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Daily consumption of *Siraitia grosvenorii* alleviated lipid accumulation and colon barrier injury *via* modulation of gut microbiota in high fat and high sugar diet C57BL/6 mice

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ABSTRACT

Siraitia grosvenorii (SG), a traditional edible medicine in China, has shown potential application in health foods due to multiple bioactivities. However, few comparative studies have investigated the effect of daily intake of SG products by traditional drinking way on alleviating metabolic disorders caused by long-term high fat and high sugar diet (HF-HSD). This study investigated the effects of 2 commercial SG products (SG juice containing comprehensive compositions and mogrosides extract containing high content of mogroside V) supplement on fat content, lipid accumulation, colon barrier and gut microbiota in a 38-week HF-HSD mice trial. Results showed that the SG intervention could decrease the weight gain and body fat content, alleviated lipid accumulation, low-grade inflammation and colon barrier injury. SG juice intake was found to increase the antioxidant enzyme activities of serum glutathione peroxidase and liver superoxide dismutase. 16S rRNA analysis found that SG could restore the gut microbiota with downregulation of harmful bacteria increased by HF-HSD and enhancement of the abundances of beneficial genus. In particular, SG juice significantly enriched taxonomic family Prevotellaceae and genus *Alloprevotella*. These results indicated that SG might be a functional additive to prevent disease risk caused by excess fat and sugar diet, and active ingredients resulting to the discrepancy of performance especially on gut microbiota are worthy of in-depth investigation.

1. Introduction

With the improvement of living standard and the change of dietary habit, long-term excessive intake of fat and sugar has become one of the main factors threatening public health. It is believed that excess fat and sugar intakes increases the risk of abnormal lipid accumulation in the blood and liver which may aggravate hyperlipidemia, nonalcoholic fatty liver disease (NAFLD), cardiovascular diseases and diabetes[1-2]. As the micro-ecological systems, gut microbiota has been proven to be not only essential for

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intestinal digestion and nutrient absorption but also closely correlation with chronic metabolic disease. Thus, the composition of gut flora directly affects the health state of host[3-4]. Excess fat and sugar intakes cause harmful metabolite exposure to liver and intestine, thereby aggravating the liver injury and damage of intestinal integrity and permeability along with the decrease of beneficial bacteria but increase of harmful bacteria[5-6]. Therefore, it is necessary to quest effective as well as safe strategy to prevent diet-induced health status.

In recent years, functional food or additives aimed at contributing to the prevention of various diseases show promising market potential[7]. Compared with chemical drugs, edible medicinal herbs and their bioactive components are considered ideal functional elements to prevent and treat chronic disease[8], which can be used in daily diet with sustained beneficial effect and lower side influence on host health. *Siraitia grosvenorii* (SG), a kind of traditional Chinese medicinal plants with usage as a popular drink in life, is rich in polysaccharides, flavonoid glycosides and mogrosides[9]. Its high sweetness mogrosides have been approved as a healthy sweetener due to the merits of low calories and nontoxicity[10-11]. Pharmacological researches have shown that SG extract, SG polysaccharides and mogrosides exhibit diverse bioactivities including anti-inflammatory[12-14], antioxidant[15], liver protection[16-17], and effect on obesity and gut microbiota[18-19]. Due to its high sweetness and pleasant taste, SG and mogrosides are usually used as additives in drink or food. However, with the expansion of the usage scope and the risk of artificial sweetener, security and functional issues have become hot attention. Whether low-dose and long-term SG intake affects the health of people with excess fat and sugar dietary habit, and the mechanism still needs investigation. On the other hand, different processed products based on SG are commercially available, but their difference in bioactivities remains unknown.

In the present work, 2 representative SG products were chosen to discuss the beneficial effects on host health. The SG juice is produced from fresh SG fruits by water extract and contains comprehensive constituents, while the mogrosides extract with high content mogroside V is a deep processed product, in which some compositions have been removed during processing. We assessed their effects on weight management, fat and lipid accumulation, antioxidant enzyme activities, inflammatory cytokines, and intestinal barrier in high fat and high sugar diet (HF-HSD) fed C57BL/6 mice. Besides, 16S rRNA sequencing was adopted to analyze the alteration of gut microbiota.

2. Materials and methods

2.1 Materials

Standard chow diet and HF-HSD (70% chow diet, 10% sucrose, 10% lard and 10% yolk powder) were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). SG juice (MCF-N2.0, with 2% mogroside V content) and mogrosides extract (MFC-E50, with 50% mogroside V content) were purchased from Monk Fruit Crop. (Guilin, China). Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Mouse tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) ELISA kits were from Elabscience Biotechnology (Wuhan, China). Bicinchoninic acid (BCA) protein assay kit was purchased from Beyotime Biotechnology (Shanghai, China). Radio immunoprecipitation assay (RIPA) protein lysate was purchased from Solarbio (Beijing, China). Antibodies against zonula occludens-1 (ZO-1) and occludin were purchased from Wuhan Sanying (Wuhan, China). Claudin-1 antibody was from Abcam (Cambridge, UK), and *β*-actin was from ELK Biotechnology (Wuhan, China).

2.2 Mice experimental design

Male C57BL/6 mice ((21.0 ± 1.5) g, 6-week-old) were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). The mice were kept in an environment at (22 ± 2) ℃ and (55 ± 5)% relative humidity in a 12-h dark/12-h light cycle with free access to food and water. The protocol was approved by the Research Ethics Committee of the Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and the Chinese Academy of Science (approval No: GXZW-20220225-1).

After 1 week of adaptation, 32 mice were randomly classified into 4 groups (n = 8): control group, mice were fed a standard chow diet and purified water; model group, mice were fed HF-HSD and purified water; MV group, mice were fed HF-HSD and given 0.4 g/L mogrosides water; SG group, mice were fed HF-HSD and given SG water (SG juice:water = 10:1, m/V). All mice were free access to food and water. The food and water were replaced and documented every day, and the body weight was recorded twice a week. After 38 weeks, all mice were fasted for 12 h and fresh feces were collected, and then mice were anesthetized with diethyl ether. Serum samples were immediately collected from eyeball and centrifuged at 2 000 r/min for 20 min at 4 ℃. Livers, colons and adipose tissues were obtained, weighted or lengthed after dissection. Partial liver samples were stored in 4% paraformaldehyde, and all of the other samples were stored at –80 ℃ for further analysis.

2.3 Chemical components measurement

High performance liquid chromatography (HPLC) analysis of SG juice and mogrosides extract was performed on Poroshell 120 EC-C_{18} (4.6 mm × 150 mm, 4 μm) column with temperature 30 ℃. The HPLC analysis was conducted in gradient mode with flow rate of 0.8 mL/min. Acetonitrile and water were used as mobile phases A and B, respectively. The gradient condition was as follows: 0–8 min, 3.0%–13.5% A; 8–35 min, 13.5%–35.0% A; 35–40 min, 3% A. The injection volume was 5 μL and detecting wavelength was 203 nm. The total flavonoid content in product was determined based on reported method[20], and kaempferol was used to calibrate the standard curve. Total polysaccharide content was determined using the phenol-sulfuric acid method[21], and glucose was used to calibrate the standard curve.

