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Licorice Flavonoids Inhibit Eotaxin-1 Secretion by Human Fetal Lung Fibroblasts *in Vitro*

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Glycyrrhiza uralensis (Gan-Cao), commonly called "licorice", is one of the most commonly used herbs in traditional Chinese medicine (TCM). In the United States, licorice products are most often consumed as flavoring and sweetening agents in food products. The licorice triterpenoid glycyrrhizin has several biological activities, including anti-inflammatory activity. Other potential anti-inflammatory constituents in *G. uralensis* have not been fully investigated. Airway eosinophilic inflammation is a major feature of allergic asthma. Eotaxin-1 (eotaxin) is involved in the recruitment of eosinophils to sites of antigen-induced inflammation in asthmatic airways. Because human lung fibroblasts are the major source of eotaxin, inhibition of eosinophil recruitment by suppression of fibroblast eotaxin production is a potentially valuable approach for the pharmacological intervention in asthma. A systematic bioassay-guided purification of *G. uralensis* yielded five flavonoids: liquiritin, liquiritigenin, isoliquiritigenin, 7,4′-dihydroxyflavone, and isoononin. The structures of the compounds were established by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and liquid chromatography−mass spectrometry (LC−MS) studies. The potential ability of these isolated pure compounds and glycyrrhizin to inhibit secretion of eotaxin-1 by human fetal lung fibroblasts (HFL-1) was tested. Liquiritigenin, isoliquiritigenin, and 7,4′-dihydroxyflavone were more effective than liquiritin, isoononin, and glycyrrhizin in suppressing eotaxin secretion. A concentration−response study showed the IC_{50} concentrations of liquiritigenin, isoliquiritigenin, and 7,4′-dihydroxyflavone were 4.2, 0.92, and 0.21 μg/mL, respectively, demonstrating that *Glycyrrhiza* flavonoids inhibit eotaxin-1 secretion *in vitro*.

KEYWORDS: *Glycyrrhiza uralensis*; flavonoids; liquiritigenin; isoliquiritigenin; 7,4′-dihydroxyflavone; eotaxin-1 inhibition

INTRODUCTION

Eosinophils are important in the pathogenesis of allergic asthma, allergic rhinitis, and other inflammatory diseases, such as inflammatory bowel disease and gastrointestinal allergic hypersensitivity (*1*–*4*). Interleukin (IL)-5 is the central mediator of eosinophilic proliferation and differentiation (*5*). Eotaxin recruits eosinophils to sites of allergic inflammation (*6*). Human lung fibroblast (HFL-1) cells are commonly used to investigate eotaxin secretion and mechanisms of action of eotaxin inhibitory agents (*7*, *8*).

Asthma is currently treated with several classes of drugs, including β2-adrenergic receptor agonists, glucocorticoids, theophylline, leukotriene modifiers, omalizumab, cromones, and anticholinergic agents. The most effective for long-term control are those that reduce the underlying inflammation (*9*). Glucocorticoids are the most effective anti-inflammatory drugs, with a good safety profile when inhaled at low doses. However, side effects when either large dose inhaled steroids or systemic steroids are required indicate the need for alternative anti-inflammatory approaches. Use of traditional herbal medicines (TM) is rapidly increasing despite the lack of conclusive scientific evidence regarding safety and efficacy of TMs. Traditional Chinese medicines (TCMs) are of increasing interest to Western researchers, and a growing number of studies are exploring their efficacy, safety, and mechanisms of action. *Glycyrrhiza uralensis* is a prominent herb in TCM and is the major constituent in several TCM formulas used to treat asthma, coughs, and peptic ulcers (*10*). An aqueous extract of *G. uralensis* rhizomes is one of the constituents in anti-asthma herbal medicine intervention (ASHMI), which has been shown to have therapeutic effects in an animal model of asthma and in a controlled clinical trial of moderate to severe asthmatics

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(*11*, *12*). The animal study found that ASHMI inhibited eosinophil accumulation in lungs of allergic mice following antigen intratracheal challenge (*11*). The present investigation was designed to determine the relative activities of individual flavonoids isolated from *G. uralensis* as inhibitors of constitutively expressed eotaxin in human lung fibroblasts *in vitro*.

MATERIALS AND METHODS

General Experimental Procedures. ^{1}H (600/500 MHz) and ^{13}C (150/125 MHz) nuclear magnetic resonance (NMR) data were recorded on INOVA VARIAN VRX 500/600 instruments using standard pulse sequences. The chemical shifts were measured in DMSO-d_6 and expressed in δ. The silica gel used for column chromatography (CC) was Merck Silica gel 60 (35−70 μm particle size). Analytical Si gel plates (20 × 20, 500 μm) were purchased from Fisher Scientific. Organic solvents for CC and liquid chromatography (LC) were purchased from Fisher Scientific (Fair Lawn, NJ). Glycyrrhizin ammonium salt was purchased from Sigma-Aldrich (St Louis, MO), and identity was confirmed by LC−mass spectrometry (MS) and NMR spectra. HFL-1 cells were purchased from American Type Collection Culture (CCL-153) and maintained in our laboratory.

