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Schisandrae Fructus oil-induced elevation in serum triglyceride and lipoprotein concentrations associated with physiologic hepatomegaly in mice

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ABSTRACT

Objective: To investigate hypertriglyceridemia and hepatomegaly caused by Schisandrae Sphenantherae Fructus (FSS) and *Schisandra chinensis* Fructus (FSC) oils in mice.

Methods: Mice were orally administered a single dose of Schisandrae Fructus oils. Serum and hepatic triglyceride (TG), triglyceride transfer protein (TTP), apolipoprotein B48 (Apo B48), very-low-density lipoprotein (VLDL), hepatocyte growth factor (HGF), alanine aminotransfease (ALT) and liver index were measured at 6-120 h post-dosing.

Results: FSS and FSC oil caused time and dose-dependent increases in serum and hepatic TG levels, with maximum increases in the liver (by 297% and 340%) at 12 h post-dosing and serum (244% and 439%) at 24-h post-dosing, respectively. Schisandrae Fructus oil treatments also elevated the levels of serum TTP by 51% and 63%, Apo B48 by 152% and 425%, and VLDL by 67% and 38% in mice, respectively. FSS and FSC oil treatments also increased liver mass by 53% and 55% and HGF by 106% and 174%, but lowered serum ALT activity by 38% and 22%, respectively. Fenofibrate pre/co-treatment attenuated the FSS and FSC oil-induced elevation in serum TG levels by 41% and 49% at 48 h post-dosing, respectively, but increased hepatic TG contents (by 38% and 33%, respectively) at 12 h post-dosing.

Conclusions: Our findings provide evidence to support the establishment of a novel mouse model of hypertriglyceridemia by oral administration of FSS oil (mainly increasing endogenous TG) and FSC oil (mainly elevating exogenous TG).

KEYWORDS: Schisandrae Fructus oils; Triglyceride; Alanine aminotransferase; Hepatomegaly; Mouse model; Hypertriglyceridemia

1. Introduction

The prevalence of the clinical condition known as metabolic syndrome (MetS) ranges between 0.3% and 26.4% worldwide, wherein the rising number of children and adolescents with MetS is apparent, depending on the criteria used for clinical diagnosis[1]. MetS, a disorder of energy usage and storage, is characterized by hyperglycemia, insulin resistance, obesity, hypertension,

Significance

We previously established a hypertriglyceridemia model by oral administration of schisandrin B. In this study, we used Sphenantherae Fructus oil (mainly increasing endogenous triglyceride) and *Schisandrae chinensis* Fructus oil (mainly elevating exogenous triglyceride) to establish two hypertriglyceridemia models. We also provided the time and dose relationships after administration of Sphenantherae Fructus oils. Our study showed two novel Schisandrae Fructus oils-induced hypertriglyceridemia models resemble the pathological features of clinical hypertriglyceridemia.

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and hyperlipidemia, especially hypertriglyceridemia (hTG), hypercholesterolemia, or both. Therefore, patients with MetS are at increased risk of type 2 diabetes mellitus (DM) and cardiovascular disease[2-5]. Moreover, it has recently been found that individuals with DM are considered to be a high-risk group for adverse morbidity and death related to pneumonia associated with COVID-19 infection[6,7]. Patients with severe hTG are known to have an increased incidence of pancreatitis[8,9]. Furthermore, epidemiological studies have shown that hyperlipidemic subjects are more prone to chronic periodontitis[10], and patients with schizophrenia have a much higher prevalence of hyperlipidemia, particularly hTG and low high-density lipoprotein (HDL) levels, as compared with the general population[11]. Women suffering from breast, lung, or gastric cancer exhibit higher levels of serum triglyceride (TG), lower levels of HDL and apolipoprotein A-1[12]. Given the adverse health consequences associated with hyperlipidemia, the search for lipid-lowering drugs has been an area of intensive research. As such, animal models of hyperlipidemia and/or MetS are needed for screening compounds with potential for drug development[13].

Schisandrae Fructus (SF) includes the dried ripe fruits of *Schisandra sphenanthera* Rehder & E.H. Wilson *Schisandra sphenanthera* Rehd. et Wils. (Nan-wuweizi in Chinese, FSS) and *Schisandra chinensis* (Turcz.) Bail. (Bei-wuweizi in Chinese, FSC), both of which are Chinese herbs that have been clinically used for thousands of years, particularly in the treatment of liver injury. Recently, research studies have shown that SF can induce CYP3A4 and CYP2E1 enzyme activities and inhibit CYP1A2 enzyme activity[14,15], and improve insulin sensitivity by stimulating PPAR-γ pathways[16]. Schisandrol, schisandrin, and schisandrol B have been found to induce the expression of drug-metabolizing enzymes *via* the activation of PXR[17,18]. The aforementioned experimental results suggest that SF could influence metabolic processes. Our previous studies have shown that schisandrin B (Sch B), an active component isolated from FSC[19], increased serum and hepatic TG levels in mice[20,21], suggesting it might provide a useful experimental model of hTG or combined hyperlipidemia/hypercholesterolemia[22,23]. In the present study, we investigated the effects of SF oils (FSS and FSC) on serum and hepatic TG levels, as well as liver size and serum alanine aminotransferase (ALT) activity, a biomarker for liver function, in mice. Fenofibrate (FF), a clinically useful lipid-lowering drug for reducing TG levels[24], was also used as a pharmacological tool to characterize the hyperlipidemic effect of SF oils as well as the interaction between SF oils and FF.

