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ORIGINAL ARTICLE

Polysaccharide-rich red algae (Gelidium amansii) hot-water extracts ameliorate the altered plasma cholesterol and hepatic lipid homeostasis in high-fat diet-fed rats

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Abstract

We have demonstrated that red algae *Gelidium amansii* (GA) hot-water extract (GHE) is a polysaccharide-rich fraction, containing 68.54% water-soluble indigestible carbohydrate polymers; the molecular weight of major polysaccharide is 892. Here, we investigated the mechanisms of GHE on plasma and hepatic lipid metabolisms in high-fat (HF) diet-fed rats. Rats were divided into: normal diet group, HF-diet group, HF-diet+5% GHE group, and HF-diet+1% cholestyramine group. GHE supplementation for 8 weeks significantly decreased plasma cholesterol, LDL-C, and VLDL-C levels and increased the fecal triglyceride and bile acid excretion in HF diet-fed rats. GHE group has lower lipid contents in the liver and adipose tissues. GHE supplementation decreased the activities of acetyl-CoA carboxylase, fatty acid synthase, and HMG-CoA reductase in the livers. The levels of increased phosphorylated AMP-activated protein kinase (AMPK), peroxisome proliferator activated receptor (PPAR)-α, farnesoid-X receptor (FXR), low density lipoprotein receptor (LDLR), and cytochrome P450-7A1 (CYP7A1) protein expression, and the decreased PPAR-γ protein expression in the livers were observed in GHE group. These results suggest that GHE supplementation is capable of interfering in cholesterol metabolism and increasing hepatic LDLR and CYP7A1 expression to decrease blood cholesterol, and activating FXR and AMPK to inhibit lipogenic enzyme activities and reduce the hepatic lipid accumulation.

Keywords: cholestyramine, high-fat diet-fed rats, lipid metabolism, polysaccharide-rich *Gelidium amansii* hot-water extract

1. Introduction

Metabolic diseases such as diabetes, dyslipidemias, cardiovascular disease, and certain cancers are global health concern. As the Body Mass Index (BMI) increases, the chances of suffering from cardiovascular disease, hypertension, osteoarthritis, type 2 diabetes, and cancer also increase [1]. These diseases are associated with obesity [2]. Obesity is also a major risk factor for cardiovascular disease, since much adipocytes increase inflammatory factors and cause the risk of developing cardiovascular disease. On the other hand, nonalcoholic fatty liver disease (NAFLD) is characterized by lipid accumulation in the liver. The NAFLD might lead to liver injury, such as non-alcohol steatohepatitis, liver fibrosis, and liver cirrhosis. Obesity is known to be associated with NAFLD [3]. Therefore, to improve and to prevent the occurrence of obesity and fatty liver have become an important and urgent issue for health.

Gelidium amansii (GA) is the edible seaweed (red algae), which is wildly distributed in Asian countries such as Korea, China, Japan, and Taiwan. The agar product (1,3-linked b-D-galactopyranose and 1,4-

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linked 3,6-anhydro-a-L-galactopyranose units) of GA [4] can be prepared to form a gel [5], which is a traditional food in Japan and Taiwan. Recent studies have indicated that GA has hypoglycemic and hypolipidemic effects in diabetic animal model [6] and patients with diabetes [7]. In addition, it has been shown that the ethanol extract of GA has a beneficial effect on decreasing body weight and reducing serum lipids in mice fed a high-fat (HF) diet [8]. Recently, we have found that GA hot-water extract (GHE) possesses the ability of anti-obesity and reducing triglyceride and cholesterol in the plasma and liver of HF diet-fed hamsters [9,10]. We have also demonstrated that GHE is a polysaccharide-rich fraction of GA and contained 68.54% carbohydrate polymers, which galactose is the major monosaccharide of water-soluble indigestible polysaccharide from GHE [10]. These water-soluble fibers of GHE may contribute to reduce lipids in the blood and liver. Although GHE may exert a down-regulation effect on hepatic lipid metabolism through AMP-activated protein kinase (AMPK) phosphorylation and up-regulation of uncoupling protein (UCP)-2 in the livers of HF diet-fed hamsters [10], the mechanism of reducing lipids in the plasma and liver by GHE still remains to be clarified. In this study, therefore, to assess the possible effect and mechanism of GHE on lipids of plasma and liver, rats fed a HF diet with GHE supplementation was investigated.

2. Materials and methods

2.1. Chemicals

Cholesterol, cholic acid, heparin, and cholestyramine were obtained from Sigma–Aldrich (St. Louis, MO, USA). The enzymatic assay kits for detection of TC and TG were provided by Audit Diagnostics (Cork, Ireland). The enzymatic assay kits for detection of AST and ALT were obtained from Randox Laboratories (Antrim, UK). A bile acid assay kit was purchased from Randox Laboratories. A glycerol assay kit was purchased from Randox Laboratories. Hematoxylin and eosin staining solution were obtained from Leica Biosystems (Richmond, IL, USA). RIPA lysis and extraction buffer was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Polyvinylidene difluoride membranes were obtained from Bio-Rad Laboratories (Hercules, CA, USA). An enhanced chemiluminescence detection kit was obtained from PerkinElmer (Waltham, MA, USA). The antibodies for AMPK and phospho-AMPK (Thr172) were purchased from Cell Signaling Technology (Danvers, MA, USA). The antibodies for farnesoid X receptor (FXR), peroxisome proliferator activated receptor (PPAR)-α, PPAR-γ, low density lipoprotein receptor (LDLR), cytochrome P450-7A1 (CYP7A1), and GAPDH were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Preparation of polysaccharide-rich hot-water extract of GA (GHE) and analysis of composition

The dry material of GA was purchased from the market at Keelung, Taiwan. The preparation of polysaccharide-rich GHE was performed as previously described [9,10]. Briefly, 100 g GA in the 2 L deionized water was autoclaved at 121 °C for 20 min, and then samples were cooling, filtered, and lyophilized. The harvest weight of GHE was 38.09 g, which the recovery rate was about 38.09%.