2.4 Biochemistry analysis

Levels of TC, TG, HDL-C, LDL-C, AST, ALT, MDA, SOD and GSH-Px in serum or liver were determined using commercially kits. The levels of TNF-α and IL-6 in the liver and colon were measured using ELISA kits. Total proteins were measured *via* BCA protein assay kit.

2.5 Real-time fluorescent quantitative PCR

The total RNA of liver tissue was extracted using TRIpure reagent (ELK Biotechnology, EP013) in accordance with the manufacturer's recommendations. The cDNAs were obtained using the EntiLink™ 1st Strand cDNA Synthesis Super Mix (EQ031, ELK Biotechnology). Real-time fluorescent quantitative PCR was performed on QuantStudio 6 Flex System PCR instrument, and qPCR reactions were performed by EnTurbo™ SYBR Green PCR SuperMix Kit (ELK Biotechnology, EQ001). The relative expression of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers sequences (Table S1) were synthesized by Hycell Biotechnology.

2.6 Western blotting

For Western blotting analysis, 2 colon samples from the same group were mixed with same weight (20 mg + 20 mg). The sample was mixed with 200 μL RIPA reagent and proteins were extracted with a tissue homogenizer at 4 °C. Then the protein concentration was determined using a BCA kit. Equivalent amounts of protein samples were loaded on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel, and subsequently transferred to a polyvinylidene difluoride membrane. The membrane was incubated with primary antibodies against ZO-1, occludin, claudin-1 and β-actin overnight at 4 °C. Then, the secondary antibody diluted with the secondary antibody diluent was added, incubated at room temperature for 30 min, and washed 4 times with TBST at room temperature for 5 min each time. The freshly prepared chemilu minescence reagent from the ECL was added dropwise to the protein side of the membrane, exposed in the dark room and then fixed the film. The film was scanned, and the optical density value of the target band was analyzed by AlphaEaseFC software.

2.7 Histological analysis

The liver tissues were fixed in 4% paraformaldehyde for 24 h. Then, tissues were dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H&E) staining. Images were viewed with a light microscope equipped with a camera (BX43, OLYMPUS, Tokyo, Japan).

2.8 Gut microbiota analysis

Total genome DNA from fecal samples was extracted using CTAB/SDS method. DNA concentration and purity was monitored on 1% agarosegels. The samples were sent for sequencing on the Illumina Miseq platform (Shanghai Origingene Bio-pharm Technology Co., Ltd., Shanghai, China). The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified and all PCR reactions were carried out in 30 μL reactions with 15 μL of Phusion®High-Fidelity PCR Master Mix (New England Biolabs, Beijing, China). PCR products were purified according to the standard protocols. Sequencing libraries were generated using NEB Next®Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, Beijing, China) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific, Shanghai, China) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina MiSeq platform and 250/300 bp paired-end reads were generated. Sequences analysis was performed by UPARSE software package using the UPARSE-OTU and UPARSE-OTUref algorithms. In-house Perl scripts were used to analyze alpha and beta diversity.

2.9 Statistical analysis

Histogram and statistical significance were performed using GraphPad Prism 5 software. All data were shown as the mean ± standard error mean (SEM). Statistical significance was determined *via* one-way ANOVA with Newman-Keuls's test or *t*-test, and $P < 0.05$ was considered statistical significance. Different lowercase letters were considered to be statistically significant. Heatmap was analyzed using origin 2022.

3. Result

3.1 Characteristics of SG juice and mogrosides extract

For the reason to simulate acceptable sweetness in daily drink, the doses of SG group and MV group were respectively set up as 10 g/L and 0.4 g/L to give the same sensory sweetness, therefore the content of mogroside V which is the representative sweet composition was equivalent. The results demonstrated that there were distinct differences presented in the HPLC spectra (Fig. 1). In comparison with the drink in MV group, that of SG group showed more constituents within the retention time of 2.5–15 min, and the frontier peak was much higher. Considering the chromatographic fingerprint of SG[22] and hot water extracted method, the frontier peak might contain constituents like polysaccharides and flavonoid glycosides which were usually believed as main functional components of herbs. Therefore, total sugar and flavonoids in the 2 experimental drinks were further determined, which occupied 1 146 and 82 mg/L in SG group drink, but no sugar and trace flavonoids were detected in MV group drink.

3.2 Effects on weight gain, liver weight and fat accumulation

The body weight, food and water intake were recorded during the experiment (Figs. 2 and S1). As shown in Fig. 2A, the body weight of HF-HSD groups obviously increased from 8th week than control group. On average, the water intake of mice in the MV group was slightly higher than that in the SG group (Fig. S1B). After 38-week trial, the weight gain of control, model, MV and SG groups were (10.70 ± 1.03), (20.85 ± 2.02), (21.16 ± 1.61) and (17.98 ± 1.67) g, respectively (Fig. 2B). Though there was no markedly difference among the HF-HSD groups, the supplement of SG juice showed lower body weight. The liver weight in model group was (1.43 ± 0.07) g, which was higher than that in control group ((1.19 ± 0.06) g) (Fig. 2C). After intervention, liver weight of MV group and SG group decreased to (1.25 ± 0.04) and (1.24 ± 0.08) g, respectively. In comparison to the model group which showed higher fat accumulation of (1.09 ± 0.04) g of perirenal fat and (2.41 ± 0.14) g of epididymal fat than control group, the drinking of SG juice exhibited noticeable decline to (0.92 ± 0.14) and (1.91 ± 0.17) g ($P < 0.05$), while MV group showed slight decrease of fat content without significant difference, indicating that SG juice could alleviate body weight gain and fat accumulation induced by HF-HSD to a certain extent.

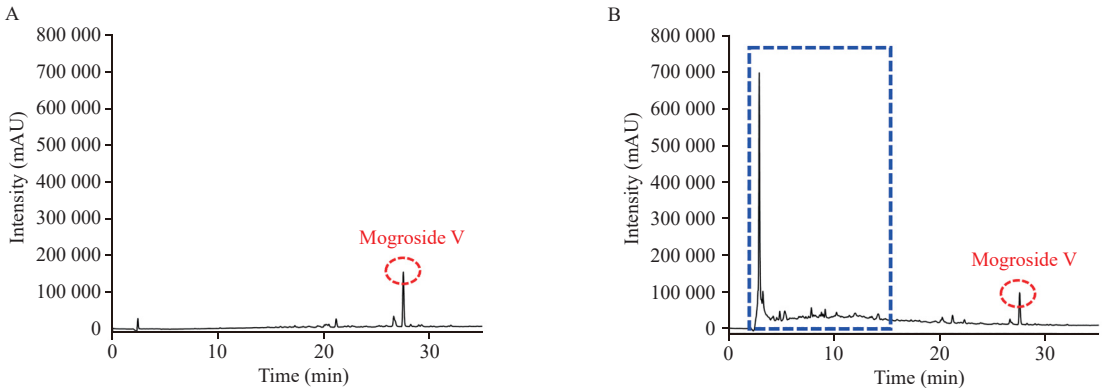

Fig. 1 HPLC spectra analysis from (A) mogrosides extract and (B) SG juice.

