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Antiobesity and lipid lowering effects of *Glycyrrhiza* chalcones: Experimental and computational studies

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ABSTRACT

Twelve flavonoids (**1–12**), isolated from *Glycyrrhiza glabra* roots were evaluated for their pancreatic lipase (PL) inhibitory activity *in vitro*. The structures of the isolated compounds were elucidated by spectroscopic methods. Amongst all the compounds **7**, **8**, **10** and **11** showed strong inhibition against PL with IC_{50} values of 7.3 μM, 35.5 μM, 14.9 μM and 37.6 μM, respectively. Molecular docking studies on the most active compound **7** revealed that it binds with the key amino acid residues of the PL active site. *In silico* absorption, distribution, metabolism and excretion (ADME) parameters were also computed on the active compounds to determine their preliminary pharmacokinetic properties. Further, investigations were carried out to determine the antiobesity and lipid lowering effects of **7** and **10** in high fat diet (HFD) fed male SD rats. In the rats supplemented with compound **7** the body weight increase was only 23.2 ± 3.6 g as compared to 64.2 ± 0.5 g in the HFD control group while in the rats treated with compound **10** showed 23.2 ± 3.6 g weight gain only. Compound **7** decreased the levels of plasma total cholesterol (TC) to 84.6 ± 1.4 mg/dl and plasma total triglycerides (TG) to 128.8 ± 6.0 mg/dl. Compound **10** also lowered the plasma TC and TG levels considerably. The results indicate the potential of the chalcone scaffold as a source of PL inhibitors for preventing obesity.

Introduction

Physiologically, obesity is a disarray of energy balance and primarily considered as a disorder of lipid metabolism (Strader et al., 1998). A growing number of enzymes involved in lipid metabolic pathways are being identified and characterized. They represent a rich pool of potential therapeutic targets for obesity (Shi and Burn, 2004; Melnikova and Wages, 2006). Inhibition of PL (triacylgycerol acyl hydrolase), the principal lipolytic enzyme, synthesized and secreted by pancreas (Mukherjee, 2003) is one of the approaches for the development of newer antiobesity drugs. Tetrahydrolipstatin (Orlistat), a commercial anti-obesity drug, is a known pancreatic lipase inhibitor (Borgstrom, 1988; Hadvary et al., 1991). Previously, we have given an account of the reported plants with antiobesity properties (Birari and Bhutani, 2007) and the various PL inhibitors reported from these natural sources (Bhutani et al., 2007). Recently, much interest has been shifted on plant flavonoids that might be beneficial in reducing the risk of obesity (Peluso, 2006). Dietary catechins and anthocyanins significantly decrease the weight of abdominal adipose tissues (Murase et al., 2002; Tsuda, 2008). Accordingly, investigation on the metabolic effects of plant flavonoids might lead to more effective strategies for the treatment of obesity. The health hazards like diabetes, obesity and metabolic related disorders are related to the dietary habits and most of the nutraceutical on the market focuses on these areas. Anthocyanin-rich berries or derived extracts, procyanidins rich grape seed, bilberry and cranberry extract are well known for their antioxidant and lipid lowering ability (Espin et al., 2007).

Roots of *Glycyrrhiza glabra* (Fabaceae/Papilionaceae), also known as licorice and sweet root has been used medicinally for the past 4000 years (Duval et al., 2007; Iritani, 1992). Historically, the dried rhizome and root of this plant were employed medicinally by the Egyptian, Chinese, Greek, Indian, and Roman civilizations as an expectorant and carminative. Several pharmacological activities, such as antiulcer, antiinflammatory, antidiuretic, antiepileptic, antiviral, antiallergic and antioxidant properties have been attributed to the licorice compound glycyrrhizin and glycyrrhizic acid (Visavadiya et al., 2009; Nassiri Asl and Hosseinzadeh, 2008). Licorice flavonoid oil (LFO) suppresses abdominal fat accumulation by regulation of rate-limiting enzyme activities related to fatty acid synthesis and oxidation in the liver (Nakagawa et al., 2004).

Licorice extract and its primary constituent glycyrrhizin are extensively used amongst US population and are considered as Generally Recognized as Safe (GRAS) for use in foods by the U.S. FDA (Isbrucker and Burdock, 2006; Nakagawa et al., 2008a,b).

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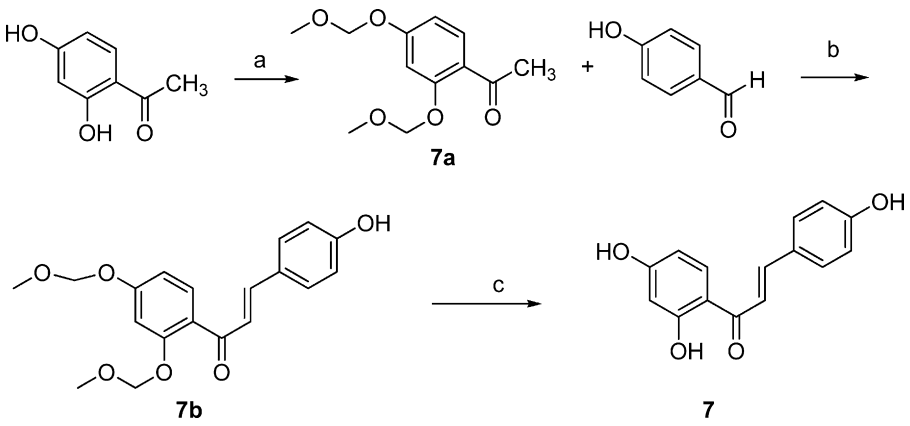

Scheme 1. Key steps in synthesis of LiOH·H_2O. Reaction conditions: (a) dry acetone, K_2CO_3, MOMCl, reflux 7 h; (b) MeOH, LiOH·H_2O, reflux 4 h and (c) 3NHCl, reflux, 30 min.

