Phenolics from *Glycyrrhiza glabra* and *G. uralensis* Roots and Their PPAR-γ Ligand-Binding Activity: Possible Application for Amelioration of Type 2 Diabetes

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**Abstract**

The EtOH extract of *Glycyrrhiza glabra* roots and the EtOAc extract of *Glycyrrhiza uralensis* roots exhibited considerable PPAR-γ ligand-binding activity. Bioassay-guided fractionation of these extracts resulted in the isolation of 52 phenolics, including 11 novel ones. The PPAR-γ ligand-binding activity of more than 10 isolated phenolics at 10 μg/mL was approximately three times greater than that of 0.5 μM triglitazone. Glycyrin (44), isolated from the EtOAc extract of *G. uralensis* roots as a PPAR-γ ligand, reduced the blood glucose levels of genetically diabetic KK-A' mice through its PPAR-γ ligand-binding activity.

**Keywords:** phenolics, *Glycyrrhiza glabra*, *Glycyrrhiza uralensis*, PPAR-γ, metabolic syndrome

**1. Introduction**

Peroxisome proliferator-activated receptor (PPAR)-γ is the primary molecular target for insulin-sensitizing thiazolidinedione drugs. These drugs activate PPAR-γ, increasing the number of small adipocytes that differentiate from preadipocytes and inducing apoptosis in large adipocytes. Because small adipocytes function normally, whereas large adipocytes hyperproduce and hypersecrete adipocytokines, an increased ratio of small adipocytes to large adipocytes improves insulin resistance. Therefore, compounds with PPAR-γ ligand-binding activity may be useful for the prevention and improvement of type 2 diabetes, a representative...
insulin resistance syndrome. We found that the EtOH extract of *Glycyrrhiza glabra* roots and the EtOAc extract of *G. uralensis* roots exhibited higher activity than did the other materials tested. Bioassay-guided fractionation of these extracts resulted in the isolation of 52 phenolics, including 11 novel ones [1, 2].

In this chapter, we describe the results of the bioassay-guided fractionation of *G. glabra* and *G. uralensis* roots using a GAL-4-PPAR-\(\gamma\) chimera assay method.

## 2. PPAR-\(\gamma\) ligand-binding activity

PPAR-\(\gamma\) ligand-binding activity was assessed using a GAL-4-PPAR-\(\gamma\) chimera assay system (Figure 1) [3]. CV-1 monkey kidney cells from the American Type Culture Collection (ATCC) were suspended in Dulbecco’s Modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 50 IU/mL Penicillin G sodium salt, 50 \(\mu\)g/mL streptomycin sulfate, and 37 mg/L ascorbic acid. The cells were then inoculated into a 96-well culture plate at 6 \(\times 10^3\) cells/well and incubated in 5% \(\text{CO}_2/\text{air}\) at 37°C for 24 h. Cells were washed with OPTI-minimum essential medium (MEM) and pM-hPPAR-\(\gamma\) and p4 \(\times\) UASg-tk-luc were transfected into cells using LipofectAMINE PLUS (Gibco). pM and p4 \(\times\) UASg-tk-luc were transfected into CV-1 cells as a mock control. Twenty-four hours after transfection, the medium was changed to DMEM containing 10% charcoal-treated FBS [4] and the cells were further cultured for 24 h. The cells were then washed with phosphate-buffered saline containing \(\text{Ca}^{2+}\) and \(\text{Mg}^{2+}\), and luciferase activity was measured using LucLite (Perkin-Elmer). Luminescence intensity was measured using a TopCount Microplate scintillation/luminescence counter. PPAR-\(\gamma\) ligand-binding activity was expressed as the relative luminescence intensity (test group/control group) determined for each sample.