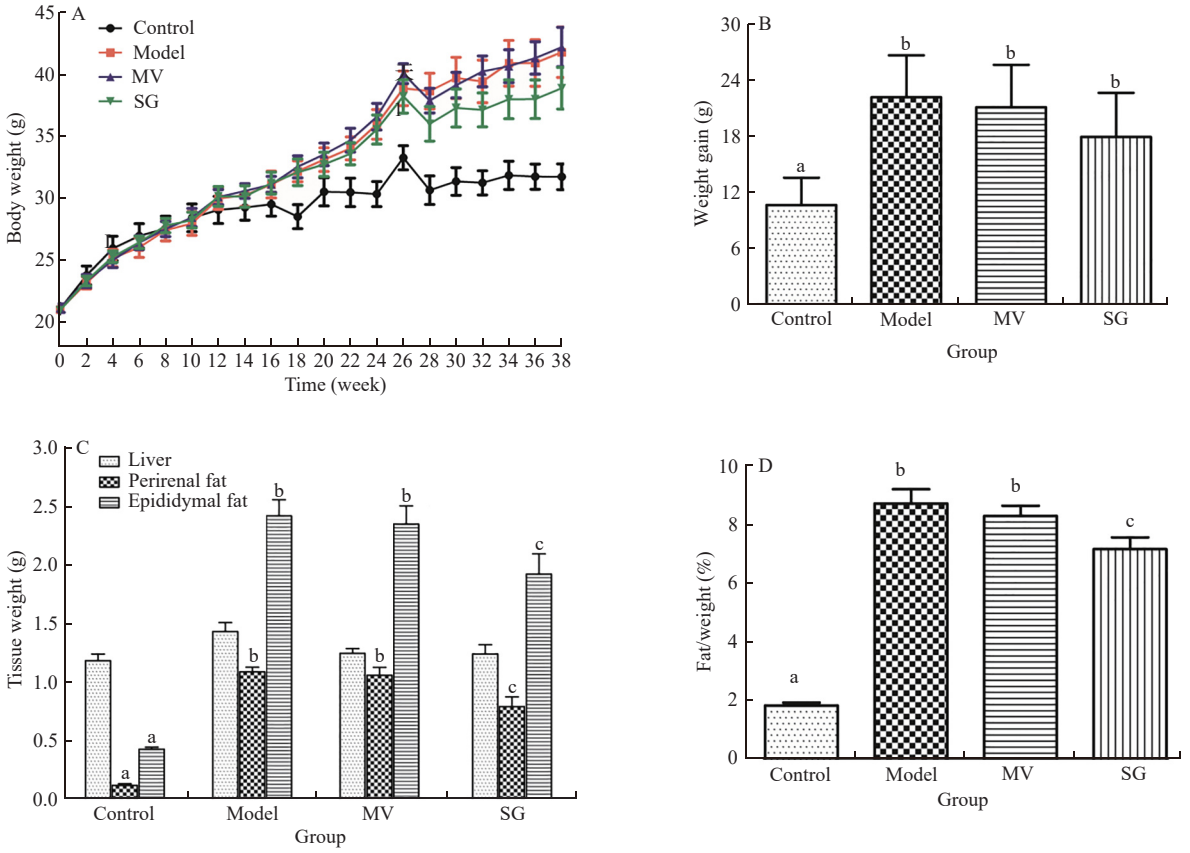

Fig. 2 (A) Body weight change. (B) Final weight gain. (C) Tissue weight. (D) Fat to weight ratio, fat = perirenal fat + epididymal fat. Data were shown as the mean ± SEM (n = 8). Different lowercase letters in same index indicated significant differences ($P < 0.05$).

3.3 Effects on lipid levels and genes related to lipid metabolisms

As shown in Table 1, long term HF-HSD increased the serum levels of TG, TC, LDL-C and HDL-C. Unexpectedly, there was no marked difference in TG and TC levels among 3 HF-HSD groups. However, the addition of mogrosides extract and SG juice significantly prevented elevation of LDL-C level. Treatment of mogrosides extract further increased the serum HDL-C level when compared to model group. As primary indicators for evaluating of liver function, level changes of AST and ALT in serum were also investigated. It was noteworthy that mogrosides extract and SG juice feeding reversed the enhancement of AST and ALT levels induced by HF-HSD. As expected, the liver TG and TC levels in model group with the values of (0.496 ± 0.043) and (67.74 ± 3.59) mmol/g pro were much higher than those in control group ((0.192 ± 0.022) and (53.62 ± 1.99) mmol/g pro). Elevated indexes were reversed to (0.355 ± 0.046) and (0.343 ± 0.025) mmol/g pro for TG and (61.65 ± 2.96) and (56.31 ± 1.45) mmol/g pro for TC upon the mogrosides extract and SG juice treatment, respectively.

In addition, we investigated the expression of lipid metabolism-related genes in the liver tissue by real-time fluorescent quantitative PCR. As shown in Figs. 3A-C, the relative expression of acetyl CoA carboxylase1 (*ACC1*) and fatty acid synthase (*FAS*) were significantly increased after HF-HSD treatment, but decreased by 20.5% and 18.8% for MV group and 30.8% and 24.6% for SG group when

Table 1
Lipid levels in serum and liver in each group.

Index	Control group	Model group	MV group	SG group
Serum TG (mmol/L)	0.383 ± 0.078	0.735 ± 0.036$^{\#\#}$	0.812 ± 0.099	0.645 ± 0.053
Serum TC (mmol/L)	2.022 ± 0.037	3.373 ± 0.089$^{\#\#\#}$	3.290 ± 0.088	3.150 ± 0.050
Serum LDL-C (mmol/L)	0.875 ± 0.058	1.919 ± 0.124$^{\#\#\#}$	1.213 ± 0.027***	1.540 ± 0.068*
Serum HDL-C (mmol/L)	2.383 ± 0.074	2.884 ± 0.066$^{\#\#\#}$	3.255 ± 0.099**	2.938 ± 0.112
Serum AST (U/L)	28.31 ± 2.61	31.81 ± 2.41	20.21 ± 1.05**	25.99 ± 1.81
Serum ALT (U/L)	32.63 ± 4.86	43.11 ± 3.29	27.20 ± 1.85**	29.57 ± 2.12**
Liver TC (mmol/g pro)	53.62 ± 1.99	67.74 ± 3.59$^{\#\#}$	61.65 ± 2.96	56.31 ± 1.45*
Liver TG (mmol/g pro)	0.192 ± 0.022	0.496 ± 0.043$^{\#\#\#}$	0.355 ± 0.046*	0.343 ± 0.025*

Note: Data were shown as mean ± SEM (n = 6–7). The t-test was used to evaluate the difference between control and model groups, or model and sample groups. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs*. control group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. model group.

compared to model group. Considering the lower LDL-C level after the treatment of SG products, the effect on low-density lipoprotein receptor (*LDL-r*) was also assayed. It could be seen that the relative expressions of *LDL-r* were remarkably increased upon mogrosides extract or SG juice treatment. Less lipid blot in MV and SG groups from liver histopathology analysis (Figs. 3D and S2) further indicated that they inhibited the lipid accumulation caused by HF-HSD.

3.4 Effects on oxidative stress and inflammatory cytokine

Liver damage might cause increasing oxidative stress, thus the MDA and antioxidant enzyme activity were assayed. As shown in Figs. 4A-E, mean values of serum MDA and SOD levels in 3 HF-HSD groups were slightly elevated but with no significant differences. Compared with control group, the serum levels of SOD in MV and SG groups were higher. When compared with model group, SG juice intervention remarkably elevated serum GSH-Px level, but mogrosides extract reduced the level. There were no significant differences in liver MDA among the 4 groups, but the liver SOD level in MV and SG groups increased distinctly.

