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Synergy Research:
Natural Products for rational comedication with
Chemotherapeutics and Antibiotics

Isobologram
 $E(dA,B) > E(dA) + E(dB)$

antagonism
additive-interaction
synergism

Curcuma longa
Camellia sinensis
Taxus baccata

Examples:
A
Curcumin
Epigallocatechin-gallate

B
Taxol
Norfloxacin

% of mice with breastcancer metastases to the lung

Group	% of mice with breastcancer metastases to the lung
Control	100
Taxol	75
Curcumin	60
Curcumin + Taxol	35

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



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ABSTRACT

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 (Mlinar 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

Abbreviations: DEPT, distortionless enhancement by polarization transfer; COSY, correlation spectroscopy; bw, body weight; AST, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; T BIL, total bilirubin; TP, total protein; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein.

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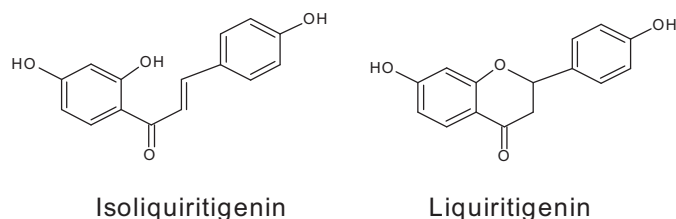


Fig. 1. Structures of isoliquiritigenin (1) and liquiritigenin (2).

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 CHCl_3 and CHCl_3 :MeOH 99:1, 97:3, and 94:6, 4 l each. Similar CHCl_3 :MeOH (97:3) fractions, 250 ml of each, monitored by TLC, provided LTG, 2, 1.02 g (0.18%, acetone-*n*-hexane). The mother liquor (1.4 g) upon flash chromatography using CHCl_3 , 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 were 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 spherisorb 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.

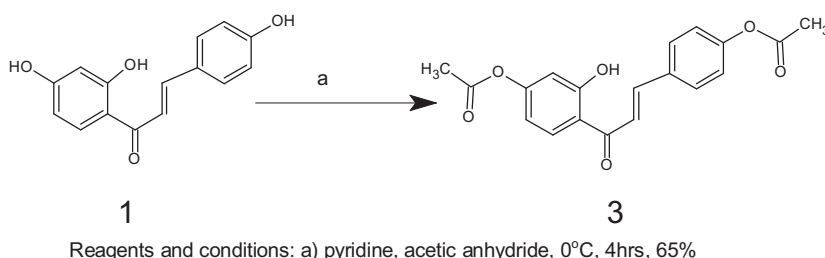
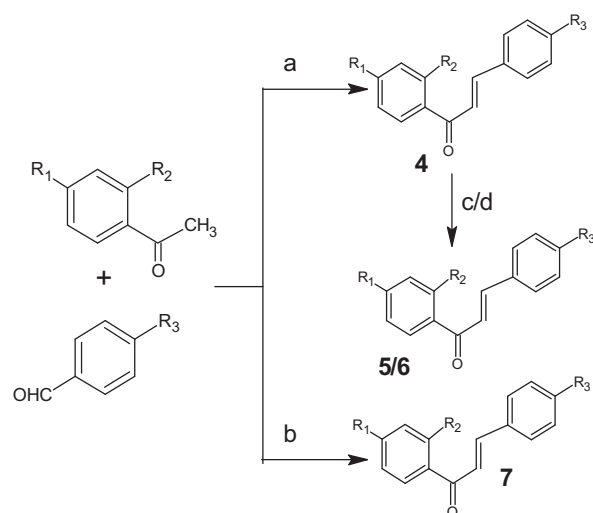


Fig. 2. Preparation of 4,4'-Diacetoxy-2'-hydroxy chalcone (3).



4, $R_1 = R_2 = \text{OMe}$, $R_3 = \text{OH}$; 5, $R_1 = R_2 = \text{OMe}$, $R_3 = \text{OAc}$; 6, $R_1 = R_2 = \text{OMe}$, $R_3 = \text{OBz}$

7, $R_1 = R_2 = \text{OMe}$, $R_3 = \text{H}$;

Reagents/conditions: (a) SOCl_2 -EtOH, RT, 2h, 87%; (b) NaOH-EtOH, RT, 2-4h, 58-93%; (c) Ac_2O - $\text{C}_5\text{H}_5\text{N}$, RT, 0.5h, 91%; (d) PhCOCl - $\text{C}_5\text{H}_5\text{N}$, RT, 0.5h, 96%

Fig. 3. Preparation of ISL (1) analogues 4–7.

