

Identification of Glyasperin B as a Lipolytic Agent with Anti-Obesity Activity from the Licorice Extract of *Glycyrrhiza uralensis*

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Abstract Obesity is recognized as a significant public health concern. Although licorice extract from the root of *Glycyrrhiza uralensis* exhibits anti-obesity effects, limited knowledge regarding its active components has hindered the development of standardized preparations. Therefore, this study aimed to identify the compound responsible for the anti-obesity activity of licorice extract. By isolating and characterizing a compound exhibiting lipolytic activity against 3T3-L1 adipocytes, glyasperin B was identified. In a diet-induced obesity mouse model, the efficacy of licorice extracts correlated with glyasperin B content, suggesting that glyasperin B is a key contributor to the anti-obesity effects of *Glycyrrhiza uralensis*. This study's findings may facilitate the standardized preparation of licorice extracts for weight management, potentially aiding in the fight against obesity.

Keywords: licorice, glyasperin B, flavonoid, lipolysis, obesity, *Glycyrrhiza uralensis*

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1. Introduction

Obesity is characterized by an excessive accumulation of body fat, which can adversely affect health and is considered a significant public health concern. Unhealthy lifestyle choices, such as excessive food intake and inadequate physical activity, contribute to body fat accumulation. Specifically, abdominal fat accumulation can lead to elevated blood pressure, high blood sugar, and/or abnormal cholesterol or triglyceride levels, thereby increasing the risk of cardiovascular disease, stroke, and type 2 diabetes [1,2,3]. Visceral fat, the intra-abdominal adipose tissue surrounding various organs, can trigger inflammation owing to the release of inflammatory cytokines from macrophages within the tissue [4]. This inflammation may result in several pathological conditions, such as insulin resistance, ultimately contributing to the aforementioned lifestyle diseases [5]. Therefore, for individuals who are overweight, weight loss, particularly through the reduction of visceral fat, is critical for restoring a healthy life.

Licorice, a perennial leguminous plant native to various arid regions, has been utilized for over 4,000 years both as a traditional medicine and a sweetener in food, rendering it one of the most popular herbal plants globally. While the medicinal use of licorice typically involves complex

formulations, occasional applications of licorice extract alone have been documented for weight management. Morikawa et al. reported that the oral intake of flavonoids extracted from the root of *Glycyrrhiza glabra* reduces body fat, including visceral fat, leading to weight loss in healthy Japanese individuals who are overweight [6]. Among the flavonoids present in this extract, glabridin, which promotes fatty acid degradation and suppresses fatty acid synthesis, has been identified as a key compound contributing to the reduction of body fat, as noted in several studies [7,8,9].

Similarly, Tsukamoto et al. found that the root extract of *Glycyrrhiza uralensis* significantly reduced abdominal body fat in healthy volunteers [10]. The continuous oral intake of this licorice extract led to decreased abdominal visceral and subcutaneous fat, resulting in weight loss among participants with a high-normal body mass index. The licorice extract presumably activates triglyceride degradation, as evidenced by increased levels of blood ketone bodies.

As regards the components of licorice extract from *Glycyrrhiza uralensis* root, Sasakawa et al. reported the anti-inflammatory activity of several flavonoids and terpenoids, which inhibit the release of monocyte chemoattractant protein-1 from 3T3-L1 adipocytes stimulated by tumor necrosis factor alpha [11]. However, there is limited information on the anti-obesity properties of compounds in *Glycyrrhiza uralensis*, thus posing

challenges for the consistent preparation of licorice extract aimed at obesity management.

Thus, the present study aimed to isolate anti-obesity compounds from the root extract of *Glycyrrhiza uralensis*. The isolation process, guided by a lipolysis assay utilizing 3T3-L1 adipocytes, led to the identification of glyasperin B. Subsequent animal studies examining various licorice extracts confirmed that glyasperin B is likely the most active compound contributing to the anti-obesity effects of *Glycyrrhiza uralensis* root extract.

2. Materials and Methods

2.1. Materials

The licorice extracts utilized in this study are detailed in Table 1. The preparation procedure involved grinding dried licorice roots using mills, followed by extraction with eight volumes (v/w) of a 1:1 (v/v) aqueous ethanol solution twice for several hours at room temperature. The resulting extracts were subsequently dried using either freeze-drying or spray-drying methods to obtain a powdered form.