2. Materials and methods

2.1. Plant materials and extraction procedure

Both FSS and FSC were purchased from the Anguo Chinese herb market in Hebei province, China, and authenticated by Dr. Guang Xi Ren in the Beijing University of Chinese Medicine. For the preparation of SF oils, 200/200 g of powdered FSS/FSC was soaked in five volumes of petroleum ether (*v/w*) for 30 min. Then they were extracted twice by reflux under a water bath for 1.5 h (the first extraction) and 2 h (the second extraction). The pooled petroleum ether extracts were dried by evaporation at 70 ℃. The final FSS (26 mL) and FSC (28 mL) oil preparations were obtained at yields of 13% and 14% (*w/v*), respectively, and they were stored at 4 ℃ before use.

2.2. HPLC analysis of SF oils

Aliquots (200 μL) of FSS and FSC oils were dissolved in 50 mL methanol. Schisandrol A (1.88 mg), schisandrin A (Sch A) (3.36 mg), and Sch B (2.59 mg) were dissolved in 10 mL methanol. All samples were filtered through a Millipore membrane with an average pore diameter of 0.22 μm, and 10 μL filtrate was injected into the HPLC system for analysis. The contents of schisandrol A, Sch A, and Sch B were quantified by reverse-phase HPLC. An Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a vacuum degasser, a quaternary pump, an autosampler, a thermostatic column compartment, and a diode array detector were used. Separation was performed on an Agilent SB C18 column (4.6 mm × 250 mm, 5 μm). A linear gradient system that consisted of A (acetonitrile) and B (water) was used. The gradient elution profile was as follows: 0-30 min, 43% A; 30-32 min, 43%-46% A; 32-53 min, 46%-50% A; 53-64 min, 50%-62% A; 64-83 min, 62% A; 83-93 min, 62%-90% A; 93-98 min, 90%-100% A. The flow rate was 1.0 mL/min, with the column temperature maintained at 35 ℃. Re-equilibration duration was 10 min between individual runs.

2.3. Chemicals and reagents

FF (Batch no. 16948) was bought from Laboratories FOURNIER S.A. (Dijon, France). The assay kit for TG was obtained from Zhongsheng Beikong Bio-technology and Science Inc. (Beijing, China). Assay kits for serum triglyceride transfer protein (TTP), apolipoprotein B48 (Apo B48), very-low-density lipoprotein (VLDL), and hepatocyte growth factor (HGF) were bought from Blue Gene Biotech (Shanghai, China). Alanine aminotransfease (ALT, certificate no.305101J), HDL, and low-density lipoprotein (LDL) assay kits were purchased from Beijing Leadman Biochemistry Co., Inc. (Beijing, China). Schisandrol A, Sch A, and Sch B were obtained from the National Institute for Food and Drug Control (Beijing, China). The purity of each standard was higher than 98%.

2.4. Animal treatment

Eight-week-old male ICR mice [Grade Ⅱ, certificate No. SCXK (jing) 2006-0009], weighing 18-20 g, were supplied by Vital River Lab Animal Co. Ltd. (Beijing, China). All animals were maintained on a 12 h light-dark cycle at 20-21 ℃, with a relative humidity of 50%-55%. Animals were allowed free access to water and food. Hepatic index was estimated by measuring the ratio of liver weight to body weight (liver tissue weight/body weight × 100). In the present study, after a week of acclimation, a single dose of FSS or FSC oil (0.3-5 g/kg, based on toxicity test and preliminary experiment) was

administered orally by gavage to mice. For determination of serum TTP, ApoB 48, VLDL, a single dose of FSS or FSC oil (0.6-5 g/kg) was administered orally by gavage to mice. Moreover, FF (0.1 g/kg, suspended in 0.5% carboxymethyl cellulose) was given to the mice by gavage once daily for 3 d. The FF-untreated groups were received the vehicle (5 mL/kg) orally for 3 d. On the fourth day of the experiment, mice were treated with FF dissolved in olive oil (5 g/kg) alone or a combination of FF and SF oils. FF-untreated animals were orally administered FSS or FSC oil (5 g/kg) only. After SF oil or FF treatment, blood and liver samples were obtained at 6, 12, 24, 48, 72, 96, and 120 h from ether-anesthetized animals which had previously been fasted for 6 h.

2.5. Toxicity assay

Mice were divided into groups of 10 animals each and orally administered FSS or FSC oils at increasing doses ranging from 10.24-20 g/kg. The mortality rate in each group of mice was determined within 7 days of post-dosing. Also, mice were treated with FSS or FSC oils at doses of 2 and 5 g/kg/day for 5 consecutive days. The median lethal dose (LD_{50}) values for FSS and FSC oils were estimated by the Bliss method.

2.6. Biochemical analyses

Serum samples were prepared by centrifuging whole blood obtained from the orbital vein for 8 min at 2000 ×*g* and stored at −70℃ until being used for biochemical analyses. Liver tissue samples were homogenized in 9 volumes of 0.9% (*w/v*) NaCl solution by two 10-s bursts of a tissue disintegrator at 13 500 rpm, and the homogenates were then centrifuged at 2000 ×*g* for 15 min to obtain the supernatants. Subsequently, 10 and 30 μL aliquots of serum and hepatic supernatants, respectively, were added into tubes to detect total cholesterol (TC) and TG levels. Serum ALT, HDL, and LDL activities were measured using a Leadman automatic biochemistry analyzer. Serum TTP, Apo B48, VLDL, and HGF were determined using a competitive enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies according to the manufacturer's instructions.

2.7. Statistical analysis

All values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA or two-way ANOVA (between FSC oil and FSS oil groups) using an SPSS (version 16.0) statistical analysis program or Graph Pad Prism version 5.0, and differences among means were analyzed using Dunnett's multiple comparison test or Tukey's multiple comparisons test. A difference was considered to be significant when $P < 0.05$.

2.8. Ethical statement

All experimental procedures were approved by the Ethics Committee of Beijing University of Chinese Medicine (No. 2013BZHYLL0101).