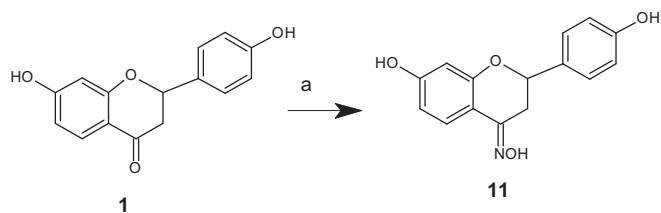
Synthesis of derivatives of isoliquiritigenin (Figs. 2 and 3)

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 Na_2SO_4 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-hydroxy 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 SOCl_2 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'-hydroxy chalcone by comparison of its mp, IR, ^1H , ^{13}C NMR and ESIMS with the reported data (Quantai et al. 2011).



Reagents and conditions: a) $\text{NH}_2\text{OH}\cdot\text{HCl}$, CH_3COONa , EtOH, reflux

Fig. 4. Preparation of LTG-oxime (**11**).

4-Acetoxy-2',4'-dimethoxychalcone (**5**)

Acetylation of compound **4** (0.198 g, 0.70 mmol) with acetic anhydride (0.5 ml) in pyridine (2 ml) afforded light orange crystals, 0.207 g, 91% yield, mp 70–71 °C, identified as 4'-acetoxy-2,4-dimethoxychalcone by comparison of its mp, IR, ^1H , ^{13}C NMR and ESIMS with the reported data (Bianco et al. 2003).

4-Benzoyloxy-2',4'-dimethoxychalcone (**6**)

Compound **4** (0.198 g, 0.70 mmol) and benzoyl chloride (0.5 ml) were stirred in pyridine (2 ml) at room temperature. After completion of benzylation, the reaction mixture was worked-up as usual which afforded a residue. The residue upon crystallization in ethyl acetate-hexane furnished dark orange crystals of derivative **6**, 0.372 g, 96% yield, mp 90–91 °C.

2',4'-Dimethoxychalcone (**7**)

2',4'-Dimethoxyacetophenone (1.80 g, 0.01 mol) and benzaldehyde (0.01 mol) were dissolved in ethanol (35 ml) and 10% NaOH in ethanol (5 ml) was added drop wise at RT with constant stirring. After completion of the reaction it was poured on ice cooled water (100 ml) and neutralized with dilute hydrochloric acid. The yellow precipitate was obtained, filtered, washed with water (200 ml) and crystallized to yield chalcone **7** as a yellow solid, 2.49 g, 93% yield, mp 65–66 °C, identified as 2',4'-dimethoxychalcone by comparison of its IR, ^1H , ^{13}C NMR and ESIMS with the reported data (Bianco et al. 2004).

Synthesis of derivatives of liquiritigenin

LTG derivatives (**8–10**)

LTG was acetylated and benzyolated as per methodology reported previously by us (Gaur et al. 2010), to LTG 7,4'-diacetate (**8**), LTG 4'-acetate (**9**) and LTG 7,4'-dibenzoate (**10**).

LTG-oxime (**11**) (Fig. 4)

It was prepared by treating (0.256 g, 0.001 mol) LTG with hydroxylamine hydrochloride (0.069 g, 0.01 mol) in ethanol and sodium acetate trihydrate (0.136 g, 0.001 mol) in water. The solution was heated on a water bath for 4 h with constant stirring. Upon cooling and addition of water, a creamy precipitate was obtained. It was filtered, washed with water, and dried. The product was crystallized from ethanol to yield 0.235 g (91.7%), mp 125–126 °C.

Experimental animals

Swiss albino male mice (body weight 25–30 g) were selected for this study. They were bred in the Animal House of the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India and maintained on a standard rodent pellets diet and water *ad libitum*. Permission and approval for animal studies was obtained from CPCSEA (Reg. No. 400/01/AB/CPCSEA, AH-2012-08), Government of India through the Institutional Animal Ethics Committee.

