Hexane/ethanol extract of Glycyrrhiza uralensis licorice exerts potent anti-inflammatory effects in murine macrophages and in mouse skin

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1. Introduction

Inflammation is a complex biological response to harmful stimuli, such as pathogens. However, epidemiological evidence has suggested that chronic inflammation predisposes individuals to several types of cancer. In addition, the cellular effectors and mediators of inflammation are crucial elements of the microenvironment surrounding the tumour. Thus, inflammation plays a crucial role in the development and progression of several types of cancer (Baud & Karin, 2009; Mantovani, Allavena, Sica, & Balkwill, 2008). There is an evidence indicating that the targeting of inflammatory mediators, interleukin (IL)-1, IL-6, and tumour necrosis factor (TNF)-α (Griennikov & Karin, 2008; Szlosarek & Balkwill, 2003; Voronov et al., 2003), and the key transcription factor involved in inflammation [nuclear factor κB (NFkB)] reduces the incidence and progression of cancer (Baud & Karin, 2009). Genes activated by NFkB include inflammatory regulators, such as several cytokines (TNFα, IL-1, IL-6), and inducible enzymes [cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS)] (Baud & Karin, 2009). In unstimulated cells, NFkB is localised in the cytoplasm and are associated with inhibitor of κB (IκB).When cells were activated by various stimuli, IκB kinase (IKK) phosphorylates the serine residues of IκB, thereby resulting in the degradation of the protein, which results in the nuclear translocation of free NFkB. NFkB binds to the promoter regions of target genes and activates their transcription (reviewed in Yamamoto & Gaynor, 2004).

Glycyrrhiza uralensis has long been utilised throughout the world as a sweetener and has been frequently prescribed in traditional folk medicine for the treatment of inflammation, liver disease, and adrenal insufficiency (Asl & Hosseinzadeh, 2008). Recently, the ethanol extract of roasted licorice was shown to inhibit lipopolysaccharide (LPS)-induced inflammatory responses in murine macrophages (Kim et al., 2006). Glycyrrhizin is the principal compound in licorice (4–10%) and was reported to inhibit LPS/galactosamine-induced liver injury (Yoshida et al., 2007) and to alleviate asthmatic features in mice (Ram et al., 2006). However, glycyrrhizin is converted, by human intestinal bacteria, to glycyrhetic acid (Hattori, Sakamoto, Kobashi, & Namba, 1983) and glycyrrethic acid has been reported to be a cause of severe hypertension and hypocalcemia, (Asl & Hosseinzadeh, 2008; van Uum, 2008).
2005). Thus, we previously prepared a hexane/ethanol (90:10, v:v) extract of *G. uralensis* (HEGU), which did not harbour measurable quantities of glycyrrhizin (Choi et al., 2008).

In the present study, we tested the effects of HEGU lacking glycyrrhizin on inflammatory responses in LPS-stimulated RAW264.7 murine macrophages and 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin swelling in male ICR mice. The present *in vitro* and *in vivo* experiments show that HEGU exerts extremely potent anti-inflammatory effects, suggesting that HEGU may be successfully developed as an anti-inflammatory agent for the treatment of skin diseases.

2. Materials and methods

2.1. Reagents

LPS, TPA, AA-861, indomethacin, and anti-β-actin antibody were purchased from Sigma Chemical Co. (St. Louis, Mo). Antibodies against iNOS and COX-2 were obtained from BD Transduction Laboratories (Palo Alto, CA). Anti-NF-κB antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-inhibitor of κB (IκB)α antibody was purchased from Cell Signaling Technology (Beverly, MA).