![Figure 1. GAL4-PPAR-\(\gamma\) chimera assay system.](image-url)
3. Isolation and structural determination of phenolic compounds from *G. glabra*

The roots of *G. glabra* (4.0 kg) were extracted twice with 20 L of 95% EtOH for 2 h at 45°C. The extracts were combined and concentrated under reduced pressure to give a 95% EtOH extract (120 g). The extract exhibited a relative luminescence intensity of 2.2 at a sample concentration of 5 μg/mL, indicating a PPAR-γ ligand-binding activity that was almost as strong as that of 0.5 μM troglitazone (TRG), a potent synthetic PPAR-γ agonist. The extract was chromatographed on a silica gel column eluted with CHCl₃-MeOH gradients (19:1; 9:1; 2:1) and finally with MeOH. After removal of solvent, the fraction eluted with CHCl₃-MeOH (19:1) yielded 85 g of material that showed notable PPAR-γ ligand-binding activity (Figure 2). A series of chromatographic separations were then performed, resulting in 10 new phenolic compounds [1] (8.0 mg), 2 (13.8 mg), 3 (1.6 mg), 4 (6.9 mg), 5 (18.5 mg), 6 (8.6 mg), 7 (18.5 mg), 8 (4.9 mg), 9 (7.3 mg), 10 (30.2 mg)] (Figure 3). In addition, 29 known phenolic compounds were obtained and identified by comparison of physical and spectral data with those reported in the literature. The following known phenolic compounds were identified in the CHCl₃-MeOH (19:1) fraction: echinatin (11, 5.4 mg) [5], lichocalcone B (12, 17.3 mg) [6], morachalcone A (13, 14.1 mg) [7], 2′,3′,4′-tri hydroxy-3′-γ,γ-dimethylallyl-6″,6″-dimethylypyrano[2″,3″:4,5]chalcone (14, 17.4 mg) [8], 1-(2′,4′-dihydroxyphenyl)-2-hydroxy-3-(4″-hydroxyphenyl)-1-propanone (15, 5.8 mg) [9], kanzonol Y (16, 2.5 mg) [10], (3R)-vestitol (17, 10.5 mg) [11], (3R)-2′,3′,7-trihydroxy-4′-methoxyisoflavan (18, 8.1 mg) [12], kanzonol X (19, 37.1 mg) [10], glabridin (20, 193 mg) [13], 4′-O-methylglabridin (21, 11.4 mg) [14], 3′-hydroxy-4′-O-methylglabridin (22, 54.7 mg) [15],

![Figure 2. PPAR-γ ligand-binding activity of *G. glabra* extract and fractions](image-url)
hispaglabridin A (23, 13.7 mg) [14], hispaglabridin B (24, 10.7 mg) [14], glabrene (25, 28.8 mg) [16], kanzonol W (26, 3.0 mg) [10], glabrocoumarin (27, 16.4 mg) [17], shinpterocarpin (28, 41.0 mg) [18], O-methylshinpterocarpin (29, 32.4 mg) [18], licoagrocarpin (30, 6.1 mg) [19], licoflavanone A (31, 4.8 mg) [20], glabrol (32, 13.5 mg) [13], shinflavanone (33, 7.8 mg) [18], euchrenone a5 (34, 1.5 mg) [21], xambioona (35, 8.0 mg) [22], gancaonin L (36, 8.8 mg) [23], glabra (37, 15.5 mg) [24], kanzonol U (38, 21.6 mg) [11], and 8,8-dimethyl-3,4-dihydro-2H,8H-pyrano[2,3-f]-chromon-3-ol (39, 17.4 mg) [25] (Figure 4). The structures of isolated compounds were classified into 12 groups: chalcones (1–4, 11–16), isoflavans (5, 6, 17–24), an isoflavone (25), 3-arylcoumarins (26, 27), pterocarpans (28–30), a flavone (31), a flavanol (7), flavanones (32–35), isoflavones (8, 36, 37), an isoflavane (9), an 2-aryl benzofuran (38), and chromones (10, 39). Compounds 13, 15, 17, 18, and 36 were isolated from G. glabra for the first time.

