

Cardioprotective Effects of *Glycyrrhiza uralensis* Extract Against Doxorubicin-Induced Toxicity

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Abstract

The purpose of the present study was to investigate the cardioprotective effects of *Glycyrrhiza uralensis* extract (GUE) against doxorubicin (DOX)-induced cardiotoxicity. Imprinting control region (ICR) mice were treated with saline, DOX (20 mg/kg intraperitoneal [ip] for once), GUE (100 mg/kg intragastric [ig] for 8 days), co-treatments with DOX and GUE (100 mg/kg ig for 8 days), and amifostine (100 mg/kg intravenous [iv] for once), respectively. Serum levels of lactate dehydrogenase (LDH) and creatine kinase isoenzyme (CK-MB), glutathione peroxidase (GSH-P_x) activity, and glutathione (GSH) level in heart tissue were measured. Histopathologic analysis of heart tissue was also performed. Treatment with GUE significantly protected the mice from DOX-induced cardiotoxicity, indicated by decreased levels of serum LDH and CK-MB, improved heart morphology and increased GSH-P_x activity and GSH level. Additionally, GUE did not compromise the tumor-inhibitory effect of DOX. In conclusion, our studies imply the potentially clinical application of GUE to overcome the cardiotoxicity of doxorubicin.

Keywords

Glycyrrhiza uralensis extract, cardiotoxicity, doxorubicin

Doxorubicin (DOX), an antitumor anthracycline antibiotic, is extremely effective in treating severe Hodgkin and non-Hodgkin lymphomas, breast cancer, and leukemia.¹ However, numerous studies have shown DOX-treatment-related free radical generation, membrane lipid peroxidation, and mitochondrial damage,^{2,3} and its therapeutic usage is rigorously limited by late-onset, acute, or chronic cardiotoxicity.

Radix Glycyrrhizae is used in foods, beverages, candies, tobacco, and dietary supplements in the United States, Europe, the Middle East, and Russia.⁴ It is considered to be the “King of Herbs”⁵ and possesses various pharmaceutical anticarcinogenic, anti-inflammatory and antiulcer properties.⁶⁻⁸ Glycyrrhizin, the main water-soluble constituent of licorice, was used as antidote for saponin, alkaloid urethan, benzene, carbon tetrachloride-induced toxicity,⁹ and exerted antigenotoxic and hepatoprotective effects.¹⁰ Isoliquiritin apioside potentially combated oxidative stress-induced genotoxicity.¹¹ Glycyrrhizic acid was shown to protect against aflatoxin-induced oxidative stress with its capacity to inhibit the metabolic activation of hepatotoxin.¹² Lee et al showed that *Glycyrrhizae inflata* attenuates the toxicity of cisplatin without compromising its antitumor effects.¹³ There are 3 species of plant material recorded as *Radix Glycyrrhizae* in Chinese Pharmacopeia. Among them, *Glycyrrhiza uralensis* is most commonly used in China. Very recently, Choi reported that hexane/ethanol extract of *Glycyrrhiza uralensis* suppresses DOX-induced

apoptosis in H9c2 rat cardiac myoblasts.¹⁴ However, few studies have been undertaken to evaluate the protective effect of *Glycyrrhiza uralensis* extracts (GUE) against DOX-induced cardiotoxicity as yet. The present study was designed to estimate the cardioprotection of GUE against DOX-induced toxicity. Meanwhile, antioxidant activity and the potential impact on antitumor activity of DOX were also studied. Amifostine, a cytoprotective agent, was also used as a positive control in our study.

Methods

Chemicals and Cell Line

Doxorubicin was provided by Hisun Pharmaceutical Co (Taizhou, China). Amifostine (AMF) was purchased from Dalian Merro Pharmaceutical Factory (Dalian, China). 2, 2-diphenyl-1-picryl-hydrazylhydrate (DPPH) was purchased from Sigma-Aldrich Co (St. Louis, Missouri). Luminol was obtained from the Institute of Analytical Science of Xi'an

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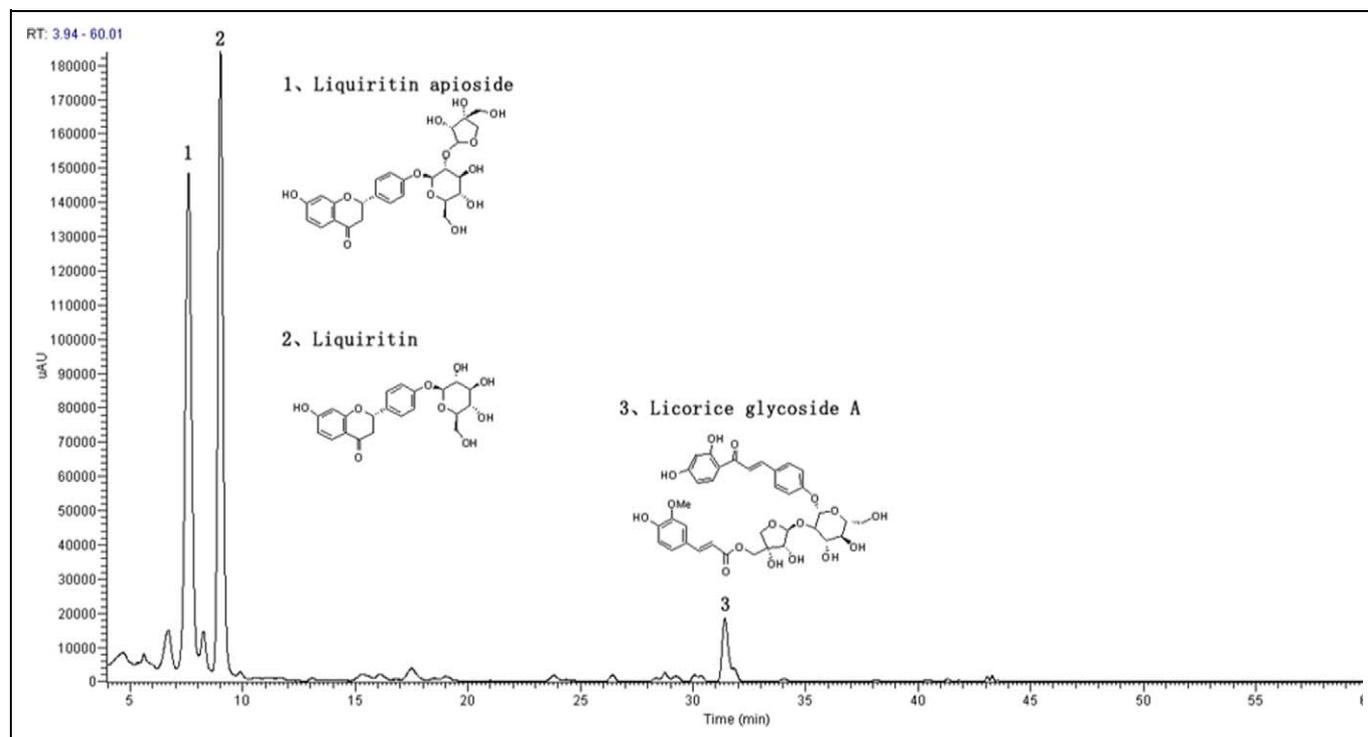