2.2. Identification of dehydroglyasperin C present in HEGU

Dried licorice roots of *G. uralensis* were purchased from a local drug store (Deaguang Medical, Chuncheon). HEGU was prepared as previously described (Choi et al., 2008). In brief, the dried and ground roots of *G. uralensis* (1 kg) were dip-extracted for 24 h with 10 l of n-hexane:ethanol (9:1,v/v) at room temperature, then filtered through Whatman No. 2 filter paper. The filtrate was then concentrated under pressure at 40 °C, yielding 90 g of HEGU. A portion of HEGU (5.2 g) was subjected to flash column chromatography with silica gel (70–230 mesh American Society for Testing and Materials; Merck, Darmstadt, Germany) and eluted by gradient systems of n-hexane-ethyl acetate (10:0 → 5:5, v/v). Fractions were collected and examined for the ability to inhibit NO production by RAW264.7 cells. The two most active fractions (700 mg) were pooled and further purified via preparative high-performance liquid chromatography (Prep-HPLC, Japan Analytical Industry Co. Ltd., Tokyo, Japan) with a silica gel column (DOS-BP-L, Japan Analytical Industry). A mixture of methanol:water (4:1, v/v) was used as the mobile phase at a flow rate of 4.0 ml/min, and detection was conducted at 245 nm. The structural identification of the compound was conducted via 1H NMR and 13C NMR. 1H and 13C NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer (Billerica, MA, USA) with tetramethylsilane as an internal standard. IR spectra were recorded on a FT-IR 4100 (JASCO Inc., Easton, USA). Mass spectra were obtained on a PolarisQ Ion Trap GC/MS (Thermo Fisher Scientific Inc., Waltham, USA). All 13C multiplicities were deduced from the 90° and 135° Distortionless Enhancement by Polarization Transfer experiments.

2.3. Cell culture and cell viability

The RAW264.7 cell line was purchased from the American Type Culture Collection (Manassas, VA) and maintained in DMEM containing 10% FBS, 100,000 units/l of penicillin, and 100 µg/ml of streptomycin. To assess the effects of HEGU and LPS on cell viability, cells were plated in 24-well plates at 50,000 cells/well with DMEM containing 10% FBS. One day later, the monolayers were serum-deprived for 24 h in DMEM containing 1% FBS. After serum deprivation, the cells were treated with various concentrations (0, 1, or 2 µg/ml) of HEGU in the absence or presence of 100 ng/ml of LPS. Viable cell numbers were estimated via an MTT assay.

2.4. Nitric oxide (NO), prostaglandin (PG)E2, and cytokine assay

Cells were treated with HEGU or dehydroglyasperin C, and media conditioned for 24 h were collected for NO, prostaglandin (PG)E2, and cytokine assays. The NO concentrations were measured using the Griess reagent system (Promega, Madison, WI), and the PGF2 concentrations were measured with a PGF2 assay kit (R&D Systems, Minneapolis, MN). The concentrations of TNF-α, IL-6, and IL-1β were measured using enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer’s instructions (eBioscience, San Diego, CA).

2.5. Western blot analysis and electrophoretic mobility shift assay (EMSA)

Raw264.7 cells were plated in 100 mm dishes at 1.5 × 10^6 cells/dish, serum-deprived, and treated with various concentrations (0, 1, or 2 µg/ml) of HEGU or dehydroglyasperin C in the absence or presence of LPS. Total cell lysates and nuclear extracts were prepared and Western blot analyses were conducted as previously described (Choi et al., 2008).

For the NFκB DNA binding assay, nuclear extracts (10 µg) were incubated with γ-32P-labelled NFκB consensus oligonucleotide (Cho et al., 2008) and 1 µg poly(dIdC) in 30 µl of binding buffer (10 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, 4% glycerol, 1 mM DTT) for 30 min at 37 °C. Each sample was subjected to 5% non-denaturing gel, and the gels were dried and visualised via autoradiography. The relative abundance of chemiluminescent detection of the blots or an autoradiography of the dried gels was quantified by the densitometric scanning of the exposed film using the Bio-Profile Bio-1D application (Vilber-Lourmat, France).

2.6. RT-PCR and real-time RT-PCR

Raw264.7 cells were plated and treated with HEGU and/or LPS as described above. Total RNA was isolated and cDNA was synthesised. For the determination of COX-2 and iNOS mRNA, sequences for PCR primer sets, annealing temperatures, and numbers of cycles used for PCR amplification were published by Park et al. (2006). The PCR products were separated on 1% agarose gel and stained with ethidium bromide.

For the quantification of TNF-α, IL-6, IL-1β, and phospholipase (PL)A2 transcripts, real-time PCR was conducted using a Rotor-gene 3000 PCR (Corbett Research, Australia) as described previously (Cho et al., 2008). The sequences for these PCR primer sets have been published previously (Schaloske, Provins, Kessen, & Dennis, 2005; Shin, Lee, Shin, Kim, & Yoo, 2004). The reaction was conducted in 20 µl of reaction mixture containing 2 µl of cDNA, 0.5 µM of both primers, and 10 µl of SYBR PCR master Mix (Qiagen, Valencia, CA). The analysis of PCR results and the calculation of the relative concentrations were conducted using Rotor-gene software (Ver. 6), and the control levels were set to 1.