Compound 1 was isolated as a yellow amorphous powder with a formula of C_{21}H_{22}O_{6} determined by high-resolution electrospray ionization mass spectrometry (HRESIMS), which showed an accurate [M+H]^+ ion at m/z 371.1487. Compound 1 had an absorption maxima at 366 and 248 nm in its UV spectrum and absorbance bands at 3427 cm^{-1} (hydroxy groups), 1625 cm^{-1} (conjugated carbonyl group), and 1595, 1507, and 1469 cm^{-1} (aromatic rings) in its IR spectrum. The ^1H NMR spectrum of 1 (acetone-d_{6}) contained signals for two trans-coupled protons at δ_{H} 7.91 and 7.67 (each d, J = 15.7 Hz), ortho-coupled aromatic protons at δ_{H} 7.25 and 6.72 (each d, J = 8.5 Hz), meta-coupled aromatic protons at δ_{H} 7.55 and 7.51 (each d, J = 1.9 Hz), and methoxy protons at δ_{H} 3.88 (s). In addition, the ^1H NMR spectrum indicated the presence of a prenyl (3-methyl-2-butenyl) group [δ_{H} 5.40 (1H, m), 3.43 (2H, br d, J = 7.3 Hz), 1.77 and 1.75 (each 3H, br s)]. These data suggested that 1 was a chalcone derivative with four hydroxy groups, a methoxy group, and a prenyl group. Long-range correlations were observed in the heteronuclear multiple bond coherence (HMBC) spectrum of 1 (Figure 5) between H-2 (δ_{H} 7.51) and C==O (carbonyl, δ_{C} 188.0)/C-3 (δ_{C} 144.6)/C-4 (δ_{C} 148.3)

Figure 3. Structures of 1–10 isolated from G. glabra roots [1].

Figure 4. Structures of 1–10 isolated from G. glabra roots [1].
and between H-6 ($\delta_H 7.55$) and C\textsuperscript{=O}/C-1 ($\delta_C 130.7$), indicating that two hydroxy groups were attached to C-3 and C-4. HMBC correlations between H-1\textsuperscript{′′} ($\delta_H 3.43$) and C-4/C-6 ($\delta_C 122.8$) and between H-2\textsuperscript{′′} ($\delta_H 5.40$) and C-5 ($\delta_C 128.2$) indicated the existence of a prenyl group at C-5. The structure of the B-ring moiety attributed to 3',4'-dihydroxy-2'-methoxyphenyl and its linkage to C-\(\beta\) of the \textit{trans}-olefinic group were determined by HMBC correlations between

Figure 4. Structures of 11–39 isolated from \textit{G. glabra} roots [1].
H-5′ (δ\textsubscript{H} 6.72) and C-1′ (δ\textsubscript{C} 121.0)/C-3′ (δ\textsubscript{C} 138.7)/C-4′ (δ\textsubscript{C} 149.0), H-6′ (δ\textsubscript{H} 7.25) and C-β (δ\textsubscript{C} 138.4)/C-2′ (δ\textsubscript{C} 148.7), and methoxy protons and C-2′. Therefore, the structure of 1 was assigned as 3,3′,4,4′-tetrahydroxy-2′-methoxy-5-prenylchalcone.

