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Original article

# Total sesquiterpene glycosides from Loquat (*Eriobotrya japonica*) leaf alleviate high-fat diet induced non-alcoholic fatty liver disease through cytochrome P450 2E1 inhibition

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease characterized by hepatic steatosis, which affects 20–40% of the population in the world. Loquat (*Eriobotrya japonica*) Leaf possesses several pharmacological actions. Many sesquiterpene glycosides were reported to be isolated exclusively from the Loquat Leaf, however, their biological activity has been rarely investigated. The present study was designed to evaluate the pharmacological effect of total sesquiterpene glycosides (TSG) in high-fat diet (HFD) induced NAFLD mice with its related mechanisms of action. Mice were fed with a normal diet or HFD for 8 weeks. TSG (25 and 100 mg/kg/day), simvastatin (10 mg/kg/day) or vehicle were orally administered for last 4 weeks of the 8-week HFD feeding period. From the result, it was showed that TSG significantly reduced the body weight and fat deposition in the liver of NAFLD mice. It also decreased total cholesterol (TC) and triglyceride (TG) contents in the serum. Compared with NAFLD mice, superoxide dismutase (SOD) and malondialdehyde (MDA) levels were increased and decreased after the administration of TSG in a dose of 100 mg/kg, respectively. TSG reduced alanine aminotransferase (ALT) activity as well. Finally, TSG was found to suppress the expression of cytochrome P450 2E1 (CYP2E1) and the phosphorylation of c-jun terminal kinase (JNK) in NAFLD mice. In summary, this study demonstrates that TSG reduces oxidative stress by downregulating of CYP2E1 expression and JNK phosphorylation in NAFLD, and alleviates NAFLD ultimately. TSG potentially serves as bioactive compounds for the treatment of NAFLD.



## 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease characterized by hepatic steatosis, which has become an international public health problem due to its high prevalence (affects 20–40% of the population) [1]. It represents a wide spectrum of liver disease including simple steatosis, fibrosis, cirrhosis, non-alcoholic steatohepatitis (NASH) and its complications (such as liver failure and hepatocellular carcinoma) [2]. NAFLD is now regarded as a manifestation of metabolic syndrome and is associated with obesity, type 2 diabetes mellitus (T2DM), and cardiovascular disease [3,4].

Although the pathophysiology of NAFLD is complex, oxidative stress, a well-known feature of NAFLD, is a crucial factor for the pathogenesis [5–7]. Mounting evidences support that cytochrome P450 2E1 (CYP2E1) makes a significant contribution to the oxidative stress in NAFLD. CYP2E1 is a member of the oxidoreductase cytochrome family, which is responsible for oxidizing a variety of substances including fatty acids, xenobiotics, ethanol and most organic solvents [8–10]. After excessive fat accumulation, with the catalytic cycle of CYP2E1, significant amounts of reactive oxygen species (ROS) such as superoxide anion are generated, which subsequently cause oxidant damage, serving as an important part of in NAFLD [5,7,8,11]. Multiple studies have shown that the increased oxidative stress by CYP2E1 overexpression was observed in NAFLD [11–14]. C-jun terminal kinase (JNK) is a kind of cytokines, which regulates hepatocellular injury. Overexpression of CYP2E1 accelerates ROS production, triggering oxidative stress associated with sustained activation of JNK-signaling cascades, which progressive hepatocellular ballooning and microvesicular

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steatosis in NAFLD [11,15–17]. Inhibition of the expression of CYP2E1 can alleviate oxidative stress to protect liver against NAFLD [18–20], and the decreased phosphorylation level of JNK may be the underlying mechanism [11]. Therefore, targeting CYP2E1 might be a new and useful therapeutic approach to reduce oxidative stress and liver injury in NAFLD.

Over the past decades, herbal medicine and active ingredients of natural products have garnered growing attentions as potential therapeutic agents to prevent and treat NAFLD, due to their high efficacy and low risk of side effects [21]. Loquat Leaf (*Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae)), has been widely used as traditional Chinese medicine for lung and stomach diseases, and it also possesses several pharmacological actions, including antioxidant [22], hypoglycemic, hypolipidemic [23], anti-inflammatory [24], antitumor [25] effect. Phytochemical studies indicate that triterpene acids, sesquiterpene glycosides, essential oils and flavonoids are the main constituents of Loquat Leaf. Sesquiterpene glycosides are constituents exclusively isolated from Loquat Leaf until now [26]. Current research mainly focuses on the pharmacological activities of triterpene acids, however, sesquiterpene glycosides related property have been rarely reported [27]. In our previous study, one sesquiterpene glycoside isolated from this herb displayed beneficial effect on glucose metabolism disorder [28], as a hepatic manifestation of the metabolic syndrome, the effect and mechanism of action of TSG on NAFLD remain unclear.