2.7. Luciferase reporter gene assay

The cells were co-transfected with pGL-miNOS-1588 (Cho, Lee, Lee, & Kim, 2005) or NFκB-luciferase reporter plasmid and pCMV II-galactosidase control vector, using Nucleofector II (Amaxa, Gaithersburg, MD). The transfected cells were plated in 6-well plates at 2 × 10^6 cells/well in a 4 ml volume. After 24 h, cells were serum-deprived for 24 h, then incubated for 6 h with 0–2 µg/ml of HEGU or 0–4 µM dehydroglyasperin C in the absence or presence of LPS. Luciferase activities were measured with the cell
lysates using a luciferase assay system (Promega). Luciferase activity was normalised to β-galactosidase activity.

2.8. In vivo inflammation model

Male ICR mice (6 weeks of age) were purchased from Koatech (Korea). The animals were maintained under standard laboratory conditions on a commercial pellet diet and water ad libitum. The dorsal surface of the mouse was shaved with electric clippers prior to the experiments. HEGU (0–2 mg) was topically administered to the shaved back 1 h prior to the topical treatment with 10 nmol of TPA. The skin application area was approximately 2 cm × 2 cm. After 24 h, the animals were sacrificed and the skin was fixed in 4% paraformaldehyde for H&E staining or frozen in liquid N2 for PGE2 assays and Western blot analyses.

Fixed skin samples were embedded in paraffin wax, and multiple 5 μm sections were prepared from the paraffin-embedded tissue blocks. The tissue sections were mounted on slides, depa-

Fig. 1. HEGU inhibits LPS-induced NO secretion via the inhibition of iNOS expression in murine macrophages. Raw264.7 cells were treated with 0–2 μg/ml of HEGU in the absence or presence of LPS. (A) NO concentrations. (B) Western blot for iNOS. (C) RT-PCR for iNOS. (D) iNOS transcriptional activity. Each bar of graph or relative abundance of each band represents the mean ± SEM (n = 3). Means without the same letter differ, P < 0.05.

Fig. 2. HEGU inhibits LPS-induced secretion of PGE2 by murine macrophages. Raw264.7 cells were treated with HEGU and/or LPS as described in Fig. 1. (A) PGE2 concentrations. (B) Western blot for COX-2. (C) RT-PCR for COX-2. (D) Real-time RT-PCR for PLA2. Each bar represents the mean ± SEM (n = 3). Means without the same letter differ, P < 0.05.

Table 1

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<th>Protein (% of control)</th>
<th>TNFα</th>
<th>IL-6</th>
<th>IL-1β</th>
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<td>0 μg/ml HEGU</td>
<td>189 ± 18 a</td>
<td>6 ± 1 d</td>
<td>5 ± 0.3 d</td>
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<td>999 ± 82 c</td>
<td>462 ± 27 c</td>
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mRNA (relative concentration) TNFα IL-6 IL-1β

<table>
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<tr>
<th>mRNA (relative concentration)</th>
<th>TNFα</th>
<th>IL-6</th>
<th>IL-1β</th>
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<td>1 c</td>
<td>1 d</td>
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<td>1874 ± 149 c</td>
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*Values are means ± SEM (n = 3). Means in a column without the same letter differ, P < 0.05.
The skin samples were homogenised in PBS containing 10 μM AA-861, 10 μM indomethacin, and protease inhibitors (20 mg/l of aprotinin, 10 mg/l of antipain, 10 mg/l of leupeptin, 80 mg/l of benzamidine HCl, and 0.2 mM phenylmethanesulfonyl fluoride). Their homogenates were centrifuged for 15 min at 14,000 g. The PGE2 concentrations in the supernatants were measured using the PGE2 assay kit. For the determination of COX-2 and iNOS protein levels, mouse skin extracts were prepared in lysis buffer and subjected to immunoblotting with their relevant antibodies.

2.9. Statistical analyses

Data were expressed as means ± SEM and analysed by ANOVA. Differences between treatment groups were analysed by Duncan’s multiple range test, using the SAS system for Windows version 8.12 (SAS Institute). Differences were considered significant at P < 0.05.

