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Research Paper

Novel action of 7-*O*-galloyl-D-sedoheptulose isolated from Corni Fructus as a hypertriglyceridaemic agent

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Abstract

Objectives We investigated the lipid-lowering activity of 7-*O*-galloyl-D-sedoheptulose, an active component of Corni Fructus, and related mechanisms.
Methods Rats were fed a high-fructose diet for 6 days, followed by treatment with 7-*O*-galloyl-D-sedoheptulose, 5, 10 or 20 mg/kg per day, or fenofibrate (positive control).
Key findings The high-fructose diet induced an increase in body weight, hypertriglyceridaemia, hyperglycaemia and hypertension. Administration of 7-*O*-galloyl-D-sedoheptulose significantly reduced the levels of triglyceride in the serum and liver (being more effective than fenofibrate) but did not lead to changes in liver weight or hepatic function, whereas fenofibrate increased the liver weight markedly. The preventive effect of 7-*O*-galloyl-D-sedoheptulose against the accumulation of triglyceride and cholesterol was related to the up-regulation of peroxisome proliferator-activated receptor α expression.
Conclusions The present study supports the role of 7-*O*-galloyl-D-sedoheptulose as a promising agent against hypertriglyceridaemia without hepatic side-effects.
Keywords cholesterol; Corni Fructus; fructose; 7-*O*-galloyl-D-sedoheptulose; hypertriglyceridaemia

Introduction

Several factors such as overeating, lack of exercise, aging and heredity leads to disorders of lipid metabolism, resulting in coronary heart diseases (CHD).[1] Major risk factors include elevation of fasting blood triglyceride and low-density lipoprotein (LDL) cholesterol levels, with a decline in the high-density lipoprotein (HDL) cholesterol level. Hypertriglyceridaemia leads to obesity,[2] CHD and diabetes, adopted as the criteria for metabolic syndrome.[3] Metabolic syndrome is associated with consumption of high-level fructose, which contributes to obesity and insulin resistance, hypertension, dyslipidaemia and a decline in the level of HDL cholesterol.[4–6] The incidence of metabolic syndrome has greatly increased over the past few decades as a result of the use of high-fructose corn syrup as a substitute for sucrose. Therefore, much effort has been made to identify safe and effective therapeutic agents for metabolic syndrome.

Corni Fructus (*Cornus officinalis* Siebold et Zuccarini) is a plant of the giant dogwood family, and is used as a galenical preparation in traditional Chinese herbal preparations such as Hachimi-jio-gan, Rokumi-jio-gan and Gosha-jinki-gan. It is known to exert several biological activities: plasma glucose-lowering action, antineoplastic activity, antimicrobial effects and improvement of liver and kidney function.[7,8] In addition, it has been reported to inhibit oxidative stress in vascular endothelial cells[9] and fat decomposition in a primary culture of adipose cells.[10] Several active components such as iridoid glycoside, morroniside, loganin and polyphenols have been reported to exhibit protective effects against hyperglycaemia, oxidative stress and cancer.[11–15] We previously reported that Corni Fructus extract and morroniside ameliorate glucose-associated metabolic disorders and the development of diabetic complications.[16] The extract exhibited antidiabetic actions, including decreases in the expression of advanced glycation end-products (AGEs) and AGE receptors induced in the presence of hyperglycaemia in the kidney.[16] Furthermore, 7-*O*-galloyl-D-sedoheptulose was isolated as an active component from a water extract of Corni Fructus.[17,18] It was reported that 7-*O*-galloyl-D-sedoheptulose has

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effects on immune function, and that it showed a beneficial effect against nephropathy.[18] However, studies on its biological activity are limited. The present study focuses on its lipid-lowering activity and related mechanisms in rats fed a high-fructose diet.

Materials and Methods

Materials

Nonidet P-40 (NP-40), phenylmethane sulfonyl fluoride (PMSF), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris (hydroxymethyl) aminomethane) and protease inhibitor mixture in DMSO (protease inhibitor cocktail) were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Dithiothreitol was purchased from BioVision Inc. (Mountain View, CA, USA). Polyclonal antibodies to peroxisome proliferator-activated receptor α (PPARα), sterol regulatory element-binding proteins (SREBP-1 and -2) and polyclonal goat anti-rabbit immunoglobulin G (IgG) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The other chemicals and reagents used were of high quality and obtained from commercial sources.

General experimental procedures

Optical rotations were measured with a digital polarimeter (Jasco DIP-370, Tokyo, Japan). ^{1}H, ^{13}C NMR, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC spectra were recorded at 27°C with a spectrometer (Varian Unity plus 500, Palo Alto, CA, USA) operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Mass spectra (MS) were recorded on a JEOL JMS-700N spectrometer; glycerol was used as the matrix for FAB–MS measurements. Column chromatography was performed with Diaion HP20SS, MCI-gel CHP 20P (75–150 μm) (Mitsubishi Chemical Co., Tokyo, Japan), Sephadex LH-20 (25–100 μm) (GE Healthcare, Piscataway, NJ, USA) and Chromatorex ODS (Fuji Silysia Chemicals Ltd, Aichi, Japan). TLC was performed on 0.2 mm precoated Kieselgel 60 F_{254} plates (Merck & Co., Whitehouse Station, NJ, USA) with benzene/ethyl formate/formic acid (1 : 7 : 1 v/v) or chloroform/methanol/water (14 : 6 : 1 v/v). Spots were detected using UV illumination and by spraying with 2% ethanolic $FeCl_3$ or 10% sulfuric acid reagent, followed by heating. Analytical HPLC was performed on a 250 × 4.6 mm i.d. Cosmosil 5C_{18}-AR II column (Nacalai Tesque Inc., Kyoto, Japan) with gradient elutions of acetonitrile in 50 mM H_3PO_4 from 10 to 30% over 30 min and 30–75% over 15 min, at a flow rate of 0.8 ml/min. Detection was with a Jasco MD-910 diode array detector.

