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Salacia oblonga ameliorates hypertriglyceridemia and excessive ectopic fat accumulation in laying hens

Jianwei Wang [a], Xianglu Rong [b], Wenlong Li [c], Johji Yamahara [d], Yuhao Li [e,f,*]

[a] Division of Metabolism, Faculty of Basic Medical Sciences, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong District, Chongqing 400016, China
[b] Department of Pharmacology, Guangzhou University of Chinese Medicine, China
[c] School of Medicine, The University of Sydney, Australia
[d] Pharmafood Institute, Kyoto, Japan
[e] Endocrinology and Metabolism group, Faculty of Pharmacy, The University of Sydney, Australia
[f] Endocrinology and Metabolism group, Sydney Institute of Health Sciences, Australia

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ABSTRACT

Ethnopharmacological relevance: Salacia oblonga root (SOR) is an *Ayurvedic* medicine for obesity and diabetes, those are associated with glucose and lipid metabolism.
Aim of the study: SOR has been demonstrated previously to improve glucose and lipid metabolism in animal models of obesity and diabetes and to be a peroxisome proliferator-activated receptor-alpha activator. However, the anti-obesogenic and anti-diabetic mechanisms of SOR are still not largely understood. Here, we investigated the effects of SOR on lipid metabolism using laying hen, a unique animal model with a very high rate of triglyceride synthesis in the liver.
Materials and methods: Laying hens and preadolescent pullets were treated with the layer ration containing 0%, 0.5%, or 1% SOR water extract for 4 weeks. Biochemical variables were determined enzymatically.
Results: Laying hens showed much higher fasted triglyceride concentrations (increased by 5–13 folds) in plasma, liver, skeletal muscle and heart than pullets. 1% SOR extract treatment inhibited body weight increase without affecting food intake. Importantly, this treatment substantially attenuated hypertriglyceridemia and inhibited increases in triglyceride contents in the non-adipose tissues. However, SOR extract did not induce change in plasma glucose concentration. Moreover, SOR extract did not alter all variables in pullets.
Conclusions: These results demonstrate that SOR ameliorates hypertriglyceridemia and excessive ectopic fat accumulation in laying hens. These findings suggest that the triglyceride-lowering property is one of the primary effects of SOR, possibly via hepatic mechanisms.

1. Introduction

Salacia species have been used as anti-diabetic and anti-obese medicines for several thousand years in *Ayurvedic* medicinal literature (Chandrasena, 1935). It has been demonstrated that the water extract from the root of *Salacia oblonga* Wall. (Celastraceae) (SOR), one of the *Salacia* species, inhibits α-glucosidase activity and improves post-prandial hyperglycemia in Zucker diabetic fatty rats (Li et al., 2004). Further, this extract also attenuates hyperlipidemia, fatty liver and cardiac steatosis in Zucker diabetic fatty rats (Huang et al., 2006a, b). These results suggest that SOR extract ameliorates derangements of glucose and lipid metabolism. However, the underlying anti-obesogenic and anti-diabetic mechanisms of SOR are still not largely understood. Recently, SOR was demonstrated to increase glucose transporter 4-mediated glucose uptake in L6 rat myotubes (Girón et al., 2009). *S. reticulata*, another *Salacia* species, was shown to inhibit differentiation of 3T3-L1 adipocytes (Shimada et al., 2011). These findings suggest that *S.* species may improve obesity and diabetes via muscular and adipose pathways. It is well known that the liver also plays an important role in regulating glucose and lipid metabolism. On the other hand, improvement of lipid metabolism may ameliorate insulin resistance in obesity and type 2 diabetes (Lettner and Roden, 2008; Postic and Girard, 2008). Therefore, we hypothesized that SOR-elicited regulation of lipid metabolism might involve a hepatic pathway, and that this regulation might be one of the primary effects of SOR.

In birds, hepatic triglyceride synthesis is strongly stimulated by estrogens (Kudzma et al., 1975; Dashti et al., 1983). Compared with mammals, laying hens have a very high rate of hepatic

Abbreviations: FA, fatty acid; HDL, High density lipoprotein; NAFLD, Nonalcoholic fatty liver disease; NEFA, Non-esterified fatty acid; PPAR, Peroxisome proliferator-activated receptor; SOR, *Salacia oblonga* root

* Corresponding author at: Endocrinology and Metabolism Group, Sydney Institute of Health Sciences, Level 5, 545 Kent Street Sydney, NSW 2000, Australia. Tel.: +61 2 9261 2289; fax: +61 2 9261 2903.
E-mail addresses: yuhao@sitcm.edu.au, yuhaoli@hotmail.com, yuhao@pharm.usyd.edu.au (Y. Li).

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synthesis of triglycerides, leading to excessive hepatic lipid accumulation and hypertriglyceridemia (Kudzma et al., 1975; Dashti et al., 1983; Walzem et al., 1999; König et al., 2008; Landers et al., 2008). To test our hypothesis, the present study investigated the effects of SOR on lipid metabolism using laying hens with the specific hepatic mechanism.