Low-grade inflammation often occurs with a HFD, thus, the inflammatory cytokine levels of IL-6 and TNF-α in liver and colon tissues were measured (Figs. 4F-I). Model group showed increasing levels of liver TNF-α and IL-6 with values of (1.81 ± 0.21) ng/mg pro and (0.31 ± 0.03) μg/mg pro, respectively, compared with control group ((1.23 ± 0.15) ng/mg pro and (0.24 ± 0.98) μg/mg pro). It was noteworthy that mogrosides intervention reduced the secretions of TNF-α and IL-6 to (1.14 ± 0.07) ng/mg pro and (0.24 ± 0.03) μg/mg pro in liver, which were close to the levels in control group. Meantime, the long-term addition of SG juice decreased level of TNF-α in liver to (1.63 ± 0.04) ng/mg pro and IL-6 to (0.26 ± 0.04) μg/mg pro. Despite no significant difference for levels of TNF-α and IL-6 in colon tissue were exhibited among the 4 groups, the mean values in model group were enhanced and mogrosides extract and SG juice feeding could still downregulate the increasing tendency.

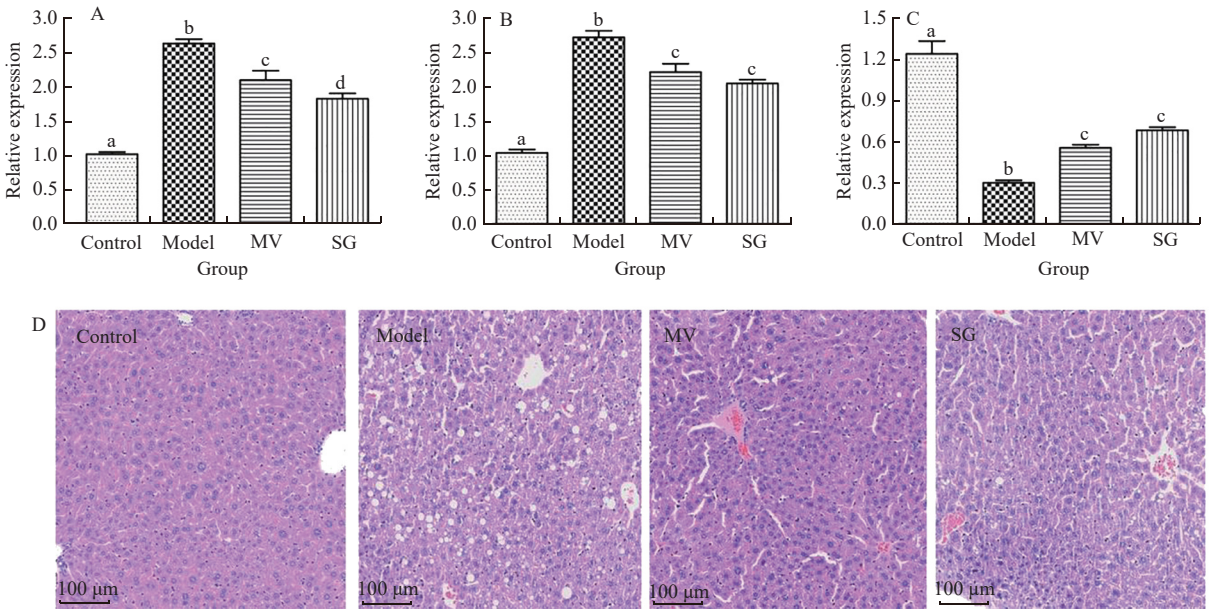

Fig. 3 Effect on mRNA relative expression of genes related to lipid metabolisms in liver tissue. Relative expression of (A) *ACC1*; (B) *FAS*; (C) *LDL-r*. (D) Representative images of H&E staining of liver paraffin sections (20×, scale bar = 100 μm). Data were shown as the mean ± SEM (n = 4). Different lowercase letters indicated significant differences ($P < 0.05$).

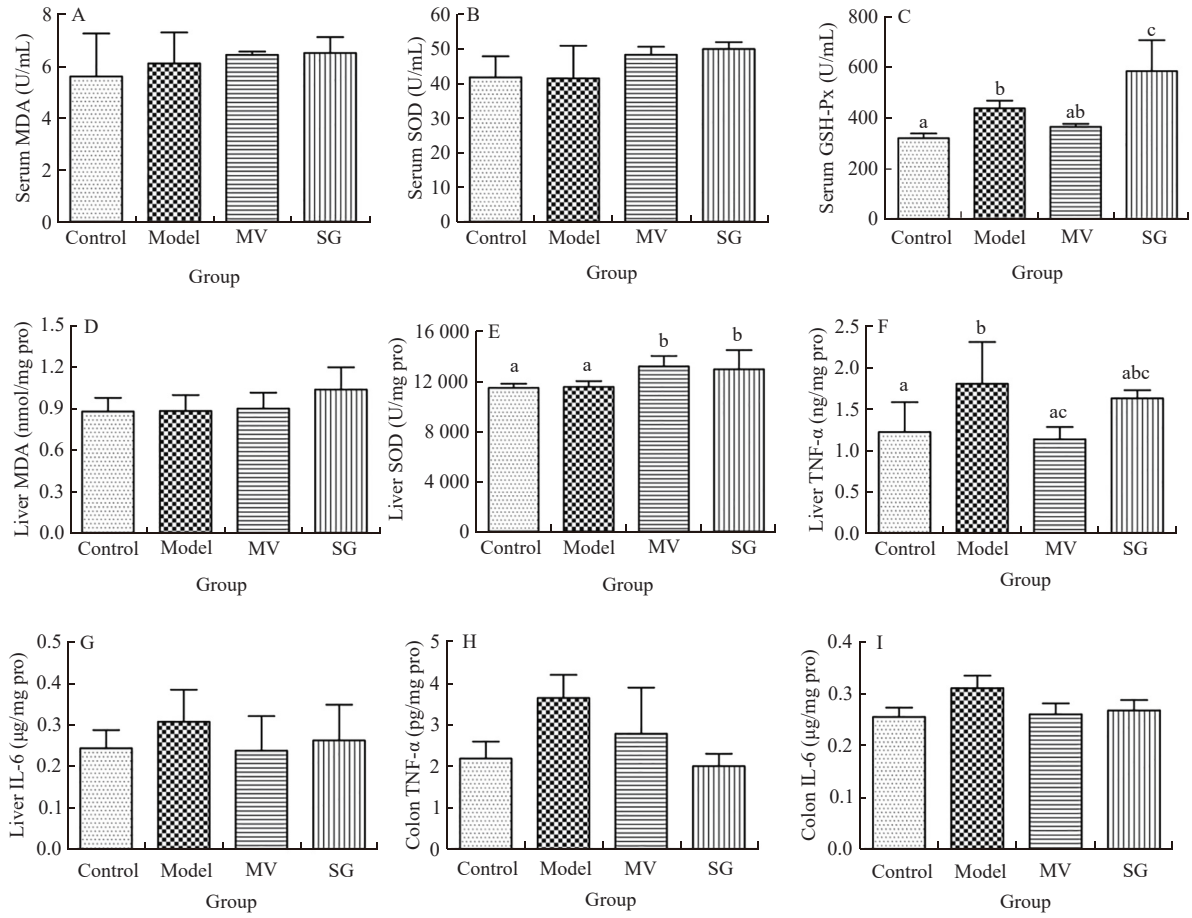

Fig. 4 Effects on oxidative stress and inflammatory cytokine. Levels of (A) serum MDA, (B) serum SOD, (C) serum GSH-Px, (D) liver MDA, (E) liver SOD, (F) liver TNF-α, (G) liver IL-6, (H) colon TNF-α, and (I) colon IL-6. Data were shown as the mean ± SEM (n = 5–7). Different lowercase letters indicated significant differences ($P < 0.05$).