Figure 1. Chromatogram of *Glycyrrhiza uralensis* extract (GUE) by HPLC/DAD/MS analysis at 254 nm. The structures of 3 representative compounds were shown. Peaks 1, Liquiritin apioside; 2, Liquiritin; 3, Licorice glycoside A.

Jiaotong University (Xi'an, China). The diagnostic kits for LDH, CK-MB were obtained from Asia-Pacific (Ningbo, China). The diagnostic kit for superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-P_x) were obtained from Jiancheng (Nanjing, China). Murine hepatoma 22 cells (H₂₂ cells) were purchased from Zhejiang Academy of Medical Sciences (Hangzhou, China). Analytical reagent-grade chemicals and double-distilled water were used to prepare all solutions.

Plant Material and Preparation of GUE

The root of *Glycyrrhiza uralensis* was purchased from Hangzhou Traditional Chinese Medicine Electuary Factory (080906, Hangzhou, China) and authorized by associate Prof Liurong Chen (College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China). A voucher specimen was deposited at the herbarium of Pharmaceutical Information Institute, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China. Dried roots of *Glycyrrhiza uralensis* was extracted with 70% ethanol (10 mL × 2) by refluxing for 1.5 hours. The solution was filtered and then evaporated to dryness under vacuum. The residue was subjected to D101 porous resin column (Tianjin Resin Factory) and eluted with water and 70% ethanol. The eluant by 70% ethanol was evaporated to dry, and the residue was obtained as GUE. The extraction efficiency was 10.8%. Using licorice glycoside as the reference compound, the content of total flavonoids in GUE was 25.8% determined

by Ultra Violet (UV) spectrophotometric method. Using glycyrrhizic acid as standard compound, the content of glycyrrhizic acid was 15.1% by High-Performance Liquid Chromatography (HPLC) analysis.

Liquid Chromatography/UltraViolet (LC/UV) chromatograms of GUE at 230 nm and 254 nm are shown in Figures 1 and 2, respectively. Analysis was carried out using a Agilent 1100 series HPLC system (Waldbronn, Germany) equipped with a quaternary pump with on-line degasser, autosampler, column oven, and diode array detector (DAD). The chromatographic conditions were as follows: Zorbax StableBond C18 column (4.6 mm × 250 mm, 5 μm, Agilent); sample injection volume: 10 μL; the temperature of column oven: 35°C; flow rate: 1 mL/min; mobile phases: water with 0.01% acetic acid (A) and methanol (B). A gradient programmer was used according to the following profile: 0-15 minutes, hold on 20% B; 15-25 minutes linear increase to 25% B; 25-35 minutes hold on 25% B; 35-45 minutes linear increase to 40% B; 45-55 minutes hold on 40% B; 55-60 minutes increase to 90% B. UV spectra wavelengths were set at 230 nm and 254 nm. The identification of major compounds in GUE are listed in Table 1. Chromatogram of GUE and structures of major compounds are shown in Figures 1 and 2.

Animal and Experimental Protocol

Male ICR mice (20-22 g) were purchased from Zhejiang Experimental Animal Center. The mice were fed with a standard laboratory diet and given free access to tap water, kept in a