Plant Material. The extracts of Gan-Cao (*G. uralensis*) (root and rhizome) were purchased from Sino-lion Pharmaceutical Company (Weifang, China). The extracts were prepared as follows: shade dried roots and rhizome of *G. uralensis* were soaked in water (1:10, w/w) and boiled for 45 min, and the decoction was collected. The process was repeated, and the combined decoction was filtered and spray-dried. The yield of extract was 22.9%. The powdered extract was shipped to Mount Sinai School of Medicine and stored at room temperature until further use.

Extraction and Isolation of Compounds. A total of 500 g of *G. uralensis* dried hot water extract was mixed with methanol (MeOH) (1 L) at room temperature with occasional stirring and filtered. The extraction was repeated several times and concentrated under reduced pressure to yield 125.0 g of MeOH extract. This extract (23.0 g) was chromatographed using a silica gel column and eluted with $CHCl_3$ and $CHCl_3$ and MeOH step gradients. A total of 50 fractions of 20 mL each were collected. On the basis of their thin-layer chromatography (TLC) profiles, fractions were combined to produce five major fractions Fr. A (2.2 g), Fr. B (1.9 g), Fr. C (4.8 g), Fr. D (5.2 g), and Fr. E (8.0 g). Fr. D contained one major compound, and it was purified by recrystallization to yield the pure compound liquritin (1.2 g). Purification of fractions B and C using silica CC yielded isoononin (150 mg) and 7,4′-dihydroxyflavone (150 mg), respectively. In addition to 7,4′-dihydroxyflavone, fraction C yielded Fr. I−III. Fr. II was subjected to CC to yield liquiritigenin (25.0 mg) and isoliquiritigenin (37.0 mg).

Characterization of Compounds. The isolated compounds were identified by ^{1}H and ^{13}C NMR and LC−MS (accurate mass) and a comparison of the spectral data with published results as liquiritin (*13*), liquiritigenin (*13*, *14*), isoliquiritigenin (*15*, *16*), 7,4′-dihydroxyflavone (*17*), and isoononin (*18*) (**Figure 1**).

High-Performance Liquid Chromatography (HPLC) Quantification. The biologically active compounds present in *Glycyrrhiza* extract were quantified by HPLC. The analyses were carried out with a Waters Alliance HT 2695 (Waters Corp., Milford, MA) HPLC instrument equipped with autosampler and photodiode array (PDA) detector with Empower software (Waters Corp., Milford, MA). A Zorbax-C_{18} (150 × 4.6 mm) column was used with mobile phase consisting of A [99.9:0.1 H_2O/H_3PO_4 (v/v)] and B (CH_3CN). The linear gradient from 2 to 46% B over 75 min and back to initial conditions (2% B) in 80 min with a flow rate of 1.0 mL/min was used for elution of the compounds. Because *G. uralensis* is a major component of the multiherbal formula ASHMI, an HPLC method was developed to monitor the presence of *G. uralensis* in ASHMI. Each run was followed by 10 min of equilibration and automatic needle wash with wash solvent (80% acetonitrile in water). Samples were initially dissolved in MeOH and diluted with H_2O. The sample injection volume was 10 μL. Each concentration was assayed 3 times. The standards required for quantification of bioactive compounds were prepared at 300, 100, 30, 10, 3, and 1 μg/mL concentration with the mobile phase.

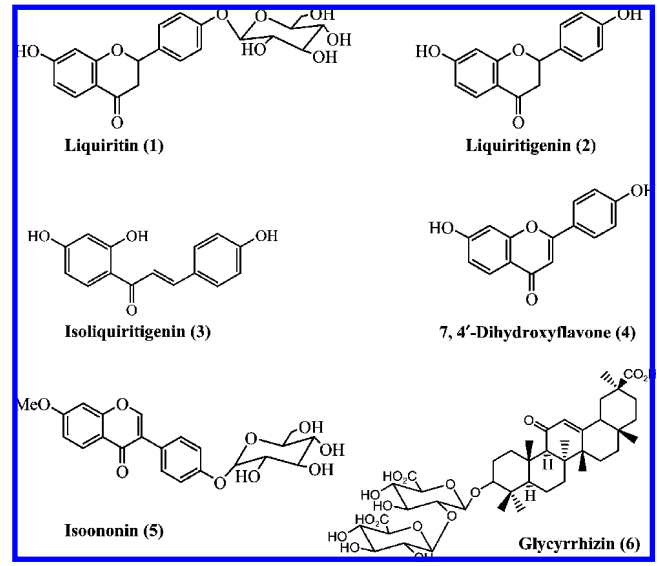

Figure 1. Structure of the compounds.

LC−MS. The accurate mass for the isolated pure compounds was determined by time of flight (TOF)−MS using LCT-Premier XE (Waters Corp., Milford, MA). For LC−MS, we developed a short elution method to monitor the compounds present in *G. uralensis* and used this method for accurate mass measurement of pure compounds. The mobile phase used was A, H_2O (0.1% HCO_2H) and B, CH_3CN (0.1% HCO_2H), with a linear gradient from 20 to 50% B over 25 min at a flow rate of 1.0 mL/min using a peak flow splitter. The LC and the mass spectrometer were controlled by MassLynx 4.1 application manager software. Leucine enkaphalin was used as a lock spray reagent for accurate mass measurements. Sodium formate (HCOONa) was used as a calibration standard. Positive electrospray ionization (ESI^+) was used to analyze the molecules with the following parameters: capillary voltage, ~3.2−3.5 kV; source temperature, 100 °C; desolvation temperature, 50 °C; desolvation gas flow, 550 L/h; cone gas flow, 50 L/h; mass range, 100−1000 amu.