Oral glucose tolerance test in normal mice

The blood glucose level of each animal was checked by glucometer using oxidase-peroxidase glucose strips (Breeze2, Bayer India) after 18 h starvation. Animals showing blood glucose levels between 60 and 80 mg/dl were divided into groups of five animals in each ($n=5$). Animals of the experimental group were administered suspension of the desired test compound orally (made in 3% cremophor EL and 0.3% carboxymethyl cellulose mixture) at a single dose of 100 mg/kg bw. Animals of the glucose control group were given an equal amount of 3% cremophor EL and 0.3% carboxymethyl cellulose mixture. An oral glucose load (2.0 g/kg bw) was given to each animal exactly after 30 min per oral administration of the test sample/vehicle. A blood glucose profile of each mouse was again determined at 15, 30, 60, 90 and 120 min post administration of glucose using glucometer (Beguino and Nigro 2012).

Dose optimization

Screening of the test compounds for hypoglycaemic activity was performed administering single dose (100 mg/kg bw) in normal healthy mice with the oral glucose tolerance test (OGTT). The same protocol was used to determine the most effective dose of selected compounds at doses of 50, 100 and 200 mg/kg bw. The minimum dose that improved glucose tolerance significantly was selected for further studies (Genta et al. 2010).

Streptozotocin-nicotinamide induced antidiabetic activity in mice

Swiss albino male mice (outbred strain) of 25–30 g body weight were acclimatized in groups of five in controlled environmental conditions (23 ± 2 °C, $55 \pm 10\%$ RH and 12 h day/night cycle), 7 days before induction of diabetes. Overnight fasted animals were administered nicotinamide (110 mg/kg bw, i.p.) dissolved in normal saline followed by streptozotocin (200 mg/kg bw, i.p.) dissolved in 100 mM citrate buffer (pH 4.5) after 15 min. Animals were fed with 5% glucose solution for 12 h to avoid hypoglycaemia. Hyperglycaemia was confirmed after 3 days. After 10 days, hyperglycaemia was reached to steady state. The blood glucose level was determined using a glucometer. Mice having blood glucose ≥ 300 mg/dl were considered as diabetic and selected for the study (Badole and Bodhankar 2010; Yadav et al. 2013). Diabetic animals were divided into five groups containing ten animals each ($n=10$). Animals of test groups were administered orally with **1**, **4** and **10** at a dose of 200, 50 and 50 mg/kg bw respectively, suspended in 3% cremophor EL and 0.3% carboxymethyl cellulose mixture for 14 days. The disease control group was given 3% cremophor EL and 0.3% carboxymethyl cellulose mixture, orally at the same volume. The standard control group was treated with metformin (200 mg/kg bw, p.o.). The normal control group was constituted using non-diabetic animals and 3% cremophor EL and 0.3% carboxymethyl cellulose mixture (vehicle) at same volume was given to them with the help of oral gavage. All animals had free access to pathogen free water and normal rodent chow (Dayal Industries, Lucknow, India) during the experimental period. Blood glucose levels were measured at 1, 7 and 14 days of the study. On the 15 day, mice were weighed and blood was collected from each mouse by heart puncture under deep ether anaesthesia. Animals were sacrificed by cervical dislocation and liver, kidney and spleen were collected from each animal and weighed. Serum was separated from the blood and used for estimation of alkaline phosphatase (AST), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (T BIL), total protein (TP), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), uric acid and creatinine using commercially available kits (Randox Laboratories Ltd., Co. Antrim, UK).

Statistical comparison is made by one way ANOVA followed by Tukey's multiple comparison tests using GraphPad PRISM version 5.01 (GraphPad software, USA). The value of $p < 0.05$ is considered statistically significant.

Results and discussion

Chemistry

ISL (**1**) and LTG (**2**) were isolated from the chloroform/ethyl acetate fraction of rhizomes as the procedure described earlier and identified by the analysis of their IR, ^1H , ^{13}C NMR, DEPT-135 and electron spray mass spectroscopy and compared with the data reported in literature (Gaur et al. 2010). Because of low quantity of ISL, the chalcone **1** was further modified to several ether and ester derivatives by directly synthesizing them. 2',4'-Dimethoxy-4-hydroxychalcone (**4**) was synthesized via aldol condensation by adding thionyl chloride to a mixture of 2',4'-dimethoxyacetophenone and 4-hydroxybenzaldehyde in ethanol at room temperature (Jaypal et al. 2010). The resulted chalcone **4** was further acetylated to 4-acetoxy-2',4'-dimethoxychalcone (**5**) and benzoylated to a new derivative, 4-benzyloxy-2',4'-dimethoxychalcone (**6**).

The natural LTG upon acetylation provided LTG 7,4'-diacetate (**8**) at room temperature and by refluxing the reaction mixture for 3 h, the diacetate was partially hydrolyzed to LTG 4'-acetate (**9**). LTG was further benzoylated to LTG 7,4'-dibenzoate (**10**).