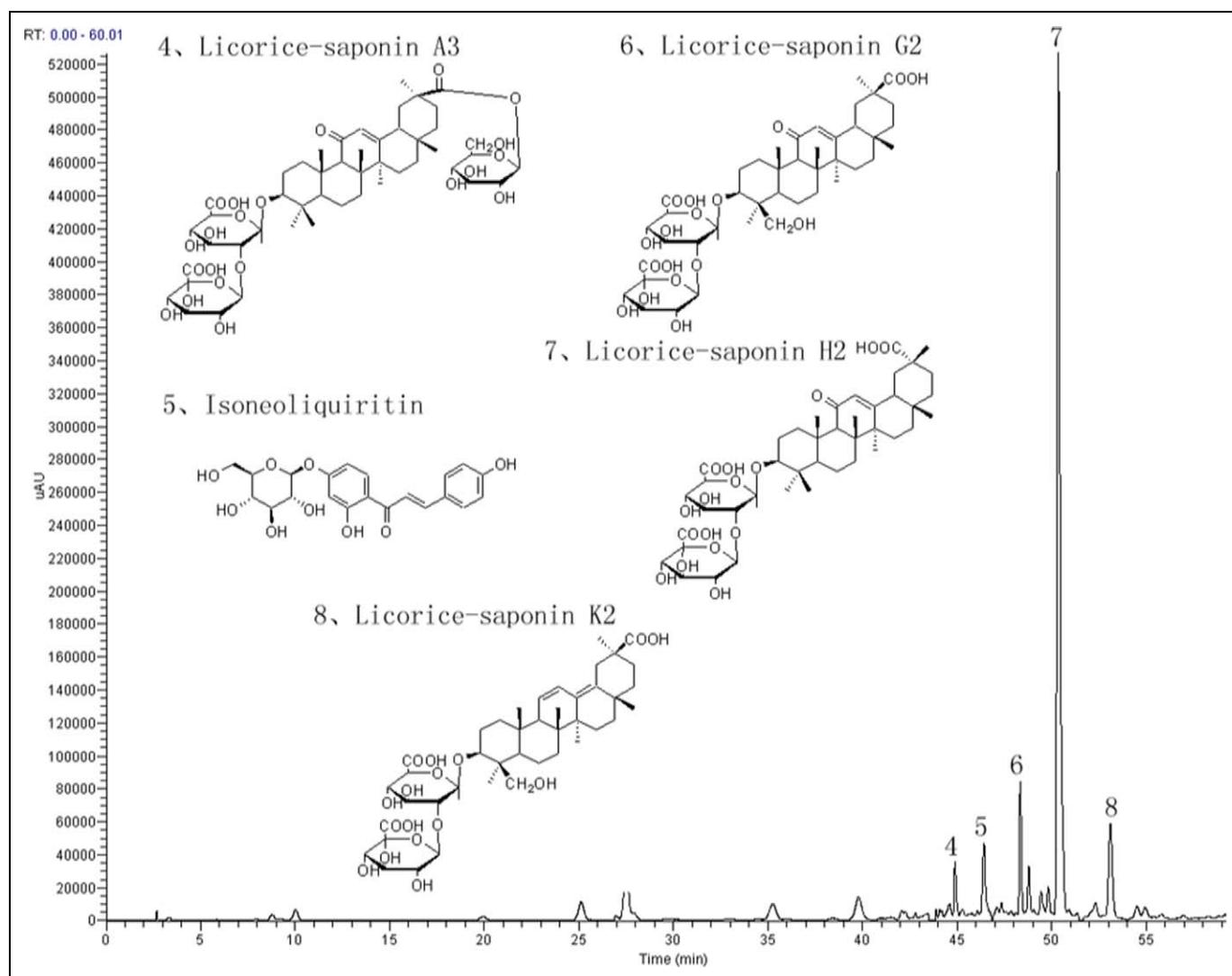


Figure 2. Chromatogram of *Glycyrrhiza uralensis* extract (GUE) by HPLC/DAD/MS analysis at 230 nm. The structures of 3 representative compounds were shown. Peaks 4, Licorice-saponin A3; 5, Isonoliquiritin; 6, Licorice-saponin G2; 7, Licorice-saponin H2; 8, Licorice-saponin K2.

controlled room temperature ($22 \pm 1^\circ\text{C}$), humidity (65%-70%), and 12 hours dark/light cycles. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Animal Ethic Review Committees of Zhejiang University approved all procedures.

Mice were divided into the following 5 groups, each group consisted of 10 mice: Control group (CON), DOX-treated group (DOX), DOX-treated group combined with amifostine (DOX + AMF), *Glycyrrhiza uralensis* extract-treated group (GUE), and DOX-treated group combined with GUE (DOX + GUE). The treatment protocol was as in Table 2.

Determination of Serum Enzymes

After 8 days of GUE treatment, blood was collected from the inferior vena cava. The serum was separated by centrifugation

Table 1. Contents of the Major Compounds in *Glycyrrhiza uralensis* Extraction

Peak No.	RT (min)	Chemical
1	7.59	Liquiritin apioside ^a
2	9.01	Liquiritin ^a
3	31.42	Licorice glycoside A ^b
4	44.89	Licorice-saponin A3 ^b
5	46.41	Isonoliquiritin ^b
6	48.33	Licorice-saponin G2 ^b
7	50.35	Licorice-saponin H2 ^b
8	53.09	Licorice-saponin K2 ^b

^a Identification with standard compounds.

^b Identification by deduction from ESI-MS/MS² data.

at 3000 rpm for 15 minutes and serum concentrations of LDH and CK-MB were determined by the use of diagnostic kit according to the manufacturer's instructions.

Table 2. Treatment Protocol

Day	CON	DOX	DOX + AMF	GUE	DOX + GUE
1	–	–	–	100 mg/kg GUE (ig)	100 mg/kg GUE (ig)
2	–	–	–	100 mg/kg GUE (ig)	100 mg/kg GUE (ig)
3	–	–	–	100 mg/kg GUE (ig)	100 mg/kg GUE (ig)
4	–	–	–	100 mg/kg GUE (ig)	100 mg/kg GUE (ig)
5	–	–	–	100mg/kg GUE (ig)	100 mg/kg GUE (ig)
6	–	–	–	100 mg/kg GUE (ig)	100 mg/kg GUE (ig)
7	–	20 mg/kg DOX (ip)	20 mg/kg DOX (ip) + 100 mg/kg AMF (iv, 15 min before of DOX ip)	100 mg/kg GUE (ig)	20 mg/kg DOX (ip) + 100 mg/kg GUE (ig, 30 min before DOX ip)
8	–	–	–	100 mg/kg GUE (ig)	100 mg/kg GUE (ig)

Abbreviations: AMF, amifostine; CON, Control Group; DOX, doxorubicin; GUE, *Glycyrrhiza uralensis* extraction; ig, intragastric; ip, intraperitoneal; iv, intravenous.

Antioxidant Activity of GUE In Vivo

Ten hearts of each group were weighed and homogenized in 10 mmol/L Tris-HCl of ice-cold phosphate-buffered saline (PBS; 50 mg/mL; pH 7.4) and centrifuged for 5 minutes at 5000 rpm. The protein content of the supernatants was determined by the Bradford protein assay. The activities of CAT and SOD were assayed by ammonium molybdate and xanthine-xanthine oxidase spectrophotometric methods. Glutathione peroxidase was assayed by enzymatic reaction and GSH was estimated at 412 nm colorimetrically by (5, 5'-dithiobis [2-nitrobenzoic acid]) (DTNB) reduction method. The GSH level and antioxidant enzymes were measured by the use of diagnostic kits according to the manufacturer's instructions. Data were normalized to the mean control value.

Histopathologic Evaluation

Another 4 hearts of each group were rapidly excised fixed in 10% formalin and embedded in paraffin. A slice containing the left and right ventricles was cut at a thickness of 5 μ m and stained with hematoxylin and eosin for morphometric analysis. The stained sections were examined at $\times 400$ magnification to analyze the severity of myocardial lesions.

DPPH[•] Scavenging Activity

DPPH[•] scavenging activity of GUE was measured by the luminol chemiluminescence (CL) system according to the method of Yao et al.¹⁵ Briefly, solutions of GUE and vitamin C with a series of concentrations (1, 10, 50, and 100 μ g mL⁻¹) were measured by an Infinite F200 microplate reader instrument (Tecan Group Ltd, Switzerland). The water and DPPH[•] solution were used as control and water was used as background. The test of DPPH[•] scavenging activity for all sample solutions, the controls, and the background were repeated 3 times. The formula of inhibition percentage of DPPH[•]-luminol CL by the sample solution was

$$\text{Inhibition (\%)} = [(I_c - I_s)/(I_c - I_b)] \times 100\%$$

I_s , I_c , and I_b represent the CL intensities for the sample solutions, the control, and the background, respectively. The

inhibition rate of GUE and vit C was plotted against the sample extract concentration, and the inhibitory concentration 50 (IC₅₀) was calculated.