Compound 5 was isolated as a yellow amorphous powder with a molecular formula of C\textsubscript{21}H\textsubscript{20}O\textsubscript{5} determined by HRESIMS. The \textsuperscript{1}H NMR spectrum of 5 indicated signals characteristic of an isoflavan skeleton at δ\textsubscript{H} 4.43 (ddd, J = 10.2, 3.4, 2.2 Hz, H-2a), 4.07 (dd, J = 10.2, 10.2 Hz, H-2b), 3.54 (m, H-3), 3.07 (dd, J = 15.5, 11.1 Hz, H-4a), and 2.90 (ddd, J = 15.5, 5.0, 2.2 Hz, H-4b). In addition, the spectrum of 5 indicated signals that we assigned to two aromatic protons at δ\textsubscript{H} 7.58 and 6.49 (each s), ortho-coupled aromatic protons at δ\textsubscript{H} 6.87 and 6.32 (each d, J = 8.2 Hz), and a 2,2-dimethylpyran ring at δ\textsubscript{H} 6.63 and 5.65 (each 1H, d, J = 9.8 Hz) and δ\textsubscript{H} 1.40 and 1.38 (each 3H, s). Compound 5 and glabridin (20) have similar features in their \textsuperscript{1}H NMR spectra. Moreover, the \textsuperscript{1}H and \textsuperscript{13}C NMR signals at δ\textsubscript{H} 9.77 and δ\textsubscript{C} 195.3 indicated the presence of a formyl group, which was attached at C-5′, as determined by the HMBC correlations between the formyl proton signal and C-4′ (δ\textsubscript{C} 163.6)/C-5′ (δ\textsubscript{C} 115.4)/C-6′ (δ\textsubscript{C} 133.9) (Figure 5). The circular dichroism (CD) profile of 5 was the same as that of synthetic 5′-formylglabridin prepared by formylation of 20, indicating that the absolute configuration at C-3 was R. Therefore, the structure of 5 was assigned as 5′-formyl glabridin. It was notable that 5 was the first naturally occurring isoflavan with a formyl group in the B-ring portion of the compound.

Compound 7 was isolated as a yellow powder with a molecular formula of C\textsubscript{20}H\textsubscript{20}O\textsubscript{5} determined by HRESIMS. Compound 7 had an absorption maxima at 313 and 276 nm in its UV.

Figure 5. Key HMBC correlations of 1, 5, 7, and 8 [1].
spectrum and absorption bands at 3374 cm\(^{-1}\) (hydroxy groups), 1673 cm\(^{-1}\) (a carbonyl group), and 1608, 1502, and 1463 cm\(^{-1}\) (aromatic rings) in its IR spectrum. The \(^1\)H NMR spectrum of 7 showed signals that we assigned to a prenyl group at \(\delta_{\text{H}} 5.39\) (1H, m), 3.38 (2H, d, \(J = 7.3\) Hz), and 1.74 and 1.72 (each 3H, br s), and two methines bearing an oxygen function at \(\delta_{\text{H}} 5.03\) and 4.59 (each d, \(J = 11.9\) Hz). Furthermore, two 1,3,4-trisubstituted aromatic rings were identified from six aromatic protons comprising two ABX-type spin-coupling systems at \(\delta_{\text{H}} 7.14\) (d, \(J = 8.6\) Hz), 6.64 (dd, \(J = 8.6, 2.2\) Hz), and 6.41 (d, \(J = 2.2\) Hz) and \(\delta_{\text{H}} 7.35\) (d, \(J = 2.0\) Hz), 7.27 (dd, \(J = 8.2, 2.0\) Hz), and 6.90 (d, \(J = 8.2\) Hz). The above data indicated that 7 was a dihydroxyflavan-3-ol derivative with a prenyl unit. The HMBC correlations between H-5 (\(\delta_{\text{H}} 5.54\)), H-8 (\(\delta_{\text{H}} 6.41\)) and C-7, H-2′ (\(\delta_{\text{H}} 7.35\)) and C-2′ (\(\delta_{\text{C}} 175.4\))/C-9 (\(\delta_{\text{C}} 159.9\), H-6 (\(\delta_{\text{H}} 6.64\)) and C-7, H-2′ (\(\delta_{\text{H}} 7.35\)) and C-2′ (\(\delta_{\text{C}} 155.8\)), H-6′ (\(\delta_{\text{H}} 7.27\)) and C-2/C-4′, and H-1′″ (\(\delta_{\text{H}} 3.38\)) and C-2′ (\(\delta_{\text{C}} 130.0\))/C-3′ (\(\delta_{\text{C}} 128.1\))/C-4′ indicated that two hydroxy groups and a prenyl group were attached to C-7, C-4′, and C-3′, respectively (Figure 5). In the CD spectrum of 7, the positive Cotton effects at 210, 240, and 334 nm and the negative Cotton effect at 304 nm indicated absolute configurations of 2R and 3R [26]. Therefore, the structure of 7 was identified as (2R,3R)-3,4′,7-trihydroxy-3′-prenylflavanone.