Preparation of Corni Fructus fractions and purification of 7-*O*-galloyl-D-sedoheptulose

Corni Fructus extract (100 g), which was produced by Tsumura & Co. (Tokyo, Japan), was fractionated by Sephadex LH-20 column chromatography (32 × 5 cm) with water containing increasing proportions of methanol (0–100%, 10% stepwise gradient elution) and finally 60% acetone to give four fractions: S1 (94.52 g), S2 (1.20 g), S3 (2.15 g) and S4 (1.55 g).[17] The S1 fraction was further separated by Diaion HP-20SS column chromatography (28 × 5 cm) with water/methanol (0–100%, 10% stepwise gradient elution) to give S1D1 (85.64 g) and S1D2 (7.88 g). TLC and HPLC analyses, performed as described above, showed that S1D1 and S1D2 mainly contained sugars and iridoid glycosides; S2, S3 and S4 contained phenolic substances (Figure 1a). A portion of S2 (150 mg) was further purified by MCI-gel CHP20P column chromatography (28 × 2 cm) with 0–10% MeOH to give 7-*O*-galloyl-D-sedoheptulose,[19] as shown in Figure 1b–d. 7-*O*-Galloyl-D-sedoheptulose (4.8 g) was also obtained on a larger scale from a methanol extract of Corni Fructus (1.5 kg) (0.32% from extract). Our previous study demonstrated that 1.32 g morroniside, the major active compound of Corni Fructus, was isolated from 100 g Corni Fructus extract.[17]

7-O-Galloyl-D-sedoheptulose

7-*O*-Galloyl-D-sedoheptulose was a white amorphous powder. HR FAB–MS *m/z*: 363.0903, $C_{14}H_{19}O_{11}$ [M+H] requires 363.0927. ^{1}H-NMR (acetone-d_6+D_2O) of major anomer δ: 7.13 (s, galloyl-H), 4.36 (m, H-4, H-7a), 4.23 (dd, J = 6.6, 11.7 Hz, H-7b), 4.09 (d, J = 6.4 Hz, H-3), 4.05 (m, H-6), 3.88 (t, J = 5.5, H-5), 3.50 (2H, br s, H-1), ^{1}H-NMR (acetone-d_6+D_2O) of major anomer δ: 167.0 (galloyl C-7), 145.9 (galloyl C-3,5), 138.7 (galloyl C-4), 121.5 (galloyl C-1), 109.8 (galloyl C-2,6), 103.7 (C-2), 83.3 (C-5), 78.0 (C-3), 77.1 (C-4), 71.1 (C-6), 66.2 (C-7), 64.4 (C-1). Other anomeric carbon signals are observed at δ 98.2, 103.7 and 109.0. Assignments of the signals were achieved by COSY, HSQC and HMBC spectral analyses.

The structure was further confirmed by the formation of an osazone derivative: a mixture of the compound (10 mg), phenylhydrazine hydrochloride (20 mg) and sodium acetate (30 mg) in water (0.5 ml) was heated at 80°C for 25 min and the resulting precipitates collected by filtration. The ^{1}H-NMR spectral data (in DMSO-d_6) and $[\alpha]_D$ value coincided with the data of the osazone derivative of 7-*O*-sedoheptulose gallate.[20]

Experimental protocol

The Guidelines for Animal Experimentation approved by the University of Toyama were followed in all experimental studies. Male Wistar rats (5 weeks old, body weight 120–130 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The rats were acclimatised in wire-bottomed cages (4–6 per cage) in a 12 h light–dark cycle at 23°C and 60% humidity. Rats were fed commercially available solid food (CE-2, CLEA Japan Inc., Tokyo, Japan) for a few days initially.

Rats were then randomly divided into two groups: one received a control casein diet (18% casein) and the other the high-fructose diet (18% casein diet containing 60% fructose) (Table 1). Each diet was available ad libitum for 6 days; daily food intake per rat was 15 g. Thereafter, rats were given 15 g per day in a paired schedule. Blood pressure was measured on day 6 and tail venous blood levels of triglyceride were determined using a commercial kit. Based on the triglyceride level, blood pressure and body weight, rats were divided into six groups: a control group (n = 7); three treatment groups, which were given 5, 10 or 20 mg/day 7-*O*-galloyl-D-sedoheptulose (n = 6, 7 and 8, respectively); a positive control group, treated with fenofibrate (n = 5); and a vehicle