Biology

Biological screening

The test compounds were screened for antihyperglycemic effect. The antihyperglycemic activity was evaluated in artificially enhanced blood glucose in albino mice called oral glucose tolerance test. ISL (**1**) and its derivatives **3**, **4**, **5**, **7** and LTG derivative **9** and **10** were found to be effective at 100 mg/kg bw in OGTT. All these compounds showed ~30.82, 25.59, 36.07, 31.74, 24.31, 40.22 and 23.44% change in blood glucose level after 120 min during OGTT, respectively. Therefore, compounds **1**, **3**, **4**, **5**, **7**, **9** and **10** were identified as potential blood glucose-lowering agents. The rest of the molecules were inefficient in the lowering of blood glucose levels. These inspiring results were further verified in streptozotocin-nicotinamide induced diabetic model in mice (Table 1).

Dose optimization

The data so far collected reveals that the compounds **1**, **3**, **4**, **5**, **7**, **9** and **10** exhibited significant antihyperglycemic activity but due to insufficient quantity of compounds **3**, **5**, **7** and **9** only **1**, **4** and **10** were selected for antidiabetic study in *in vivo* model using mice and also evaluated through OGTT to ascertain the lowest dose required for antidiabetic effect. After 15, 30, 60, 90 and 120 min post-administration of ISL (**1**) (200 mg/kg bw), ~12.53, 40.42, 48.85, 46.81, and 36.13% improvement was noticed in glucose tolerance, respectively (Fig. 5a). Compound **4** (50 mg/kg bw) exhibited ~25.79, 34.65, 20.81, 14.87, and 27.05% improvement in glucose tolerance, after 15, 30, 60, 90 and 120 min respectively (Fig. 5b), whereas compound **10** (50 mg/kg bw) displayed ~12.42, 43.43, 38.60, 29.22 and 23.92% improvement in glucose tolerance, respectively (Fig. 5c). Hence, minimum effective dose of compounds **1**, **4** and **10** was calculated as 200, 50 and 50 mg/kg bw, respectively.

Antidiabetic activity in Swiss albino mice

Compounds **1**, **4** and **10** were administered to hyperglycaemic albino mice once daily for 14 days at a dose of 200, 50 and 50 mg/kg bw, respectively. All the tested compounds recovered ($p < 0.001$) from the hypoglycaemic state by ~53.20% (**1**), ~54.56% (**4**) and