Table 1. The contents of schisandrol A, Sch A, and Sch B in Schisandra Fructus (SF) oils.

Analytes	Regression equation	Correlation (R^2)	Contents (mg/mL)	
			FSC oil	FSS oil
Schisandrol A	Y=9 486.300X+3.525	0.999	2.000	NA
Sch A	Y=8 140.100X+5.048	0.999	0.817	0.627
Sch B	Y=8 301.300X+4.320	0.999	0.993	NA

Schisandrin A: Sch A; Schisandrin B: Sch B; FSC: *Schisandra chinensis* Fructus; FSS: Schisandrae Sphenantherae Fructus; NA: not available.

3. Results

3.1. Characterization of SF oils

HPLC analysis showed that schisandrol A, Sch A, and Sch B were present in FSC oil (Figure 1). The contents of schisandrol A, Sch A, and Sch B in FSC oil were 2.000, 0.817, and 0.993 mg/mL, respectively (Table 1). Moreover, the contents of schisandrol A and Sch B were not detectable in FSS oil and the content of Sch A was 0.627 mg/mL (Figure 1).

3.2. Serum TG levels

Mice were administered FSS oil or FSC oil (5 g/kg). After 6, 12, 24, 48, 72, and 96 h, serum TG levels were determined. Both FSS oil and FSC oil caused time-dependent increases in serum TG levels, which reached a maximum of 244% and 439%, respectively, at 24 h post-dosing, when compared with the control (Figure 2A). As SF oils caused a more prominent elevation in serum TG levels at 24 post-treatment, dose-response studies were performed with FSS oil or FSC oil 24 h following treatment. Mice were intragastrically administered increasing doses (0.3, 0.6, 1.25, 2.5, and 5 g/kg) of either type of SF oils. As shown in Figure 2B, FSS and FSC oil treatments caused dose-dependent increases in serum TG levels up to 355% and 673%, respectively, when compared with the control. The FSC oil-induced hTG was more prominent than that of FSS oil at the same dosage (1.25 and 5 g/kg).

Theoretically, hTG caused by SF oils should be inhibited by FF pretreatment. Therefore, mice were intragastrically administered FF 0.1 g/kg/day for 3 d, and on the fourth day, FF in combination with FSS oil or FSC oil was given by gavage. FF alone caused decreases in serum TG at 24- and 48-h after the last dose by 58% and 48%, respectively, when compared with the corresponding control group. FSS oil treatment alone elevated serum TG levels by 219% and 63% at 24 and 48 h post-dosing, respectively, when compared with the control. FSC oil treatment alone elevated serum TG levels by 465% and 111% at 24 and 48 h post-dosing, respectively, when compared with the control. Pre/co-treatment with FF and FSC oil reduced serum TG levels by 35% and 49% at 24 and 48 h post-dosing, respectively, when compared with the corresponding FSC oil group. FF pretreatment did not affect TG levels at 24 h after FSS oil treatment, but it inhibited the elevation of serum TG level by 41% at 48 h post-treatment, when compared with that of the FSS oil-treated group (Figure 3).

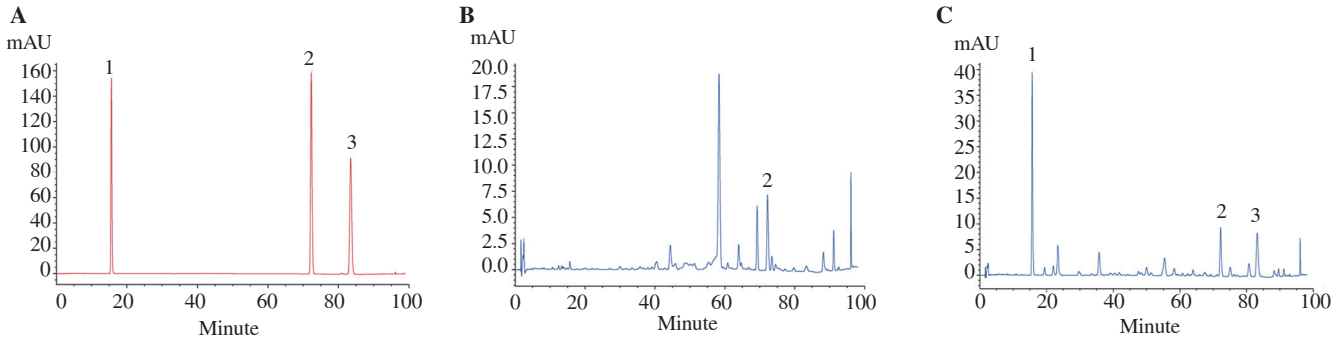

Figure 1. HPLC chromatograms of SF oils. (A) HPLC chromatogram of schisandrol A, Sch A and Sch B. (B) HPLC chromatogram of FSS oil and (C) HPLC chromatogram of FSC oil. Peaks 1, 2, and 3 represent schisandrol A, Sch A, and Sch B, respectively.