3.5 Effect on colonic barrier

As shown in Figs. 5A and S3, the colon lengths in control, model, MV and SG groups were (7.35 ± 0.50), (6.56 ± 0.17), (6.68 ± 0.17) and (6.96 ± 0.14) cm, respectively, indicating HF-HSD without MV and SG intervention shorted colon length. To explore the protective effect on colonic barrier of mogrosides extract and SG juice, the tight junction protein expressions of ZO-1, occludin and claudin-1 were investigated (Figs. 5B and S4). The Western blotting results showed that HF-HSD significantly reduced the protein expressions of ZO-1, occludin and claudin-1 when compared with control group. Both treatments could restore the colonic barrier by increasing the expression levels of the 3 proteins.

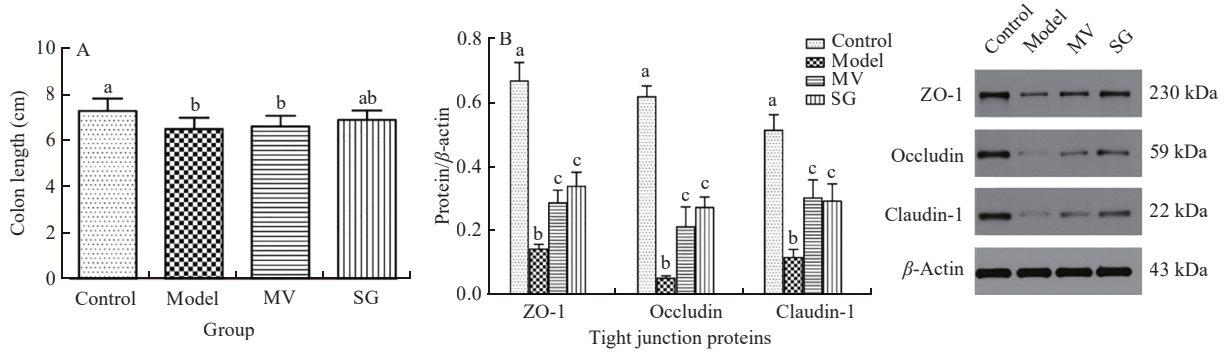

Fig. 5 Effect on colonic barrier. (A) Colon length. (B) Western blot analysis of tight junction proteins. Data were shown as the mean ± SEM (n = 4). Different lowercase letters in same index indicated significant differences ($P < 0.05$).

3.6 Effect on gut microbiota

3.6.1 Diversity analysis

16S rRNA high-throughput sequencing analysis was performed to evaluate the effect on gut microbiota. The Chao1, Shannon and Simpson indices were used to evaluate α-diversity from the perspective of species abundance and species diversity (Figs. 6A-C). The Chao1 index were 1 081 ± 171, 1 276 ± 196, 1 237 ± 139 and 1 320 ± 128 in control, model, MV and SG groups, respectively, among which SG group showed a significant increase when compared with control group. The Simpson index showed no significance among the 3 HF-HSD groups with values of 0.019 ± 0.003, 0.014 ± 0.003 and 0.018 ± 0.006 for model, MV and SG groups, but remarkably differed from that in the control group (0.029 ± 0.009). No significant difference of Shannon index was observed among the 3 HF-HSD groups with values of 5.09 ± 0.19, 5.24 ± 0.21 and 5.19 ± 0.22 for model, MV and SG groups, respectively, but higher than that in the control group (4.69 ± 0.33). These results suggested that dietary intake of SG containing comprehensive component was more associated with the increase of gut microbiota abundance. Principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) analysis based on Bray-curtis distance exhibited the β-diversity assessment among the 4 groups. Distinct clustering separation between control and model groups and significant differences were observed among three HF-HSD groups (Figs. 6D and E), indicating that the long-term HF-HSD changed the gut microbiota composition, and the 2 SG products could restore the composition but with difference.

3.6.2 Composition of gut microbiota

At the phylum level (Figs. 7A and S5), the main microbiota included Bacteroidota, Firmicutes, Campylobacterota, Desulfobacterota, Actinobacteriota, Proteobacteria, Spirochaetota and Cyanobacteria, among which Bacteroidota and Firmicutes were more than 92%. Compared with the control and model groups, MV and SG groups showed lower Firmicutes/Bacteroidota (F/B) ratio, but no significant difference. Moreover, compared with model group, the relative abundances of Desulfobacterota and Spirochaetota in MV group decreased from 1.98% to 0.75% and 1.57% to 0.32%, and the abundance of Proteobacteria increased from 0.49% to 0.96%, while the relative abundances of corresponding bacteria in SG group decreased to 0.81% and 0.49%, and increased to 0.95%.

At the family level (Fig. S6), Muribaculaceae, Lachnospiraceae and Prevotellaceae were the top 3 compositions. Compared with three other groups, SG juice supplement markedly increased the relative abundance of Prevotellaceae. Furthermore, HF-HSD induced a notably increase in the abundances of Oscillospiraceae and Spirochaetaceae, but mogrosides extract and SG juice interventions decreased their relative abundance. Increasing relative abundances of Desulfovibrionaceae and Lactobacillaceae in model group as well as decreasing tendency in MV and SG groups were also observed, but with no significant difference. Notably, compared with the control and model groups, the relative abundances of Marinifilaceae and Rikenellaceae in MV and SG groups were markedly elevated. Besides, mogrosides extract and SG juice enriched the abundance of Ruminococcaceae. Moreover, SG juice supplement also significantly enhanced the beneficial bacteria Bifidobacteriaceae.