Therefore, the aim of the present study was to investigate the beneficial effects of TSG, a fractionationed extract of Loquat leaves, on a high-fat diet (HFD)-induced mouse model of NAFLD and explore its mechanism based on hepatic lipogenesis and oxidative stress response. We hypothesized that after 4-week treatment of TSG, hepatic steatosis and oxidative stress response would get improved. To test this hypothesis, we investigated several parameters including hepatic lipid content, biochemical indicators of oxidative stress injuries and histopathologic changes, protein expression of CYP2E1 and relative signaling molecule JNK were also examined to figure out the mechanism of TSG effect on NAFLD.

## 2. Materials and methods

### 2.1. Plant material

The leaves of *E. japonica*, which were collected from Suzhou of Jiangsu Province in China, were identified by Prof. Ronglin Guo. The voucher specimen (No. 328636) was deposited at the Herbarium of the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences.

### 2.2. Preparation and analysis of the TSG from Loquat leaf

TSG from the Loquat Leaf was prepared in our lab according to the former reported method [29]. Nerolidol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnpyranosyl-(1→6)]-β-D-glucopyranoside (I) was purified as standard compound and its chemical structure (Fig. 1B) was identified by comparing the MS and NMR spectral data with those reported in literature [28,29]. For the chemical analysis of TSG obtained, HPLC analysis was performed on a Dionex Ultimate 3000 HPLC systems (Thermo Fisher Scientific Inc., Germany), equipped with a quaternary solvent delivery system, an autosampler, and a DAD detector. Separations were carried out on an Acclaim 120 C18 column (250 mm × 4.6 mm, 5 μm) detecting at 210 nm with isocratic elution mode, using methyl alcohol (A) and water (B) at 30° C. The elution profile was 70% A. For quantification of compound I in TSG samples, the same solvent and conditions were applied to the analytical HPLC system. For each TSG sample, three replicate assays were performed.

### 2.3. Animals and their treatment

Six-week-old, male ICR (Institute of Cancer Research) mice, weight 17–20 g, were purchased from the Animal Experiment Center in China Pharmaceutical University. The animals were kept in a 12 h light-dark cycle animal room with a temperature maintained at 23 ± 2° C. All the animals were allowed free access to diet and tap water. After a week of adaptation, mice were treated with regular diet, which contained 20% (weight/weight) flour, 10% rice flour, 20% corn, 26% drum head, 20% bean, 2% fish powder, and 2% bone powder (XieTong Organism Inc., China) or high fat diet (HFD), which were prepared in our lab (18% lard stearin (w/w), 5% egg powder, 1% cholesterol, 20% sucrose, 0.1% bile salt, and 55.9% normal diet) [30] for 4 weeks. Fifty mice were separated into five random groups with ten mice in each group and treated as follows: regular diet-fed mice (CON), high-fat diet-fed (HFD), simvastatin (10 mg/kg/day)-treated mice (HFD + simvastatin), low-dose TSG (25 mg/kg)-treated HFD mice (HFD + $TSG_{25}$), high-dose TSG (100 mg/kg)-treated HFD mice (HFD + $TSG_{100}$). Group of HFD + simvastatin, HFD + $TSG_{25}$ and HFD + $TSG_{100}$ received a HFD and were administered with oral gavages of simvastatin, 25 and 100 mg/kg TSG respectively. Correspondingly, the CON and HFD

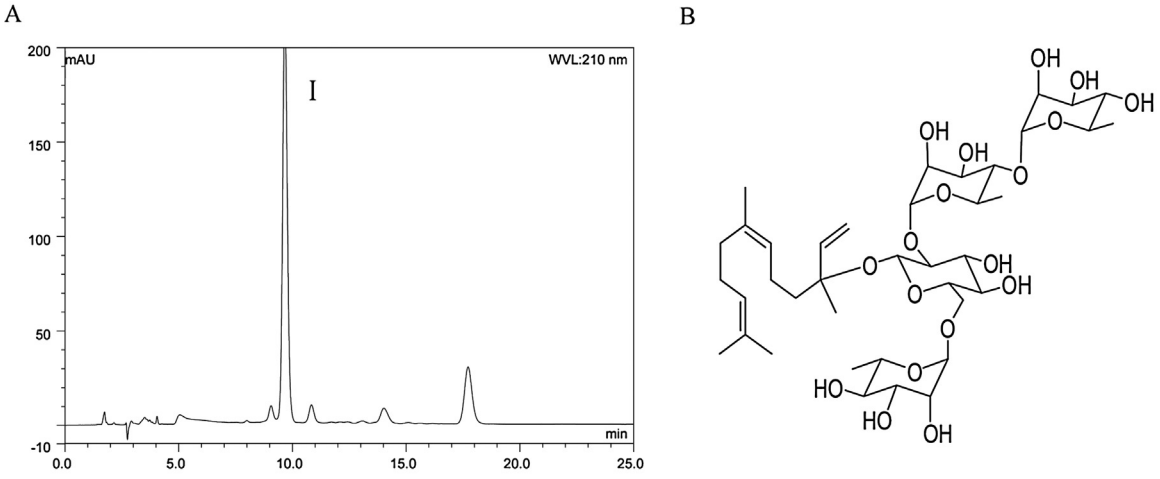


**Fig. 1.** Quantification and identification of TSG by HPLC analysis.