hsiao et al., 2003; Wang et al., 2004; Song et al., 2003). In particular, emodin, which is isolated from C. tora L. with inhibitory activity on benzo[a]pyrene-mediated DNA damage in HepG2 cells, has been reported to exhibit protective effects on CCl_4 as well as D-galactosamine-induced hepatotoxicity (Wu et al., 2001; Lin et al., 1996).

Alaternin and emodin showed their hepatoprotective activities on tacrine-induced cytotoxicity with the EC50 values of 4.02 μM and 2.37 μM, respectively (Table II). Silymarin, an antihepatotoxic agent used as a positive control exhibited the EC50 value of 2.00 μM. Even though the antioxidative potentials of alaternin were stronger than emodin, especially in regards to the ·OH inhibitory/scavenging activities, the hepatoprotective activity of emodin was two times more important than that of alaternin.

In final remark, it can be concluded that these results demonstrated that both alaternine and emodin have antioxidant and hepatoprotective activities. However, the detailed mechanisms of the relationship between the antioxidative and hepatoprotective acitivities remains to be solved.

ACKNOWLEGEMENT

REFERENCES

Asha, V. V., Akhila, S., Wills, P. J. and Subramoniam, A., Futher studies on the antihepatotoxic activity of Phyllanthus maderaspatensis Linn. J. Ethnopahrmacol., 92, 67-70 (2004).

Benoit, G. G., Naud, C. F., Simard, M. A., and Astier, A. L., Noninterference of cytochrome P4501A2 in the cytotoxicity of tacrine using genetically engineered V79 Chinese hamster cells for stable expression of the human or rat isofrom an two human hepatocyte cell lines. Chem. Pharmacol., 53, 423-427 (1997).

Bruck, R. B., Aeed, H., Shirin, H., Matas, Z., Zaidel, L., Avni, Y. and Halpern, Z., The hydroxyl radical scavengers dimethyl-sulfoxide and dimethylthiourea protect rats against thioaceta-mide-induced fulminant hepatic failure. J. Hepatol., 31, 27-38 (1999).

Chen, C. W., He, B., and Chen, Q., Biochemical study of Chinese rhubarb anthraquinone derivatives on the inhibition of NADH oxidase and succinate oxidase of the mitochondrion. Shenwu Hauxue Zazhi, 4, 36-41 (1988).

Cho, H., Jun, J. Y., Song, E. K., Kang, K. H., Baek, H. Y., Ko, Y. S., and Kim, Y. C., Bakuchiol: a hepatoprotective compounds of Psoralea corylifolia on tacrine-induced cytotoxicity in Hep G2 cells. Planta Med., 67, 750-751, (2001).

Choi, J. S., Chung, H. Y., Jung, H. A., Park, H. J., and Yokozawa, T., Comparative evaluation of antioxidant potential of alaternin (=2-hydroxyemodin) and emodin. J. Agric. Food Chem., 48, 6347-6351 (2000).

Choi, J. S., Lee, H. J., and Kang, S. S., Alaternin, cassiaside and rubrofusarin gentiobioside, radical scavenging principles from the seeds of Cassia tora on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Arch. Pharm. Res., 17, 462-466 (1994).

Choi, J. S., Lee, H. J., Park, H. J., Kim, H. G., Young, H. S., and Mun, S. I., Screening for antioxidant activity of plant and marine algae and its active principles from Prunus davidiana. Kor. J. Pharmacogn., 24, 299-303 (1993).

Demierezer, L. Ö., Kuruüzüm-Uz, A., Bergere, I., Schiewe, H. J., and Zeeck, A., The structures of antioxidant and cytotoxic agents from natural sources: anthraquinones and tannins from roots of Rumex patientia. Phytochemistry, 58, 1213-1217 (2001).

Doucet-Personeni, C., Bentley, P. D., Fletcher, R. J., Kinkaid, A., Kryger, G., Pirard, B., Taylor, A., Taylor, R., Viner, R., Silman, I., Sussman, J. L., Greenblatt, H. M., and Lewis, T., A structure-based design approach to the development of novel, reversible AchE inhibitors. J. Med. Chem., 44, 3203-3215 (2001).

Galm, O., Fabry, U., Efferth, T., and Osieka, R., Synergism between rViscumin and cisplatin is not dependent on ERCC-1 expression. Cancer Lett., 187, 143-151 (2002).

Halliwell, B. and Gutteridge, J. M. C., Biologically relevant metal ion-dependent hydroxyl radical generation. An update. FEBS Lett., 307, 108-112 (1992).

Halliwell, B. and Gutteridge, J. M. C., Oxygen free radical and iron in relation to biology and medicine; some problems and concepts. Arch. Biochem. Biophys., 246, 501-514 (1986).

Hipeli, S. and Elstner, E. F., OH-radical type reactive oxygen species: a short review on the mechanisms of OH-radical

and peroxynitrite toxicity. *Z. Naturforsch.*, 52C, 555-563 (1997).

