In vivo anti-diabetic activity of derivatives of isoliquiritigenin and liquiritigenin

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A B S T R A C T

Isoliquiritigenin (ISL), a chalcone and liquiritigenin (LTG), a flavonoid found in licorice roots and several other plants. ISL displays antioxidant, anti-inflammatory, antitumor and hepatoprotective activities whereas LTG is an estrogenic compound, acts as an agonist selective for the β-subtype of the oestrogen receptor. Both the phenolics were isolated from the rhizomes of Glycyrrhiza glabra. Five derivatives from ISL and four derivatives from LTG were synthesized. All the compounds were established by extensive spectroscopic analyses and screened through oral glucose tolerance test to gain preliminary information regarding the antihyperglycemic effect in normal Swiss albino male mice. ISL (1), ISL derivatives 3, 4, 5, 7 and LTG derivatives 9 and 10 showed significant blood glucose lowering effect. The structure–activity relationship indicated that the presence of ether and ester groups in ISL and LTG analogues are important for exhibiting the activity. Compounds 1, 4 and 10 were selected for in vivo antidiabetic activity and found to be potential candidates for treatment of diabetes. It is the first report on antidiabetic activity of ISL derivative 4 and LTG derivative 10.

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Introduction

Diabetes is a metabolic disease which has become a serious problem of the society due to the severe long term health complications associated with it. Type 2 diabetes mellitus (T2DM) is the most encountered form of diabetes, accounting for more than 80% of the total cases (Milnar et al. 2007) and is expected to increase by 5.4% in 2025 (Kim et al. 2006). Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high levels of glucose in the blood due to the non-secretion of insulin (ADA 2007). The plants have been used since ancient times to treat diabetes and prevent conditions associated with diabetes (Soumyanath 2006). The roots of Glycyrrhiza spp. is one of the oldest and most frequently used crude drugs in traditional Chinese medicines for its extensive pharmacological effects (Songpei et al. 2010). Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world and popularized as nutraceuticals. In addition, many of the currently available drugs have been derived directly or indirectly from plant source. The discovery of the widely used hypoglycemic drug metformin also came from the traditional approach of using Galega officinalis (Akpan et al. 2007).

Isoliquiritigenin (1, ISL), a chalcone and liquiritigenin (2, LTG), a flavonoid found in licorice root and several other plants. ISL displays antioxidant, anti-inflammatory and antitumor activities as well as hepatoprotection against steatosis-induced oxidative stress (Gaur et al. 2010). LTG is an estrogenic compound, acts as an agonist selective for the β-subtype of the oestrogen receptor (Mersereau et al. 2008). Chalcones possess a broad spectrum of biological activities and have been shown the promising compounds for the prevention or treatment of diabetic complications (Hsieh et al. 2012). The mechanism is most often not completely understood, but more and more studies are being conducted to elucidate the mechanisms of action of different plants and natural compounds. Biosynthetically and structurally, 1 is the precursor and an isomer of 2 (Jayaprakasam et al. 2009). During the early stages of the biosynthesis of these flavonoids, chalcone isomerase (CHI) catalyzes the intramolecular cyclization of chalcone 1 into flavanone 2 (Liu and Dixon 2001). Both the phenolics were isolated from the rhizomes of Glycyrrhiza glabra. The present study was undertaken to investigate the antidiabetic effect of derivatives of...
isoliquiritigenin (ISL) and liquiritigenin (LTG) (Fig. 1) on STZ-induced diabetic mice.

Materials and methods

Plant material

Rhizomes of Glycyrrhiza glabra L. (Fabaceae) were collected from the research farm of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India in March 2011. The plant was identified by a taxonomist in Botany and Pharmacognosy Department, CSIR-CIMAP, Lucknow and a voucher specimen #7401 was deposited.

Extraction and isolation

Fresh rhizomes were cut into small pieces without peeling the bark, dried at 35 °C in oven and powdered. The powder (625 g) was extracted with hot acetone (1.5 l) for eight hours. The acetone extract, after concentration, was fractionated with n-hexane (3 × 400 ml, 12.6 g) and ethyl acetate (4 × 400 ml, 45.1 g). The ethyl acetate extract (40 g) was column chromatographed (silica gel, 60–120 mesh, 6.5 cm × 120 cm), eluting with CHCl₃ and CHCl₃:MeOH 99:1, 97:3, and 94:6, 41 each. Similar CHCl₃:MeOH (97:3) fractions, 250 ml of each, monitored by TLC, provided LTG, 1.02 g (0.18%, acetone-n-hexane). The mother liquor (1.4 g) upon flash chromatography using CHCl₃, with a flow rate of 2.5 ml/min and a 2 min per tube collection time, yielded ISL 1 (122 mg, 0.022%), and LTG (605 mg, 0.11%). The purity of isolates was found to be 94–97%, detected by their HPLC analysis (chromatographic conditions are shown below).

HPLC analysis

HPLC analysis was performed using a Shimadzu LC-10AD liquid chromatography equipped with two LC-10A pumps controlled by a CBM-10 interface module, SPD-M10A VP diode array detector, and a SIL-10ADVP auto injector. Data were collected and analyzed using a class LC-10 Work Station. The samples were analyzed by using reverse phase chromatography on waters spheroςorb ODS2 (250 × 4.6 mm i.d., 10 mm) column using binary gradient elution with acetonitrile and water containing 0.1% TFA mobile phase (30:70) at a flow rate of 0.6 ml/min, a column temperature of 25 °C and UV detection at λ 254 nm.

Synthesis of derivatives of isoliquiritigenin (Figs. 2 and 3)

4,4′-Diacetoxy-2′-hydroxy chalcone (3)

To a solution of ISL (300 mg, 1.17 mmol) in pyridine (10 ml) acetic anhydride (1 ml, 10.53 mmol) was added drop wise over a period of 10 min at 0 °C. The reaction mixture was allowed to stir for 4 h at room temperature and monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice cold 10% HCl solution (25 ml). The mixture was extracted with ethyl acetate (3 × 25 ml). The organic phase was washed with solution of sodium bicarbonate (20 ml), brine (20 ml), dried over Na₂SO₄ and concentrated under reduced pressure to give an oily residue which was crystallized in methanol to afford yellow crystals in 65% (w/w) yield, mp 88–90 °C (Figs. 2 and 3).

2′,4′-Dimethoxy-4′-hydrox y chalcone (4)

2′,4′-Dimethoxyacetophenone (1.80 g, 0.01 mol) and 4-hydroxybenzaldehyde (0.01 mol) were dissolved in ethanol (35 ml) and 0.05 ml SO₂Cl₂ was added at RT with constant stirring. The reaction was completed in 2 h and the solid obtained was separated by filtration, dried and crystallized in ethyl acetate hexane to provide chalcone 4, (2.47 g, 87% yield), mp 91–92 °C, identified as 2,4-dimethoxy-4′-hydroxychalcone by comparison of its mp, IR, 1H, 13C NMR and ESIMS with the reported data (Guantai et al. 2011).