The analysis of molecular weight of polysaccharides was determined as previously described by Kazlowski et al. [11,12]. The polysaccharide sample was analyzed by HPLC (Hitachi L-2130) with an Asahipak SB-804 HQ (7.5 × 300 mm) column and pure water as the mobile phase.

The methods for analysis of carbohydrate content and monosaccharide composition were performed as previously described [10]. A colorimetric method was used to determine the carbohydrate content and a high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to analyze the monosaccharide composition.

The contents of reducing sugar were determined as previously described by Miller using a dinitrosalicylic acid reagent [13]. The amounts of sulfate present in sugar were detected as previously described by Terho and Hartiala using a sodium rhodizonate reagent, which formed a red compound in the presence of barium [14].

2.3. Animals

The Animal House Management Committee of the National Taiwan Ocean University approved this animal study. The experimental animal management was in accordance with the guidelines for the care and use of laboratory animals [15]. The male Sprague–Dawley (SD; 6-week-old) were purchased from BioLASCO (Taipei, Taiwan). Rats were individually maintained in cages at an environmental condition of 23 ± 1 °C and 40–60% relative humidity with a 12 h light/12 h dark cycle. Rats had a 1-week acclimation period and free access to a standard laboratory diet (5001 rodent diet, LabDiet, St. Louis, MO, USA) and deionized water. Rats were

randomly divided into four groups: normal control diet (5001 rodent diet; NC group), HF diet (HF group), HF diet + 5% GHE (GHE group), and HF diet + 1% cholestyramine (CH group) and each group fed the experimental diets for 8 weeks. The compositions of these experimental diets were listed in Table 1. Rats were free access to diet and water during the experimental period. Body weight was weighed per week. During the final 3 days in week 8, fecal samples were collected, which were further dried and weighed. Cholestyramine, a bile acid sequestrant, was as a positive control for hypolipidemic function.

2.4. Collection of samples from blood and tissues

Rats were euthanized under anesthesia at the end of the experiment. Blood, liver, and perirenal and para-epididymal adipose tissues were collected. The preparation of plasma was performed by centrifugation at 1750×*g* for 20 min (4 °C). All samples were immediately frozen and stored at −80 °C until further analysis.

2.5. Analysis of plasma lipids, lipoproteins, and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Both plasma TC and TG levels were analyzed by using the enzymatic assay kits for TC and TG (Audit Diagnostics). A density gradient by an ultracentrifuge (Hitachi, Tokyo, Japan) with 194,000×*g* at 10 °C for 3 h was used to isolate and analyze the plasma low-density lipoprotein (LDL), high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL); the lipoproteins were then collected by tube slicing. The AST and ALT activities were determined by the AST and ALT enzymatic assay kits (Randox). The absorbance at 340 nm was determined by a Hitachi U2800A spectrophotometer (Tokyo, Japan).

Table 1. Composition of experimental diets (%).

Ingredient (%)	NC	HF	GHE	CH
Chow diet	100	89.3	82.8	89.3
Lard		10	11.5	10
Cholesterol		0.5	0.5	0.5
Cholic acid		0.2	0.2	0.2
Gelidium amansii hot-water extract			5	
Total	100	100	100	100
Cholestyramine				1
Total calories (kcal/100g)	336.20	394.7	396.4	394.7
Carbohydrate (% kcal)	57.94	44.07	43.21	44.07
Protein (% kcal)	28.67	21.81	20.14	21.81
Fat (% kcal)	13.39	34.12	36.65	34.12

NC: Normal control diet (Chow diet); HF: High fat diet (Chow diet + 10% lard); GHE: High fat diet + *Gelidium amansii* hot-water extract; CH: High fat diet + 1% Cholestyramine.

2.6. Analysis of liver lipids and fecal lipids and bile acid

The extractions of both liver and fecal lipids were performed as previously described by Folch et al. [16]. Both TG and TC levels were analyzed as previously described by Carlson and Goldfarb [17]. The extraction and detection of fecal bile acids were determined as previously described by Cheng and Lai [18].

2.7. Detection of lipolysis rate

The detection of lipolysis rate was performed as previously described by Berger and Barnard [19]. Briefly, the samples of adipose tissues (0.2 g) were minced, and then incubated in 2 mL of 25 mM N-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.4) containing 1 μM isoproterenol at 37 °C. The glycerol levels were determined by a glycerol assay kit (Randox Laboratories) after 1, 2, and 3 h of incubation. The absorbance at 520 nm was measured by a Hitachi U2800A spectrophotometer. The equation of micromoles of glycerol released per gram of adipose tissue per hour was used to indicate the lipolysis rate.

2.8. Detection of lipoprotein lipase (LPL) activity

The activity of LPL in the adipose tissues was analyzed as previously described by Kusunoki et al. [20]. Briefly, the samples of adipose tissues (0.1 g) were minced, and then incubated in Krebs–Ringer bicarbonate buffer (pH 7.4) containing 10 units/mL heparin for 60 min at 37 °C. The heparin solution was reacted with an equal volume of p-nitrophenyl butyrate (2 mM). The absorbance at 400 nm was measured by a Hitachi U2800A spectrophotometer. The amount of p-nitrophenol formation over the 10 min incubation was used to indicate the LPL activity.

2.9. Histological examination of liver

The hepatic histological examination was performed as previously described [21]. The 5-μm thick paraffin sections of liver samples were used to stain hematoxylin and eosin (H&E). A photo microscope (Nikon Eclipse TS100, Nikon Instruments, Melville, NY, USA) equipped with a digital camera (Nikon

D5100, Nikon Instruments) was used to observe and image the stained tissue sections.