O₂^{-•} Scavenging Activity

O₂^{-•} scavenging activity of GUE was assayed by SOD diagnostic kit. Briefly, solutions of GUE with a series of concentrations (20, 100, 200, 400, and 1000 μ g mL⁻¹) and vit C with a series of concentrations (4, 20, 100, 200, and 400 μ g mL⁻¹) were measured at 550 nm. The reaction system without xanthine oxidase and sample was used as 100% clearance (A_{100}), the reaction system only without sample was used as 0% clearance (A_0). The test of O₂^{-•} scavenging activity for all sample solutions was repeated 3 times. The formula of clearance rate of O₂^{-•} by the sample solution was

$$\text{Clearance rate (\%)} = [(A_0 - A_s)/(A_0 - A_{100})] \times 100\%$$

A_s represents the absorbance for the sample solutions. The clearance rate of GUE and vit C was plotted against the sample extract concentration, and the inhibitory concentration 50 (IC₅₀) was calculated.

The Effect on Antitumor Activity of DOX In Vivo

An independent experiment was conducted to evaluate the potential effect of GUE on antitumor activity of DOX. H₂₂ cells were propagated by serial intraperitoneal transplantation in an aseptic environment, then, 8×10^5 H₂₂ ascites tumor cells were inoculated subcutaneously (sc) on the left side of the axilla of mice, according to the methods of Chen et al.¹⁶ *Glycyrrhiza uralensis* extract treatment was started when the tumor size was approximately 100 mm³. The first treatment day was designated as day 1. Treatment and control groups consisted of 10 mice each. Treatments were as follows: control group, no treatment; DOX group, 7.5 mg/kg of DOX intraperitoneal (ip) on days 4 and 5; DOX + AMF group, 7.5 mg/kg of DOX ip on days 4 and 5, 100 mg/kg amifostine iv on days 4 and 5 (15 minutes before DOX injection); DOX + GUE group 7.5 mg/kg of DOX ip on days 4 and 5, 100 mg/kg GUE intragastric (ig) once daily consecutively for 8 days (30 minutes before DOX injection); GUE group, 100 mg/kg GUE ig once daily

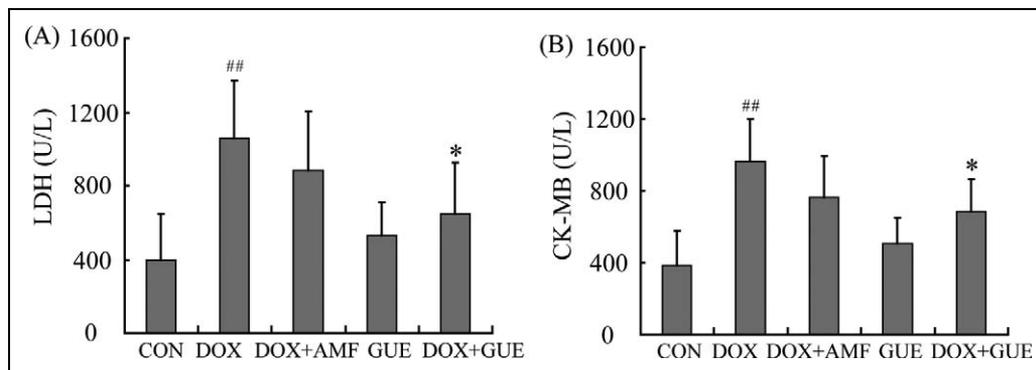


Figure 3. Serum enzyme level in experimental groups. (A) lactate dehydrogenase (LDH) level; (B) creatine kinase isoenzyme (CK-MB) level. *Glycyrrhiza uralensis* extract (GUE) alone group had no statistical difference in the serum level of LDH and CK-MB compared with control group. Compared with doxorubicin (DOX) group, DOX + GUE group significantly decreased the activity of LDH and CK-MB, while DOX + amifostine (AMF) group had no statistically significant differences. Values are means \pm SD. $n \geq 7$. ## $P < .01$ compared to control group; * $P < .05$ compared to DOX group.

consecutively for 8 days. After 8 days of GUE treatment, the tumors were weighed. The antitumor activity was assessed on the basis of tumor weight and the tumor inhibition, the tumor inhibition was calculated as:

$$\text{Tumor inhibition (\%)} = [(C - T)/C] \times 100\%$$

C and T represent the tumor weight means of control and treatment group, respectively.

Data Analysis

All data were expressed as means \pm SD and n , the number of individual experiments. One-way analysis of variance (ANOVA) was used for statistical analysis. A value of $P < .05$ was considered significant. All statistical analyses were performed using MINITAB 14 (Mintab Inc, State College, Pennsylvania).

Results

Effect of GUE on Serum LDH and CK-MB Level

Creatine kinase isoenzyme and LDH were measured in the serum as indicators of cardiovascular damage. Figure 3 shows the effect of GUE on DOX-induced biochemical changes in the serum of mice. Compared with control group, administration of DOX significantly elevated serum level of LDH and CK-MB ($P < .01$, respectively), which indicated DOX-induced cardiotoxicity. Injection of AMF reduced the level of LDH and CK-MB, but there were no statistically significant differences. Oral treatment with GUE for 8 days significantly prevented the increase of LDH and CK-MB caused by DOX ($P < .05$, respectively; Figure 3).

Antioxidant Activity of GUE In Vivo

We also investigated the effect of GUE on the activity of antioxidant enzymes in heart tissue in vivo. With the exception of the increased level of SOD in GUE + DOX group, there was

no significant change in the activities of CAT and SOD among these groups. In contrast to CAT and SOD, the level of GSH and the activity of GSH- P_x were notably reduced in DOX group compared with control group ($P < .05$, respectively; Figure 4). Compared with DOX group, the activity of GSH- P_x and the level of GSH were markedly restored in the AMF-treated group ($P < .01$ and $P < .05$; Figure 4). *Glycyrrhiza uralensis* extract significantly increased the activities of GSH- P_x and the level of GSH in DOX-treated mice ($P < .01$ and $P < .05$; Figure 4).