3. Results

3.1. HEGU inhibits LPS-induced NO and PGE2 production in Raw264.7 cells

We first attempted to determine whether HEGU has cytotoxic effects on Raw264.7 cells. The cells were treated with various concentrations of HEGU in the presence of LPS, and cell viability was evaluated using the MTT assay. As cell viability was not affected significantly by HEGU up to 2 μg/ml (data not shown), we used concentrations of 0–2 μg/ml of HEGU for the subsequent experiments. In order to determine whether HEGU inhibits the secretion of NO and PGE2, media conditioned for 24 h were collected. HEGU inhibited LPS-induced NO production (IC50 = 1.84 ± 0.15 μg/ml) in a dose-dependent manner (Fig. 1A). In order to determine whether iNOS expression is regulated by HEGU, Western blot analysis and RT-PCR were conducted. As is shown in Fig. 1B and C, HEGU suppressed LPS-induced increases in iNOS protein and mRNA levels in a concentration-dependent manner. In addition, a reporter gene assay, using cells transfected with the plasmid harbouring the iNOS promoter and the luciferase structural gene, showed that HEGU inhibited LPS-induced iNOS transcriptional activity (Fig. 1D). These results demonstrate that HEGU inhibits NO secretion via the down-regulation of iNOS expression.

In addition to NO, HEGU also inhibited LPS-induced PGE2 production (IC50 = 0.59 ± 0.03 μg/ml) in a dose-dependent fashion (Fig. 2A). However, HEGU exerted no detectable effect on the levels of COX-2 protein or mRNA (Fig. 2B and C). We reported previously that the mRNA of phospholipase (PLA)2 is increased in LPS-stimulated macrophages (Cho et al., 2008). Real-time RT-PCR analysis revealed that HEGU suppressed the LPS-induced increase in PLA2 transcripts (Fig. 2D). These results indicate that the down-regulation of PLA2 expression probably contributed to the reduced PGE2 secretion in HEGU-treated cells.

Fig. 3. HEGU inhibits LPS-stimulated NFκB signalling in murine macrophages. Raw264.7 cells were treated with various concentrations (0–2 μg/ml) of HEGU for 18 h and then treated with LPS for a further 15 min. (A) Western blot for NFκB signalling. (B) NFκB DNA binding activity. The adjusted mean ± SEM (n = 3) of each band is shown above each blot. Means without the same letter differ, P < 0.05.
3.2. HEGU decreases LPS-induced production of pro-inflammatory cytokines in Raw264.7 cells

Treatment of cells with LPS markedly increased the secretion of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β), and the increased production of these cytokines was inhibited by HEGU in a dose-dependent manner (Table 1). In addition, real-time RT-PCR analysis showed that HEGU inhibited LPS-induced increases in TNF-α, IL-6, and IL-1β transcripts (Table 1).

3.3. HEGU inhibits LPS-induced activation of NFκB in Raw264.7 cells

Because LPS-induced iNOS expression and the secretion of pro-inflammatory cytokines, we subsequently assessed the effects of HEGU on NFκB signalling. Western blot analysis demonstrated that HEGU prevented LPS-induced IkBα degradation and the nuclear translocation of p65 (Fig. 3A). EMSA showed that LPS-induced NFκB DNA binding activities were significantly inhibited by HEGU (Fig. 3B).

3.4. HEGU suppresses TPA-induced inflammatory responses in mouse skin

In an effort to confirm the anti-inflammatory effects of HEGU in vivo, we topically applied TPA to the dorsal skin of the mice. After 24 h, skin samples were stained with hematoxylin and eosin. The TPA treatment increased skin swelling and the infiltration of inflammatory cells (white arrow), which were suppressed by pretreatment with HEGU (Fig. 4A and B). Because we noted previously that 2 mg of licorice extract markedly (approximately 90%) inhibited TPA-induced ear oedema formation in mice (unpublished results by Chung WY and Park JHY), we utilised HEGU at doses of 1–2 mg in the present experiments. HEGU pre-treatment also inhibited TPA-induced PGE2 production (Fig. 4C) and the expression of iNOS and COX-2 proteins (Fig. 4D).