2.10. Measurement of hepatic acetyl-CoA carboxylase (ACC) activity

The analysis of ACC activity was determined as previously described [22]. Briefly, the reagents (50 mM Tris–HCl buffer, 10 mM $MgCl_2$, 10 mM potassium citrate, 3.75 mM glutathione, 12.5 mM $KHCO_3$, 0.675 mM BSA, 0.125 mM acetyl-CoA, 3.75 mM ATP, liver cytosol preparations, and 10 mM NADPH) were mixed and reacted in 96-well microplates. The absorbance at 340 nm was measured by a VersaMax microplate reader (Molecular Devices, San Jose, CA, USA).

2.11. Measurement of hepatic fatty acid synthase (FAS) activity

The analysis of ACC activity was determined as previously described [22]. Briefly, the reagents (0.2 M K_2HPO_4 buffer, 20 mM dithiothreitol (DTT), 0.25 mM acetyl-CoA, 60 mM EDTA·2Na, 0.39 mM malonyl-CoA, liver cytosol preparations, and 6 mM NADPH) were mixed and reacted in 96-well microplates. The absorbance at 340 nm was measured by a VersaMax microplate reader (Molecular Devices, San Jose, CA, USA).

2.12. Measurement of hepatic HMG-CoA reductase (HMGCR) activity

The preparation of liver microsomes fraction was performed as previously described by Krüner and Westernhagen [23]. Briefly, the reagents (0.2 M KCl, 0.16 M KH_2PO_4, 0.004 M EDTA, 0.01 M DTT, 0.1 mM HMG-CoA, liver microsomal preparations, and 0.2 mM NADPH) were mixed and reacted in 96-well microplates. The absorbance at 340 nm was measured by a VersaMax microplate reader (Molecular Devices, San Jose, CA, USA).

2.13. Immunoblot analysis

The analysis of protein expression by Western blot was performed as previously described [10,21]. Briefly, the proteins in the livers were lysed and extracted by a RIPA lysis and extraction buffer (Thermo Fisher Scientific). The proteins (50–100 μg) were added into 8% or 10% sodium dodecyl sulfate polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories). After blocking for 1 h, the membranes were reacted overnight at 4 °C with primary antibodies for AMPK, phospho-AMPK (Thr172) (Cell Signaling Technology), farnesoid X receptor (FXR), peroxisome proliferator activated receptor (PPAR)-α, PPAR-γ, low density lipoprotein receptor (LDLR), cytochrome P450-7A1 (CYP7A1), and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and then incubated with horseradish peroxidase linked secondary antibodies for 1 h at room temperature. The cross-reactivity was determined by an enhanced chemiluminescence kit (PerkinElmer). The densitometric analysis was determined by an ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.14. Statistical analysis

Data are presented as mean ± standard deviation (S.D.). The statistical analysis was assessed by one-way analysis of variance (ANOVA) and post-hoc Duncan test using a statistical software IBM SPSS statistics 22.0 (Armonk, NY, USA). The $p < 0.05$ is considered as statistically significant difference.

Table 2. Analysis of carbohydrate content and monosaccharide composition of Gelidium amansii hot-water extract (GHE).

General composition	GHE (%)[a]
Moisture	6.5
Protein	6.7
Total lipids	0.25
Ash	4.6
Nitrogen free extract	81.95
Sugar	**GHE (per mg)[b]**
Total sugar	0.84 mg
Reduced sugar	0.79 mg
Sulfate content	4.11%
Carbohydrate content	**GHE (%)[a]**
Carbohydrate polymers	68.54
Molecular weights of major component in polysaccharides[c]	**GHE (kDa)**
	892
Monosaccharide Composition[d]	**GHE (%)[a]**
Galactose	86.0
Fucose	8.3
Glucuronic acid	2.0
Mannose	1.5
Xylose	1.1
Glucose	0.6
Rhamnose	0.5

[a] Data are cited from our previous study by Yang et al. (2019) [10].
[b] Partial data are cited from our previous study by Yang et al. (2017) [9].
[c] The molecular weight of polysaccharide was analyzed by HPLC.
[d] Values for monosaccharide composition analysis were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

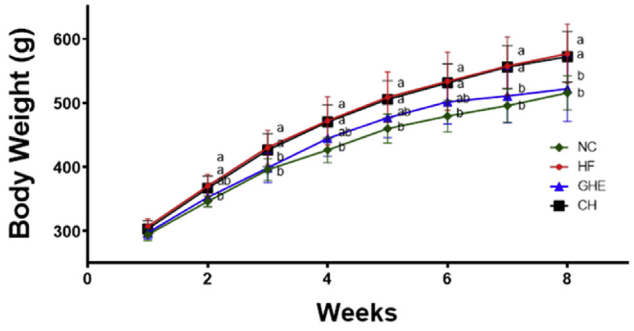

Fig. 1. Effects of GHE on body weight in SD rats fed different experimental diets for 8 weeks. Data are presented as mean ± SD for each group (n = 8). Values with different letters indicate statistical significance ($p < 0.05$). NC: Normal control diet; HF: High-fat diet; GHE: High-fat diet + Gelidium amansii hot-water extract; CH: High-fat diet +1% Cholestyramine.

3. Results

3.1. Analysis of polysaccharide content, molecular weight, and monosaccharide composition in GHE

We have demonstrated that GHE contains 6.5% moisture, 4.6% ash, 0.25% crude fat, 6.7% crude protein, and 81.95% nitrogen free extract [9]. We have also found that GHE contains 68.54% carbohydrate polymers and 86.0% Galactose, which is a major monosaccharide of the water-soluble indigestible polysaccharides from GHE [10]. We further found that GHE contained 4.11% of sulfate content in sugar. The polysaccharide samples had three main major components with retention time at 6.00, 8.49, and 9.15 min, and their molecular weights were estimated as 892, 26.5, and 10.5 kDa, respectively. These data were summarized in Table 2.