Effect of GUE on Histopathological Change

Microscope analysis of the heart tissues was performed in Control, DOX, DOX + AMF, GUE, and DOX + GUE groups. Normal myocardium morphology was observed in control and GUE groups (Figures 5A and 5E), whereas cytoplasmic vacuolation in DOX-treated animals was very prominent (Figure 5B). After GUE and AMF treatment in DOX-administrated mice, a significant reduction in cytoplasmic vacuolation was observed (Figure 5C and 5D), indicating a protective effect of GUE on DOX-induced cardiotoxicity.

Measurements of DPPH \cdot and O $_2^{\cdot-}$ scavenging activity

We investigated the radical (DPPH \cdot) scavenging activities of GUE (a series of concentrations) by DPPH \cdot -luminol CL inhibition rate (%), and the result was compared with a standard antioxidant, vitamin C. Vitamin C had stronger radical (DPPH \cdot) scavenging activities than GUE (Figure 6A). The IC $_{50}$ of GUE and vitamin C were 3.71 and 0.56 $\mu\text{g/mL}$ respectively.

The results for O $_2^{\cdot-}$ scavenging activity showed that vitamin C as a standard antioxidant had much higher clearance rate of O $_2^{\cdot-}$ than GUE (Figure 6B). The IC $_{50}$ of vitamin C is 5.07 $\mu\text{g/mL}$, and IC $_{50}$ of GUE is 599.80 $\mu\text{g/mL}$.

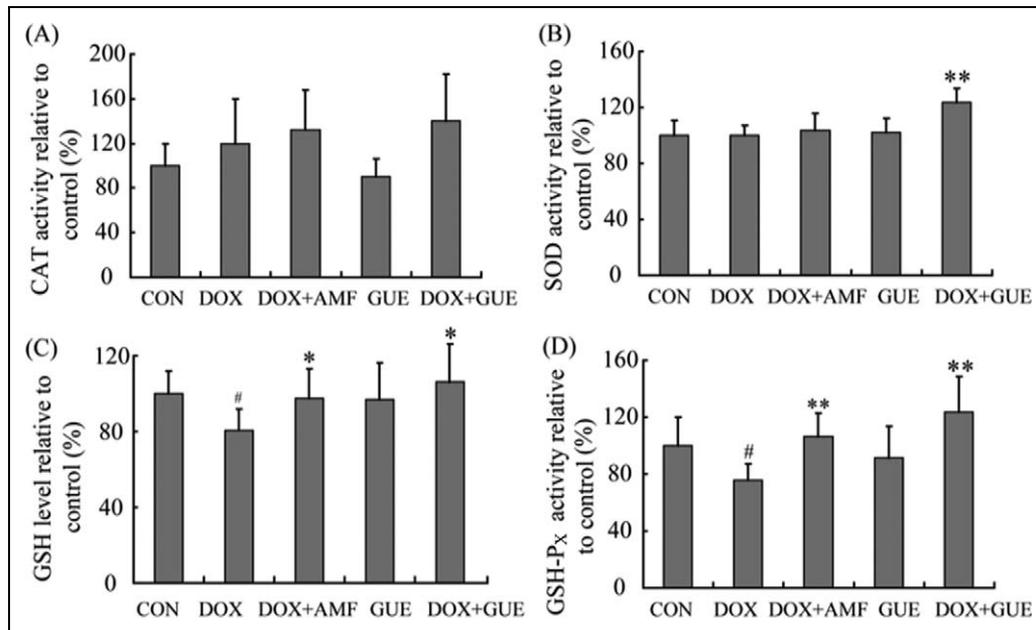


Figure 4. Antioxidant activity of *Glycyrrhiza uralensis* extract (GUE) *in vivo*. (A) catalase (CAT) activity; (B) superoxide dismutase (SOD) activity; (C) glutathione (GSH) level. *Glycyrrhiza uralensis* extract significantly increased the level of GSH in doxorubicin (DOX)-treated mice; (D) glutathione peroxidase (GSH-Px) activity. The activity of GSH-Px was markedly increased in GUE-treated group compared with DOX group. Data are normalized to the mean control value. Values are means \pm SD. $n \geq 7$. # $P < .05$ compared to control group; ** $P < .01$ compared to DOX group; * $P < .05$ compared to DOX group.

Effect of GUE on antitumor activity of DOX

No spontaneous regression was observed in the mice injected with H₂₂ cells. Compared with the control, treatment of DOX significantly inhibited the tumor weight ($P < .01$), indicating an arrest of tumor growth (Figure 7). The tumor weight in DOX + GUE and DOX + AMF groups also decreased compared with control group ($P < .01$ respectively) (Figure 7). The tumor inhibitions of DOX, DOX + AMF and DOX + GUE group were 47.22%, 38.50%, and 48.39% respectively. The result showed that GUE did not compromise the antineoplastic activity of DOX.

Discussion

In this study, GUE alleviated DOX-induced cardiotoxicity *in vivo* by decreasing of serum LDH and CK-MB levels, both of which were indicators of cardiac myocyte injury, and preventing morphological changes in heart structure. Though GUE did not exert the stronger DPPH[•] and O₂^{-•} scavenging activities compared to vitamin C, it recovered the activity of GSH-Px and the level of GSH reduced by DOX. The result suggested that the protective effect of GUE on DOX-induced cardiotoxicity might be caused by increasing the activity of GSH-Px and the level of GSH *in vivo*, which are more likely effects of less oxidative stress. Furthermore, our study indicated that GUE did not interfere with the antineoplastic effect of DOX on H₂₂ tumors in mice.