The following suggested that compound 8 (C\(_{21}\)H\(_{20}\)O\(_{5}\)) was an isoflavone derivative: a UV absorption maximum at \(\lambda_{\text{max}} 263\) nm [27], a proton resonance at \(\delta_{\text{H}} 8.16\) (1H, s), and a corresponding oxygen-bearing olefinic carbon signal at \(\delta_{\text{C}} 152.2\). The \(^1\)H NMR spectrum of 8 contained signals for an aromatic proton at \(\delta_{\text{H}} 7.54\) (s), p-disubstituted aromatic protons at \(\delta_{\text{H}} 7.55\) and 6.98 (each d, \(J = 8.8\) Hz), and methoxy protons at \(\delta_{\text{H}} 3.84\) (3H, s). In addition, the \(^1\)H NMR spectrum implied the presence of a prenyl unit [\(\delta_{\text{H}} 5.41\) (1H, m), 3.56 (2H, d, \(J = 7.3\) Hz), and 1.76 (3H × 2, br s)]. In the HMBC spectrum of 8 (Figure 5), correlation peaks were observed between H-5 (\(\delta_{\text{H}} 7.54\)) and C-4 (\(\delta_{\text{C}} 175.4\))/C-7 (\(\delta_{\text{C}} 148.7\))/C-9 (\(\delta_{\text{C}} 145.7\))/C-1′″ (\(\delta_{\text{C}} 28.4\)), methoxy protons (\(\delta_{\text{H}} 8.16\)) and C-4′ (\(\delta_{\text{C}} 159.9\)), and H-2 (\(\delta_{\text{H}} 8.16\)) and C-8 (\(\delta_{\text{C}} 145.7\)), indicating that two hydroxy groups were attached to C-7 and C-8, a methoxy group to C-4′, and a prenyl group to C-6. Therefore, the structure of 8 was found to be 7,8-dihydroxy-4′-methoxy-6-prenylisoflavanone.

In the same way, the structures of 2–4, 6, 9, and 10 were established as shown in Figure 3. Compounds 3, 4, and 9 showed neither specific rotation nor Cotton effects in their CD spectra, indicating that these compounds were racemates.

4. PPAR-γ ligand-binding activity of compounds 1–39 isolated from G. glabra

Compounds 5, 7, 11, 18, 19, 26, 28, 31–33, 36, and 37 showed significant PPAR-γ ligand-binding activity. Among these compounds, the prenylflavone derivative licoflavanone A (31) was the most potent (Figure 6). These active compounds likely contributed the most to the PPAR-γ ligand-binding activity of the EtOH extract. The isoflavone derivative, kanzonol X (19), and flavanone derivative, glabrol (32), both had two prenyl units and exhibited potent ligand-binding activity. Hispaglabridin B (24) and xambioona (35), in which two prenyl units were cyclized to form two six-membered rings, exhibited weaker ligand-binding activities than 19 and 32 did, suggesting
that the two non-cyclic prenyl moieties were necessary for the potent activity of these compounds (Figure 7). Taking together all the above data, the PPAR-γ ligand-binding activity of the phenolic compounds was affected by slight differences in the substitution groups on the aromatic rings.

**Figure 6.** PPAR-γ ligand-binding activity of compounds 1–39 at 2 (□) and 10 (□) μg/mL with a GAL-4-PPAR-γ chimera assay [1]. Troglitazone (TRG) at 0.5, 1.0, and 2.0 μM was used as a positive control, and dimethyl sulfoxide at 1 mL/L was used as a solvent control. Values are means ± SD, n = 4 experiments. Statistical significance is indicated by * (p < 0.05) or ** (p < 0.01) as determined by Dunnett’s multiple comparison test.
5. Isolation and structural determination of phenolic compounds from *G. uralensis*