3.5. Dehydroglyasperin C is one of the active compounds present in HEGU

We collected 29 fractions via flash column chromatography with the n-hexane–ethyl acetate gradient and examined their ability to inhibit NO production by Raw264.7 cells. The abilities of F-22, F-24, F-28, and F-29 proved more profound than those of the other fractions at a concentration of 2 μg/ml (Fig. 5A). F-28 and F-29 (0.7 g) were pooled and purified further by Prep-HPLC to generate compound 1 (7.28 mg). The structure of the compound was elucidated as dehydroglyasperin C (Fig. 5B) via comparison of the spectral data with an authentic sample, as described in the literature (Kitagawa, Hori, Motozawa, Murakami, & Yoshikawa, 1998). We obtained 1.4 mg of dehydroglyasperin C from 1 g of HEGU.
3.6. Dehydroglyasperin C inhibits NO secretion via the inhibition of iNOS expression in murine macrophages

We then assessed the effects of dehydroglyasperin C on inflammatory responses in Raw264.7 cells. Dehydroglyasperin C, at 2 μM concentration (0.708 μg/ml), inhibited LPS-induced NO production (IC50 = 5.54 ± 1.02 μM) and the expression of iNOS protein (Fig. 6A and B). However, the compound exerted no effect on COX-2 protein expression. In order to determine whether HEGU and dehydroglyasperin C inhibit LPS-stimulated NFκB activation, we measured NFκB transcriptional activity using the NFκB-luciferase reporter plasmid. As is shown in Fig. 6C, LPS-induced NFκB transcriptional activity was inhibited by HEGU and dehydroglyasperin C. These results show that dehydroglyasperin C is one of the active compounds present in HEGU, and that the compound exerts anti-inflammatory effects via the inhibition of NFκB signalling.

4. Discussion

Licorice is widely utilised in soft drinks, tobacco products, and candies, as a sweetener and flavouring agent. In addition, licorice is the most frequently prescribed plant in traditional Chinese medicine. Recently, several studies have reported that licorice exerts anti-inflammatory and anti-carcinogenic effects. For example, the ethanol extract of licorice has been shown to induce apoptosis and inhibit cell cycle progression in MCF-7 human breast cancer cells (Jo et al., 2005), and it also inhibited tumour growth and reduced cisplatin-induced toxicity in a mouse xenograft model (Lee, Park, Lim, Park, & Chung, 2007). The ethanol extract of roasted licorice, which contains glycyrrhizin, has also been shown to inhibit LPS-induced inflammation in murine macrophages and to increase the survival rate in LPS-treated mice (Kim et al., 2006). Glycyrrhizin, a major compound in the licorice root, evidences profound anti-inflammatory activities, such as the inhibition of LPS/o-galactosamine-induced liver injury (Yoshida et al., 2007), the prevention of free fatty acid-induced hepatic lipotoxicity (Wu et al., 2008), and the attenuation of carrageenan-induced lung injury (Menegazzi et al., 2008). However, chronic glycyrrhizin ingestion can induce severe hypertension (Asl & Hosseinzadeh, 2008; van Uum, 2005), which precludes us from using licorice extract containing glycyrrhizin as an anti-inflammatory agent. We reported previously that HEGU harboured no measurable quantities of glycyrrhizin and protected cardiac myoblasts against doxorubicin-induced apoptosis (Choi et al., 2008), which suggests that HEGU may be employed as an anti-inflammatory agent. In the present study, we show that HEGU evidences very potent anti-inflammatory properties in both in vivo and in vitro experimental models. Using LPS-stimulated murine macrophages, we demonstrated that HEGU, at very low (1–2 μg/ml) concentrations, reduces, (1) the secretion of NO, PGE2, TNF-α, IL-6, and IL-1β, (2) iNOS protein expression, (3) mRNA levels of iNOS and PLA2 and (4) degradation of IκBα, nuclear translocation of p65, NFκB DNA binding activity, and NFκB transcriptional activity. Using a TPA-stimulated mouse skin inflammation model, we have also demonstrated that HEGU
inhibits skin swelling, PGE2 production, and iNOS and COX-2 protein expression.