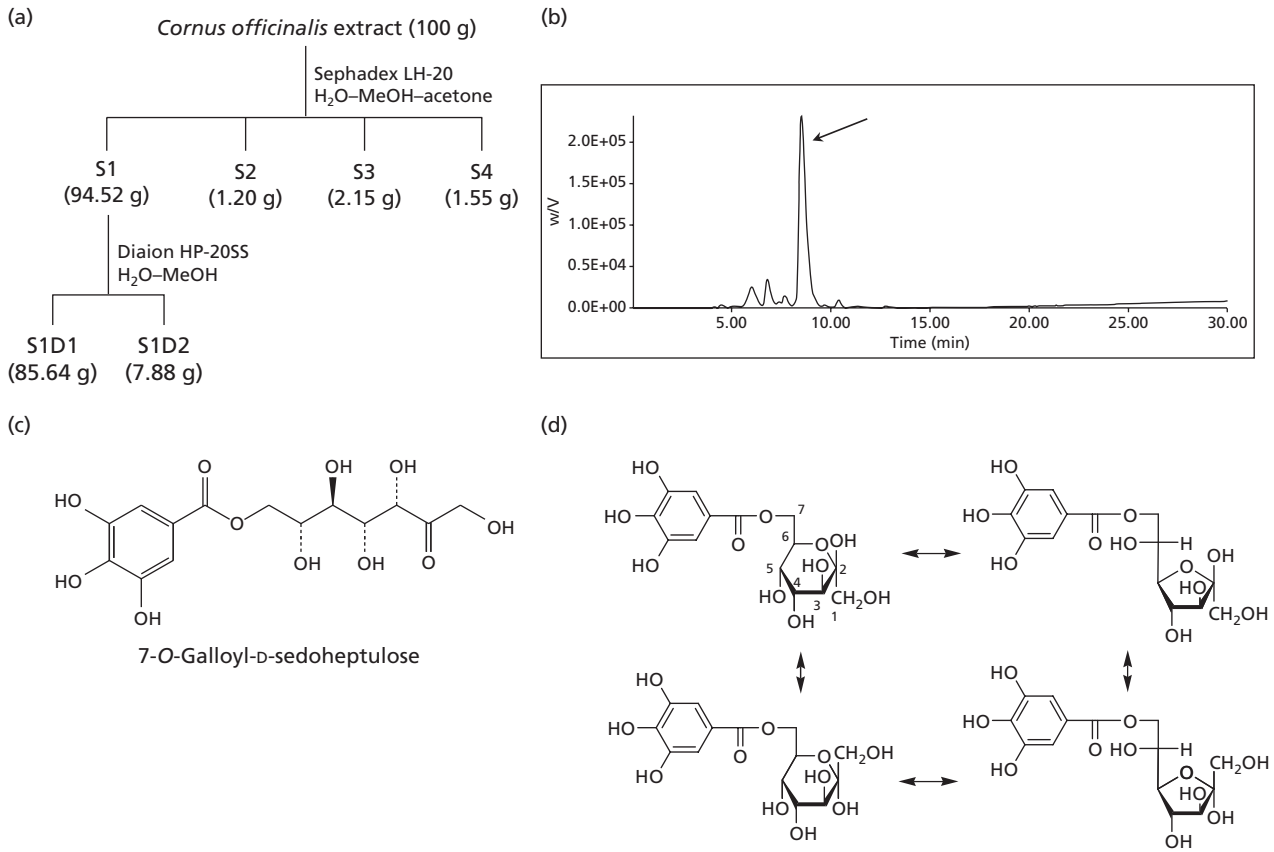

Figure 1 (a) Fractionation of *Cornus officinalis* and (b) its HPLC profile. The large peak indicated by the arrow represents 7-*O*-galloyl-D-sedoheptulose, the structure of which is shown in (c); the other peaks represent its four isomers, shown in (d).

Table 1 Composition of diets

Ingredients	18% Casein diet (g/100 g)	60% Fructose diet (g/100 g)
Casein	18.0	18.0
α-Cornstarch	57.9	12.6
Sucrose	15.0	–
Soybean oil	2.0	2.0
Salt mixture	4.0	4.0
Vitamin mixture	1.0	1.0
Cellulose powder	2.0	2.0
Choline chloride	0.1	0.1
Fructose	–	60.0
DL-Methionine	–	0.1

control group ($n = 5$). These groups all received the high-fructose diet. The diet control group was given the 18% casein diet and water.

7-*O*-Galloyl-D-sedoheptulose was dissolved in water and administered using a gastric tube daily for 20 days. Fenofibrate was suspended in 0.5% methylcellulose, which was administered daily using a gastric tube at 50 mg/kg per day until day 10 and at 20 mg/kg per day thereafter. The vehicle control group was given 0.5% methylcellulose.

Blood was collected through the tail vein on day 10 to measure the serum triglyceride level. Blood pressure was determined on day 19. On day 20, laparotomy was performed under ether anaesthesia and blood was collected from the abdominal aorta. Subsequently, the liver was perfused with physiological saline and then isolated. Blood samples were centrifuged at 1670*g* for 15 min to separate the serum. The liver was frozen and stored at −80°C until analysis.

Measurement of blood pressure

Blood pressure was measured by the tail-cuff method using an automatic blood pressure monitoring system (UR-5000, UETA, Tokyo, Japan). The animals were kept at 37°C for 30 min before measurement of blood pressure. The average of five consecutive readings was used.

Determination of serum components

The levels of serum triglyceride, total cholesterol, glucose and free fatty acids were determined using commercial reagents (Triglyceride E-Test Wako, Cholesterol E-Test Wako, Glucose CII-Test Wako, and NEFA C-Test Wako, respectively, obtained from Wako Pure Chemical Industries).

Measurement of hepatic triglyceride and total cholesterol content

The liver of each rat was homogenised, and total lipids were extracted with a mixture of chloroform and methanol (2 : 1 v/v) according to the method of Folch *et al.*[21]

Triglyceride and total cholesterol contents were determined using the Wako kits described above.

Determination of hepatic functional parameters

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using commercial reagents (GPT-UV Test Wako obtained from Wako Pure Chemical Industries).

Preparation of nuclear fractions

Nuclear fractions were prepared according to the method of Sakurai and colleagues.[22] Hepatic tissue was homogenised with 10 mM 2-[4-(2-hydroxyethyl)-1-piperazyl] ethanesulfonic acid (HEPES) buffer (pH 7.9) containing 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.5 mM PMSF and protease inhibitor cocktail. The homogenate was chilled on ice for 15 min and 10% NP-40 added to 1% of the final volume. The mixture was vortexed vigorously for 10 s and the supernatant obtained (cytoplasmic fraction) by centrifugation at 15 000*g* for 5 min at 4°C. The pellet was resuspended in 20 mM HEPES buffer (pH 7.9) containing 0.4 M NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM PMSF and protease inhibitor cocktail. The mixture was left on ice for 15 min with frequent agitation. Nuclear extract was prepared by centrifugation at 15 000*g* for 5 min at 4°C. The protein concentration of each fraction was quantified using a commercial kit (Bio-Rad Laboratories, Hercules, CA, USA).