3.2. Effects of GHE on body and tissue weights and plasma, liver, and fecal lipids and biochemistry in HF diet-fed rats

Male Sprague–Dawley rats were divided into: normal control diet group, HF diet group, HF diet+5% GHE group, and HF diet+1% cholestyramine (as a positive control) group. As shown in Fig. 1 and Table 3, there was a significant increase in body weight in rats fed a HF diet for 8 weeks as compared to the normal control diet (NC) group. Supplementation of GHE, but not cholestyramine, significantly decreased body weight in HF diet-fed rats. The average food intake in the HF group was significantly lower than that in the NC group, but the feed efficiency ratio in the HF group was significantly higher than that in the NC group (Table 3). Supplementation of GHE, but not cholestyramine, significantly reduced the food intake in HF diet-fed rats, although it did not affect the feed efficiency ratio (Table 3). Moreover, the liver weights were significantly increased in HF diet-fed rats, which could be significantly reversed by both GHE and cholestyramine supplementation (Table 3). Supplementation of GHE, but not cholestyramine, could significantly reduce the increased adipose tissues weights in HF diet-fed rats (Table 3).

The changes in the levels of plasma lipids, AST/ALT, and glucagon like peptide-1 (GLP-1) were shown in Table 4. The levels of Total cholesterol (TC), VLDL-C, LDL-C, TC/HDL-C ratio, AST, and ALT were significantly increased in HF diet-fed rats, which could be significantly reversed by both GHE and cholestyramine supplementation. The decreased HDL-C/(LDL-C + VLDL-C) ratio could also be significantly reversed by GHE, but not

Table 3. Effects of GHE on body weights, food intake and tissue weights in rats fed with HF diets for 8 weeks.

Parameters	NC	HF	GHE	CH
Initial body weight (g)	233.5 ± 8.8	239.4 ± 8.5	234.0 ± 6.9	237.6 ± 8.0
Final body weight (g)	507.1 ± 23.8^{b}	573.4 ± 47.9^{a}	519.1 ± 47.6^{b}	570.4 ± 36.1^{a}
Body weight gain (g)	273.6 ± 24.8^{b}	334.0 ± 42.1^{a}	285.1 ± 45.5^{b}	332.8 ± 36.6^{a}
Food intake (g/day)	30.6 ± 1.89^{a}	28.5 ± 1.9^{b}	26.0 ± 2.1^{c}	27.2 ± 1.5^{bc}
Feed efficiency ratio[1]	9.0 ± 1.0^{b}	11.7 ± 1.3^{a}	10.9 ± 1.3^{a}	12.21 ± 0.9^{a}
Liver weight (g)	15.2 ± 1.1^{c}	31.8 ± 5.3^{a}	26.7 ± 3.6^{b}	26.8 ± 2.8^{b}
Relative liver weight (g/100 g BW)	3.0 ± 0.2^{c}	5.5 ± 0.5^{a}	5.1 ± 0.4^{b}	4.7 ± 0.4^{b}
Perirenal adipose weight (g)	7.6 ± 1.8^{b}	13.2 ± 3.0^{a}	8.2 ± 3.1^{b}	9.8 ± 5.0^{ab}
Epididymal adipose weight (g)	6.7 ± 1.4^{b}	10.0 ± 1.9^{a}	6.6 ± 1.7^{b}	9.1 ± 2.8^{a}
White adipose tissue weight (g)	14.4 ± 2.4^{b}	23.1 ± 3.7^{a}	14.8 ± 4.8^{b}	18.8 ± 7.7^{ab}
Relative white adipose tissue weight (g/100 g BW)	2.8 ± 0.5^{b}	4.0 ± 0.6^{a}	2.8 ± 0.7^{b}	3.3 ± 1.2^{ab}

Data are presented as mean ± SD for each group (n = 8). [1]Feed efficiency ratio = [body weight gain (g/day) ÷ food intake (g/day)]. Significant differences were determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple rand test. Values with different letters indicate statistical significance ($p < 0.05$). NC: Normal control diet (Chow diet); HF: High fat diet (Chow diet + 10% lard); GHE: High fat diet + *Gelidium amansii* hot-water extract; CH: High fat diet + 1% Cholestyramine.

Table 4. Effects of GHE on plasma lipids, AST/ALT, and GLP-1 in rats fed with HF diets for 8 weeks.

Parameters	NC	HF	GHE	CH
Total cholesterol (mg/dL)	51.0 ± 14.1^{b}	78.8 ± 23.9^{a}	56.9 ± 21.0^{b}	47.1 ± 12.4^{b}
HDL-C (mg/dL)	37.1 ± 8.8^{a}	25.4 ± 7.5^{b}	25.8 ± 7.5^{b}	21.4 ± 3.6^{b}
VLDL-C (mg/dL)	7.8 ± 2.1^{b}	23.8 ± 7.1^{a}	14.1 ± 11.0^{b}	12.3 ± 7.7^{b}
LDL-C (mg/dL)	6.1 ± 3.9^{b}	29.6 ± 18.2^{a}	17.0 ± 12.0^{b}	13.4 ± 2.9^{b}
LDL-C + VLDL-C	13.9 ± 3.7^{b}	53.5 ± 22.8^{a}	31.1 ± 17.9^{b}	25.7 ± 9.6^{b}
TC/HDL-C ratio	1.4 ± 0.2^{c}	3.3 ± 1.1^{a}	2.2 ± 0.7^{b}	2.2 ± 0.3^{b}
HDL-C/(LDL-C + VLDL-C) ratio	2.8 ± 0.6^{a}	0.5 ± 0.2^{c}	1.0 ± 0.4^{b}	0.9 ± 0.3^{bc}
Triglyceride (mg/dL)	36.5 ± 12.9^{a}	33.9 ± 13.2^{a}	18.2 ± 8.0^{b}	27.7 ± 11.3^{ab}
AST (U/L)	50.1 ± 11.4^{b}	94.6 ± 24.3^{a}	42.7 ± 10.7^{b}	46.5 ± 13.7^{b}
ALT (U/L)	40.7 ± 15.4^{b}	67.7 ± 35.1^{a}	35.1 ± 6.3^{b}	43.9 ± 13.3^{b}
GLP-1 (pM)	5.2 ± 1.1^{b}	5.4 ± 1.5^{b}	12.0 ± 11.3^{a}	4.5 ± 0.9^{b}