The mechanism of cardiotoxicity induced by anthracyclines is not clearly known. Some investigations suggest that apoptosis

plays a crucial role in the pathogenesis of DOX-induced cardiac damage.^{17,18} The sensitization of Ca²⁺ release from sarcoplasmic reticulum channels¹⁹ and alterations in the myocardial energy metabolism²⁰ are also involved in DOX-induced cardiomyopathy. Among these hypotheses, increased oxidative stress associated with an impaired antioxidant defense status is most widely accepted.^{21,22} In cells or tissue, oxidative stress results in the enhanced generation of reactive oxygen species (ROS) and a depletion of the antioxidants in the defense system. Reactive oxygen species generated in the tissue can be efficiently scavenged by the enzymatic antioxidant system and nonenzymatic antioxidants.²³ Heart is particularly susceptible to free radical injury, due to lower levels of free radical detoxifying substances than are found in organs such as liver or kidney.²⁴

The glutathione redox cycle is one of the most important intracellular antioxidant systems. GSH-Px and GSH are the essential compounds for maintaining cell integrity of structure and function, and the elevation of GSH-Px activity and GSH synthesis has been shown to be associated with oxidative stress.²⁵ Selenium (Se) possesses important biochemical functions in GSH-Px.²⁶ GSH is the most abundant cellular thiol and an important protective antioxidant against oxidative stress that plays a role in numerous detoxifications, bioreduction, and conjugation reactions.^{27,28} It is a substrate and cofactor of GSH-Px in degrading hydrogen peroxide (H₂O₂). Low level of GSH may cause increased oxidative stress.²⁹ H₂O₂ is known to be cytotoxic and causes damage to lipids. GSH-Px catalyzes the H₂O₂ to water, thus GSH-Px can inhibit tissue injury by removing H₂O₂.³⁰ In addition, GSH-Px can act on hydroperoxides of unsaturated fatty acids, thus it plays an

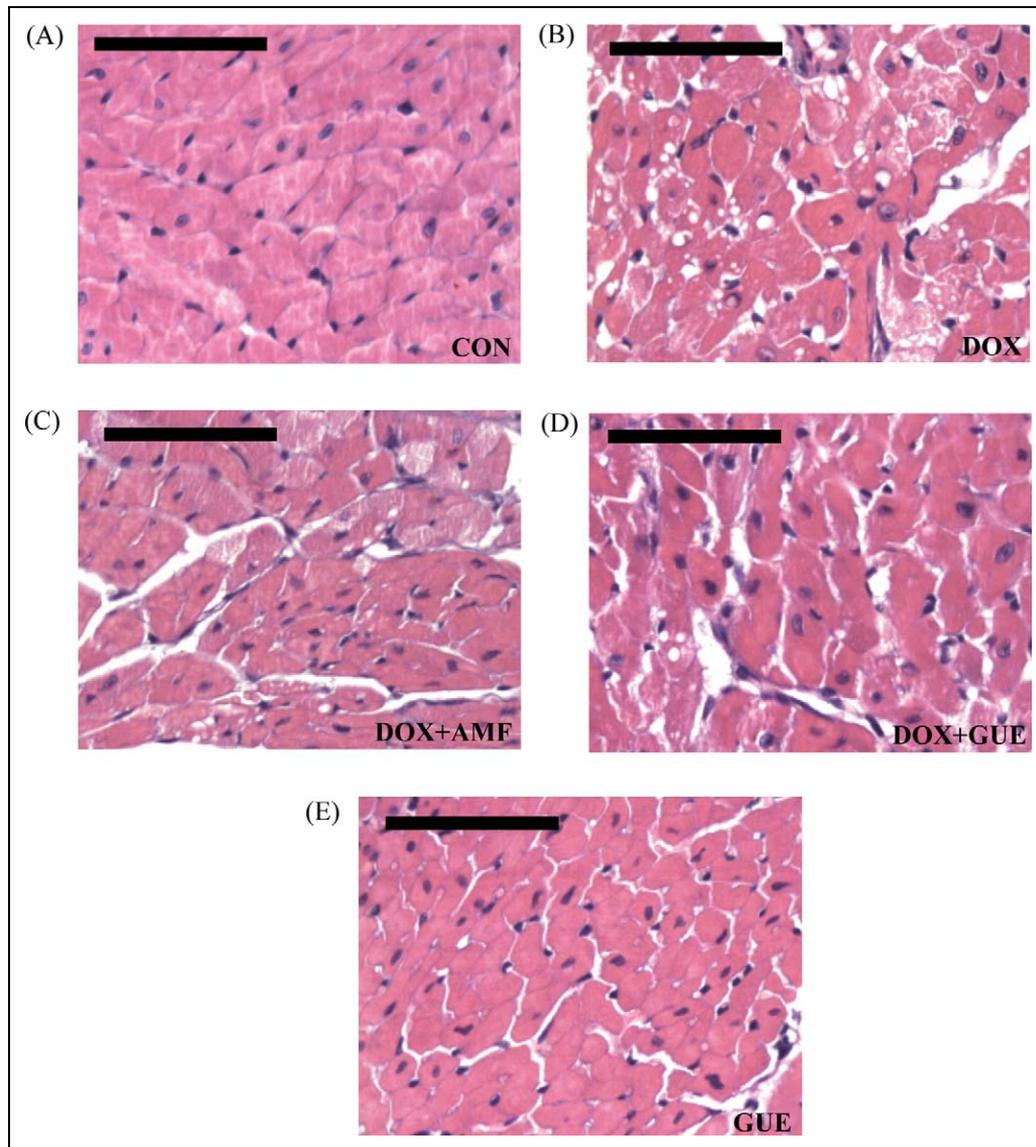


Figure 5. Histopathological examination. Hematoxylin and Eosin (H&E) staining for (A) Control, (B) doxorubicin (DOX), (C) DOX + amifostine (AMF), (D) DOX + GUE, and (E) *Glycyrrhiza uralensis* extract (GUE) group. Normal myocardium morphology was shown in control group, a significant reduction in cytoplasmic vacuolation formation was observed in GUE and AMF treatment group compared with DOX group. Original magnification $\times 400$. Scale bar = 100 μm .

important role in protecting cell membranes from oxidative disintegration.²⁶ Our study also showed that GUE protected the heart from DOX-induced injury by increasing the activity of GSH-P_x and the level of GSH.