Protein expression

Western blot analysis was performed with 30 μg protein from the nuclear fraction. The protein was separated by SDS-PAGE. Separated proteins were electrophoretically transferred to a membrane, blocked with 5% non-fat dry milk solution for 1 h and then incubated with the corresponding primary anti-PPARα, SREBP-1, SREBP-2 and β-actin antibodies overnight at 4°C. The blots were then washed and incubated with goat anti-rabbit and/or goat anti-mouse IgG horseradish peroxidase-conjugated secondary antibodies for 90 min at room temperature. Antigen–antibody complexes were visualised using enhanced chemiluminescence Western blotting detection reagents and detected by chemiluminescence with LAS-1000plus (Fujifilm, Tokyo, Japan). The identification of each protein was estimated by comparison with the protein markers of known molecular weight. Band densities were determined by Scion Image software (Scion Corporation, Frederick, MD, USA) and quantified as a ratio to the density of the β-actin band.

Statistical analysis

Results are expressed as means ± SEM. The effect of 7-*O*-galloyl-D-sedoheptulose on each parameter was examined using one-way analysis of variance. Individual differences between groups were analysed by Dunnett's test. *P* values below 0.05 were considered significant.

Results

Body weight and hepatic weight

Figure 2 shows the changes in body and liver weights during the experimental period. Body weight increased gradually in all experimental groups given the high-fructose diet but there was no significant difference in body weight between groups. Liver weight was significantly higher in rats fed a high-fructose diet than in those fed the casein diet. There were no marked differences between the control, 7-*O*-galloyl-D-sedoheptulose-treated and vehicle groups. By contrast, in the fenofibrate-treated group, liver weight was 1.8 times higher than that in the vehicle group and 2.5 times higher than that in the casein-diet group.

Triglyceride and total cholesterol in serum and hepatic tissue

The effect of 7-*O*-galloyl-D-sedoheptulose on triglyceride levels in serum and hepatic tissue is shown in Figure 3. Serum triglyceride levels in the fructose-fed rats were approximately twice the level in the casein-diet rats. Levels were significantly reduced on days 10 and 20 in a dose-dependent manner in rats treated with 7-*O*-galloyl-D-sedoheptulose. Serum triglyceride levels on day 20 in rats treated with 7-*O*-galloyl-D-sedoheptulose at 10 or 20 mg/kg per day were significantly lower than pretreatment values and levels in the fenofibrate group, and were 30% lower than in the the control group. Treatment with fenofibrate did not lead to a significant decrease in the serum triglyceride concentration on day 20, but showed a significant change on day 10 (Figure 3).

Hepatic levels of triglyceride after 20 days were 1.4 times higher in the fructose-fed rats than in the casein-fed rats (Figure 3b). This parameter decreased significantly in the groups treated with 7-*O*-galloyl-D-sedoheptulose at 10 or 20 mg/kg per day, and in the group given the higher dose was similar to that in the casein-fed rats. By contrast, administration of fenofibrate increased the hepatic triglyceride concentration by 1.9 fold compared with casein-fed rats and 1.4 times compared with the fructose-diet control rats. It was also higher than the value in the vehicle group.

As shown in Figure 3c, the serum total cholesterol level after 20 days' treatment was 1.4 times higher in the fructose-fed rats than in the casein-fed rats. The level was reduced in the groups treated with 7-*O*-galloyl-D-sedoheptulose and fenofibrate at 20 mg compared with the control group.

Total cholesterol level in the liver was 1.2 times higher in the fructose-fed rats than in the casein-fed rats, while treatment with 20 mg 7-*O*-galloyl-D-sedoheptulose lowered this level slightly. By contrast, fenofibrate treatment elevated the level by 1.2 and 1.4 times compared with values in the vehicle group and the fructose-fed control groups, respectively (Figure 3d).

Hepatic functional parameters, blood glucose and serum free fatty acids

AST and ALT levels were higher in rats fed the high-fructose diet than in casein-fed rats (Table 2). Administration of 7-*O*-galloyl-D-sedoheptulose did not alter the values significantly, although the AST level was slightly decreased at the 10 and 20 mg doses. Fenofibrate-treated rats did not show a significant change in these hepatic functional parameters.