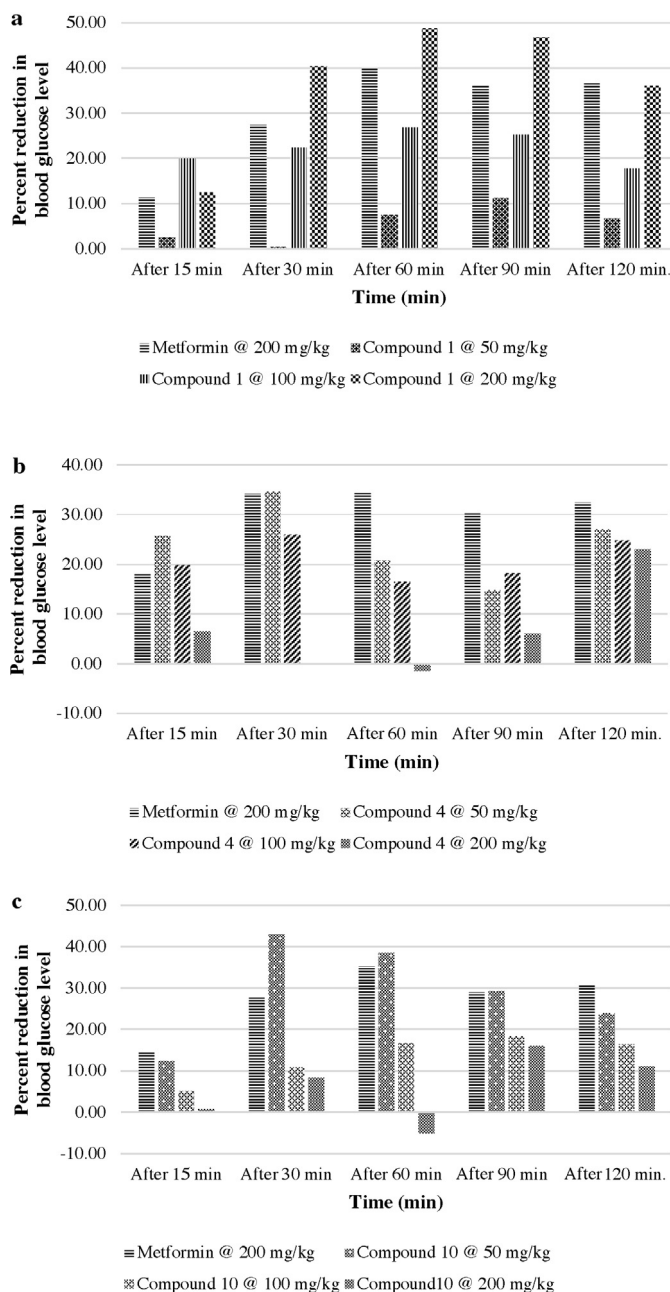


Fig. 5. Dose optimization of compound **1** (Isoliquiritigenin), compound **4** (2',4'-Dimethoxy-4-hydroxychalcone) and compound **10** (liquiritigenin 7,4'-dibenzoate) by oral glucose tolerance test (OGTT) in normal 18 h fasted mice, (a) mice administered with compound **1**: 50 mg/kg bw/10 ml; 100 mg/kg bw/10 ml; 200 mg/kg bw/10 ml, (b) mice administered with compound **4**: 50 mg/kg bw/10 ml; 100 mg/kg bw/10 ml; 200 mg/kg bw/10 ml, and (c) mice administered with compound **10**: 50 mg/kg bw/10 ml; 100 mg/kg bw/10 ml; 200 mg/kg bw/10 ml, before 30 min of glucose load (2 g/kg bw/10 ml). All the treatments were given orally. Data is expressed as percent reduction in blood glucose level. $n = 5$ mice/group.

~73.24% (**10**) towards normal levels after 14 days of treatment. The standard drug metformin also significantly ($p < 0.001$) recovered random blood glucose level by ~32.93% (Fig. 6).

Effect on biochemical parameters

The effects of compounds **1**, **4** and **10** on liver glycogen contents as well as serum biochemical parameters of liver, lipid and kidney were also scrutinized in experimental mice to see further beneficial effect of the compounds. The liver glycogen content is in

Table 1

Percent change in blood glucose level of normal mice after single oral administration of test compounds (100 mg/kg bw) during glucose tolerance test.

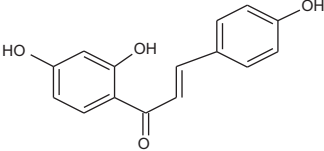
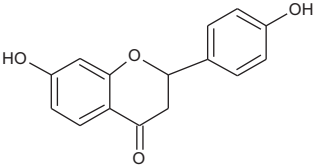
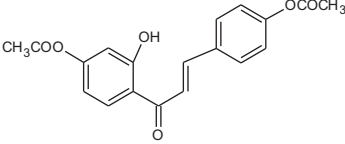
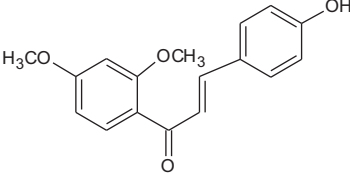
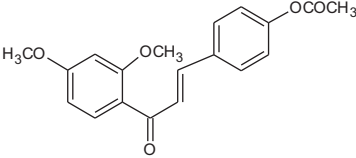
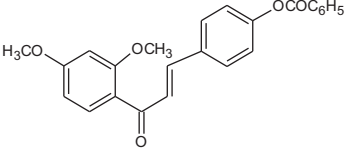
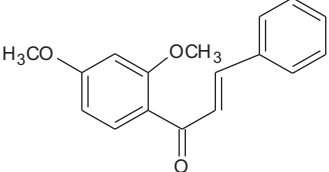
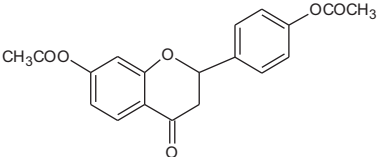
Test compound number	Test compound name	Structure of compound	Percent change in blood glucose level after				
			15 min	30 min	60 min	90 min	120 min
	Metformin		38.13	41.80	50.55	52.09	55.12
1	Isoliquiritigenin		24.72	40.72	32.93	35.73	30.82
2	Liquiritigenin		21.43	10.55	-5.56	-4.07	1.09
3	4,4'-Diacetoxy-2'-hydroxy chalcone		26.66	50.22	41.12	38.97	25.59
4	2',4'-Dimethoxy-4-hydroxychalcone		22.89	49.94	41.73	49.74	36.07
5	4-Acetoxy-2',4'-dimethoxychalcone		27.32	44.88	47.70	34.02	31.74
6	4-Benzoyloxy-2',4'-dimethoxychalcone		26.69	11.59	15.58	8.38	6.85
7	2',4'-Dimethoxychalcone		47.36	30.84	35.04	25.07	24.31
8	Liquiritigenin-7,4'-diacetate		6.80	-1.20	-8.19	-4.24	1.96