Glyasperin B, employed as a standard chemical for high-performance liquid chromatography (HPLC) analysis, was purchased from Nagara Science Co., Ltd. (Gifu, Japan), while glabridin, used for the same purpose, was procured from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Table 1. Licorice extracts prepared in this study

Extract	Licorice species	Glyasperin B (μg/g)	Glabridin*
A	<i>Glycyrrhiza uralensis</i>	122.9	ND
B	<i>Glycyrrhiza uralensis</i>	39.3	ND
C	<i>Glycyrrhiza uralensis</i>	100.0	ND
D	<i>Glycyrrhiza glabra</i>	ND	Detected
E	<i>Glycyrrhiza uralensis</i>	148.1	ND
F	<i>Glycyrrhiza uralensis</i>	137.5	ND

ND: not detected

*Glabridin was analyzed solely to determine its presence.

2.2. Lipolysis Assay

Mouse 3T3-L1 preadipocytes (JCRB9014; JCRB Cell Bank, Osaka, Japan) were seeded at a density of 1.0×10^4 /cm² in 24- or 48-well plates and subsequently incubated overnight at 37°C under 5% CO₂ in Dulbecco's modified Eagle medium (DMEM; NacalaiTesque, Kyoto, Japan) supplemented with 10% newborn calf serum. The following day, the cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) for 3 days and subsequently transitioned to adipocyte differentiation medium (0.5 mM isobutyl-methylxanthine, 1 μM dexamethasone, and 1 μg/mL insulin in DMEM supplemented with 10% FBS) for an additional 2 days. Thereafter, the cells were maintained in DMEM supplemented with 10% FBS and 1 μg/mL insulin for 6–9 days prior to the lipolysis assay.

The lipolysis assay procedure for 3T3-L1 adipocytes involved stimulating the cells overnight (16–24 h) with each test substance dissolved in phenol-red-free DMEM

supplemented with 10% FBS and 1 μg/mL insulin. Where necessary, to minimize background interference from test substances, the cells were incubated for an additional 4 h in fresh, subject-free medium. The glycerol released into the culture medium (either during overnight stimulation or the subsequent short-term incubation, depending on the experiment) was quantified using the Lab Assay™ Triglyceride kit (FUJIFILM Wako Pure Chemical, Osaka, Japan) to evaluate lipolysis in the cultured cells.

2.3. HPLC

The Waters 2695 Separations Module (Waters, Milford, USA), equipped with a Waters 2487 dual lambda absorbance detector, was used in the present study. To fractionate licorice extract and isolate glyasperin B, the following conditions were employed: column: TSK gel ODS-100V (4.6 mm ID × 150 mm, 5 μm; TOSO, Tokyo, Japan), column temperature: 40°C, mobile phase: acetonitrile/water (60:40 or 45:55), flow rate: 1.0 mL/min, and detection wavelength: 290 nm. To determine glyasperin B content in licorice extract, the following conditions were applied: column: YMC-Pack Pro C18 RS (4 mm ID × 250 mm, 5 μm; YMC, Kyoto, Japan), column temperature: 40°C, mobile phase: acetonitrile/water/TFA (425:575:1), flow rate: 1.0 mL/min, and detection wavelength: 290 nm.

2.4. Chromatographic Fractionation of Licorice Extract

Licorice extract (MG Pharma) was initially fractionated using Sep-pak® C18 Environmental Cartridges (Waters) with a step gradient solvent system comprising acetonitrile/water (20:80, 50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 85:15, and 90:10) or absolute methanol. The fractions were dried *in vacuo* and subsequently suspended in dimethyl sulfoxide for the lipolysis assay. The active fractions in the lipolysis assay (acetonitrile/water 55:45) were further fractionated via HPLC under the previously mentioned conditions for subsequent analysis.