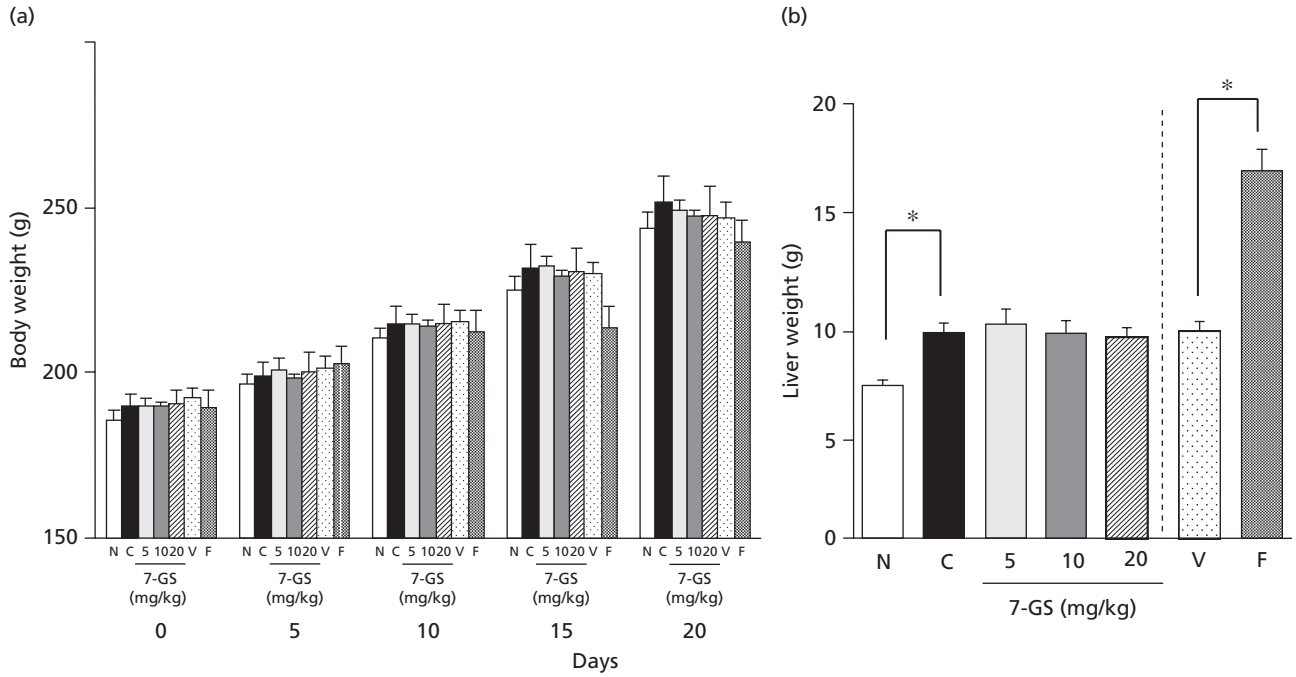

Figure 2 Changes in (a) body weight and (b) liver weight. N, normal; C, control; 7-GS, 7-*O*-galloyl-D-sedoheptulose; V, vehicle; F, fenofibrate, 20 mg/kg. All groups except N were fed the 60% fructose diet. $^{*}P < 0.05$ vs fructose-fed control rats.

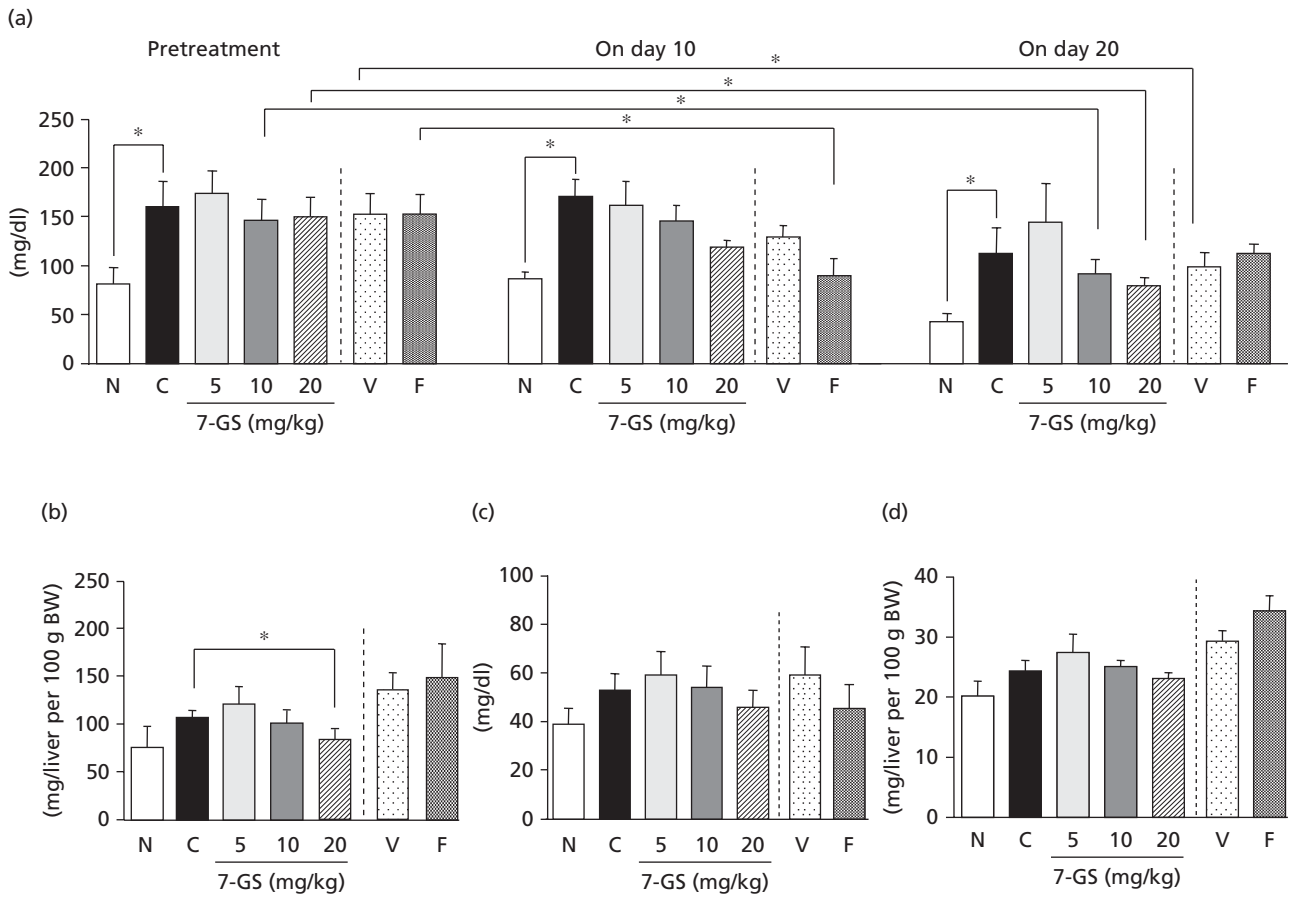

Figure 3 Triglyceride levels in (a) serum and (b) hepatic tissue and total cholesterol levels in (c) serum and (d) hepatic tissue. N, normal; C, control; 7-GS, 7-*O*-galloyl-D-sedoheptulose; V, vehicle; F, fenofibrate (20 mg/kg; 50 mg/kg pretreatment and day 10 in (a)). All groups except N were fed the 60% fructose diet. $^{*}P < 0.05$ vs fructose-fed control rats or pretreatment values.