Data are presented as mean ± SD for each group (n = 8). Values with different letters indicate statistical significance ($p < 0.05$). NC: Normal control diet; HF: High-fat diet; GHE: High-fat diet + *Gelidium amansii* hot-water extract; CH: High-fat diet +1% Cholestyramine.

Table 5. Effects of GHE on hepatic and fecal lipid profile in rats fed with HF diets for 8 weeks.

Parameters	NC	HF	GHE	CH
Liver				
Total cholesterol (mg/g liver)	4.53 ± 2.17^{c}	102.23 ± 26.13^{a}	57.93 ± 15.29^{b}	68.62 ± 14.15^{b}
Total cholesterol (g/liver)	0.07 ± 0.04^{c}	3.30 ± 1.19^{a}	1.55 ± 0.53^{b}	1.85 ± 0.46^{b}
Triglyceride (mg/g liver)	14.66 ± 6.60^{c}	103.73 ± 11.85^{a}	82.82 ± 21.22^{b}	86.57 ± 14.20^{b}
Triglyceride (g/liver)	0.22 ± 0.10^{c}	3.28 ± 0.57^{a}	2.25 ± 0.79^{b}	2.35 ± 0.63^{b}
Feces				
Total cholesterol (mg/g feces)	4.9 ± 0.7^{d}	10.1 ± 0.9^{a}	7.4 ± 0.9^{b}	6.2 ± 1.1^{c}
Total cholesterol (mg/day)	30.5 ± 6.1^{c}	58.1 ± 9.2^{a}	47.8 ± 7.5^{b}	31.1 ± 7.1^{c}
Triglyceride (mg/g feces)	3.4 ± 0.5^{a}	3.0 ± 0.7^{a}	3.6 ± 0.7^{b}	2.7 ± 0.6^{ac}
Triglyceride (mg/day)	20.5 ± 2.6^{ab}	17.8 ± 5.7^{ac}	22.3 ± 2.72^{b}	13.9 ± 2.9^{ac}
Bile acid (μmol/day)	2.8 ± 1.9^{ab}	1.6 ± 1.0^{b}	4.2 ± 3.1^{a}	4.1 ± 2.1^{a}

Data are presented as mean ± SD for each group (n = 8). Values with different letters indicate statistical significance ($p < 0.05$). NC: Normal control diet; HF: High-fat diet; GHE: High-fat diet + *Gelidium amansii* hot-water extract; CH: High-fat diet +1% Cholestyramine.

cholestyramine, supplementation in HF diet-fed rats. GHE, but not cholestyramine, supplementation could significantly decrease the levels of plasma triglyceride (TG) and significantly increased the levels of GLP-1 in HF diet-fed rats.

The changes in the contents of hepatic and fecal lipids were shown in Table 5. The contents of TC and TG in the liver of HF diet-fed rats were significantly enhanced, which could be significantly reversed by both GHE and cholestyramine supplementation. Both GHE and cholestyramine supplementation significantly decreased the fecal TC contents, but only GHE supplementation significantly increased the fecal TG contents in HF diet-fed rats. Moreover, both GHE and cholestyramine supplementation could also significantly increase the fecal bile acid contents in HF diet-fed rats (Table 5).

We further observed the hepatic morphology for lipid accumulation. As shown in Fig. 2, the morphology of hepatic cells in the NC group were complete and compact; but in the HF group, there were obvious fat vacuoles and the morphology of hepatic cell was incomplete and the nuclei were squeezed to the edge of the cells, which could be significantly improved by both GHE and cholestyramine supplementation.

3.3. Effects of GHE on adipose tissue TG contents and lipolysis rate and lipoprotein lipase (LPL) activity and hepatic lipid metabolism-related protein expressions in HF diet-fed rats

Rats fed a HF diet showed significantly increased adipose tissue TG contents and significantly decreased the lipolysis rate, which could be significantly reversed by GHE supplementation (Fig. 3A, 3B, and 3D). Cholestyramine supplementation could also improve the increased perirenal adipose tissue TG contents and the decreased perirenal adipose tissue lipolysis rate in HF diet-fed rats (Fig. 3B and 3D). Both GHE and cholestyramine supplementation did not affect the LPL activity in the adipose tissues of HF diet-fed rats (Fig. 3C).