2.5. Isolation of Active Compound

Licorice extract (3 g; MG Pharma) was partitioned between ethyl acetate and water (10 mL each). The organic layer was dried over magnesium sulfate, evaporated *in vacuo*, and chromatographed using silica gel (silica gel 60 [70–230 mesh; Merck, Germany]) with a hexane/ethyl acetate solvent system (1:1). Following analytical HPLC verification, the fractions containing the target compound were dried *in vacuo* and subjected to preparative HPLC. The resulting semi-pure compound underwent mass spectrometry analysis. For nuclear magnetic resonance (NMR) analysis, an additional preparative purification was carried out by Nagara Science Co., Ltd. (Gifu, Japan).

2.6. Spectroscopic Analysis

High-resolution electrospray ionization (ESI) mass

spectrometry was performed using a JMS-T100LP mass spectrometer (JEOL, Tokyo, Japan), while electron ionization (EI) mass spectrometry was conducted using a GCMS-QP2010 Ultra gas chromatograph–mass spectrometer (Shimadzu, Kyoto, Japan). ^1H and ^{13}C NMR spectra were acquired using a JEOL ECA 500 spectrometer (JEOL, Tokyo, Japan).

2.7. Animal Studies

This study adhered to the ethical guidelines for animal experimentation established by MG Pharma Inc. (Approval No. 20160926-1), consistent with the Declaration of Helsinki. Healthy 4-week-old male C57BL/6J mice from Japan SLC (Shizuoka, Japan) were utilized in the experiments. The mice, which exhibited good pelage condition, were housed in an air-conditioned environment ($23 \pm 2^\circ\text{C}$, $50 \pm 10\%$ relative humidity) with a 12-h light/dark cycle (light hours: 7:00–19:00). Before commencing the experiment, the mice were acclimatized for 7 days in an experimental animal room with *ad libitum* access to a mouse formula (MF) diet (Oriental Yeast, Tokyo, Japan; 5% fat, 55% carbohydrate [55% cornstarch], and 23% protein) and water.

At 5 weeks of age, the mice were randomly assigned to one of eight groups ($n=6$ each): normal, control, A, B, C, D, E, or F (Table 1). Each group was fed a specific diet for 8 weeks. The normal group received the MF diet, while the control group was administered a high-fat high-sucrose (HFS) diet (D12079BM; Research Diets, New Brunswick, NJ, USA; 21% fat, 50% carbohydrate [34% sucrose], and 20% protein). The experimental groups were administered the HFS diet supplemented with various extracts of licorice, as outlined in Table 1. Following the 8-week dietary period, body weight and visceral fat mass were measured, with visceral fat mass defined as the total weight of the epididymal, perirenal, and mesenteric fat tissues.

2.8. Statistical Analysis

Results are presented as the mean $\pm$ standard error of the mean. Statistical analysis was performed using one-way analysis of variance, followed by Dunnett's test (single-step method), with the control group serving as the reference [12]. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Fractionation and Characterization of the Lipolytic Compound from Licorice Extract

We initially fractionated licorice extract using a cartridge column (Sep-pak[®] C18 Environmental Cartridges), followed by HPLC. The resulting fractions

were subsequently subjected to a lipolysis assay. The fraction exhibiting lipolytic activity underwent further purification via HPLC under modified conditions, and the purified compound was spectroscopically analyzed to elucidate its structure. The molecular formula was determined to be $\text{C}_{21}\text{H}_{22}\text{O}_6$, corresponding to a peak detected at m/z 393.13059 ($\text{C}_{21}\text{H}_{22}\text{O}_6\text{Na}$ requires 393.13086) through high-resolution ESI mass spectrometry. A literature search for licorice metabolites based on the estimated chemical formula identified glyasperin B as a candidate, anticipated to be the target compound [13]. The fragmentation patterns observed in the EI-MS spectrum supported this hypothesis, as they closely resembled previously reported data (data not shown). To definitively confirm that the target compound was glyasperin B, we conducted ^1H and ^{13}C NMR analyses. The NMR spectra were consistent with those reported in the literature, leading to the identification of the target compound as glyasperin B (Figure 1). The obtained NMR spectra were as follows: ^1H NMR (acetone- d_6) ppm: 1.63 (3H, s), 1.74 (3H, s), 3.24 (2H, d, $J=7.0$ Hz), 3.90 (3H, s), 4.27 (1H, dd, $J=5.5$, and 10.5 Hz), 4.48 (1H, dd, $J=5.0$, and 11.5 Hz), 4.62 (1H, t, $J=10.5$ Hz), 5.17 (1H, t, $J=7.0$ Hz), 6.13 (1H, s), 6.33 (1H, dd, $J=2.0$ and 8.5 Hz), 6.45 (1H, d, $J=2.0$ Hz), 6.94 (1H, d, $J=8.5$ Hz), 8.30 (1H, bs), 8.63 (1H, bs), and 12.48 (1H, s); ^{13}C NMR (acetone- d_6) ppm: 17.6, 21.4, 25.7, 47.2, 56.2, 71.0, 91.3, 103.5, 103.7, 107.6, 109.7, 113.5, 123.3, 131.1, 131.5, 156.8, 158.7, 161.1, 162.7, 165.8, and 198.7.