Table 2 Hepatic functional parameters, blood glucose and serum free fatty acids

Group	AST (Karmen units)	ALT (Karmen units)	Blood glucose (mg/dl)	Free fatty acids (mEq/l)
18% Casein diet	53.7 ± 2.9	13.1 ± 0.7	165.5 ± 8.7*	0.60 ± 0.03
60% Fructose diet				
Control	58.1 ± 9.4	17.4 ± 2.9	217.7 ± 16.9	0.63 ± 0.04
7-*O*-Galloyl-D-sedoheptulose (5 mg/kg)	62.4 ± 10.2	21.8 ± 4.4	214.8 ± 21.8	0.64 ± 0.11
7-*O*-Galloyl-D-sedoheptulose (10 mg/kg)	50.8 ± 1.5	18.8 ± 1.5	210.0 ± 14.5	0.61 ± 0.04
7-*O*-Galloyl-D-sedoheptulose (20 mg/kg)	53.7 ± 2.2	18.9 ± 1.6	202.7 ± 9.7	0.65 ± 0.04
Vehicle	45.0 ± 2.9	18.1 ± 2.2	239.5 ± 26.7	0.63 ± 0.05
Fenofibrate (20 mg/kg)	58.1 ± 18.9	18.9 ± 2.9	210.5 ± 12.1	0.63 ± 0.10

ALT, alanine aminotransferase; AST, aspartate aminotransferase. *$P < 0.05$ vs fructose diet controls.

The blood glucose level in the fructose-fed rats was 1.3 times higher than in the casein-fed rats. However, no significant change was observed with the administration of 7-*O*-galloyl-D-sedoheptulose or fenofibrate.

The concentration of free fatty acids in serum was not significantly different between the experimental groups (Table 2).

Blood pressure

Figure 4 show the effect of 7-*O*-galloyl-D-sedoheptulose on blood pressure. In the fructose-fed rats, the systolic, mean and diastolic blood pressures were increased by approximately 10% compared with the casein-fed rats. Systolic blood pressure was higher in the high-fructose control group than in the normal group. Changes in the groups treated with 7-*O*-galloyl-D-sedoheptulose were similar to those seen in the control group. Systolic blood pressure was also increased in the vehicle and fenofibrate-treated groups, but there were no significant changes in the mean and diastolic blood pressures.

Hepatic expression of SREBP-1, SREBP-2 and PPARα proteins

Figure 5 shows the effect of 7-*O*-galloyl-D-sedoheptulose on the expression of proteins related to lipid metabolism. The expression of SREBP-1 was slightly higher in the fructose-fed rats than in the casein-fed rats. However, expression was decreased in the group treated with 20 mg/kg 7-*O*-galloyl-D-sedoheptulose. SREBP-1 expression was 1.8 times higher in the fenofibrate-treated group than in the vehicle group. A 1.2-fold increase in SREBP-2 expression was observed in the fructose-fed rats compared with casein-fed rats. Treatment with 10 mg 7-*O*-galloyl-D-sedoheptulose led to a further increase, whereas 20 mg decreased the expression of SREBP-2. SREBP-2 expression was markedly increased in the fenofibrate-treated group (20 mg), to 2.2 times higher than that in the vehicle group.

The expression of PPARα in fructose-fed rats was decreased by approximately 15% compared with the casein-fed rats but was increased by treatment with 7-*O*-galloyl-D-sedoheptulose: PPARα expression was higher in the groups treated with 10 mg and 20 mg than in the casein-fed rats. Treatment with fenofibrate elevated the value by 1.3 times compared with that in the vehicle group (Figure 5).

Discussion

The induction of metabolic syndrome – hyperglycaemia, hyperinsulinaemia, hypertension and hypertriglyceridaemia – by a high-fructose diet has been well established. Hyperglycaemia induced by a high-fructose diet is related to the decrease in insulin sensitivity of the liver and skeletal muscle, which is primarily involved in the effects of insulin on carbohydrate metabolism.[23,24] In agreement with this

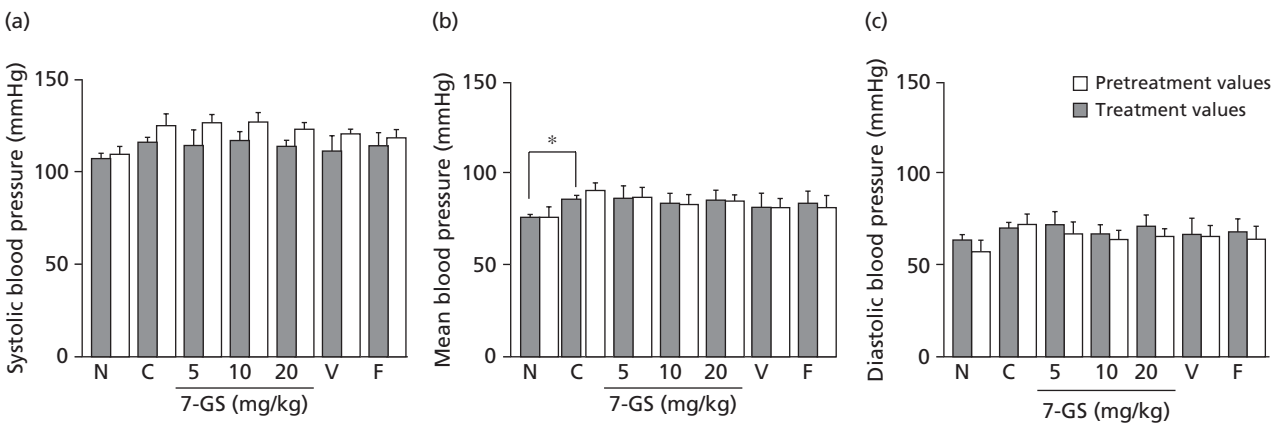

Figure 4 Systolic (a), mean (b) and diastolic (c) blood pressures. N, normal; C, control; 7-GS, 7-*O*-galloyl-D-sedoheptulose; V, vehicle; F, fenofibrate, 20 mg/kg. All groups except N were fed the 60% fructose diet. *$P < 0.05$ vs fructose-fed control rats or pretreatment values.