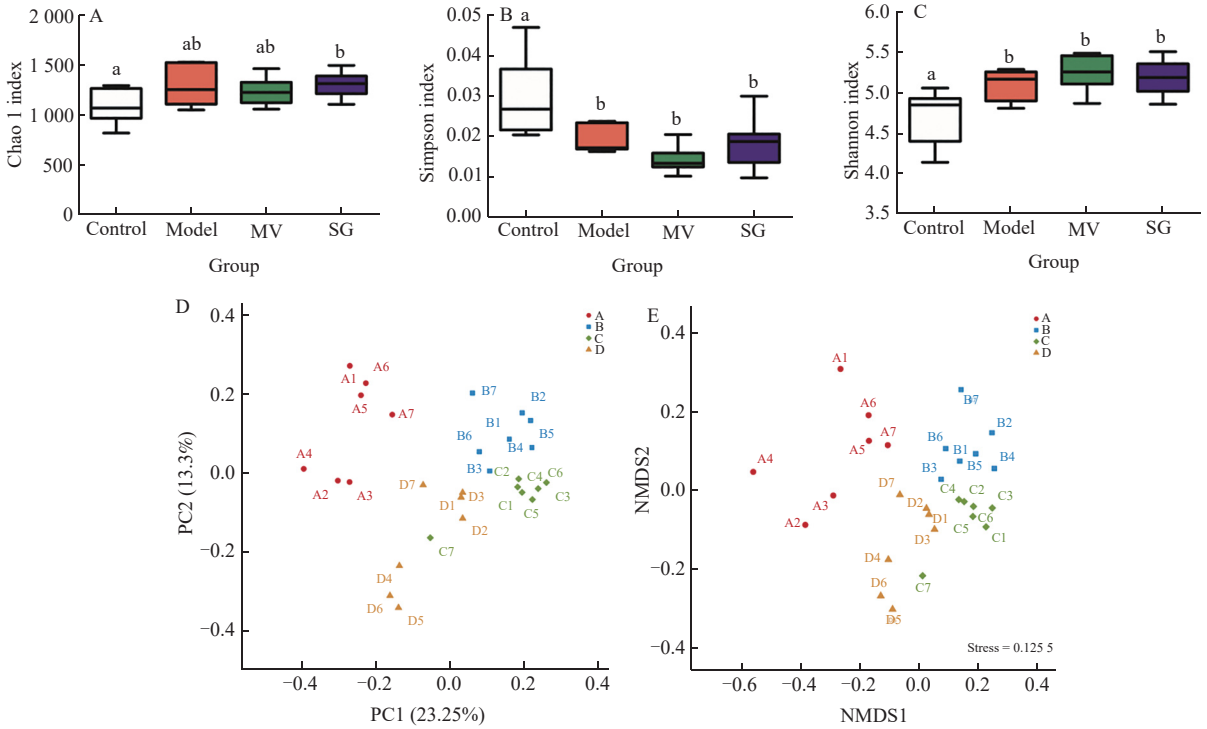

Fig. 6 Diversity of gut microbiota. (A) Chao1 index. (B) Simpson index. (C) Shannon index. (D) PCoA analysis. (E) NMDS score plot (A, control group; B, model group; C, SG group; D, MV group). Data were shown as the mean ± SEM (n = 7). Different lowercase letters indicated significant differences (P < 0.05).

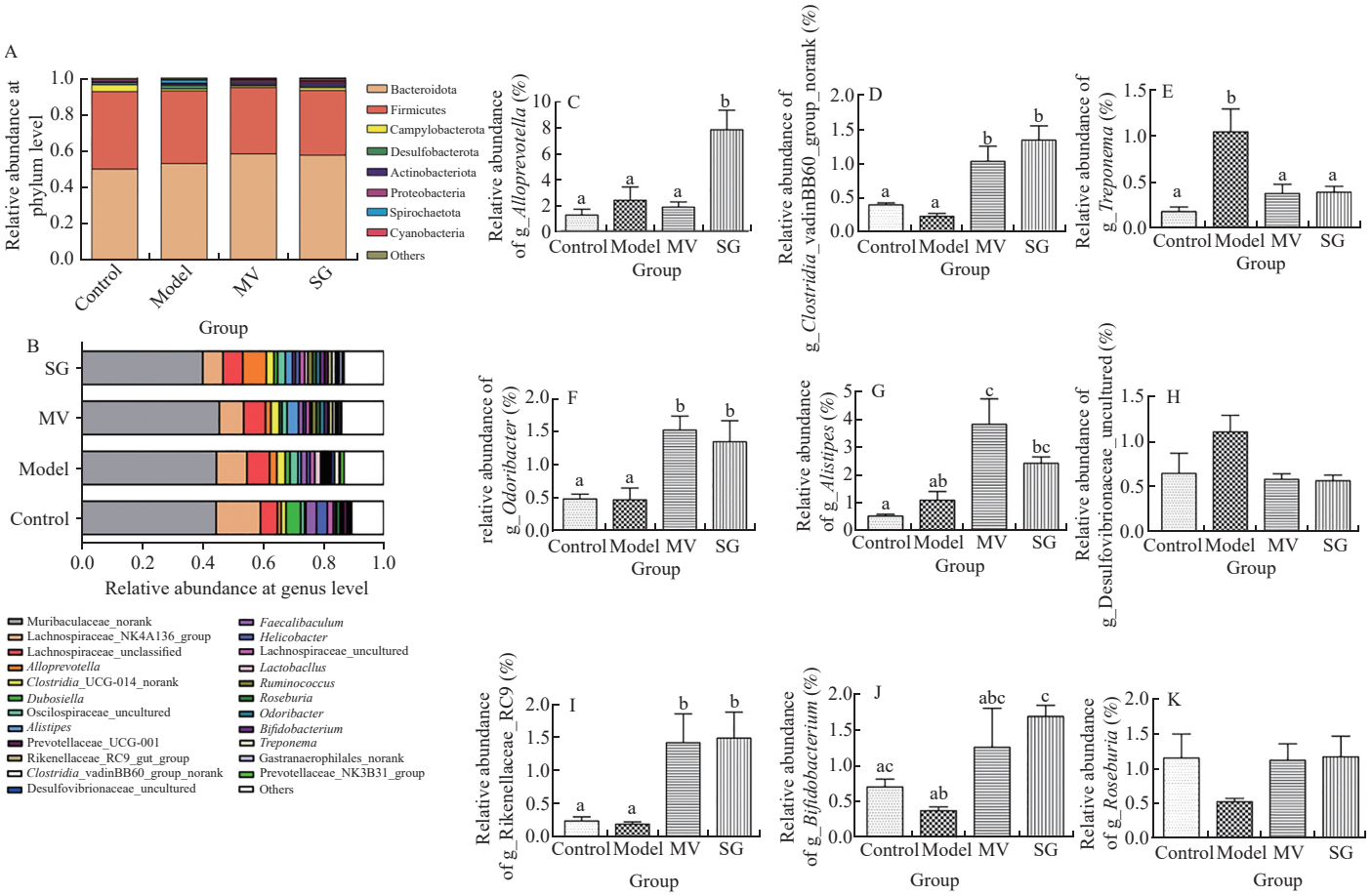

Fig. 7 Composition of gut microbiota. (A) Phylum level. (B) Genus level. (C-K) Representative taxonomic genus. Data were shown as the mean ± SEM (n = 7). Different lowercase letters indicated significant differences ($P < 0.05$).

At the genus level (Figs. 7B-K), taxonomic genus Muribaculaceae_norank, Lachnospiraceae_NK4A136_group and Lachnospiraceae_unclassified were three top abundance bacteria that accounted for 53%–64% in all groups. To be specific, in comparison to the control group, HF-HSD enriched the abundance of *Lactobacillus*, Desulfovibrionaceae_uncultured and *Treponema*, meantime decreased the abundance of *Roseburia* and *Clostridia*_vadinBB60_group_norank. However, mogrosides extract and SG juice interventions reversed above changes. Besides, the abundance of *Bifidobacterium*, Rikenellaceae_RC9_gut_group, *Alistipes* and *Odoribacter* in model group was similar with control group, but MV and SG groups showed significantly increased. Interestingly, different from the other 3 groups, SG group showed significant enrichment of *Alloprevotella* which was consistent with change of taxonomic family Prevotellaceae.