HFL-1 Cell Culture and Eotaxin-1 Inhibition Assay. Eotaxin-1 inhibitory assays were conducted by using human fetal lung fibroblast (HFL-1) cells. HFL-1 (CCL-153) cells were grown in F-12K medium (ATCC, Rockville, MD) containing 10% fetal bovine serum (Gibco BRL, Grand Island, NY) and 1% penicillin−streptomycin (BD Bio, San Jose, CA). The linearly growing cells were detached using trypsin−ethylenediaminetetraacetic acid (EDTA) and transferred to 24-well culture plates, 8 × 10^4 cells per well. The cells between passages 10−14 were used for the assay. After 48 h, the growth medium was replaced with medium containing test compounds at different concentrations. Compounds were initially dissolved in dimethylsulfoxide (DMSO) and added to the medium. The final DMSO concentration in the assay well was 0.1%. Serial dilutions (1:1) were made to establish the concentration−response curves. Each concentration was tested in triplicate. Supernatants were harvested at 96 h after the addition of the compounds. Then, cells were detached from wells with trypsin−EDTA, centrifuged, and resuspended in 2.0 mL of medium. Cell suspensions (50 μL) were mixed 50 μL of trypan blue (Mediatech, Inc., Manassas, VA). Cell viability was determined by direct counts of live and dead cells using a microscope.

Enzyme-Linked Immunosorbent Assay (ELISA). Eotaxin-1 levels were determined in cell supernatants by ELISA (R and D Systems) according to the directions of the manufacturer. Briefly, the capture antibody (2.0 μg/mL) was diluted and coated on 96-well microplates and incubated overnight at room temperature. The next day, plates were blocked with 300 μL of reagent diluent [1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS)] for 1 h, and cell supernatants (100 μL), standards (1000−15.6 pg/mL), and QCs were incubated for 2 h. Then, detection antibody (100 ng/mL) was incubated (2 h) followed by strepadivin−horseradish peroxidase (HRP) (20 min). At each step, plates were washed with 350 μL of wash buffer. A total of 100 μL of substrate solution was added to the plates and incubated for 30 min,