We further investigated the activities of the key enzymes for triglyceride synthesis [acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)] and the enzyme of rate-limiting step for cholesterol synthesis [HMG-CoA reductase (HMGCR)] in the livers. As shown in Fig. 4, rats fed a HF diet

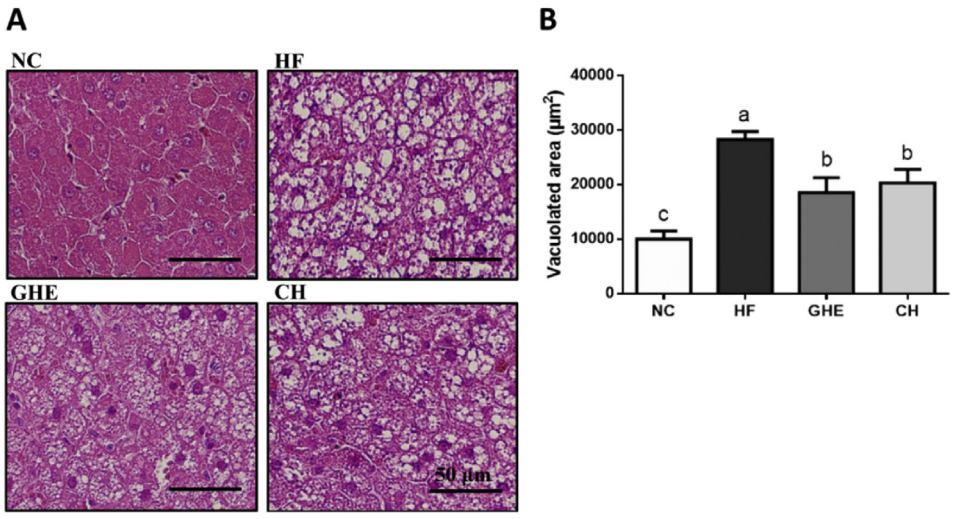

Fig. 2. Effects of GHE on hepatic morphology in rats fed different experimental diets for 8 weeks. (A) Representative hematoxylin and eosin (H&E) stained images in the livers were shown. Scale bar: 50 μm. (B) The fat vacuoles in the livers were quantified. Data are presented as mean ± SD for each group (n = 8). Values with different letters indicate statistical significance (p < 0.05). NC: Normal control diet; HF: High-fat diet; GHE: High-fat diet + Gelidium amansii hot-water extract; CH: High-fat diet +1% Cholestyramine.

exhibited significantly increased enzyme activities of ACC, FAS, and HMGCR in the livers, which could be significantly reversed by GHE, but not cholestyramine, supplementation.

We next tested the lipid metabolism-related protein expressions in the liver. The levels of protein expression of phosphorylated adenosine monophosphate (AMP)-activated protein kinase (AMPK), farnesoid X receptor (FXR), and peroxisome proliferator-activated receptor (PPAR)-α were significantly decreased, and the protein expression of PPAR-γ was significantly increased in the livers of HF diet-fed rats, which could be significantly reversed by GHE supplementation (Fig. 5). Cholestyramine supplementation could improve the decreased protein expression of FXR and PPAR-α in the livers of HF diet-fed rats, but it did not affect the HF diet-induced alteration in phosphorylated AMPK and PPAR-γ protein expression in the rat livers (Fig. 5). Both GHE and cholestyramine supplementation could also increase the protein expression of low density lipoprotein receptor (LDLR) and cytochrome P450-7A1 (CYP7A1) in the livers of HF diet-fed rats (Fig. 5).

4. Discussion

In the present study, we demonstrate that GHE supplementation effectively ameliorates the altered plasma cholesterol and hepatic lipid homeostasis in a HF diet-fed rat model. GHE supplementation decreases plasma TC, TG, LDL-C, and VLDL-C levels, decreases adipose tissue TG levels, increases fecal TG and bile acid excretion, decreases the enzyme activities of hepatic ACC, FAS, and HMGCR, and induces the protein expression of hepatic phosphorylated AMPK, FXR, PPAR-α, LDL receptor, and CYP7A1 in HF diet-fed rats.

GHE supplementation has been shown to increase fecal cholesterol and bile acid contents, and decreases plasma LDL-C in HF diet-fed hamsters [9,10]. Similarly, the present study found that both GHE and cholestyramine supplementation increased fecal bile acid excretion and decreased plasma LDL-C in HF diet-fed rats. However, both GHE and cholestyramine supplementation significantly decreased fecal cholesterol contents in HF diet-fed rats. This is a different phenomenon from the finding in hamster. We speculated that the increased bile acid contents in the intestine may enhance to increase the absorption of cholesterol and to decrease fecal cholesterol contents. Cholestyramine, a bile acid chelator with positive charge, can combine with negatively charged bile acids in the intestine to form an insoluble complex, which is not absorbed and can be excreted [24]. This mechanism causes massive excretion of bile acids in feces that triggers a negative feedback to activate the LDLR activity, which enhances the uptake of blood cholesterol into the liver and reduces blood cholesterol levels; a feedback may further induce the activity of CYP7A1 in the liver and metabolize cholesterol into bile acid [24]. It has been shown that dietary fibers may reduce blood lipids by improving enterohepatic circulation [25]. Dietary fiber has also been shown to play a major role in regulating the