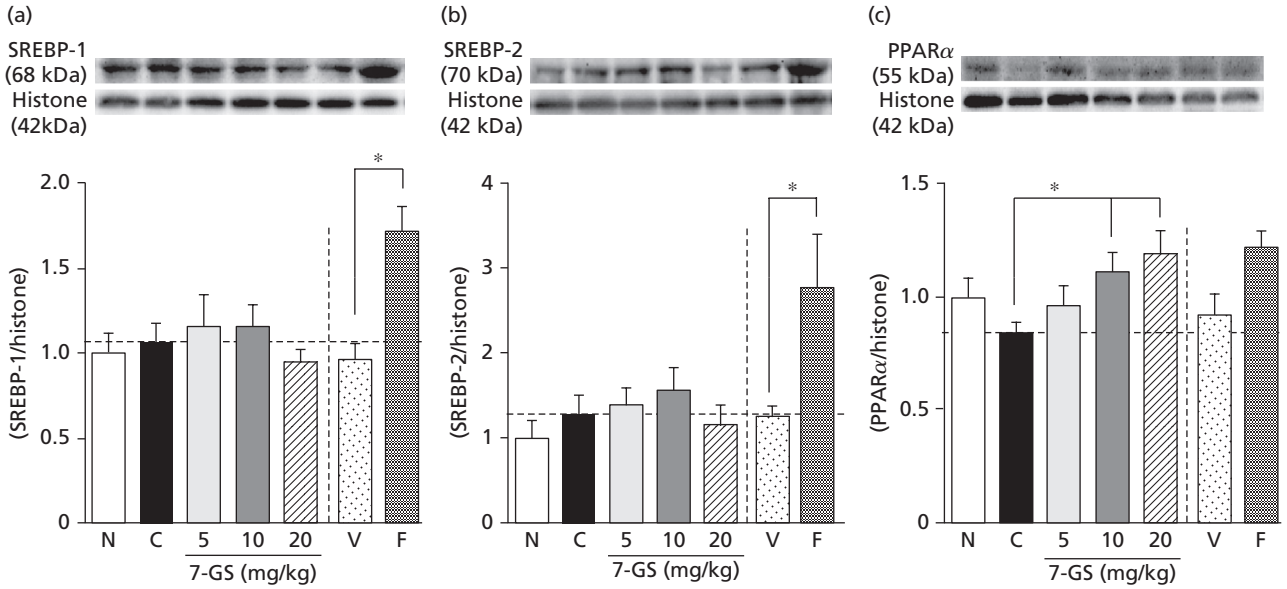

Figure 5 SREBP-1 (a), SREBP-2 (b) and PPARα (c) protein levels in hepatic tissue. N, normal; C, control; 7-GS, 7-*O*-galloyl-D-sedoheptulose; V, vehicle; F, fenofibrate, 20 mg/kg. All groups except N were fed the 60% fructose diet. $^{*}P < 0.05$ vs fructose-fed control rats.

evidence, the present results also demonstrated that a high-fructose diet led to hyperglycaemia, dyslipidaemia and hypertension.

Fenofibrate, one of the most commonly used fibrates worldwide, inhibis the development of atherosclerosis through the treatment of dyslipidaemia and hypercholesterolaemia.[25,26] Fenofibrate was therefore used as a positive control in the present study, to compare the effects of 7-*O*-galloyl-D-sedoheptulose.

Our previous studies demonstrated that Corni Fructus extract, active fractions and morroniside ameliorated the disorders of glucose metabolism via the attenuation of hyperglycaemia and diabetic complications.[16,17] S1D2 and S2, the active fractions of Corni Fructus, containing morroniside and 7-*O*-galloyl-D-sedoheptulose, respectively, showed beneficial effects against diabetes-related pathological conditions in a rat-based diabetes model, but via different mechanisms.[17] The active fraction containing morroniside had an effect on intracellular AGE formation and/or accumulation but a weak effect on oxidative stress, whereas the fraction containing 7-*O*-galloyl-D-sedoheptulose exerted a protective effect mainly against oxidative stress. In the present study, the administration of 7-*O*-galloyl-D-sedoheptulose did not lead to a significant change in the blood glucose level (Table 2). In addition, our previous investigation also showed that the fraction containing 7-*O*-galloyl-D-sedoheptulose did not affect the serum glycosylated protein level in diabetic rats.[17] This indicates that the beneficial effect of 7-*O*-galloyl-D-sedoheptulose against hypertriglyceridaemia is not related to glucose metabolism.

The high-fructose diet induced hypertriglyceridaemia with an increase in the cholesterol level (Figure 3). The possibility of metabolic derangement and induction of oxidative damage by dietary fructose is supported by numerous recent studies.[27–30] In humans and rats, fructose is more lipogenic than glucose or starch, leading to an increase in hepatic triglyceride synthesis and overproduction of very-low-density lipoprotein (VLDL).[31,32] Hypertriglyceridaemia and the presence of triglyceride-rich lipoproteins are part of the metabolic syndrome frequently encountered in individuals with early-onset CHD.[33] Accordingly, a high-fructose diet could increase the occurrence of aortic atherosclerotic plaques. In the present study, we observed a significant elevation of serum triglyceride levels in fructose-fed rats, indicating that an increased flux of free fatty acids into the liver may contribute to VLDL overproduction. However, the administration of 7-*O*-galloyl-D-sedoheptulose at 20 mg/kg led to a decline in the levels of triglyceride and cholesterol (Figure 3). This suggests that 7-*O*-galloyl-D-sedoheptulose may play a protective role against CHD through the attenuation of hypertriglyceridaemia and hyperlipidaemia induced by a high-fructose diet. This preventive activity of 7-*O*-galloyl-D-sedoheptulose against triglyceride accumulation was more effective than that of fenofibrate.