We noted that HEGU reduced NO production, iNOS protein and mRNA expression, and iNOS transcriptional activity (Fig. 1). These data show that HEGU inhibits the LPS-induced secretion of NO via the downregulation of iNOS expression. However, HEGU was also shown to inhibit PGE2 secretion in murine macrophages without changes in COX-2 expression (Fig. 2). Because we have previously observed that LPS increases PLA2 transcripts, we conducted real-time RT-PCR analysis. The reduction in PLA2 transcripts noted in the HEGU-treated cells suggests that the reduction in PLA2 expression may have resulted in decreased arachidonic acid release which contributed to the decrease in PGE2 secretion in the HEGU-treated macrophages. By way of contrast to the results observed in the macrophages, HEGU significantly reduces COX-2 expression in TPA-treated mouse skin. Future studies will be required to resolve the discrepancy between the in vitro and in vivo experiments.

LPS (lipid A portion) binding to the extracellular domain of toll-like receptor 4 (TLR4) initiates a complex signal-transduction cascade, induces the activation of the transcription factor NFkB, and increases the transcription of pro-inflammatory cytokines, as well as other pro-inflammatory proteins, such as iNOS and COX-2 (Miller, Ernst, & Bader, 2005; Palsson-McDermott & O’Neill, 2004). In addition, previous studies have shown that NFkB inhibition induces a reduction in pro-inflammatory cytokine expression (Cho et al., 2008; Kwon et al., 2008). In the present study, HEGU was shown to prevent the LPS-induced degradation of inhibitor of kBα, nuclear translocation of p65, NFkB DNA binding activity (Fig. 3), and NFkB transcriptional activity (Fig. 6). We also noted that HEGU inhibited the LPS-induced expression of the pro-inflammatory cytokines, TNF-α, IL-6, and IL-1β, as well as iNOS and PLA2 expression. It has also been reported that PLA2 expression is regulated by NFkB signalling (Jaulmes, Janvier, Andreani, & Raymond-jean, 2005). These data show that HEGU suppresses LPS-induced inflammatory responses via the inhibition of NFkB signalling.

The topical application of TPA to mouse skin generates inflammatory responses, and this is a well-established experimental model for the evaluation of the effects of various agents on inflammation. TPA treatment causes histological alterations in skin, resulting in an increase in epidermal thickness and inflations of inflammatory cells (Rahman et al., 2008). In addition, it is generally accepted that the application of TPA induces COX-2 and iNOS expression via the activation of NFkB signalling (Chun et al., 2003). The results from our in vivo experiments clearly demonstrate that HEGU suppressed TPA-induced skin swelling and inflammatory cell infiltration, as well as the production of PGE2 and iNOS and COX-2 protein expression. These results demonstrate that HEGU can be utilised as a topical anti-inflammatory agent.

It has been reported that licochalcone-A, another major compound in licorice root, suppresses LPS-stimulated inflammatory response (Kwon et al., 2008). In addition, glycyrrhin and glycyrl have been reported to have anti-inflammatory activity in both in vitro and in vivo experimental models. In the current study, we identified dehydroglyasperin C as an active compound in HEGU. To the best of our knowledge, the biological effects of dehydroglyasperin C have not been elucidated, except by Mae et al. (2003) who noted that licorice ethanol extract effectively prevents and ameliorates metabolic syndrome (diabetes, abdominal obesity and hypertension), and dehydroglyasperin C is one of the active compounds that evidences PPARγ ligand-binding activity. In the current study, dehydroglyasperin C was shown to effectively inhibit LPS-induced NO production, iNOS expression, and NFkB transcriptional activity in murine macrophages. Our data reveal that dehydroglyasperin C exerts anti-inflammatory effects via the inhibition of NFkB signalling. Future studies will be required to determine whether more active anti-inflammatory compounds are present in F-22 and F-24 (Fig. 5A).

In summary, we have demonstrated herein that HEGU inhibits LPS-induced increases in pro-inflammatory cytokines and enzymes in murine macrophages, which may be mediated via the inhibition of NFkB signalling. These in vitro observations indicate that HEGU lacking glycyrrhizin can be employed as an effective anti-inflammatory agent. We identified dehydroglyasperin C, which effectively inhibits NO production, iNOS expression and NFkB transcriptional activity, in HEGU. Future studies will be necessary in order to determine the bioavailability of this preparation and metabolites in the blood. In addition, the results of in vivo studies show that HEGU can be utilised as a topical anti-inflammatory agent. As licorice has been used for 4000 years without any toxicity except severe hypertension and because hypertension is a major
health problem worldwide. HEGU lacking glycyrrhizin evidences tremendous potential as a possible anti-inflammatory agent.

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References