Hsiao, G. H., Shen, M. Y., Lin, K. H., Lan, M. H., Wu, L. Y., Chu, D. S., Lin, C. H., Su, C. H., and Sheu, J. R., Antioxidative and hepatoprotective effects of *Antrodia camphorata* extract. *J. Agric. Food Chem.*, 51, 3302-3308 (2003).

Huang, S. S., Yeh, S. F. and Hong, C. Y., Effect of anthraquinone derivative on lipid peroxidation in rat heart mitochondria: structure-activity relationship. *J. Nat. Prod.*, 58, 1365-1371 (1995).

Komarov, A. M., Hall, J. M., and Weglicki, W. B., Azidothymidine promotes free radical generation by activated macrophage and hydrogen peroxide-iron-mediated oxidation in a cell-free system. *Biochim. Biophysic. Acta.*, 1688, 257-264 (2004).

Lebel, C. P. and Bondy, S. C., Sensitive and rapid quantitation of oxygen reactive species formation in rat synaptosomes. *Neurochem. Int.*, 17, 435-441 (1990).

Li, L., Abe, Y., Kanagawa, K., Ushi, N., Imai, K., Mashino, T., Moshizuki, M., and Miyata, N., Distinguishing the 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO)-OH radical quenching effect from the hydroxyl radical scavenging effect in the ESR spin-trapping method. *Anal. Chim. Acta*, 512, 121-124 (2004).

Lin, C. C, Chang, C. H., Yang, J. J., Namba, T., and Hattori, M., Hepatoprotective effects of emodin from *Ventilago leiocarpa*. *J. Ethnopharmacol.*, 52, 107-111 (1996).

Lloyd, R. V., Hanna, P. M., and Mason, R. P., The origin of the hydroxyl radical oxygen in the fenton reaction. *Free Radic. Biol. Med.*, 22, 885-888 (1997).

Moraes, J. E., Quina, F. H., Nascimento, C. A. O., Silva, D. N. and Chiavone-Filho, O., Treatment of saline wastewater contaminated with hydrocarbons by the photo-fenton process. *Environ. Sci. Technol.*, 38, 1183-1187 (2004).

Morita, T., Jinno, K., Kawagishi, H., Arimoto, Y., Suganuma, H., Inakuma, T., and Sugiyama, K., Hepatoprotective effect of myristin from nutmeg (*Myristica fragrans*) on lipopolysaccharide/D-galactosamine-induced liver injury. *J. Agric. Food Chem.*, 51, 1560-1565 (2003).

Mothilal, K. K., Inbaraj, J. J., Gandhidasan, R., and Murugesan, R., Photosensitization with anthraquinone: optical and EPR spin trapping studies of photogeneration of reactive oxygen species. *J. Photochem. Photobiol. A: Chem.*, 162, 9-16 (2004).

Nagashima, H., Nakamura, K., and Goto, T., Hepatotoxin rubratoxin B induced the secretion of TNF-, IL-8 and MCP-1 in HL60 cells. *Biochem. Biophy. Res. Comm.*, 287, 829-832 (2001)

Ng, T. B., Liu, F., Lu, Y., Cheng, C. H. K., and Wang, Z., Antioxidant activity of compounds from the medicinal herb *Aster tataricus*. *Comparative Biochem. Physiol. Part C*, 136, 109-115 (2003).

Noro, T., Noro, K., Milyase, T., Kuroyanagi, M., Umehara, K., Ueno, A., and Fukushima, S., Inhibition of xanthine oxidase by anthraquinone. *Chem. Pharm. Bull.*, 35, 4314-4316 (1987).

Oh, H. C., Kim, J. S., Song, E. K., Cho, H., Kim, D. H., Park, S. E., Lee, H. S., and Kim, Y. C., Sesquiterpenes with hepatoprotective activity from *Cnidium monnieri* on tacrine-induced cytotoxicity in Hep G2 cells. *Planta Med.*, 68, 748-749 (2002a).

Oh, H. C., Lee, H. S., Kim, T. W., Chai, K. Y., Chung, H. T., Kwon, T. O., Jun, J. Y., Jeong,O. S., Kim, Y. C., and Yun, Y. G., Fucocoumarines from *Angelica dahurica* with hepatoprotective activity on tacrine-induced cytotoxicity in Hep G2 cells. *Planta Med.*, 68, 463-464 (2002b).

Osseni, R. A., Debbasch, C., Christen, M. O, Rat, P., and Warnet, J. M., Tacrine-induced reactive oxygen species in a human liver cell line: the role of anethole dithilethione as a scavenger. *Toxicol. In Vitro*, 13, 683-688 (1999).

Oturan, M. A., Peiroten, J., Chartrin, P., and Acher, A. J., Complete destruction of *p*-nitrophenol in aqueous medium by electro-fenton reaction. *Environ. Sci. Technol.*, 34, 3474-3476 (2000).