Table 1 (Continued)

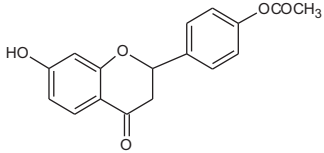
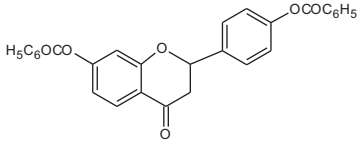
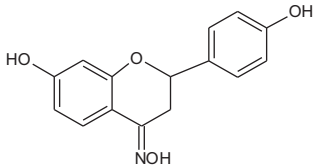
Test compound number	Test compound name	Structure of compound	Percent change in blood glucose level after				
			15 min	30 min	60 min	90 min	120 min
9	Liquiritigenin-4'-acetate		7.35	34.06	35.56	47.29	40.22
10	Liquiritigenin-7,4'-dibenzoate		14.17	45.31	34.83	29.40	23.44
11	Liquiritigenin-oxime		26.07	24.12	28.25	2.44	12.42

Table 2
Effect of test compounds on serum biochemical parameters in STZ-induced diabetic mice.

	Normal control	Diabetic control	Metformin	Compound 1	Compound 4	Compound 10
Liver glycogen (mg/100 mg)	347.83 ± 20.21	172.53 ± 15.39 ^{###}	244.78 ± 28.72	260.04 ± 8.04 [*]	217.60 ± 21.02	222.82 ± 12.91
Liver parameters						
ALP (IU/L)	257.09 ± 12.54	356.86 ± 9.14 ^{###}	281.19 ± 10.07 ^{***}	296.23 ± 12.38 ^{**}	289.35 ± 10.79 ^{**}	299.05 ± 11.26 [*]
ALT (IU/L)	57.35 ± 6.17	190.66 ± 8.60 ^{###}	88.98 ± 5.08 ^{***}	92.10 ± 7.17 ^{***}	101.48 ± 6.42 ^{***}	124.98 ± 5.06 ^{***}
AST (IU/L)	136.89 ± 14.78	273.16 ± 13.84 ^{###}	160.10 ± 12.77 ^{***}	195.73 ± 13.81 ^{**}	234.20 ± 11.42	233.70 ± 10.19
T BIL (mg%)	0.27 ± 0.04	1.16 ± 0.07 ^{###}	0.83 ± 0.04 [*]	0.81 ± 0.07 ^{**}	0.78 ± 0.07 ^{**}	0.80 ± 0.07 ^{**}
TP (g%)	5.13 ± 0.18	4.45 ± 0.21	4.91 ± 0.19	4.77 ± 0.24	4.95 ± 0.35	4.55 ± 0.18
Lipid parameters						
TG (mg%)	98.10 ± 13.88	177.33 ± 14.06 ^{###}	108.01 ± 12.06 ^{**}	109.22 ± 7.32 ^{**}	111.11 ± 8.62 ^{**}	105.75 ± 9.17 ^{**}
HDL (mg%)	32.95 ± 2.10	19.37 ± 2.47 [#]	31.69 ± 4.23	30.15 ± 3.02	28.67 ± 3.29	31.33 ± 1.83
LDL (mg%)	35.96 ± 1.72	31.33 ± 3.27	32.18 ± 1.37	33.14 ± 1.98	32.66 ± 1.33	34.51 ± 2.55
Kidney parameter						
Uric acid (mg%)	2.92 ± 0.28	3.81 ± 0.28	3.01 ± 0.31	3.45 ± 0.37	3.66 ± 0.42	3.37 ± 0.36
Creatinine (mg%)	0.37 ± 0.04	0.46 ± 0.03	0.36 ± 0.03	0.42 ± 0.08	0.36 ± 0.04	0.33 ± 0.02

Each value presents mean ± S.E.M., n = 10.