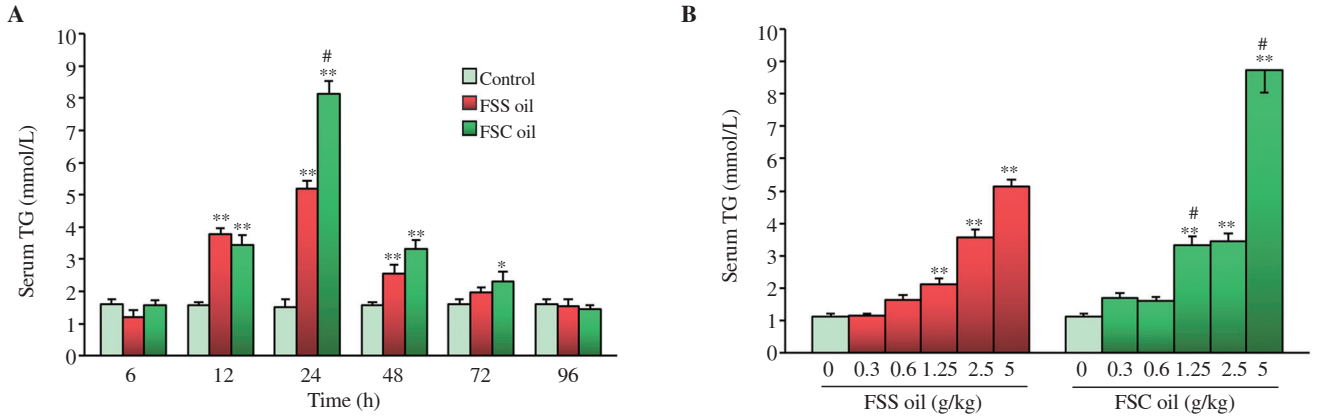

Figure 2. Time and dose response of SF oil-induced elevation in serum triglyceride (TG) levels in mice. Animals were orally administered a single dose of Schisandrae Sphenantherae Fructus (FSS) oil or *Schisandra chinensis* Fructus (FSC) oil. (A) Time-response study: serum TG levels were measured at 6, 12, 24, 48, 72, and 96 h after FSS and FSC oil treatment (5 g/kg). (B) Dose-response study: serum TG levels were determined 24 h after administration of FSS and FSC oil at increasing doses of 0.3, 0.6, 1.25, 2.5, and 5 g/kg. Control mice received the vehicle (olive oil 5 g/kg). Values are expressed as mean ± SEM, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* the control group using one-way ANOVA followed by Dunnett's *post-hoc* analysis; $^{\#}P < 0.01$ *vs.* the FSS oil group at the corresponding dose using two-way ANOVA followed by Tukey's multiple comparisons test.

3.3. Serum TG-rich lipoprotein levels

Levels of TTP, VLDL, and Apo B48, which are involved in TG transportation and formation, were measured at 24 h after administration of SF oils. FSS oil and FSC oil (0.6, 1.25, 2.5, and 5 g/kg) dose-dependently increased serum TTP by 7%-51% and 5%-63%, respectively (Figure 4A). FSS oil and FSC oil treatments (1.25, 2.5, and 5 g/kg) increased serum Apo B48 levels by 80%, 122%, and 152% as well as 122%, 262%, and 425%, respectively, when compared with the vehicle control group (Figure 4B). Both FSS oil and FSC oil treatments also caused increases in serum VLDL levels by 10%-67% and 4%-38%, respectively, when compared with the control (Figure 4C). However, FSC treatment at a dose of 1.25 g/kg decreased serum VLDL level by 10%, when compared with the control. The extent of the FSC oil-induced increase in Apo B48 levels was significant than that of FSS oil ($P < 0.01$). In contrast, the degree of FSS oil-induced increase in serum VLDL levels was higher than that of FSC oil ($P < 0.01$). However, there was no difference between FSS oil and FSC oil treatments in terms of serum TTP levels.

3.4. Serum TC, HDL, and LDL

We also determined the levels of serum TC, HDL, and LDL after administration of SF oils after 6, 12, 24, 48, 72, and 96 h. The results showed that both FSS oil and FSC oil treatments did not significantly affect the level of serum TC or HDL at any time point (Figures 5A and B, $P > 0.05$). Moreover, FSC oil treatment did not result in any detectable changes in serum LDL level at any time point. However, FSS oil treatment significantly increased serum LDL only at 48 h ($P < 0.01$) (Figure 5C).

3.5. Hepatic TG content

FSS and FSC oils at a dose of 5 g/kg elevated hepatic TG content by up to 297% and 340%, respectively, at 6-96 h post-treatment, when compared with the untreated control group, with the maximum level being observed at 12 h post-treatment (Figure 6A). As SF oils caused a more prominent elevation in hepatic TG content at 12 h post-treatment, dose-response studies of both SF oils were performed at 12 h post-treatment with SF oils given at a dose of 0.3-5 g/kg. Significant changes in hepatic TG content were only detectable in mice receiving FSS oil at doses of 2.5 g/kg by 202% and 5 g/kg by 300% and FSC oil at a dose of 5 g/kg by 342% (Figure 6B).

The effect of FF pre/co-treatment (0.1 g/kg/day for 4 d) on the SF oils-induced elevated hepatic TG content was investigated. FF itself did not alter hepatic TG content at 12, 24, or 48 h after the last dosing, when compared with the control group. FSS oil increased hepatic TG content by 287%, 46%, and 14% at 12, 24, and 48 h post-dosing, respectively, when compared with the control. Moreover, FSC oil increased hepatic TG content by 407%, 47%, and 37% at 12, 24, and 48 h post-dosing, respectively. Pre/co-treatment with FF and FSS oil further increased hepatic TG content by 38%, 19%, and 41% at 12, 24, and 48 h, respectively, when compared with FSS oil treatment alone (Figure 7). Pre/co-treatment with FF and FSC oil elevated hepatic TG content by 33%, 18%, and 34%, at 12, 24, and 48 h, respectively, when compared with FSC oil treatment alone (Figure 7).