3.6.3 Differential analysis

Linear discriminant analysis effect size (LEfSe) analysis based on Kruskal-Wallis (KW) sum-rank test revealed the key altered bacterial taxa among the 4 groups (Fig. 8). The enrichment in Firimicutes taxa including *Bacilli* and *Dubosiella* was observed in control group. The model group significantly enriched *Desulfovibrio* and Spirochaetota including Spirochaetaceae, *Treponema* and Spirochaetales. Mogrosides extract supplementation gave increasing abundance of Bacteroidota including Rikenellaceae, *Alistipes*, Marinifilaceae, *Odoribacter* and *Muribaculum*. The key bacteria that were altered by SG juice included Prevotellaceae, *Alloprevotella*, Rikenellaceae_RC9_gut_group, *Cyanobacteria*, Gastranaerophilales_g_norank, *Vampirivibrionia*, *Bifidobacterium*, Bifidobacteriaceae, Actinobacteria and *Clostridia*_vadinBB60_group_g_norank. Notably, results showed that the mice of MV and SG groups had significant enrichment of identical probiotics including Rikenellaceae and Ruminococcaceae, but also differences such as Prevotellaceae, *Alloprevotella* and *Bifidobacterium*.

3.7 Correlation of microbiota communities and biological indicators

Spearman correlation analysis was conducted to investigate the relationship between key gut microbiota and different biological indicators (Fig. 9). It was obvious that fat content, lipid indicators including serum ALT and LDL-C, liver TG and TC, and liver TNF-α were positive correlated with Desulfovibrionaceae_uncultured and *Treponema*, but negative correlated with *Roseburia*, *Clostridia*_vadinBB60_group_norank, Rikenellaceae_RC9_gut_group, *Odoribacter*, and *Bifidobacterium*. Among which, remarkable negative correlation between liver weight and *Clostridia*_vadinBB60_group_norank and *Bifidobacterium* were shown (r = –0.57 and r = –0.54). Fat content was significant positive correlated with *Treponema* and Desulfovibrionaceae_uncultured (r = 0.72 and r = 0.71). Serum

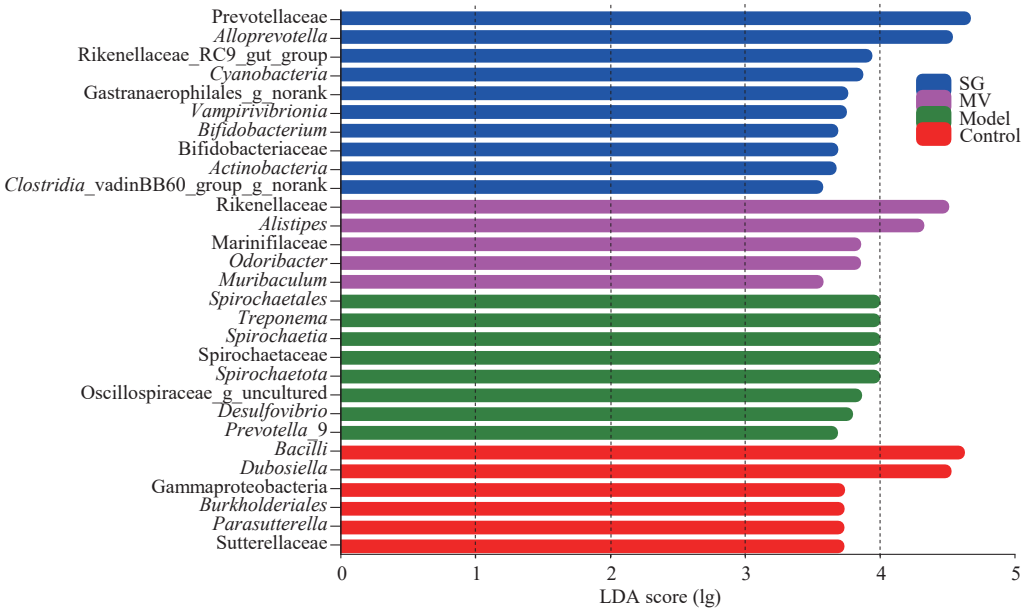

Fig. 8 LEfSe analysis of taxonomic association from 4 groups (LDA > 3.5).

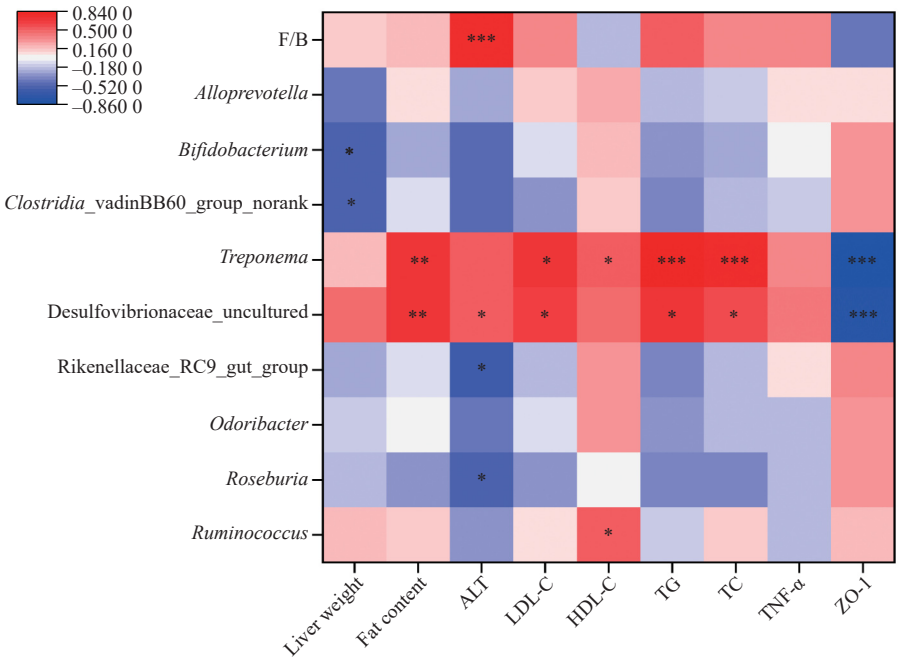

Fig. 9 Heatmap of the Spearman correlations between the abundance microbiota and key parameters in mice. Data were based on the control, model and SG groups. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

LDL-C level, liver TG and TC levels were significant positive correlated with *Treponema* (r = 0.72, r = 0.84 and r = 0.76) and Desulfovibrionaceae_uncultured (r = 0.66, r = 0.69 and r = 0.58). There was markedly positive correlation between HDL-C and *Ruminococcus* and *Treponema* (r = 0.53 and r = 0.51). Serum ALT level was significant negative correlated with *Roseburia* and Rikenellaceae_RC9_gut_group (r = –0.53 and r = –0.60), and significant positive correlated with F/B ratio (r = 0.78). Besides, expression level of ZO-1 in colon was observed remarkable negative correlation with *Treponema* and Desulfovibrionaceae_uncultured (r = –0.86 and r = –0.77). *Alloprevotella* was negative with liver weight, level of serum ALT and levels of liver TG and TC, and positive with serum HDL-C level.