High dietary fructose intake enhances fatty acid synthesis in the liver, with an elevation of the level of LDL via release of VLDL into the blood.[34] In this study, we measured hepatic expression of SREBP-1, which activates the transcription of an enzyme involved in fatty acid synthesis.[35] In the group treated with 20 mg 7-*O*-galloyl-D-sedoheptulose, expression was lower than in the control group (Figure 5), consistent with changes in the serum triglyceride level. The serum triglyceride level in the fenofibrate-treated group was similar to that in the control group. However, the expression of SREBP-1 was markedly increased. In addition, SREBP-2 activates the transcription of an enzyme related to cholesterol synthesis.[36] The administration of 7-*O*-galloyl-D-sedoheptulose at 20 mg/kg led to a decline in the protein expression of SREBP-2, although this was not statistically significant (Figure 5). By contrast, hepatic expression of SREBP-2 was markedly

increased in the fenofibrate-treated group, although the serum total cholesterol level was decreased compared with that in the group treated with 20 mg 7-*O*-galloyl-D-sedoheptulose.

PPARα plays an important role in the metabolic homeostasis of fatty acids through the regulation of target genes that encode enzymes for fatty acid oxidation and fatty acid transporters.[37,38] It promotes the transcription of enzymes that break down fatty acids and HDL cholesterol apolipoproteins, resulting in a decline in serum levels of triglyceride and cholesterol.[39,40] In the present study, the elevation of serum and hepatic triglyceride levels caused by dietary fructose corresponded to the decrease in PPARα. Fenofibrate is used in clinical practice to decrease both serum triglyceride and cholesterol levels.[41] Hepatic expression of PPARα was increased in the fenofibrate-treated group. Nagai and colleagues[42] have also reported that treatment of fructose-fed rats with fenofibrate increased the liver expression of PPARα. The increase may have led to decreases in serum triglyceride and cholesterol. Similarly, 7-*O*-galloyl-D-sedoheptulose elevated the expression of PPARα in a dose-dependent manner, to the level in the fenofibrate group, with both the 10 and 20 mg/kg doses. These findings suggest that both 7-*O*-galloyl-D-sedoheptulose and fenofibrate control serum lipid levels via an increase in the expression of PPARα. Although 7-*O*-galloyl-D-sedoheptulose down-regulated the protein expressions of SREBP-1 and -2 only slightly, regulation of lipid metabolism is mainly attributed to the elevation of PPARα expression. Liver X receptors (LXRs) are also lipid-activated transcription factors that have emerged as key regulators of lipid metabolism and inflammation.[43] LXRs are activated by cholesterol metabolites. The ability of these nuclear receptors to integrate metabolic and inflammatory signalling makes them attractive targets in intervention for metabolic diseases such as atherosclerosis and diabetes. Several studies have reported that PPARs inhibit inflammatory gene expression by several mechanisms, including a direct interaction with activator protein 1 and nuclear factor (NF)-κB, the nucleocytoplasmic redistribution of the p53 subunit of NF-κB and modulation of p38 mitogen-activated protein kinase activity.[43–46] Further study into the mechanisms by which 7-*O*-galloyl-D-sedoheptulose affects lipid metabolism is needed to explain its protective role against hypertriglyceridaemia.

To estimate the side-effects or toxicity of 7-*O*-galloyl-D-sedoheptulose on the liver, the primary organ responsible for cholesterol homeostasis, the activities of AST and ALT, well-known marker enzymes of liver function, were determined. Treatment with 7-*O*-galloyl-D-sedoheptulose did not have any significant effects on these values (Table 2) whereas in the fenofibrate group there was a marked increase in the liver weight but no significant changes in AST or ALT. Peters and colleagues[47] reported that the administration of fibrate agents, including fenofibrate, to rodents resulted in hepatocellular proliferation, causing liver cancer. Furthermore, a clinical study indicated that fenofibrate caused hepatopathy.[48,49] Several adverse effects have also been reported, such as gastrointestinal discomfort, headache, anxiety, dizziness, sleep disorders, arthralgia, rash, pruritus, urticaria, blurred vision and renal problems.[26] However, it is not known whether these side-effects are of clinical importance. In this experiment, there was no hepatic hypertrophy in the groups treated with 7-*O*-galloyl-D-sedoheptulose. Thus, in contrast to fenofibrate, 7-*O*-galloyl-D-sedoheptulose did not cause any adverse effects on the liver, suggesting the safety of this component in humans. Furthermore, our previous study also demonstrated that the active fraction containing 7-*O*-galloyl-D-sedoheptulose improved metabolic parameters associated with renal damage.[17] This evidence suggests that 7-*O*-galloyl-D-sedoheptulose is a promising agent for use against hypertriglyceridaemia without hepatic or renal side-effects, although further studies on its safety and toxicity are required to support its clinical application.

Conclusions

7-*O*-Galloyl-D-sedoheptulose decreased levels of triglyceride and cholesterol in the serum and liver, and was more effective than fenofibrate. The preventive effect of 7-*O*-galloyl-D-sedoheptulose against the accumulation of triglyceride and cholesterol was related to the up-regulation of PPARα expression. The present study supports the protective role of 7-*O*-galloyl-D-sedoheptulose as a promising agent against hypertriglyceridaemia.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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