Some free radical scavengers listed by Wouters et al³¹ have been shown to protect the heart against DOX-induced cardiotoxicity. In the heart, iron ions can react with H₂O₂ to form reactive hydroxyl radicals and play a crucial role in the generation of oxygen free radicals. In the clinic, iron chelators such as dexrazoxane, also known as ICRF-187, which is the only cardioprotective drug currently available,³² is limited in clinical use because of hematological toxicity and renal toxicity.³³ Amifostine, a broad-spectrum cytoprotective agent, has been extensively used clinically against chemotherapy-related

toxicity. The experiments showed that amifostine can reduce DOX-associated cardiotoxicity by scavenging oxygen radicals in cultured neonatal myocytes and in mice.^{34,35}

Radix Glycyrrhizae has been widely used in food and medicinal remedies for thousands of years. Recently, glycyrrhetic acid was found to induce actin disruption and had tumor cell-selective toxic properties, thus, it could be utilized as a promising chemopreventive and therapeutic antitumor agent.^{36,37} Glycyrrhizin prevented human endometrial cancer by significant decrease in the COX-2, IL-1 α , and TNF- α mRNA expression.³⁸ The antiangiogenic activity of licochalcone A, a component of *Radix Glycyrrhizae*, supports it as a potential supplemental source for cancer therapy.³⁹ These advantages suggest that the addition of *Radix Glycyrrhizae* and

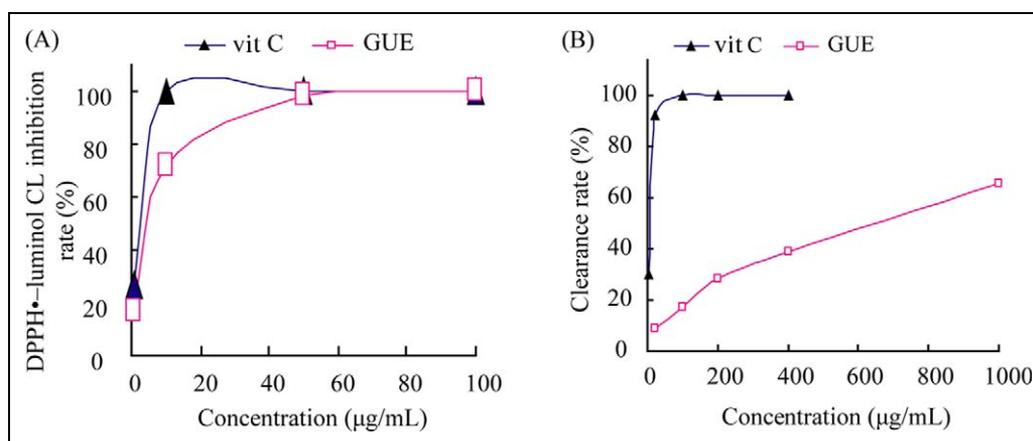


Figure 6. Effect of *Glycyrrhiza uralensis* extract (GUE) on DPPH• and $O_2^{\cdot-}$ scavenging activity. (A) DPPH• scavenging activity of GUE and vit C with a series concentrations; (B) $O_2^{\cdot-}$ scavenging activity of GUE and vit C with a series concentrations. The radical (DPPH•) scavenging activities and clearance rate of $O_2^{\cdot-}$ induced by GUE was less than vit C.

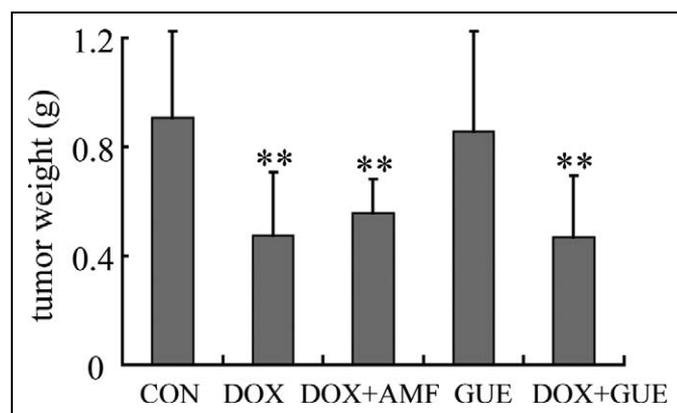


Figure 7. Effect of *Glycyrrhiza uralensis* extract (GUE) on tumor weight. GUE did not compromise the antineoplastic activity of doxorubicin (DOX). Values are means \pm SD. $n \geq 8$. ** $P < .01$ compared to control group.

its active compounds may have favorable protective effects during DOX chemotherapy.

The present findings demonstrate that GUE protects against DOX-induced cardiotoxicity without compromising the antitumor activity of DOX. Therefore, GUE may be considered as a potentially useful candidate in the combination with DOX chemotherapy.

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Declaration of Conflicting Interests

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

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References

- Pacher P, Liaudet L, Bai P, et al. Potent metalloporphyrin peroxynitrite decomposition catalyst protects against the development of doxorubicin-induced cardiac dysfunction. *Circulation*. 2003; 107(6):896-904.
- Xu MF, Tang PL, Qian ZM, Ashraf M. Effects by doxorubicin on the myocardium are mediated by oxygen free radicals. *Life Sci*. 2001;68(8):889-901.
- Oliveira PJ, Wallace KB. Depletion of adenine nucleotide translocator protein in heart mitochondria from doxorubicin-treated rats—relevance for mitochondrial dysfunction. *Toxicology*. 2006; 220(2-3):160-168.
- Lin SP, Tsai SY, Hou YC, Chao PD. Glycyrrhizin and licorice significantly affect the pharmacokinetics of methotrexate in rats. *J Agric Food Chem*. 2009;57(5):1854-1859.
- Kim DC, Choi SY, Kim SH, et al. Isoliquiritigenin selectively inhibits H(2) histamine receptor signaling. *Mol Pharmacol*. 2006;70(2):493-500.
- Sheela ML, Ramakrishna MK, Salimath BP. Angiogenic and proliferative effects of the cytokine VEGF in Ehrlich ascites tumor cells is inhibited by *Glycyrrhiza glabra*. *Int Immunopharmacol*. 2006;6(3):494-498.
- Kim JY, Park SJ, Yun KJ, Cho YW, Park HJ, Lee KT. Isoliquiritigenin isolated from the roots of *Glycyrrhiza uralensis* inhibits LPS-induced iNOS and COX-2 expression via the attenuation of NF-kappaB in RAW 264.7 macrophages. *Eur J Pharmacol*. 2008;584(1):175-184.
- Ji XL, Jiang YX, Ren BB, Liu K, Liu G. Effect of bismuth glycyrrhizate on experimental gastric ulcers and its mechanisms. *Zhongguo Zhong Yao Za Zhi*. 2007;32(14):1429-1432.
- Agarwal R, Wang ZY, Mukhtar H. Inhibition of mouse skin tumor-initiating activity of DMBA by chronic oral feeding of