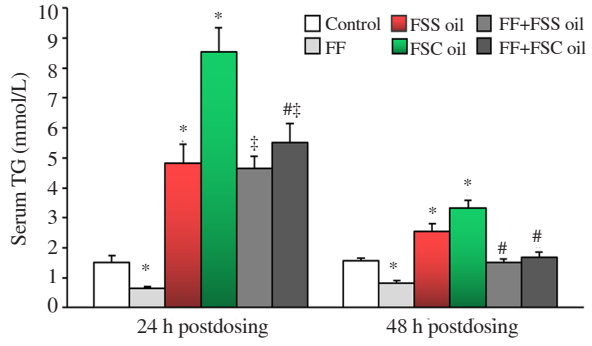

Figure 3. Effects of fenofibrate (FF) pre/co-treatment on serum TG levels in mice treated with SF oils. Mice were pretreated with FF (0.1 g/kg/day for 3 d, *p.o.*). On the fourth day of the experiment, they were co-treated with FF and FSS oil or FSC oil (5 g/kg). Control mice received the vehicle. Serum samples were collected and TG contents were measured at 24 and 48 h after the last dosing. Values are expressed as mean ± SEM, $n = 10$. $^{*}P < 0.01$ *vs.* the control group; $^{\#}P < 0.01$ *vs.* the corresponding FSS oil or FSC oil group without FF pre/co-treatment; $^{\ddagger}P < 0.01$ *vs.* FF treatment alone. Significant differences were determined using one-way ANOVA followed by Dunnett's *post–hoc* analysis.

3.6. Hepatic mass and hepatic function

The liver plays an important role in lipid metabolism. In our previous study, it was shown that SF and its related compounds can affect hepatic size and serum ALT activity. Therefore, in the present study, the effects of FSS oil and FSC oil on hepatic size and function were investigated. Supplementary Figure 1A shows that hepatomegaly occurred at 24 h after FSS oil or FSC oil treatment by 19% or 28%, respectively and the extent of increase was reduced at 120 h by 11% or 8%, respectively. FSS oil and FSC oil (0.3-5 g/kg) dose-dependently increased the hepatic index by 11%-53% and 13%-55% at 48 h post-treatment, respectively (Supplementary Figure 1B). Moreover, FSS oil lowered serum ALT activity by 29%-38% at 24-72 h, but a significant reduction of serum ALT activity by 22% was observed at 72 h after FSC oil treatment (Supplementary Figure 1C).

Treatment with SF oils or FF increased hepatic mass. SF oils lowered ALT activity, but only FF elevated serum ALT activity. FF alone increased hepatic index by 46% and 36% and ALT activity by 39% and 42% at 24 and 48 h post-dosing, respectively, when compared with the corresponding control group. When compared with FF treatment alone, co-treatment of FF, as well as FSS and FSC oils, lowered serum ALT levels by 35% and 19% at 48 h post-dosing, respectively. However, the hepatic index was elevated by up to 45% with both SF oil treatments (Supplementary Figure 2).

3.7. Serum HGF

Given that HGF plays a fundamental role in liver development, regeneration, and function, as well as in the development of hepatomegaly, serum HGF levels were also determined at 48 h after SF oil treatment when the extent of FF-induced hepatomegaly was maximal. Results showed that FSS and FSC oils at doses of 0.6, 1.25, 2.5, and 5 g/kg significantly elevated serum HGF levels up to 106% and 174%, respectively, when compared with the control group. The increased HGF levels of FSC oil treatment were more significant than those of FSS oil treatment at 1.25 and 2.5 g/kg (Supplementary Figure 3).

3.8. Toxicity

The LD_{50} values of FSS oil and FSC oil were (13.87±2.91) g/kg and (13.26±1.82) g/kg, respectively. In addition, the body weight gain of mice was suppressed by both SF oils, with the extent of inhibition of FSC oil being larger than that of the FSS oil. The body weight gain in FSS oil-treated mice (5 g/kg) was suppressed by 109% and 26% after the first and fifth doses, respectively, when compared with the control group. Moreover, the body weight gain in FSC oil-treated mice (5 g/kg) was suppressed by 206% and 33% after the first and fifth doses, respectively, when compared with the control group. (Supplementary Figure 4).