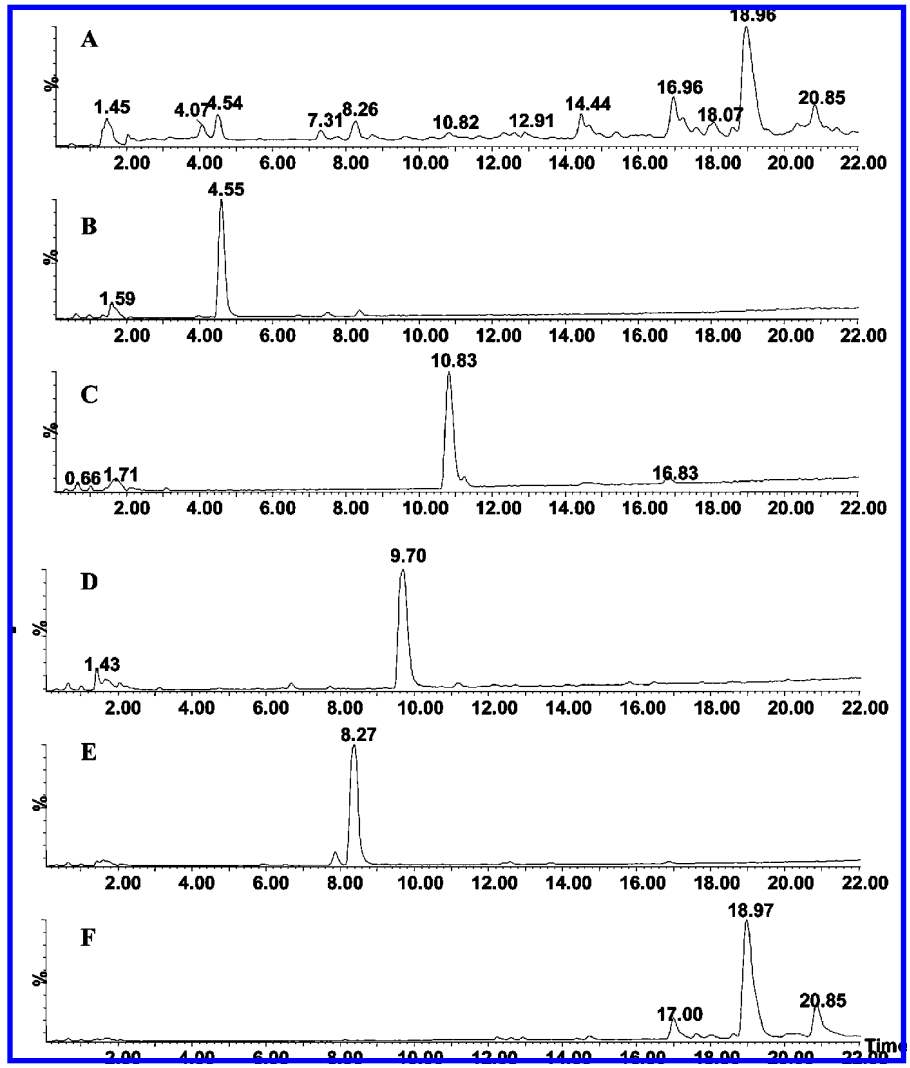

Figure 2. TIC of water extracts of (A) *G. uralensis*, (B) liquiritin, (C) liquiritigenin, (D) 7,4′-dihydroxyflavone, (E) isoononin, and (F) glycyrrhizin.

and the reaction was stopped by stop solution (2 N H_2SO_4). The optical density (OD) was recorded at 570 nm using a Vmax Kinetic Microphate Reader and Softmax pro. 3.0 (Molecular Device Corp., Sunnyvale, CA). The amount of eotaxin was calculated automatically by the software from the standard curve. The percent inhibition was calculated by a comparison to the controls. The IC_{50} values were calculated by nonlinear regression analysis using GraphPad Prism 4.01 (San Diego, CA).

MTT Cell Viability Assay. Cell viability was determined according to a previously published procedure (*19*). HFL-1 cells were detached from growth plates with trypsin−EDTA, centrifuged, and resuspended in medium. An aliquot of 100 μL containing 8×10^3 cells was transferred to each well of a 96-well cell-culture plate. After 48 h, compounds of required concentration as described in the cell-culture assay were added. After 96 h of incubation, 25 μL of MTT (4.0 mg/mL) was added to the wells and incubated for 4 h. The medium along with MTT was aspirated; formozan crystals were solubilized in DMSO; and OD was read at 570 nm.

RESULTS AND DISCUSSION

LC−MS Analysis of *G. uralensis* Extract and Its Purified Compounds. Figure 2 represents the total ion chromatogram (TIC) of the crude formulation of *G. uralensis* and isolated pure compounds. MS spectra showed the presence of flavonoids and triterpenoids in *G. uralensis*. The isolated pure compounds and glycyrrhizin were also analyzed along with *G. uralensis* extract, and selected data are represented in **Figure 3**. The molecular mass for the compounds in the extract was displayed <5.0 ppm deviation with calculated mass. When the retention time is compared to the MS spectrum, the peak at 4.54 min was identified as liquiritin, with a molecular mass of 419.1337. The peak at 4.07 min had the same molecular formula as liquiritin, indicating that it was an isomer of liquiritin. Because this is a minor compound, we could not isolate it in pure form. The UV spectrum together with an accurate mass measurement identified the structure of the compound as isoliquiritin. Peaks at 10.83 and 14.44 min were identified as liquiritigenin and isoliquiritigenin. A minor peak at 9.70 min was identified as 7,4′-dihydroxyflavone. Our attempts to isolate the major peak at 18.96 min were unsuccessful. This may be due to instability of the molecule under our isolation conditions. However, it was identified as glycyrrhizin by high-resolution mass spectrometry followed by comparing the retention time to a commercially available standard. The structures of the isolated compounds were also confirmed by ^1H and ^{13}C NMR spectra and a comparison to the published results.

Quantification. The bioactive flavonoids present in the *G. uralensis* were quantified by HPLC. The calibration curves were generated for pure compounds by plotting the peak areas as a function of the concentration for six concentrations ranging from 300 to 1 μg/mL. The isomers, liquiritigenin and isoliquirtigenin, were present in approximately equal quantities, about 30 μg/100 mg of extract. The most potent compound, 7,4′-dihydroxyflavone, is a minor herbal constituent [8 μg/100 mg (w/w)], whereas liquiritin is the most abundant [184 μg/100