Various genotoxic studies have indicated that licorice extracts and glycyrrhizin is neither teratogenic nor mutagenic, and may even possess anti-genotoxic properties under certain conditions (Nakagawa et al., 2008a,b; Kaur et al., 2009). Clinical studies have shown that LFO is safe because no clinically significant adverse events occurred when it was given daily to healthy or overweight subjects for up to 12 weeks. These reports suggest the relative safety of licorice extracts and the chalcone when administered orally in animals as well as humans (Aoki et al., 2007). Isoliquiritigenin the major chalcone from *G. glabra* also has several pharmacological effects such as antioxidant, anti-inflammatory, anti-tumor, anti-platelet, and anti-peptic ulcer actions (Haraguchi et al., 1998). Recently it has shown to have protective effects on mitochondrial cells against arachidonic acid and iron induced oxidative stress (Choi et al., 2010). Isoliquiritigenin was reported as quite safe and well tolerated when given orally (Lee et al., 2008).

In our continuing research on identification of newer antiobesity leads from natural sources, many plant extracts have been screened for their PL inhibition (Birari et al., 2009). Recently our group has published the antiobesity and lipid lowering effects of *Murraya koenigii* (L.). Spreng leaves extracts and mahanimbine on high fat diet induced obese rats (Birari et al., 2010). In the present work, an integrative *in vivo-in vitro-in silico* approach was followed to study the antiobesity properties of *Glycyrrhiza* flavonoids. Twelve flavonoid aglycones and glycosides isolated from *Glycyrrhiza* roots were tested for their PL inhibitory action *in vitro*. In order to understand the mode of binding of these compounds with the active site of the PL enzyme, molecular docking approach was employed. *In silico* ADME parameters were also computed on the active compounds to determine their preliminary pharmacokinetic properties. Animal studies on most active compounds **7** and **10** demonstrated the potential of these types of compounds in the development of antiobesity therapeutics.

Materials and methods

Plant material

G. glabra (root) was procured from local market of Chandigarh, India and identified by qualified botanist and voucher specimen has been preserved in the herbarium of Department of Natural Products, NIPER, SAS Nagar, India.

Animals and grouping

Forty-eight male SD rats of weight 175 ± 15 g were procured from Central Animal Facility, NIPER. The animals were housed under standard environmental conditions (temperature 22 ± 2 °C; humidity 55 ± 5%) with a 12 h light/dark cycle at the animal house. All the animals were housed in polypropylene cages in groups of 3 per cage and had free access to water *ad libitum*. The protocol of this experiment was approved by the Institutional Animal Ethics Committee (IAEC) and the experiments were carried out in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA, India) given on animal experimentation.

Extraction and isolation

Dried and powdered roots of *G. glabra* (1 kg) were subjected to sequential extraction with hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) to prepare the respective extracts. The DCM extract of *G. glabra* is expected to contain the prenylated flavonoids and hence, a reported method was followed (Vaya et al., 1997) to give compound **1**–**4**.

EtOAc extract (GGE; 10 g) was purified on silica gel (#60-120). Elution was carried out using hexane, $CHCl_3$ and MeOH in increasing order of polarity. Fractions were pooled according to TLC pattern to give 5 fractions. Non-polar fractions (GGE-1 to GGE-3) were further purified on silica gel (#60-120) to give aglycones **5–9**. The polar fractions (GGE-4 and GGE-5) were purified on Sephadex LH-20 to give the glycosides **10**–**12**.

MeOH extract (45 g) was dissolved in water and partitioned with butanol (BuOH). BuOH fraction (27 g) was taken for purification. It was fractionated by vacuum liquid chromatography on silica gel G (#200-400), using $CHCl_3$/MeOH gradient to yield 5 major fractions. Purification of Fraction 4 (FrM4) yielded compound **10**.

For *in vivo* studies large quantity of compound **7** was required hence its synthesis was carried out. A mixture of 2,4-dihydroxyacetophenone (0.76 g, 5 mmol), anhydrous K_2CO_3 (2.070 g, 15 mmol), and MOM chloride (0.48 g, 12 mmol) was refluxed in dry acetone (25 ml) for 7 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated and the residue subjected to column chromatoghraphy with pet ether/EtOAc 1:1 as an eluent to yield **7a** (68%). Base catalyzed Claisen condensation of **7a** with 4-hydroxy benzaldehyde in presence of LiOH·H_2O (Bhagat et al., 2006) gave compound **7b** which upon deprotection (Vogel et al., 2008) yielded compound **7** (Scheme 1).

Measurement of PL inhibitory activity

PL inhibitory activity was measured using p-nitro phenol palmitate (PNPP) as a substrate. The samples were screened against the PL using standard protocol previously published from our labora-