The roots of *G. uralensis* (1.2 kg) were extracted with EtOAc (5.5 L) at room temperature for 7 days. The extract was then filtered and concentrated (74.0 g). The EtOAc extract of *G. uralensis* roots exhibited potent PPAR-γ ligand-binding activity and its relative luminescence intensity was 2.8 at a sample concentration of 30 μg/mL. This was almost equivalent to that of 1.0 μM TRG. The EtOAc extract was chromatographed on a silica gel column eluted with CHCl₃–MeOH gradients (19:1; 9:1; 4:1) and finally with MeOH to provide four fractions. PPAR-γ ligand-binding activity was concentrated in the CHCl₃–MeOH (19:1) eluate (relative luminescence intensity of 2.8 at 30 μg/mL). The CHCl₃–MeOH (19:1) eluate (55.4 g) was subjected to multiple chromatographic steps on a silica gel column eluted with CHCl₃–MeOH, an ODS silica gel column eluted with MeOH-H₂O and MeCN-H₂O, and to reversed phase HPLC using MeOH-H₂O and MeCN-H₂O mobile phases to obtain compounds 40–52 (22.2, 28.3, 58.7, 225, 80.7, 12.1, 43.5, 17.5, 11.8, 74.5, 51.0, 22.1, and 40.1 mg, respectively). Compounds 41–52 were identified by comparison of their physical and spectral data with those of reported compounds as dehydroglyasperin C (41) [28], glyasperin D (42) [29], glycyicumarin (43) [30], glycyerin (44) [31], glyasperin B (45) [29], glycyrol (46) [32], isoglycyrol (47) [32], glicoricone (48) [33], licoricone (49) [34], licocoumarone (50) [30], gancaonin I (51) [35], and liquiritigenin (52) [36] (Figure 8). This was the first isolation of glyasperin B (45) from *G. uralensis*. The structure of the new compound, 41, was determined by 1D and 2D NMR spectroscopic analysis and

Figure 7. PPAR-γ ligand-binding activity of 19, 24, 34, and 35 isolated from *G. glabra* roots [1]. Values in parentheses are the relative luminescence intensities at 10 μg/mL.

Figure 8. Structures of 40–52 isolated from *G. uralensis* roots [2]. Values in parentheses are the relative luminescence intensities at 5 μg/mL.
HRESIMS as 3-(2,4-dihydroxyphenyl)-5,7-dimethoxy-6-(3-methyl-2-butenyl)-2H-chromene and was named dehydroglyasperin D.

6. PPAR-γ ligand-binding activity of compounds 40–52 isolated from G. uralensis

Of the isolated compounds, the new compound 40 and known compounds 41–45 exhibited significant PPAR-γ ligand-binding activity (Figure 6). The activity of 40 at 5.0 μg/mL (=13.6 μM) was stronger than that of 2.0 μM TRG (relative luminescence intensity of 3.7). The coumestan derivative 46, which was less active than 40, was structurally similar to the active compound 43, and the only detected difference between 43 and 46 was the formation of a five-membered ether ring between C-4 and C-2′ in 46. This suggested that the presence of a hydroxy group at C-2′ in the isoflavan, isoflavene, or arylcoumarin skeleton is necessary for PPAR-γ ligand-binding activity. Furthermore, the isoflavones, 48 and 49, which have a hydroxy group at C-2′ and no isoprenyl group at C-6, did not exhibit activity, suggesting that the isoprenyl group at C-6 was also involved in PPAR-γ ligand-binding activity. In conclusion, the isoprenyl group at C-6 and the C-2′ hydroxy group in the aromatic C ring of the isoflavan, isoflavene, or arylcoumarin skeleton were structural requirements for PPAR-γ ligand-binding activity (Figure 9).