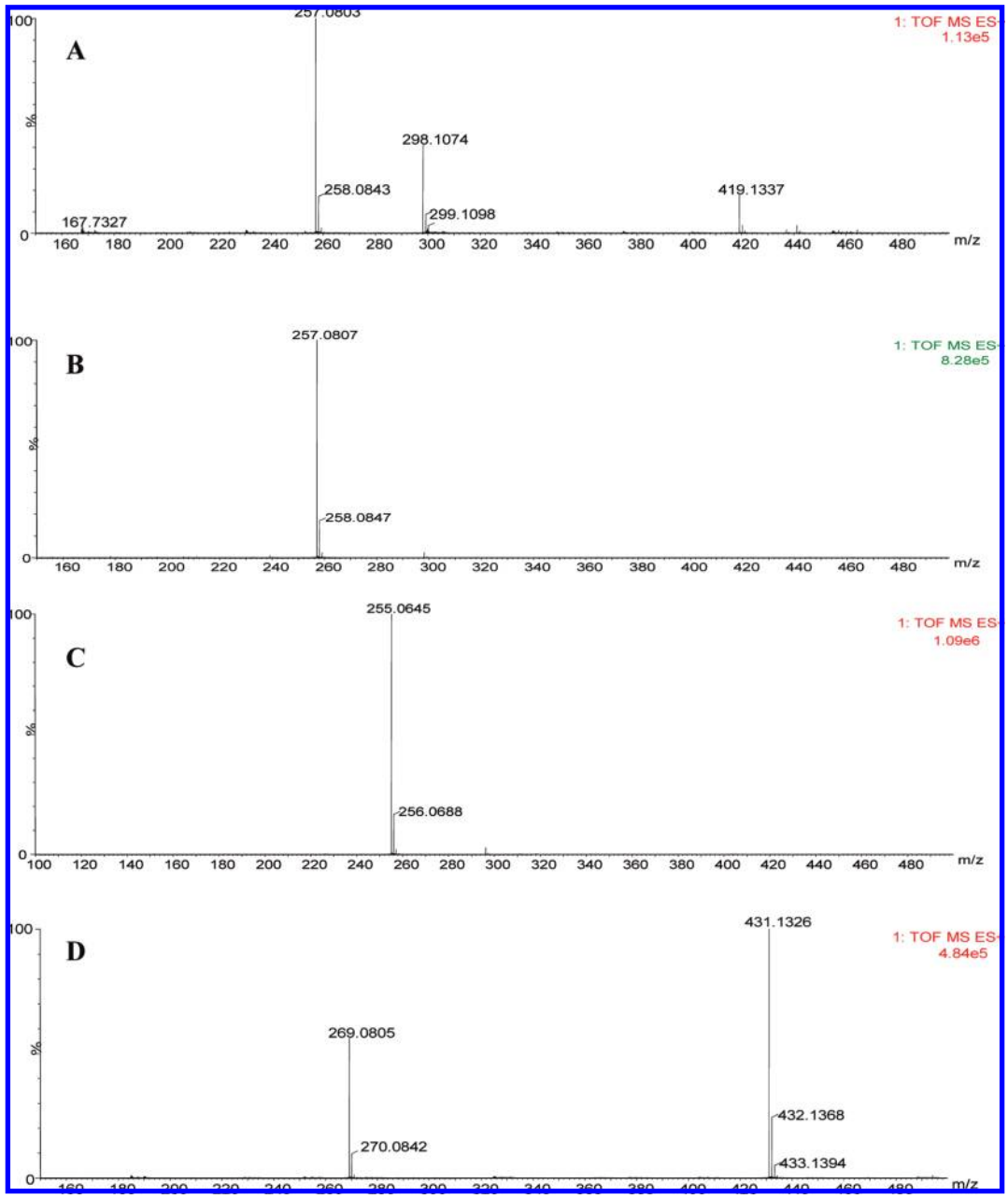

Figure 3. Mass spectrum of (A) liquiritin, (B) liquiritigenin, (C) 7,4′-dihydroxyflavone, and (D) isononin. The accurate mass (high resolution) of the compounds was measured by a TOF instrument.

Table 1. Amounts and IC_{50} Values of Tested Compounds

compound	concentration (μg/100 mg of extract)	IC_{50} (μg/mL)
liquiritin	184	25.2 ± 1.0
liquiritigenin	28	4.2 ± 0.07
isoliquiritigenin	30	0.92 ± 0.05
7,4′-dihyroxyflavone	8	0.21 ± 0.06
isoononin	36	ND[a]
glycyrrhizin	ND[a]	ND[a]

[a] ND = not determined.

mg (w/w)] in our formulation (**Table 1**). Even though active compounds are present in lower quantities, considering the IC_{50} values, they may play major roles in the overall observed activity of *G. uralensis*.

Inhibitory Effect of Licorice Flavonoids on Eotaxin Secretion. To determine the potential anti-inflammatory effects of the licorice flavonoids identified above and because eotaxin is the key chemokine involved in eosinophil migration into the lung in asthma and other diseases, we tested the effects of these licorice flavonoidal compounds on HFL-1 eotaxin production. A previous study showed that the concentration of glycyrrhizin and its derivatives that produced approximately 50% inhibition of stimulation-induced eotaxin and other inflammatory cytokines was between 25 and 100 μg/mL (*7*). For our initial activity screen, we tested 25 μg/mL concentrations of the five isolated compounds and glycyrrhizin (**Figure 4A**). Liquiritin inhibited eotaxin-1 production by approximately 50%, and isoononin inhibited eotaxin-1 production by approximately 20%, which is similar to glycyrrhizin (**Figure 4A**) in our assay. Liquiritigenin completely abolished eotaxin production without cytotoxicity, demonstrating effective antieotaxin activity. Isoliquiritigenin and 7,4′-dihydroxyflavone also abolished eotaxin production; however, both compounds exhibited significant cytotoxicity at the 25 μg/mL concentration used (**Figure 4B**). Because lower concentrations might significantly inhibit eotaxin production