Pan, S. Y., Han, Y. F., Carlier, P. R., Pang, Y. P., Mak, D. H. F., Lam, B. Y. H., and Ko, K. M., Schisandrin B protects against tacrine- and bis(7)-tacrine-induced hepatotoxicity and enhances cognitive function in mice. *Planta Med.*, 68, 217-220 (2002).

Rimbach, G., Weinberg, P. D., de Pascual-Teresa, S., Alonso, M. G., Ewins, B. A., Turner, R., Minihane, A. M.,Botting, N., Firley, B., Matsugo, S., Uchida, Y., and Cassidy, A., Sulfation of genistein alters its antioxidant properties and its effect on platelet aggregation and monocyte and endothelial function. *Biochim. Biophys. Acta*, 1670, 229-237 (2004).

Rota, C., Chignell, C. F., and Mason, R. P., Evidence for free radical formation during the oxidation of 2',7'-dichloro-fluorescin to the fluorescent dye 2',7'-dichlorofluorescein by horseradish peroxidase: possible implications for oxidative stress measurements. *Free Radic. Biol. Med.*, 27, 873-881 (1999).

Shao, Z. H., Xie, J. T., Vanden Hoek, T. L., Mehendale, S., Aung, H., Li, C. Q., Qin, Y., Schumacker, P. T., Becker, L. B., and Yuan, C. S., Antioxidant effects of American ginseng berry extract in cardiomyocytes exposed to acute oxidant stress. *Biochim. Biophysic. Acta*, 1670, 165-171 (2004).

Song, E. K., Cho, H., Kim, J. S., Kim, N, Y., An N. H., Kim, J. A., Lee, S. H., and Kim, Y. C., Diarylhepatonoids with free radical scavenging and hepatoprotective activity *in vitro* from *Curcuma longa*. *Planta Med.*, 67, 876-877 (2001).

Song, E. K., Kim, J. H., Kim, J. S., Cho, H., Nan, J. X., Sohn, D. H., Ko, G. I., Oh, H. C., and Kim, Y. C., Hepatoprotective phenolic constituents of *Rhodiala sachalinensis* on tacrine-induced cytotoxicity in HepG2 cells. *Phytother. Res.*, 17, 563-565 (2003).

Song, T. Y. and Yen, G. C., Protective effects of fermented filtrate from *Antrodia camphorata* in submerged culture against CCl_4-induced hepatic toxicity in rats. *J. Agric. Food Chem.*, 51, 1571-1577 (2003).

Stohs, S. J. and Bagchi, D., Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.*, 18, 321-336 (1995).

Ueda, J. I., Saito, N., Shimazu, Y., and Ozawa, T., A comparison of scavenging abilities of antioxidants against hydroxyl radicals. *Arch. Biochem. Biophys.*, 333, 377-384 (1996).

Wang, B. J., Liu, C. T., Tseng, C. Y., Wu, C. P., and Yu, Z. R., Hepatoprotective and antioxidant effects of Bupleurum kaoi Liu (Chao et chuang) extract and its fractions fractionated using supercritical CO_2 on CCl_4-induced liver damage. *Food. Chem. Toxicol.*, 42, 609-617 (2004).

Watkins, P. B., Zimmerman H. J., Knapp, M. J., Gracon, S. I., and Lewis, K. W., Hepatotoxic effects of tacrine administration in patients with Alzheimers disease. *J. Am. Med. Assoc.*, 271, 992-998 (1994).

Wu, C. H., Hsieh, C. L., Song, T. Y., and Yen, G. C., Inhibitory effects of *Cassia tora* L. on benzo[a]pyrene-mediated DNA damage toward HepG2 cells. *J. Agric. Food Chem.*, 49, 2579-2586 (2001).

Yang, C. F., Shen, H. M., and Ong, C. N., Protective effect of ebselen against hydrogen peroxide-induced cytotoxicity and DNA damage in $HepG_2$ cells. *Biochem. Pharmacol.*, 57, 273-279 (1999).

Yen, G. C., Chen, H. W., and Duh, P. D., Extraction and identification of an antioxidative compound from jue ming zi (*Cassia tora* L.). *J. Agric. Food Chem.*, 46, 820-824 (1998).

Yen, G. C., Chen, H. Y., and Peng, H. H., Antioxidant and pro-oxidant effects of various tea extracts. *J. Agric. Food Chem.*, 45, 30-34 (1997).

Yen, G. C., Duh, P. D., and Chuang, A. Y., Antioxidant activity of anthraquinones and anthrone. *Food Chem.*, 70, 437-441 (2000).

Yoshimura, Y., Inomata, T., Nakazawa, H., Kubo, H., Yamaguchi, F., and Ariga, T., Evaluation of free radical scavenging activities of antioxidants with an H_2O_2/NaOH/DMSO system by electron spin resonance. *J. Agric. Food Chem.*, 47, 4653-4656 (1999).