- glycyrrhizin in drinking water. *Nutr Cancer*. 1991;15(3-4):187-193.
10. Isbrucker RA, Burdock GA. Risk and safety assessment on the consumption of Licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regul Toxicol Pharmacol*. 2006;46(3):167-192.
 11. Kaur P, Kaur S, Kumar N, Singh B, Kumar S. Evaluation of antigenotoxic activity of isoliquiritin apioside from *Glycyrrhiza glabra* L. *Toxicol In Vitro*. 2009;23(4):680-686.
 12. Chan HT, Chan C, Ho JW. Inhibition of glycyrrhizic acid on aflatoxin B1-induced cytotoxicity in hepatoma cells. *Toxicology*. 2003;188(2-3):211-217.
 13. Lee CK, Park KK, Lim SS, Park JH, Chung WY. Effects of the licorice extract against tumor growth and cisplatin-induced toxicity in a mouse xenograft model of colon cancer. *Biol Pharm Bull*. 2007;30(11):2191-2195.
 14. Choi HJ, Seon MR, Lim SS, Kim JS, Chun HS, Park JH. Hexane/ethanol extract of *Glycyrrhiza uralensis* licorice suppresses doxorubicin-induced apoptosis in H9c2 rat cardiac myoblasts. *Exp Biol Med (Maywood)*. 2008;233(12):1554-1560.
 15. Yao H, Wu B, Cheng YY, Qu HB. High throughput chemiluminescence platform for evaluating antioxidative activity of total flavonoid glycosides from plant extracts. *Food Chem*. 2009;115(1):380-386.
 16. Chen J, Zhang W, Lu T, Li J, Zheng Y, Kong L. Morphological and genetic characterization of a cultivated *Cordyceps sinensis* fungus and its polysaccharide component possessing antioxidant property in H22 tumor-bearing mice. *Life Sciences*. 2006;78(23):2742-2748.
 17. Fujimura L, Matsudo Y, Kang M, Takamori Y, Tokuhisa T, Hatano M. Protective role of Nd1 in doxorubicin-induced cardiotoxicity. *Cardiovasc Res*. 2004;64(2):315-321.
 18. Jang YM, Kendaiah S, Drew B, et al. Doxorubicin treatment in vivo activates caspase-12 mediated cardiac apoptosis in both male and female rats. *FEBS Lett*. 2004;577(3):483-490.
 19. Pessah IN. Calcium release channel of sarcoplasmic reticulum: an important target for doxorubicin-mediated cardiotoxicity. *Adv Exp Med Biol*. 1992;311:409-410.
 20. Tokarska-Schlattner M, Zaugg M, Zuppinger C, Wallimann T, Schlattner U. New insights into doxorubicin-induced cardiotoxicity: the critical role of cellular energetics. *J Mol Cell Cardiol*. 2006;41(3):389-405.
 21. Dalloz F, Maingon P, Cottin Y, Briot F, Horiot JC, Rochette L. Effects of combined irradiation and doxorubicin treatment on cardiac function and antioxidant defenses in the rat. *Free Radic Biol Med*. 1999;26(7-8):785-800.
 22. Yagmurca M, Fadillioglu E, Erdogan H, Ucar M, Sogut S, Irmak MK. Erdosteine prevents doxorubicin-induced cardiotoxicity in rats. *Pharmacol Res*. 2003;48(4):377-382.
 23. Seo HJ, Jeong KS, Lee MK, et al. Role of naringin supplement in regulation of lipid and ethanol metabolism in rats. *Life Sci*. 2003;73(7):933-946.
 24. Kelishomi RB, Ejtemaemehr S, Tavangar SM, Rahimian R, Mobarakeh JI, Dehpour AR. Morphine is protective against doxorubicin-induced cardiotoxicity in rat. *Toxicology*. 2008;243(1-2):96-104.
 25. Frank L, Massaro D. Oxygen toxicity. *Am J Med*. 1980;69(1):117-126.
 26. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hofeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science*. 1973;179(73):588-590.
 27. Anderson ME. Glutathione: an overview of biosynthesis and modulation. *Chem Biol Interact*. 1998;111-112:1-14.
 28. Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J*. 1999;13(10):1169-1183.
 29. Injac R, Radic N, Govedarica B, et al. Acute doxorubicin pulmonary toxicity in rats with malignant neoplasm is effectively treated with fullereneol C60(OH)24 through inhibition of oxidative stress. *Pharmacol Rep*. 2009;61(2):335-342.
 30. Faine LA, Diniz YS, Almeida JA, Novelli EL, Ribas BO. Toxicity of ad lib. overfeeding: effects on cardiac tissue. *Food Chem Toxicol*. 2002;40(5):663-668.
 31. Wouters KA, Kremer LC, Miller TL, Herman EH, Lipshultz SE. Protecting against anthracycline-induced myocardial damage: a review of the most promising strategies. *Br J Haematol*. 2005;131(5):561-578.
 32. Speyer JL, Green MD, Kramer E, et al. Protective effect of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. *N Engl J Med*. 1988;319(12):745-752.
 33. Herman EH, el-Hage A, Ferrans VJ. Protective effect of ICRF-187 on doxorubicin-induced cardiac and renal toxicity in spontaneously hypertensive (SHR) and normotensive (WKY) rats. *Toxicol Appl Pharmacol*. 1988;92(1):42-53.
 34. Dorr RT, Lagel K, McLean S. Cardioprotection of rat heart myocytes with amifostine (Ethyol) and its free thiol, WR-1065, in vitro. *Eur J Cancer*. 1996;32A(suppl 4):S21-S25.
 35. Balanehru S, Nagarajan B. Intervention of adriamycin induced free radical damage. *Biochem Int*. 1992;28(4):735-744.
 36. Yamaguchi H, Noshita T, Yu T, et al. Novel effects of glycyrrhetic acid on the central nervous system tumorigenic progenitor cells: induction of actin disruption and tumor cell-selective toxicity. *Eur J Med Chem*. 2010;45(7):2943-2948.
 37. Yu T, Yamaguchi H, Noshita T, Kidachi Y, Umetsu H, Ryoyama K. Selective cytotoxicity of glycyrrhetic acid against tumorigenic r/m HM-SFME-1 cells: potential involvement of H-Ras downregulation. *Toxicol Lett*. 2010;192(3):425-430.
 38. Niwa K, Lian Z, Onogi K, et al. Preventive effects of glycyrrhizin on estrogen-related endometrial carcinogenesis in mice. *Oncol Rep*. 2007;17(3):617-622.
 39. Kim YH, Shin EK, Kim DH, Lee HH, Park JH, Kim JK. Antiangiogenic effect of licochalcone A. *Biochem Pharmacol*. 2010;80(8):1152-1159.