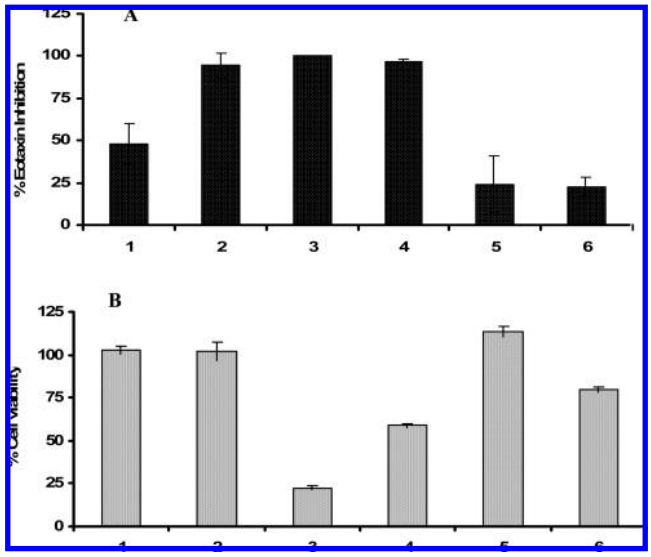

Figure 4. Effect of compounds on (A) eotaxin inhibition and (B) cell viability at 25 μg/mL concentration. Compounds were incubated with HFL-1 cells for 96 h, and the amount of eotaxin secreted was estimated by the ELISA assay. A MTT calorimetric assay was used to determine the cell viability of compounds as described in the Materials and Methods. The percent inhibition was calculated with respect to controls. Data represent the mean $\pm$ standard error of the mean (SEM) ($n = 3$).

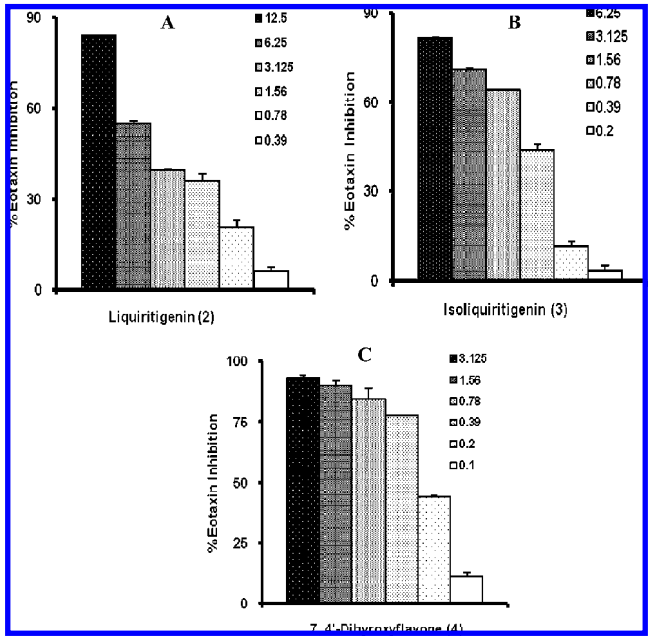

Figure 5. Concentration-dependent inhibition of eotaxin-1 by (A) liquiritigenin, (B) isoliquiritigenin, and (C) 7,4′-dihyroxyflavone. Percent inhibition was calculated by a comparison of the eotaxin-1 levels of treated groups with controls. Data represents the mean $\pm$ SEM ($n = 3$). The compounds did not show any cytotoxicity, even at the maximum tested concentrations, and cells were very healthy in concentration−response studies as determined by both MTT and trypan blue exclusion assays.

without concomitant cytotoxicity, we further investigated the three compounds that were most active in the initial screen.

Concentration−response curves for inhibition of eotaxin release by liquiritigenin, isoliquiritigenin, and 7,4′-dihydroxyflavone demonstrated significant inhibition at nontoxic levels, with IC_{50} values of 4.2, 0.92, and 0.21 μg/mL, respectively (**Figure 5** and **Table 1**). The cells were >95% viable, as determined by both MTT and trypan blue exclusion assays, at the maximal concentrations tested [12.5 μg/mL (liquiritigenin), 6.25 μg/mL (isoliquiritigenin), and 3.125 μg/mL (7,4′-dihydroxyflavone)] (data not shown). These results suggest that liquiritigenin, isoliquiritigenin, and 7,4′-dihydroxyflavone in *G. uralensis* may produce potent anti-inflammatory effects.