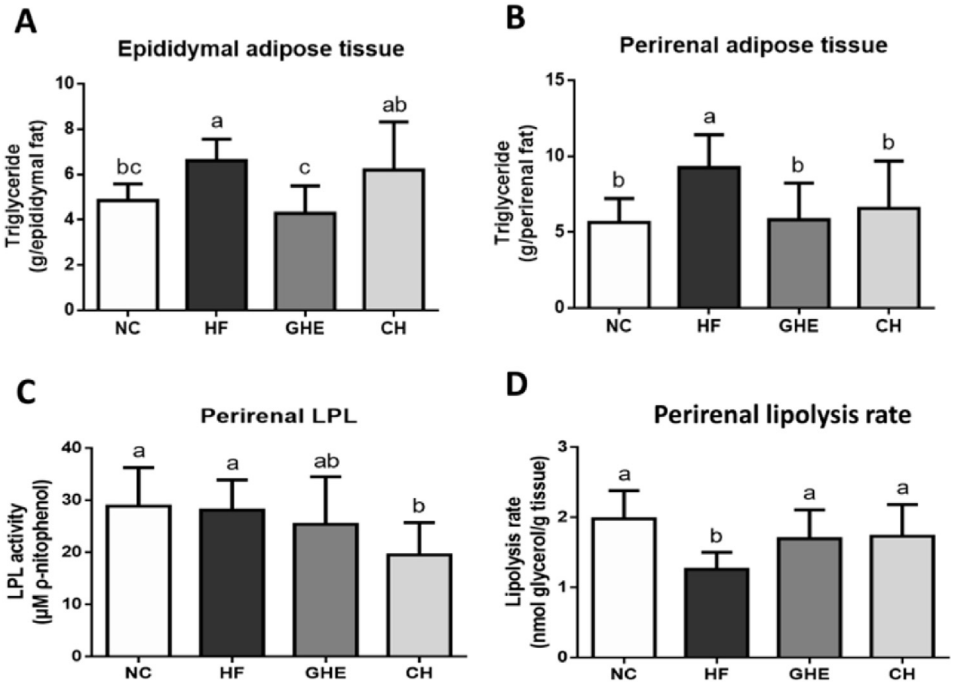

Fig. 3. Effects of GHE on adipose tissue triglyceride levels and lipoprotein lipase (LPL) activity and lipolysis rate in SD rats fed different experimental diets for 8 weeks. The levels of triglyceride in para-epididymal (A) and perirenal (B) adipose tissues were shown. The lipoprotein lipase (LPL) activity and lipolysis rate in perirenal adipose tissues were shown in (C) and (D), respectively. Data are presented as mean ± SD for each group (n = 8). Values with different letters indicate statistical significance (p < 0.05). NC: Normal control diet; HF: High-fat diet; GHE: High-fat diet + Gelidium amansii hot-water extract; CH: High-fat diet +1% Cholestyramine.

metabolism of bile acids [26]. In the present study, we found that HF diet-fed rats supplemented with both GHE and cholestyramine induced an increase in LDLR and CYP7A1 protein expressions. Therefore, dietary soluble fiber-rich GHE lowered plasma TC and LDL-C might be due to the increased excretion of bile acids and the increased LDLR and CYP7A1 protein expressions. However, the difference between GHE and cholestyramine supplementation is that GHE can inhibit the hepatic lipogenic enzyme activities such as FAS and ACC in HF diet-fed rats, while cholestyramine does not, which lead to decrease plasma TG in GHE group, but not in CH group.

FXR, a nuclear receptor protein, is mainly expressed in the liver and intestine, and has important regulatory functions for bile acid balance and liver lipid metabolism [27]. FXR can activate small heterodimer partner (SHP) to inhibit CYP7A1 expression and regulate bile acid synthesis. During liver lipid metabolism, FXR can also inhibit the activity of SREBP1c by activating SHP to reduce the

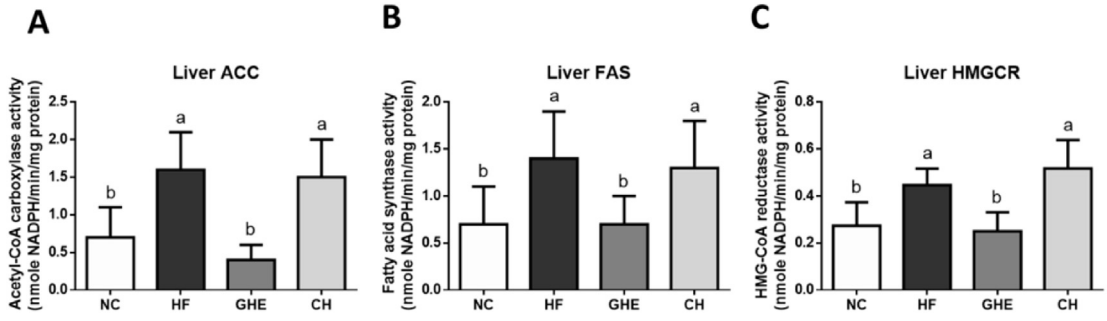

Fig. 4. Effects of GHE on hepatic enzyme activities of lipid biosynthesis in SD rats fed different experimental diets for 8 weeks. The activities of acetyl-CoA carboxylase (ACC) (A) fatty acid synthase (FAS) (B) and HMG-CoA reductase (HMGCR) (C) in the livers were shown. Data are presented as mean ± SD for each group (n = 8). Values with different letters indicate statistical significance (p < 0.05). NC: Normal control diet; HF: High-fat diet; GHE: High-fat diet + Gelidium amansii hot-water extract; CH: High-fat diet +1% Cholestyramine.