Compound **1**, **4** and **10** refers to Isoliquiritigenin, 2',4'-Dimethoxy-4-hydroxychalcone and liquiritigenin-7,4'-dibenzoate, respectively.

- ^{*} Statistical significance vs. diabetic control ($p < 0.05$).
- ^{**} Statistical significance vs. diabetic control ($p < 0.01$).
- ^{***} Statistical significance vs. diabetic control ($p < 0.001$).
- [#] Statistical significance vs. normal control ($p < 0.05$).
- ^{###} Statistical significance vs. normal control ($p < 0.001$).

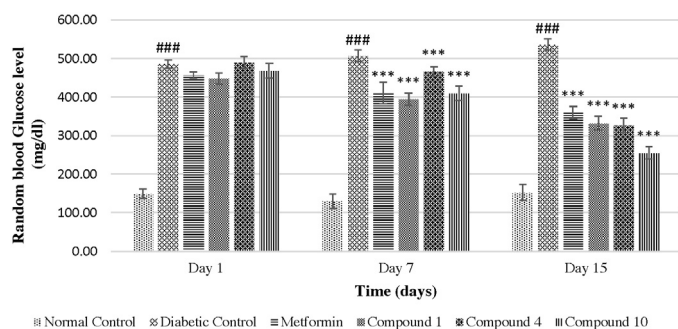
proportion to insulin deficiency which is the primary intracellular storable form of glucose (Chandramohan et al. 2008). Only ISL (**1**) was able to modify liver tissue glycogen level significantly ($p < 0.05$) by ~49.92%.

The disturbances in carbohydrate, lipid and protein metabolisms together with oxidative stress under hyperglycaemic condition possibly affect hepatic and renal functions. An increase in liver marker enzymes activities reflects active liver damage (Yadav et al. 2010). Increased liver enzymes level under insulin deficiency has been related with increased gluconeogenesis and ketogenesis during diabetes (Nabi et al. 2013). The oral administration of test compounds **1** (200 mg/kg bw), **4** (50 mg/kg bw) and **10** (50 mg/kg bw) for the period of 14 consecutive days recovered ($p < 0.05$) the serum ALP levels by ~60.77, 67.67 and 57.94%, respectively. Significant ($p < 0.001$) recoveries were also observed in ALT levels by ~73.93, 66.90 and 49.27%, respectively. The levels of AST was significantly ($p < 0.01$) recovered only in the

compound **1** group by ~56.82%. Significant recovery of T BIL levels were observed in all treatment groups (Compound **1**, **4** and **10**) by ~39.33, 42.70 and 40.45%, respectively.

Enhanced proteolysis and lowered protein synthesis occurs due to distinct metabolic renal alterations in experimental diabetes (Tuvemo et al. 1997) but no significant differences were noticed for TP levels, uric acid and creatinine levels during experimentation in any group.

STZ-induced diabetes also developed hyperlipidaemia which represents a risk factor for coronary heart diseases. Due to insulin deficiency or its resistance, a variety of alterations in metabolic and regulatory mechanisms occur, which are responsible for the accumulation of lipids (Sancheti et al. 2013). In this experiment, it was observed that compound **1**, **4** and **10** significantly ($p < 0.01$) recovered the TG levels by ~85.96, 83.58 and 90.34% respectively. No significant changes were perceived in HDL and LDL levels.



Each value presents Mean \pm S.E.M., n=10,

Represents statistical significance vs. normal control ($p < 0.001$),

*** Represents statistical significance vs. diabetic control ($p < 0.001$).

Fig. 6. Effect of compound **1** (Isoliquiritigenin), compound **4** (2',4'-Dimethoxy-4-hydroxychalcone) and compound **10** (liquiritigenin-7,4'-dibenzoate) on random blood glucose level of STZ induced diabetic mice.

The antihyperglycemic activity of compound **1**, **4** and **10** was compared with metformin which also significantly ($p < 0.05$) recovered the ALP, ALT, AST, T BIL and TG by ~ 75.84 , 76.27 , 82.97 , 37.08 and 87.49% respectively, towards normal levels (Table 2).

Effect on body weight of mice

STZ-induced diabetes is characterized by a severe loss in body weight. The decrease in body weight is due to the increased muscle destruction or degradation of structural proteins (Stephen et al. 2012). Repeated administration of test compounds **1**, **4** and **10** for 14 consecutive days at oral dose of 200, 50 and 50 mg/kg bw, respectively showed significant increase in animal body weight by ~ 28.76 , 24.23 and 24.38% respectively, compared to the toxin treated group. Metformin treatment also increased the body weight of mice significantly by $\sim 23.08\%$ as compared to diabetic control group (Table 3).