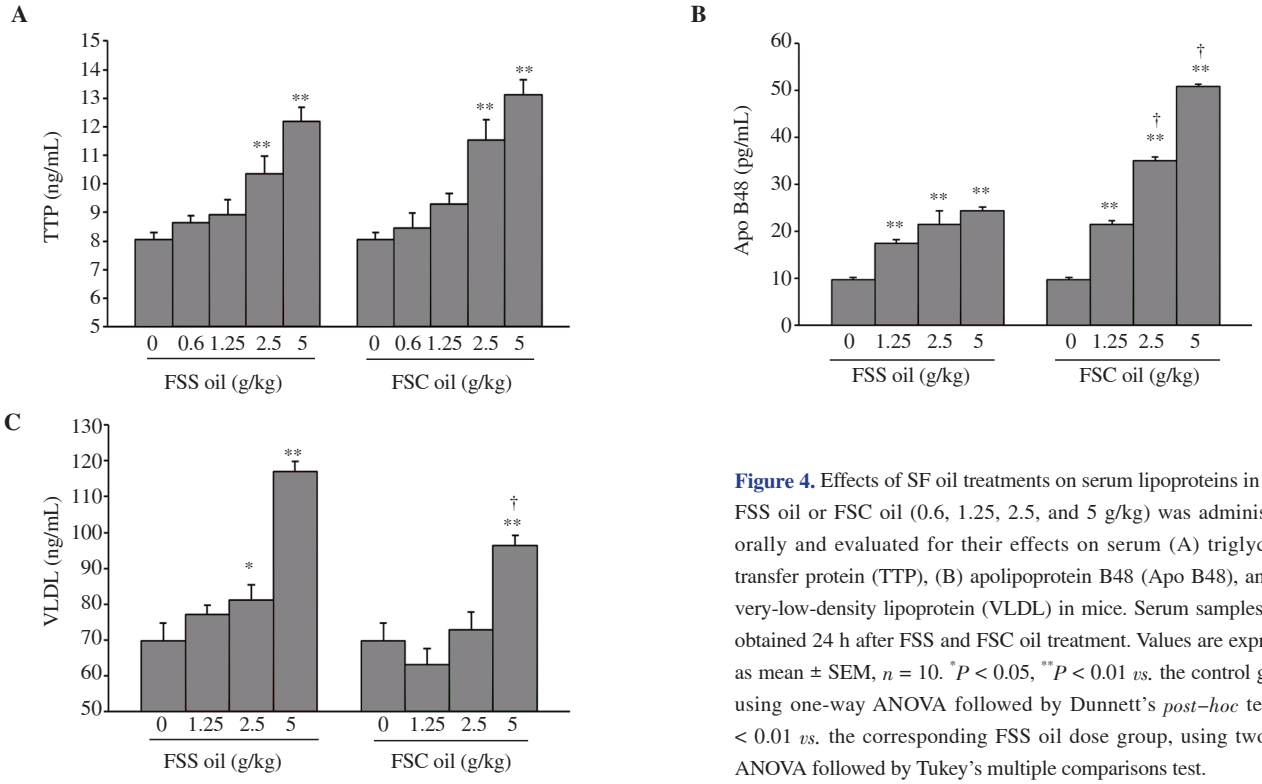

Figure 4. Effects of SF oil treatments on serum lipoproteins in mice. FSS oil or FSC oil (0.6, 1.25, 2.5, and 5 g/kg) was administered orally and evaluated for their effects on serum (A) triglyceride transfer protein (TTP), (B) apolipoprotein B48 (Apo B48), and (C) very-low-density lipoprotein (VLDL) in mice. Serum samples were obtained 24 h after FSS and FSC oil treatment. Values are expressed as mean ± SEM, n = 10. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* the control group, using one-way ANOVA followed by Dunnett's *post-hoc* test; $^{\dagger}P < 0.01$ *vs.* the corresponding FSS oil dose group, using two-way ANOVA followed by Tukey's multiple comparisons test.

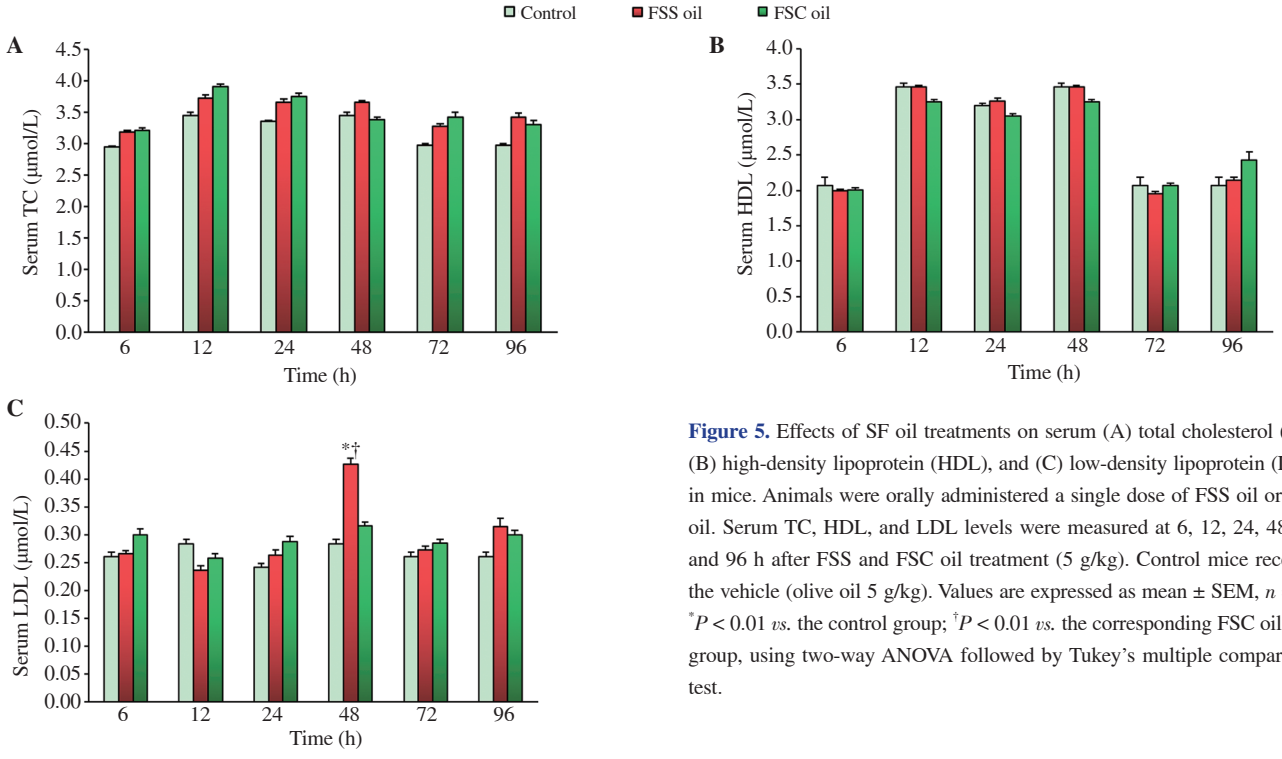

Figure 5. Effects of SF oil treatments on serum (A) total cholesterol (TC), (B) high-density lipoprotein (HDL), and (C) low-density lipoprotein (LDL) in mice. Animals were orally administered a single dose of FSS oil or FSC oil. Serum TC, HDL, and LDL levels were measured at 6, 12, 24, 48, 72, and 96 h after FSS and FSC oil treatment (5 g/kg). Control mice received the vehicle (olive oil 5 g/kg). Values are expressed as mean ± SEM, n = 10. $^{*}P < 0.01$ *vs.* the control group; $^{\dagger}P < 0.01$ *vs.* the corresponding FSC oil dose group, using two-way ANOVA followed by Tukey's multiple comparisons test.