In many parts of the world, licorice products are most often used as flavoring and sweetening agents in food products (*20*–*22*). Previous phytochemical investigations of this plant identified several flavonoids, triterpenoids, and polysaccharides (*23*). The triterpenoids glycyrrhizin and its aglycone glycyrhetinic acid are the major compounds exhibiting anti-inflammatory activities, including decreased IL-4 and IgE levels in murine asthma models (*24*). 18α-Glycyrrhizin, with an IC_{50} value of 25 μg/mL for inhibition of eotaxin-1, has been reported to be more effective than its 18β counterpart in HFL-1 cells (*7*). However, in the present study, glycyrrhizin exhibited only a slight effect at 25 μg/mL. Recent studies also showed that glycyrrhizin and its derivative, 3β-[(2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranuronosyl)oxy]-olean-11,13(18)-dien-30-ol (hetero-30-OH-glycyrrhizin) inhibited activation of STAT-6 and NF-kB, the major transcription factors involved in upregulation of eotaxin synthesis (*8*). Because eotaxin-1 is one of the major chemokines involved in recruitment of eosinophils, inhibition of eotaxin-1 secretion would be expected to have a beneficial effect on eosinophil-associated inflammatory disorders, such as asthma, eosinophilic esophagitis, and inflammatory bowel disease. The antiallergic effects of *G. uralensis* have mainly been attributed to glycyrrhizin; however, other constituents have not been systematically evaluated. In the present study, liquiritin, liquiritigenin, isoliquiritigenin, and 7,4′-dihydroxyflavone, structurally related flavonoidal compounds, showed potent inhibition of eotaxin-1 secretion. The rank order potency for inhibition of eotaxin production was 7,4′-dihydroxyflavone > isoliquiritigenin > liquirtitigenin > liquiritin. Although substantial quantities of liquirtin are present in *G. uralensis*, it did not exhibit promising biological activities in earlier studies. Its aglycone liquiritigenin potently inhibited IgE-induced degranulation of RBL-2H3 cells *in vitro* and blocked anaphylactic reactions in mice (*25*). Liquiritigenin also showed protective effects against acetaminophen-induced acute liver injuries (*26*). Isoliquiritigenin is an isomer of liquiritigenin well-known for antitumor activity, which induces apoptosis in human prostate cancer cells (*27*). Isoliquirtigenin also decreased the production of inflammatory prostaglandin, PGE2, and NO through suppression of COX-2 and inducible nitric oxide synthase (iNOS) protein expression (*28*). 7,4′-Dihydroxyflavone, the dehydro derivative of liquiritigenin, which was the most potent constituent evaluated in this study, has not been extensively studied in bioassays. In fact, except for baicalein (IC_{50} value of 1.8 μg/mL), none of the flavonoidal compounds have been tested for eotaxin-1 inhibitory activity (*29*). Biosynthetically, isoliquiritigenin is the precursor of liquiritigenin, which in turn is the precursor for 7,4′-dihydroxyflavone. Liquiritin, a major constituent of *G. uralensis*, is less active than its aglycone, liquiritigenin. The formation of double bonds at the 2 and 3 positions in compound 7,4′-dihydroxyflavone enhanced eotaxin inhibitory activity. Even though the compounds examined are too few to draw extensive conclusions about the relationships between structure and activity, the results do show that the double bond at the 2 and 3 positions plays a major role in inhibition of eotaxin-1.

Our *in vitro* study shows that *Glycyrrhiza* flavonoids potently inhibit eotaxin-1 secretion. Because *G. uralensis* is widely consumed in herbal medications and food products, exploring the biological active components in this plant will benefit human

health. Our study, together with earlier studies, shows the potential of *G. uralensis* compounds as anti-inflammatory agents for asthmatics. However, further studies are needed to determine the efficacy and safety of purified components of this herb *in vivo*. Our study is the first demonstration of inhibition of eotaxin-1 by the reported compounds.

LITERATURE CITED

(1) Maddox, L.; Schwartz, D. A. The pathophysiology of asthma. *Annu. Rev. Med*. **2001**, *53*, 477–498.

(2) Mould, A. W.; Matthaei, K. I.; Young, I. G.; Foster, P. S. Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J. Clin. Invest*. **1997**, *99*, 1064–1071.

(3) Rankin, S. M.; Conroy, D. M.; Williams, T. J. Eotaxin and eosinophil recruitment: Implications for human disease. *Mol. Med. Today* **2000**, *6*, 20–27.

(4) Wardlaw, A. J. Eosinophil trafficking in asthma. *Clin. Med*. **2001**, *1*, 214–218.

(5) Clutterbuck, E. J.; Hirst, E. M.; Sanderson, C. J. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: Comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. *Blood* **1989**, *73*, 1504–1512.

(6) Mould, A. W.; Ramsay, A. J.; Matthaei, K. I.; Young, I. G.; Rothenberg, M. E.; Foster, P. S. The effect of IL-5 and eotaxin expression in the lung on eosinophil trafficking and degranulation and the induction of bronchial hyperreactivity. *J. Immunol*. **2000**, *164*, 2142–2150.

(7) Matsui, S.; Matsumoto, H.; Sonoda, Y.; Ando, K.; Aizu-Yokota, E.; Sato, T.; Kasahara, T. Glycyrrhizin and related compounds down-regulate production of inflammatory chemokines IL-8 and eotaxin 1 in a human lung fibroblast cell line. *Int. Immunopharmacol*. **2004**, *4*, 1633–1644.

(8) Matsui, S.; Sonoda, Y.; Sekiya, T.; Aizu-Yokota, E.; Kasahara, T. Glycyrrhizin derivative inhibits eotaxin 1 production via STAT6 in human lung fibroblasts. *Int. Immunopharmacol*. **2006**, *6*, 369–375.

(9) Expert Panel Report 3 (EPR-3). Guidelines for the Diagnosis and Management of Asthma-Summary Report. *J. Allergy Clin. Immunol*. **2007**, *120*, S94−S138.

(10) Zheng, X. Y. *Pharmacopoeia of the People's Republic of China*; Chemical Industry Press: Beijing, China, 2000.

(11) Li, X. M. Traditional Chinese herbal remedies for asthma and food allergy. *J. Allergy Clin. Immunol*. **2007**, *120*, 25–31.