4. Discussion

Unhealthy diet results in not only weight gain and body fat accumulation, but also hyperlipidemia, liver disease and intestinal dysfunction. In order to explore potentially beneficial effect of SG on abnormal biomarkers induced by HF-HSD, a 38-week mice trial was performed.

Our research indicated that daily administration of SG products in drinks could reduce weight gain, liver weight and fat content causing by long-term excessive fat and sugar intake. It seemed that SG juice performed better on the weight management. This might be due to the more diverse functional substances intake such as polysaccharides and flavonoid glycosides in the SG juice. It is well known that high

fat diet is more likely to cause hyperlipidemia[23]. In the study, we had observed abnormal indicators including higher levels of TG, TC, LDL-C and HDL-C in serum from the model mice fed with slightly higher fat and sugar diet. Both SG juice and mogrosides extract reversed the changing trend of LDL-C level, and mogrosides extract could significantly increase the level of HDL-C, indicating the protective effect against hyperlipidemia. In addition, obesity and hyperlipidemia usually are accompanied with hepatic damage. Excessive fat intake would weak the function of the liver, leading to increased blood lipid levels and fat deposition in the liver[24]. As expected, we found that the levels of AST and ALT in serum had an increase with HF-HSD, as well as increasing TG and TC levels in liver tissue. The intervention of two SG products markedly decreased serum ALT and liver TG levels, while the AST level in MV group and liver TC level in SG group were also significantly downregulated, which suggested that SG could alleviate pathology of liver. Lower expression of *ACC1* and *FAS* genes, higher expression of *LDL-r* gene together with less lipid blot in liver tissue from MV and SG groups shown in the H&E staining further verified the hepatic protection effect.

No matter obesity or hepatic damage, inflammation and oxidative stress were important indicators[25-26]. Lü et al.[19] had investigated that SG extract supplement could lower the level of inflammatory cytokines in adipose tissues. Mogrosides was reported to exhibit anti-inflammatory activities in ALI model and DSS-induced colitis[27-28]. As expected, mogrosides extract contributed to the significant level decrease of IL-6 and TNF-α in liver, and a decline tendency in colon. Qi et al.[29] had reported that supplementation with mogrosides extract at low dose did not affect the activities of antioxidant enzymes in alloxan-induced diabetic mice. In this work, except for the increasing reactivation of liver SOD in MV and SG groups, no significant changes were observed neither MDA concentration nor activities of antioxidant enzymes in all groups. These results suggested that daily SG drink was helpful to alleviate the low-grade inflammation but less protection against oxidative stress.

Intestinal integrity is the primary condition for maintaining intestinal function[30]. Studies had proposed that the tight junction between cells is a membrane protein complex formed by occludins, claudins and ZO which play an important role in the connection between epithelial cells and endothelial cells and help to maintain the integrity and homeostasis of intestinal barrier[31-32]. In the present trial, we could observe that HF-HSD remarkably downregulated the expression of occludin, ZO-1 and claudin-1 in the colon, but daily administration of SG juice and mogrosides extract reversed these protein levels, indicating the SG intervention could enhance the intestinal barrier through affecting the tight junctions.

To further explore the effect on intestine microenvironment, the change in gut microbiota was investigated. Though it was apparent that the variety and richness of microbiota species were both changed through the diversity analysis after HF-HSD and drink feeding, there were some obvious differences in the abundance of some bacteria, which might result from the composition difference. Results of the LEfSe analysis indicated that mogrosides extract and SG juice intervention enhanced the abundance of potential probiotics and decreased the abundance of harmful taxa. It was reported that Firmicutes contained more metabolism-related genes which could promote absorption of calories, while Bacteroides played a key role in carbohydrate metabolism[33]. Thus, it was widely accepted that the high ratio of Firmicutes to Bacteroides was positive associated with body weight and fat accumulation[34]. In our study, increased abundance in Bacteroides and decreased enrichment in Firmicutes were observed after supplementation with mogrosides extract or SG juice in the HF-HSD mice. In addition, the consumption of SG juice or mogrosides extract reversed the increased abundance of harmful phyla Spirochaetota induced by HF-HSD, further indicating the beneficial effect of SG on health maintenance. Precisely, genus *Roseburia* was enriched after SG juice and mogrosides extract addition. Evidences have shown that *Roseburia* produces short-chain fatty acids especially butyric acid and decomposes indigestible carbohydrates, thus plays an important role in the metabolism of carbohydrate and fat[35]. Petersen et al. identified a specific bacterium named *Clostridia* from the intestine which prevented mice from getting fat[36]. As shown, genus *Clostridia*_vadinBB60_group_norank was decreased after HF-HSD, but treatment of SG products restored the abundance. Besides, as a beneficial bacterium, *Bifidobacterium* has important physiological functions such as enhancement of gastrointestinal function, liver protection and chronic diarrhea[37]. Rikenellaceae_RC9, a well-known probiotic, can use succinic acid, lactic acid or acetic acid as substrates to produce propionic acid and butyric acid, thus improving the intestinal environment and inhibiting obesity and diabetes[38]. From our data, *Bifidobacterium* and Rikenellaceae_RC9_gut_group in SG group, and Rikenellaceae_RC9_gut_group in MV group showed significant abundance enhancement, meanwhile exhibited negative relation with lipid levels (serum LDL-C, liver TG and TC) and liver TNF-α, suggesting the SG products had beneficial effect on enrichment of prebiotics. In addition, family Ruminococcaceae have also been reported to stimulate the short chain fatty acids producing, and thus exert anti-inflammatory effect[39]. We observed the enrichment of genus *Ruminococcus* in MV and SG groups, as well as negative correlation with serum AST level and liver TNF-α liver. In contrast, HF-HSD promoted the growth of Desulfovibrionaceae_uncultured and *Treponema* in model mice. These bacteria genera are well known to relate with inflammation[40-41]. Several clinical studies have confirmed that the increase in the abundance of Desulfovibrionaceae is an important feature of ulcerative colitis[42]. According to present result, treatment of SG juice and mogrosides extract remarkably decreased abundance of *Treponema* that was upregulated by HF-HSD, meantime decreased average abundance of Desulfovibrionaceae_uncultured. The positive correlation with levels of serum LDL, liver TG, liver TC and liver TNF-α was in line with previous reports. In particular, SG juice treatment significantly enriched the abundance of taxonomic family Prevotellaceae and genus *Alloprevotella*, which was distinctly different from MV group. It was reported that *Prevotella* contributed to lose weight and lower cholesterol levels[43]. To some extent, the higher Prevotellaceae and *Alloprevotella* abundances in mice of SG group explained the lower weight gain and fat content.

5. Conclusion

The study indicated the beneficial effect of daily intake of SG juice from whole fresh fruit or sweetener product of mogrosides for the alleviating of fat accumulation, liver disease and inflammation which were induced by long-term excess fat and sugar diet.

According to results, SG juice showed more powerful regulation capacity to weight/fat management and enrichment of potential probiotics especially *Alloprevotella*. The different efficiency between MV and SG groups might origin from the different component of the two products, and we will further explore the hypothesis.

Declaration of competing interest

Hongwei Liu is an editorial board member for *Food Science and Human Wellness* and was not involved in the decision to publish this article. The authors declare no conflict of interest.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250241.

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