7. Ameliorative effects on diabetic KK-A′ mice

The ameliorative effects of glycyrin (44) in KK-A′ mice, an animal model of genetic type 2 diabetes, were studied using pioglitazone as a positive control. There was no difference in the food intake or body weight of mice between the treated groups and the control group. Test compound intake, calculated from the food intake and body weight of the mice, was approximately 100 mg/(kg day) in the glycyrin and glycyrol (46) groups and 23 mg/(kg day) in the pioglitazone group. Blood glucose levels significantly decreased after 4 days of feeding in both the glycyrin- and pioglitazone-treated groups compared to that in the control group, whereas the blood glucose levels of the glycyrol-treated group were comparable to those of the control group (Table 1).
Pioglitazone, a potent PPAR-γ agonist that activates PPAR-γ, resulted in the improvement of insulin resistance and type 2 diabetes mellitus. Glycyrrin exhibited significant PPAR-γ ligand-binding activity and appeared to reduce the blood glucose levels of KK-A\textsubscript{Y} mice by the same biological mechanism as pioglitazone. This finding was supported by the observation that glycyrol, structurally related to glycyrin but lacking PPAR-γ ligand-binding activity, failed to improve the hyperglycemia of KK-A\textsubscript{Y} mice.

### 8. Conclusion

Fractionation of the EtOH extract of \textit{G. glabra} roots and the EtOAc extract of \textit{G. uralensis} roots, guided by a GAL-4-PPAR-γ chimera assay method, resulted in the isolation of 52 phenolics, including 11 new compounds. The structures of the new compounds were determined by spectroscopic analysis. Of the isolated compounds, more than 10 phenolics exhibited significant PPAR-γ ligand-binding activity and the prenylflavone derivative, licoflavanone A (31), exhibited the most potent ligand-binding activity. The activity of these compounds at a sample concentration of 10 μg/mL was approximately three times greater than that of 0.5 μM TRG. Six phenolics were isolated from the EtOAc extract of \textit{G. uralensis} roots as PPAR-γ ligands and one, glycyrin (44), reduced the blood glucose levels of genetically diabetic KK-A\textsubscript{Y} mice through its PPAR-γ ligand-binding activity. We have therefore discovered a possible new application of \textit{G. glabra} and \textit{G. uralensis} roots and their constituents for the amelioration of type 2 diabetes, a representative insulin resistance syndrome that is becoming a serious worldwide public health problem.

### Table 1. Effect of feeding glycyrin (44) on KK-A\textsubscript{Y} mice in experiments for the preventing diabetes [2].

|                     | Control | Glycyrrin (0.10%) | Glycyrol (0.10%) | Pioglitazone (0.02%) |
|---------------------|---------|-------------------|------------------|----------------------|
| **Body weight (g)** |         |                   |                  |                      |
| Day 0               | 52.6 ± 0.53 | 54.1 ± 1.78 | 52.6 ± 1.07    | 55.1 ± 0.69         |
| Day 4               | 48.9 ± 0.48 | 50.4 ± 1.58 | 49.1 ± 1.02    | 53.6 ± 1.07         |
| Day 7               | 50.4 ± 0.56 | 51.9 ± 1.62 | 50.3 ± 0.91    | 55.8 ± 1.50         |
| Day 10              | 46.9 ± 0.42 | 48.1 ± 1.57 | 46.8 ± 0.08    | 52.5 ± 11.41        |
| **Average food intake (g/mouse/day)\textsuperscript{b}** | 5.43 | 5.34 | 5.47 | 6.34 |
| **Average test compound intake (mg/kg/day)\textsuperscript{c}** | 0 | 102 | 108 | 23 |
| **Blood glucose level (mg/dL)** | | | | |
| Day 0               | 476 ± 22 | 474 ± 27 | 427 ± 24    | 486 ± 26            |
| Day 4               | 420 ± 14 | 278 ± 14\textsuperscript{**} | 421 ± 19     | 191 ± 6\textsuperscript{**} |

\textsuperscript{a}Body weights and blood glucose levels are expressed as means ± SE of five mice.

\textsuperscript{b}Calculated as (total food intake) (number of mice day).

\textsuperscript{c}Calculated as (average food intake/average body weight of mice).

Statistical significance is indicated as \textsuperscript{**} (P < 0.01) as determined by Dunnett’s multiple comparison test.
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