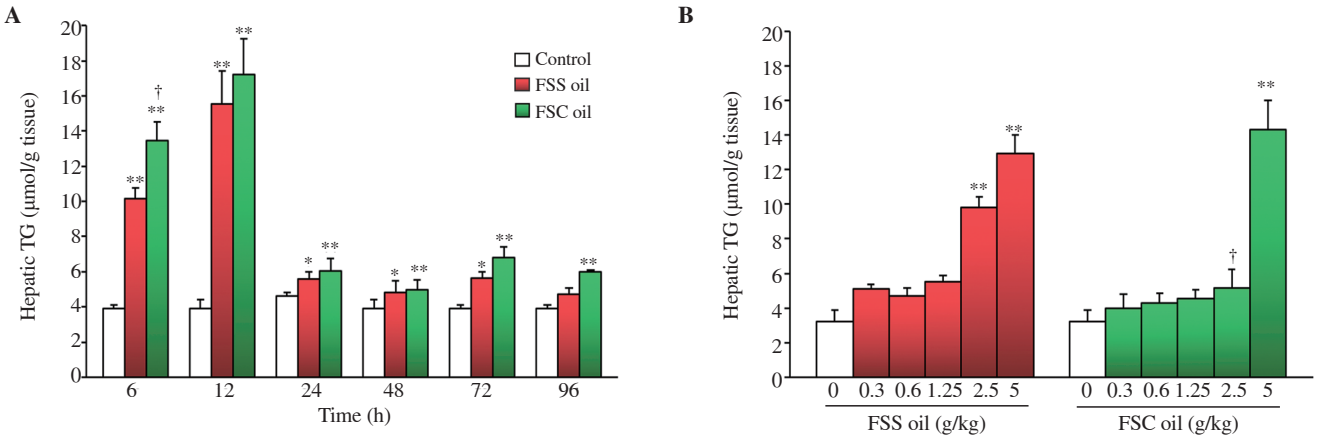

Figure 6. Time and dose response of SF oil-induced elevation in hepatic TG levels in mice. The (A) time and (B) dose-response relationship are shown respectively. Values are expressed as mean ± SEM, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* the control group, using one-way ANOVA followed by Dunnett's t-test; $^{\dagger}P < 0.01$ *vs.* the corresponding FSS oil group, using two-way ANOVA followed by Tukey's multiple comparisons test.

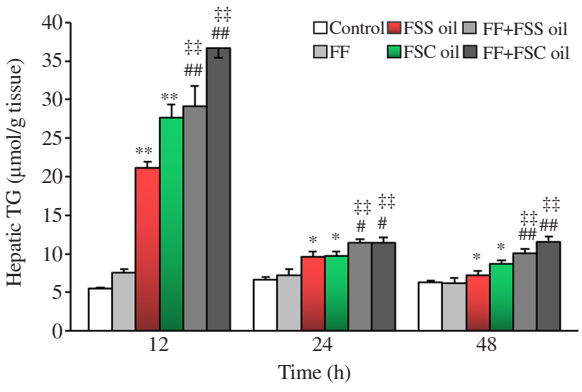

Figure 7. Effects of FF pre/co-treatment on hepatic TG levels in mice treated with SF oils. Liver samples were collected for measuring TG contents at 12, 24, or 48 h after the last treatment. Values are expressed as mean ± SEM, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* the corresponding FSS oil or FSC oil group without FF pre/co-treatment; $^{\ddagger\ddagger}P < 0.01$ *vs.* the FF treatment alone. Significant differences were determined using one-way ANOVA followed by Dunnett's *post–hoc* analysis.

4. Discussion

TG, the main constituent of animal fats and vegetable oils, is the major storage form of fats in the human body. Because dietary TG cannot be absorbed by the duodenum, it must be broken down into fatty acids (FA) and monoglycerides *via* a lipolytic process catalyzed by pancreatic lipase before being transported to the small intestine. After crossing the intestinal barrier, TG fragments in enterocytes are re-formed and packaged together with cholesterol (6%-12%), phospholipids (6%-12%), and proteins (1%-2%) into lipoproteins called chylomicrons (CM), intestinal TG-rich (85 enterocytes -92%) lipoproteins[25,26]. Therefore, CM comprises the exogenous source of TG. Also, the liver can synthesize TG using FA that is either derived from the blood circulation or by synthesis from glucose. TG synthesized in the liver is secreted into the bloodstream in the form of VLDL, which consists of 0.5% phospholipids, 30.8% free cholesterol, 15% cholesterol esters, and 33.7% TG[27]. Therefore, the bulk of circulating TG is present in the form of CM (an exogenous TG biomarker) or VLDL (an endogenous TG biomarker). As Apo B48 is a structural protein of CM, it is usually used as an indirect measurement of CM concentration in the bloodstream[28,29].

In the current study, both SF oils markedly increased serum and hepatic TG levels associated with elevations in the serum level of TG-rich lipoproteins such as VLDL, Apo B48, and TTP (a molecule responsible for the assembly and secretion of Apo B-containing lipoproteins from the intestine and liver). However, serum TC, HDL, and LDL levels did not change significantly after SF oil administration, indicating that SF oils specifically affect the metabolism of both exogenous and endogenous TG. The extent of FSS oil-induced elevation in serum VLDL levels was greater than that produced by FSC oil, suggesting that FSS oil mainly increased endogenous TG synthesis when compared to FSC oil. However, the prominent increase in serum Apo B48 levels following the administration of FSC oil was more than that of FSS oil, suggesting that exogenous TG plays an essential role in the hTG produced by FSC oil. The difference between FSS and FSC oils in elevating serum TG and Apo B48 levels may be attributed to different chemical components present in FSS and FSC, with the latter containing a higher level of Sch B. The finding of no detectable difference in TTP levels between FSS and FSC oil-treated mice could be explained by the fact that TTP participates in the assembly and secretion of both VLDL and CM, which are stimulated by FSS and FSC, respectively[30].