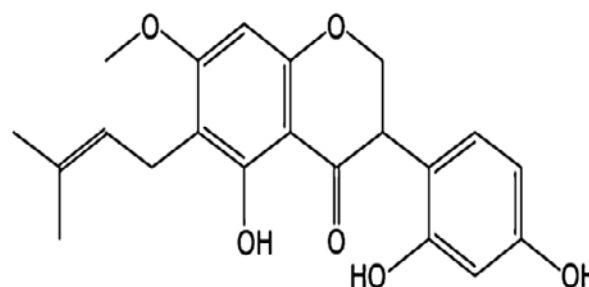
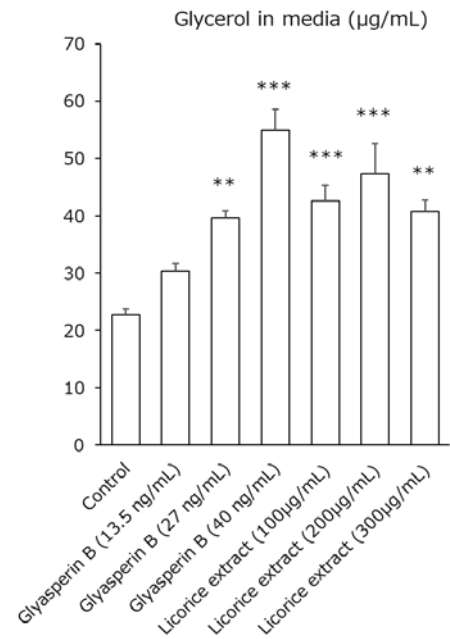


Figure 1. Structure of glyasperin B

3.2. Lipolysis Assay

The lipolytic activity of the purified glyasperin B was evaluated alongside the original licorice extract using a bioassay with 3T3-L1 adipocytes. To facilitate comparison, the concentrations of glyasperin B in the test solutions were standardized. As depicted in Figure 2, at low concentrations (glyasperin B: 13.5 ng/mL or licorice extract: 100 $\mu\text{g/mL}$), glyasperin B exhibited insufficient activity compared to the original licorice extract. However, at intermediate (glyasperin B: 27 ng/mL or licorice extract: 200 $\mu\text{g/mL}$) and high (glyasperin B: 40 ng/mL or licorice extract: 300 $\mu\text{g/mL}$) concentrations, the lipolytic activity of glyasperin B was comparable to or exceeded that of the original extract.



Statistically significant differences were observed between the test and control groups (** p<0.01, *** p<0.001), as determined by Dunnett’s test.

Figure 2. Results of lipolysis assay using 3T3-L1 adipocytes

Table 2. Effects of licorice extract on body and visceral fat weights in HFS-diet-fed mice

Group	Diet	Extract	Final body weight (g)		Body weight gain (g/8 weeks)			Visceral fat weight (g)			Dietary energy intake (kcal/day/head)
A	HFS	A	29.99	± 0.98	9.78	± 0.67		1.88	± 0.16		13.5
B	HFS	B	30.89	± 1.11	10.64	± 0.78		2.20	± 0.25		13.6
C	HFS	C	30.62	± 1.21	10.38	± 0.78		2.03	± 0.26		13.5
D	HFS	D	30.52	± 0.56	10.29	± 0.36		2.16	± 0.11		13.3
E	HFS	E	27.32	± 0.42	7.09	± 0.50	*	1.13	± 0.14	**	13.3
F	HFS	F	28.09	± 0.65	7.88	± 0.38		1.48	± 0.11		13.3
Control	HFS	none	30.35	± 1.11	10.13	± 0.99		2.14	± 0.34		13.1
Normal	MF	none	26.66	± 0.87	6.39	± 0.49	**	0.62	± 0.06	***	12.2

Significant differences were observed between the test and control groups (* p<0.05, ** p<0.01, *** p<0.001), as determined by Dunnett’s test.