(12) Wen, M. C.; Wei, C. H.; Hu, Z. Q.; Srivastava, K.; Ko, J.; Xi, S. T.; Mu, D. Z.; Du, J. B.; Li, G. H.; Wallenstein, S.; Sampson, H.; Kattan, M.; Li, X. M. Efficacy and tolerability of anti-asthma herbal medicine intervention in adult patients with moderate−severe allergic asthma. *J. Allergy Clin. Immunol*. **2005**, *116*, 517–524.

(13) Fu, B.; Li, H.; Wang, X.; Lee, F. S. C.; Cui, S. Isolation and identification of flavonoids in licorice and a study of their inhibitory effects on tyrosinase. *J. Agric. Food Chem*. **2005**, *53*, 7408–7414.

(14) Yahara, S.; Nishioka, I. Flavonoid glucosides from licorice. *Phytochemistry* **1984**, *23*, 2108–2109.

(15) Vaya, J.; Belinky, P. A.; Aviram, M. Anti-oxidant constituents from licorice roots: Isolation, structure elucidation, and anti-oxidative capacity toward LDL oxidation. *Free Radical Biol. Med*. **1997**, *23*, 302–313.

(16) Ma, C.-J.; Li, G.-S.; Zhang, D.-L.; Liu, K.; Fan, X. One step isolation and purification of liquiritigenin and isoliquiritigenin from *Glycyrrhiza uralensis* Risch. using high-speed counter-current chromatography. *J. Chromatogr., A* **2005**, *1078*, 188–192.

(17) Park, Y.; Moon, B.-H.; Lee, E.; Lee, Y.; Yoon, Y.; Ahn, J.-H.; Lim, Y. Spectral assignments and reference data: ^{1}H and ^{13}C-NMR data of hydroxyflavone derivatives. *Magn. Reson. Chem*. **2007**, *45*, 674–679.

(18) Haznagy, A.; Toth, G.; Tamas, J. Constituents of the aqueous extracts from *Ononis spinosa*. *L. Arch. Pharm*. **1978**, *311*, 318–323.

(19) Jayaprakasam, B.; Zhang, Y.; Seeram, N. P.; Nair, M. G. Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. *Life Sci*. **2003**, *74*, 125–132.

(20) Kitagawa, I. Licorice root. A natural sweetener and an important ingredient in Chinese medicines. *Pure Appl. Chem*. **2002**, *74*, 1189–1198.

(21) Kinghorn, A. D.; Compadre, C. M. Less common high potency sweeteners. In *Alternative Sweeteners*, 3rd ed.; O'Brien Nabors, L., Ed.; Marcel Dekker: New York, 2001; pp 209−231.

(22) U.S. Department of Agriculture. GRAS status of licorice (*Glycyrrhiza*), ammoniated glycyrrhizin, and monoammonium glycyrrhizinate. Federal Regulation 50, 52104321045, 19852.

(23) Wang, Z. Y.; Nixon, D. W. Licorice and cancer. *Nutr. Cancer* **2001**, *39*, 1–11, and references cited therein.

(24) Ram, A.; Mabalirajan, U.; Das, M.; Bhattacharya, I.; Dinda, A. K.; Gangal, S. V.; Ghosh, B. Glycyrrhizin alleviates experimental allergic asthma in mice. *Int. Immunopharmacol*. **2006**, *6*, 1468–1477.

(25) Shin, Y.-W.; Bae, E.-A.; Lee, B.; Lee, S. H.; Kim, J. A.; Kim, Y.-S.; Kim, D.-H. In vitro and in vivo antiallergic effects of *Glycyrrhiza glabra* and its components. *Planta Med*. **2007**, *73*, 257–261.

(26) Kim, Y. W.; Ki, S. H.; Lee, J. R.; Lee, S. J.; Kim, C. W.; Kim, S. C.; Kim, S. G. Liquiritigenin, an aglycone of liquiritin in *Glycyrrhizae radix*, prevents acute liver injuries in rats induced by acetaminophen with or without buthionine sulfoximine. *Chem.-Biol. Interact*. **2006**, *161*, 125–138.

(27) Jung, J. I.; Chung, E.; Seon, M. R.; Shin, H. K.; Kim, E. J.; Lim, S. S.; Chung, Y.-W.; Park, K.-K.; Park, J. H. Y. Isoliquiritigenin (ISL) inhibits ErbB3 signaling in prostate cancer cells. *BioFactors* **2006**, *28*, 159–168.

(28) Takahashi, T.; Takasuka, N.; Iigo, M.; Baba, M.; Nishino, H.; Tsuda, H.; Okuyama, T. Isoliquiritigenin, a flavonoid from licorice, reduces prostaglandin E_2 and nitric oxide, causes apoptosis, and suppresses aberrant crypt foci development. *Cancer Sci*. **2004**, *95*, 448–453.

(29) Nakajima, T.; Imanishi, M.; Yamamoto, K.; Cyong, J.-C.; Hirai, K. Inhibitory effect of baicalein, a flavonoid in *Scutellaria* root, on eotaxin production by human dermal fibroblasts. *Planta Med*. **2001**, *67*, 132–135.

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