It is well known that exogenous FA travel through the portal vein to the liver, where they are used for the synthesis of endogenous TG that is eventually transported in the bloodstream *via* VLDL. This is consistent with the observation that hepatic TG levels began to rise 6 h after SF oil treatment, which was followed by an increase in serum TG levels at 12 h post-treatment. While the extent of FSC oil-

induced formation of exogenous TG (ApoB 48) was greater than that of FSS oil, the degree of stimulation of endogenous TG (VLDL) production by FSS oil was larger than that of FSC oil. Besides, at the same dosage of FSC and FSS oil, the degree of hTG was found to be higher in FSC-treated mice.

In the present study, FSS and FSC oil treatments increased hepatic size, but reduced serum ALT activity, elevations of which are an indicator of liver injury, suggesting that the SF oil-induced increase in liver mass is not pathologically relevant. It was also found that both SF oils lowered serum ALT activity in the mice pre/cotreated with FF, a lipid-lowering drug that can cause liver injury[31], but FF-induced hepatomegaly was amplified by FSS and FSC oil treatments. It has been reported that the main chemical components in SF oils, such as Sch A and Sch B, have a potential liver-protective action[32–34]. Therefore, we speculate that SF oils may have potential ability to protect against liver injury. It is well known that HGF produced by mesenchymal cells serves to regulate cell growth, including cell division and migration[35]. Defective functioning of HGF can lead to embryonic lethality, a decrease in liver size, and an extensive loss of parenchymal cells[36]. In this study, high levels of serum HGF were observed at 48 h after FSS and FSC oil administration. Therefore, HGF seems to play an essential role in the SF oil-induced increase in liver mass/size. Unfortunately, the time-dependence of the SF oil-induced increase in serum HGF was not determined in the present study. Our futher investigation will be conducted to determine the time-dependence of serum HGF after SF oil administration.

It has been well established that FF alone or in combination with other medications is clinically effective in lowering serum TG in patients with dyslipidemia, especially those with MetS[37]. The biochemical mechanism underlying this lipid-lowering action involves the activation of PPAR , with a resultant altered transcription of several relevant genes[38,39]. Given that FF is an important therapeutic agent in the treatment of abnormal blood TG levels, in this study, FF was used to determine if it can reverse SF oil-induced hTG, and thus support the establishment of hTG models using SF oils. It was found that FF treatment decreased serum TG, but it did not affect hepatic TG levels. While FF pre/co-treatment attenuated the hTG caused by FSS oil at 48 h (but not 24 h) post-dosing, the increase in serum TG levels produced by FSC oil was suppressed in FF pre/co-treated mice at both 24- and 48-h post-dosing. Moreover, FF pre/co-treatment stimulated, rather than inhibited, TG accumulation in the liver of mice treated with SF oils. Although the mechanism underlying the complex interaction between FF and SF oils remains to be investigated, the differential effect of FSS and FSC oils on exogenous and endogenous-derived TG may play an important role. Conceivably, FF may preferably eliminate exogenous TG generated by FSC oil rather than endogenous TG induced by FSS oil.

In addition, there have two main differences between SF oil-induced hTG models and our previous hTG animal model produced by Sch B. Firstly, Sch B treatment only elevated exogenous TG production, while SF oil administration promoted both exogenous and endogenous TG production. Secondly, the serum TC level of patients with hTG is usually normal in clinical situations[40]. Sch B-induced hTG in mice was accompanied by increased serum TC levels, but SF oil administration did not alter serum TC levels, suggesting that SF oil-induced hTG in mice may closely resemble the pathological features of clinical hTG.

There are two limitations of our study that should be mentioned. Firstly, we only evaluated the changes of lipids and lipoproteins after SF oil treatment. However, the underlying molecular mechanisms of this action were not investigated. Future investigations will be designed to explore the molecular mechanism of SF oils-induced hTG. Secondly, as the pathophysiology of hTG is very complex, further studies are required to use metabolomics to find the potential biomarkers for SF oil-induced hTG.

In conclusion, Both FSS and FSC oil treatments markedly elevated serum TG (FSC oil > FSS oil), and hepatic TG contents, as well as liver mass in a time/dose-dependent manner. Also, FS oil-induced hTG was associated with an increase in serum TTP (FSS oil ≅ FSC oil), VLDL (FSS oil > FSC oil), and Apo B48 (FSC oil > FSS oil). Taken together, these results showed that FSS oil-induced hTG might mainly involve the stimulation of endogenous TG synthesis, whereas FSC oil-induced hTG might be related to the stimulation of exogenous TG production. Therefore, hTG caused by SF oils would involve exogenous TG and endogenous TG synthesis. In addition, both SF oils increased serum HGF levels (FSC oil > FSS oil) and lowered serum ALT activity in normal and FF-pre/co-treated mice (FSS oil > FSC oil) (Supplementary Figure 5). Although MetS is causally related to lifestyle factors and individual dietary patterns, the oral administration of SF oils may prove to be useful in developing an animal model of MetS, especially with hTG associated with hyperlipoproteinemia shown as elevations in serum CM and VLDL, in humans[41,42].

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

SYP and YZ contributed to the conceptualization, funding acquisition and project administration. XLS, ZHL, QY, XYW, PLZ, NS, and ZSC conducted the biological investigation. GL performed

UPLC analysis. YZ and HCT analyzed data. YZ and SYP performed data visualization. SYP and YZ drafed the original manuscript. ZLY and KMK revised the manuscript.

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