3.3. Animal Study

To elucidate the relationship between the efficacy and content of glyasperin B, the anti-obesity effects of licorice extract were examined in a model of HFS diet-induced obesity. The control group exhibited significant increases in both body and visceral fat weights (Table 2). Among the test groups, the most pronounced reductions in body weight and visceral fat were observed in group E, achieving statistical significance. Although a similar trend was noted in group F, the reductions did not reach statistical significance (body weight gain: p<0.1, visceral fat weight: p=0.12). The results for the remaining test groups were comparable to those for the control group.

The glyasperin B content of the licorice extract samples was determined using analytical HPLC, with results presented in Table 1. The highest concentration was detected in extract E (148.1 µg/g), followed closely by extract F (137.5 µg/g). This pattern aligns with observations from the animal study, which indicated decreases in both body weight and visceral fat. Although glabridin was

analyzed simultaneously, it was exclusively detected in extract D, with no presence in the other samples.

4. Discussion

Numerous studies have reported the direct effects of flavonoid compounds on adipocytes [14,15]. The compounds present in Ural licorice, which is known for its diverse flavonoid content [16], presumably exert lipolytic effects on 3T3-L1 adipocytes. The characterization of the active compound in the assay led to the successful isolation of glyasperin B. Furthermore, the licorice extracts demonstrated a capacity to suppress weight and body fat gain in a diet-induced obesity mouse model, correlating with the glyasperin B content.

Glyasperin B was initially isolated from *Glycyrrhiza aspera* [13] and subsequently re-isolated by Inami et al. as an antimutagenic component against N-methyl-N-nitrosourea [17]. Nevertheless, no reports have documented its lipolytic activity in the existing literature.

To date, our search has revealed no studies on glyasperin B, rendering this the first report of its isolation as a compound with lipolytic properties.

Regarding the mode of action of glyasperin B, it ostensibly activates triglyceride degradation in adipose tissue, as suggested by *in vitro* test results. Certain flavonoids, such as quercetin and catechin, which enhance lipolysis in 3T3-L1 adipocytes [14,15], demonstrate their effects solely in the presence of noradrenaline. These compounds induce lipolysis by activating hormone-sensitive lipase, rendering noradrenaline essential for triggering lipolysis in adipocytes. In contrast, glyasperin B stimulated lipolysis independently of noradrenaline, suggesting a potentially different mechanism of action compared to these flavonoids, although the precise mechanism warrants further investigation.

Several licorice extracts were evaluated for their anti-obesity effects using a diet-induced obesity mouse model, with the observed effects appearing to correlate with the glyasperin B content in each extract. To ascertain the presence of other compounds within the licorice extracts, glabridin, a known anti-obesity compound, was analyzed via HPLC. The results indicated that the licorice extracts exhibiting efficacy did not contain glabridin (Table 1 and Table 2). Notably, all effective licorice extracts in this study were sourced from Ural licorice (*Glycyrrhiza uralensis*), which is not expected to contain glabridin [18]. The absence of glabridin, despite its recognized anti-obesity properties, further corroborates the conclusion that the anti-obesity effects observed in this study are attributable to glyasperin B. Further studies aimed at identifying and quantifying other lipolytic agents would help to better understand the mechanisms underlying the anti-obesity effects of licorice extracts.

5. Conclusion

The isolation and characterization of a compound with lipolytic activity from *Glycyrrhiza uralensis* extract led to the identification of glyasperin B. In a diet-induced obesity mouse model, licorice extract demonstrated efficacy associated with the glyasperin B content, suggesting that it should be a key contributor to the anti-obesity effects of licorice extract. The findings of this study potentially facilitate the standardized and consistent preparation of licorice extracts for obesity management.

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Statement of Competing Interests

All authors of this study are affiliated with MG Pharma Inc., the manufacturer of the licorice extract utilized in this research.

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