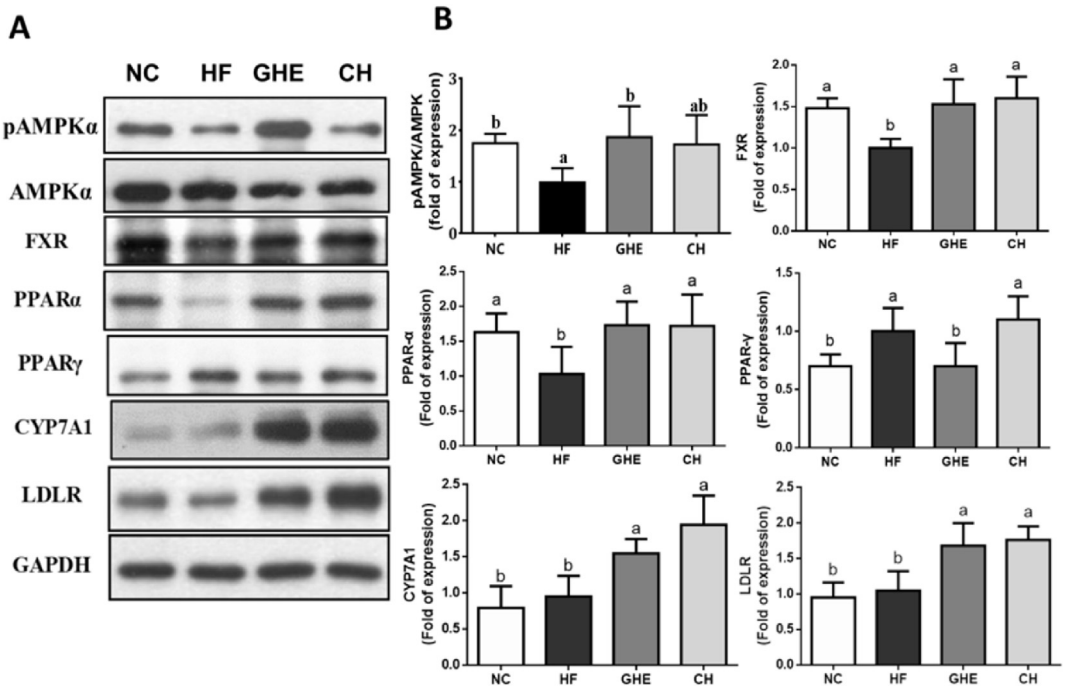

Fig. 5. Effects of GHE on hepatic lipid metabolism-related protein expression in SD rats fed different experimental diets for 8 weeks. (A) The levels of protein expression of pAMPKα/AMPK, FXR, PPARα, PPARγ, CYP7A1, and LDLR in the livers were measured by Western blotting. (B) Densitometric analyses for protein levels corrected to each internal control were shown. Data are presented as mean ± SD for each group (n = 4–6). Values with different letters indicate statistical significance (p < 0.05). NC: Normal control diet; HF: High-fat diet; GHE: High-fat diet + Gelidium amansii hot-water extract; CH: High-fat diet +1% Cholestyramine.

activity of lipid biosynthetic enzymes FAS and ACC, or directly activates PPARα to promote fatty acid oxidation [27] and activates LDLR [28] to reduce plasma LDL-C. In the present study, GHE supplementation induced the hepatic CYP7A1 protein expression and increased bile acid secretion, which may lead to a feedback regulation to increase the FXR protein expression to regulate the bile acid metabolism. The increased FXR by GHE supplementation could also activate PPARα to promote fatty acid oxidation. Furthermore, GHE supplementation could induce FXR and AMPK signaling activation to inhibiting SREBP1c and PPARγ signaling that further inhibited the activities of lipogenic enzymes such as ACC, FAS and HMGCR, and thereby reducing the production of TC and TG in the liver of HF diet-fed rats.

Increased body weight and adipose tissue and liver weights were observed in rats fed a HF diet. Supplementation of GHE induced a significant decrease in body weight and adipose tissue and liver weights. The lower body weight by GHE supplementation might be related to the decreased adipose tissue and liver weights. However, HF diet-fed rats supplemented with GHE had lower food intake that may be one of the reasons for lower body weight. Most of dietary fibers do not reduce appetite or energy intake, but some types and doses of dietary fibers are effective in reducing appetite and energy intake [29]. It has been shown that water-soluble dietary fibers can increase the concentration of GLP-1 [30,31]. GLP-1 can inhibit the satiety and food intake through acting on the hypothalamus of the central nervous system, and can increase satiety, suppress appetite, and delay gastric emptying [32,33]. In the present study, an increase in plasma GLP-1 level was observed in rats fed a HF diet with GHE supplementation. Thus, the lower food intake in rats fed a HF diet with GHE supplementation might be due to the increased GLP-1 level. Moreover, the lower adipose tissue weight in rats fed a HF diet with GHE supplementation might be due to the increased activity of adipose tissue hormone-sensitive lipase (HSL, increasing lipolysis rate) through AMPK activation, because the increased AMPK phosphorylation can promote HSL activation in adipose tissue [34].

Kang et al. (2016) have found that mice fed a HF diet treated with ethanol extract of GA (1 and 3%) for 12 weeks exhibit effectively decreased body weight, which may be due to decreased adipogenesis [8]. Yang et al. (2017) have reported that hamsters fed a HF diet supplemented with GHE (1.5%) for 6 weeks show significantly decreased

body weight and improved lipid metabolism, which GHE may activate AMPK and decrease SREBP-1 and SREBP-2 protein levels in the livers to reducing hepatic lipogenesis [9]. Moreover, Yang et al. (2019) have recently shown that supplementation with 3% GHE for 9 weeks in HF diet-induced obese hamsters, which previously feed a HF diet for 5 weeks to induce obesity, prevents against diet-induced obesity and altered TC and TG in the plasma and liver; they further demonstrated that GHE ameliorated the dysregulation of hepatic lipid metabolism through AMPK activation and up-regulation of PPARα and UCP-2 [10]. In the present study, a HF diet-fed rat model was used to demonstrate that supplementation of 5% GHE for 8 weeks ameliorated the altered plasma TC and hepatic lipid homeostasis by increasing hepatic LDLR and CYP7A1 expression and activating FXR and AMPK. These findings from different animal models suggest that ethanol or hot-water extracts of GA possess the potential for anti-obesity or preventing dysregulation of lipid metabolism from high-fat diet feeding.

5. Conclusions

Based on these results, GHE supplementation to HF diet-fed rats can interfere in cholesterol metabolism and increase hepatic LDLR and CYP7A1 expression to decrease blood cholesterol, and induce FXR and AMPK signaling activation to inhibit lipogenic enzyme activities and reduce the lipid accumulation in the livers. To clarify the preventive role of polysaccharides fraction of GHE in high-fat diet-induced alteration in plasma cholesterol and hepatic lipid homeostasis, the polysaccharides fraction by precipitating GHE with alcohol may be used to further investigation in the future.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

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