2. Materials and methods

2.1. Preparation and identification of S. oblonga root extract

SORs were collected in Tamil Nadu, India. A voucher specimen (voucher specimen no. PS0075) was deposited in Pharmafood Institute, Kyoto, Japan. SOR was identified carefully, and extracted as described previously (Li et al., 2004; Huang et al., 2006a, 2008). Briefly, dried SOR materials were ground into crude powder and extracted with 5 volumes of hot water (85–90 °C) 3 times. The water was evaporated under reduced pressure below 55 °C. The yield of the extract was 6.5%. Mangiferin, a prominent component of SOR, has been found to have pleiotropic bioactivities (Li et al., 2004; Huang et al., 2006a, b, 2008; He et al., 2011). Therefore, mangiferin is one of the compounds responsible for the activity of this particular plant. It has been reported that various Salacia species contain mangiferin, and the quantitative HPLC analysis of mangiferin is considered suitable for the quality control of *Salacia* species and its products (Yoshikawa et al., 2001). The SOR extract was characterized by HPLC methods to determine the content of mangiferin (Huang et al., 2008). A HPLC profile was performed on a Shimadzu SPD-M10 AVP variable wavelength instrument with diode array detector and SPD-10ADVP autoinjector operated through a Shimadzu CBM-10A communication module and with LC-10 workstation software. The chromatography was carried out on a Phenomenex Synergi 4 μm, $250 \times 4.6\ mm^2$ MAX-RP80A column (35 °C) at a flow rate of 1.0 ml/min with detection at 270 and 360 nm. The sample injection volume was 50 ml (1.0 mg/ml in H_2O). The mobile phase gradient consisted of a mixture of acetonitrile and 0.1% (v/v) phosphoric acid water solution (0 min, 10:90; 5 min, 15:85; 15 min, 20:80; 30–35 min, 40:60). The major peak in SOR was identified as mangiferin by comparison of the retention time (tR ¼ 13.2 min) and coincidence (similarity ¼ 0.9995) of the UV–visible spectra (200–400 nm) of the peak and a mangiferin standard by diode array detection. The content of mangiferin was quantified by comparison of the area under curve of the sample with an injection of a standard solution of mangiferin. The result showed that the content of mangiferin was 1.4% in the extract, which is within the previously reported range (Japanese patent no. P2002-267655).

2.2. Chemicals and reagents

Fenofibrate and the kits for determination of glucose, triglyceride, total cholesterol and non-esterified fatty acid (NEFA) were purchased commercially (Wako, Osaka, Japan).

2.3. Animals and treatment protocols

All experimental procedures were carried out in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society (http://plaza.umin.ac.jp/JPS1927/jps/Animal.pdf).

2.3.1. Experiment 1: study in laying hens

24 Boris Brown hens aged 17 weeks (Kiwa Laboratory Animals, Wakayama, Japan) were caged individually in wire cages ($25 \times 40\ cm^2$) with individual feed-troughs but a common water-trough. The cages were placed in an open-sided house. Laying hens were provided *ad libitum* access to layer ration and water. After two weeks of acclimation, they were randomly assigned to 4 groups (6 birds/group): (a) *ad libitum* access to the standard layer ration (control), (b) *ad libitum* access to the layer ration mixed with 0.5% SOR extract (w/w), (c) *ad libitum* access to the layer ration mixed with 1% SOR extract (w/w), and (d) *ad libitum* access to the layer ration mixed with 0.3% fenofibrate (w/w). Body weights were comparable between groups before treatments commenced (Fig. 1A). Laying hens were weighed every week, and food intake was measured in week 4. After 4-week treatment, hens were fasted (free access to water) for 15 h, and blood samples were collected for determination of plasma concentrations of glucose, total cholesterol, triglyceride and NEFA. Then, laying hens were sacrificed by prompt cervical dislocation, the livers and hearts were collected and weighed. Breast (*pectoralis major*) muscles were also collected. Segments of liver, heart and muscle were snap frozen in liquid nitrogen and stored at −20 °C for subsequent determination of total cholesterol and triglyceride contents.

2.3.2. Experiment 2: study in preadolescent pullets

15 Boris Brown pullets aged 9 weeks (Kiwa Laboratory Animals, Wakayama, Japan) were used as preadolescent animals, and also acclimated for two weeks. Pullets were randomly assigned to 3 groups (5 birds/group): (a) *ad libitum* access to the standard layer ration (control), (b) *ad libitum* access to the layer ration mixed with 0.5% SOR extract (w/w), and (c) *ad libitum* access to the layer ration mixed with 1% SOR extract (w/w). Body weights were comparable between groups before treatments commenced (Fig. 1B). The other procedures were the same as described in *Experiment* 1.

2.4. Biochemical determination

Plasma concentrations of glucose, total cholesterol, triglyceride and NEFA were determined using enzymatic methods. Total cholesterol and triglyceride contents in liver, heart and skeletal muscle were determined as described previously (Rong et al., 2009, 2010a, b). Briefly, 100 mg of tissue was homogenized and extracted with 2 ml of isopropanol. After centrifugation (3000 rpm), total cholesterol and triglyceride contents in the supernatant were determined enzymatically.

2.5. Data analysis

All results are expressed as means ± SEM. Data were analyzed by one-way ANOVA using the StatView software. If a difference was detected (F-ratio), the Student-Newman-Keuls test was performed to locate the differences between groups. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects on body weight and food intake in laying hens and preadolescent pullets

Laying controls were heavier than preadolescent controls (Fig. 1A and B). There was no significant difference in food intake between the groups (Fig. 1C). Treatment with the layer ration containing 1% SOR extract for 4 weeks slightly, but significantly, decreased body weight increase in laying hens (Fig. 1A). 0.5% SOR extract and 0.3% fenofibrate were not effective. Both two dosages of SOR extract also showed no effect in pullets (Fig. 1B). There was no significant difference in food intake between untreated and treated groups either in laying hens or in pullets (Fig. 1C).