The toxicity of some drugs may only result in animal organ weight or organ coefficient changes during the process of non-clinical drug safety evaluation (Piao et al. 2013). Liver, kidney and spleen coefficient were measured in this study but no significant change was observed in any treatment groups. Only liver coefficient of diabetic control group was significantly changed as compared to a normal control group (Fig. 7).

The antidiabetic activity of *G. glabra* extracts have also been reported earlier (Gupta et al. 2011; Sharma et al. 2007). The efforts to obtain antidiabetic compounds, structural modifications at C-4, C-2' and, 4' positions on ISL and C-7, C-4' position on LTG were focused.

Table 3

Effect of test compounds on body weight of diabetic mice.

Groups	Day 1	Day 15
Normal Control	26.63 \pm 1.53	27.60 \pm 1.60
Diabetic Control	28.18 \pm 1.27	19.89 \pm 0.61##
Metformin	25.96 \pm 1.33	24.48 \pm 0.82*
Compound 1	27.70 \pm 1.12	25.61 \pm 1.02**
Compound 4	26.10 \pm 1.53	24.71 \pm 1.33*
Compound 10	27.24 \pm 0.51	24.74 \pm 0.86*

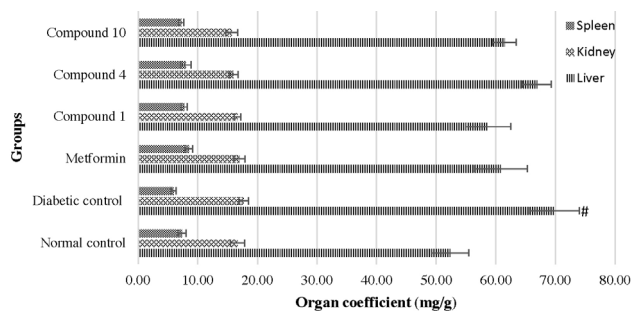
Each value presents mean \pm S.E.M., n = 10.

Compound **1**, **4** and **10** refers to Isoliquiritigenin, 2',4'-Dimethoxy-4-hydroxy-chalcone) and liquiritigenin-7,4'-dibenzoate, respectively.

Statistical significance vs. normal control ($p < 0.01$).

* Statistical significance vs. diabetic control ($p < 0.05$).

** Statistical significance vs. diabetic control ($p < 0.01$).



Each value presents Mean \pm S.E.M., n=10, and calculated as [organ coefficient = (weight of organ (mg)/body weight (g))

* Represents statistical significance vs. normal control ($p < 0.05$).

Compound **1**, **4** and **10** refers to isoliquiritigenin; 2',4'-dimethoxy-4-hydroxychalcone and liquiritigenin-7,4'-dibenzoate, respectively.

Fig. 7. Effect of compound **1**, compound **4** and compound **10** on organ coefficient of diabetic mice.

The starting synthones for our synthesis, ISL and LTG were isolated from ethyl acetate extract of fresh rhizomes of *G. glabra* as per process reported by us (Gaur et al. 2010).

Ethyl acetate extract of *G. uralensis* containing LTG is also reported to have antidiabetic activity (Kuroda et al. 2003). ISL and LTG were also previously reported as anti-inflammatory (Kobayashi et al. 1995), hepatoprotective (Gaur et al. 2010) antimicrobial and antitumor agents (Jaypal et al. 2010). However, this is the first report on *in vivo* antidiabetic activity of their derivatives.

The recent findings in literature reveal that antidiabetic activity of chalcone derivatives is mediated via stimulation of PPAR- γ (Hsieh et al. 2012) and flavonoids, which are based on 2-phenylchromone or 2-phenyl benzopyrone, have shown beneficial effects in the treatment of hyperglycemic disease probably through changes in the activity of intracellular enzymes, such as glucosidase enzyme (Zhang et al. 2012). Therefore it is likely that isoliquiritigenin (ISL), a chalcone and liquiritigenin (LTG), a flavonoid and their derivatives may act by above means to exert its antidiabetic effect.

The present study has shown that the ISL type chalcones as well as LTG derived flavonoids could be used as possible leads for the control of blood glucose level/diabetes. Therefore, plants are excellent source or information centre of diverse molecules to be modified or synthesized for the discovery of new therapeutics.

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