Preventive role of Pastinaca sativa in mitigating metabolic dysfunction- associated steatotic liver disease via modulation of metabolic endotoxemia

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a major contributor to liver disorders worldwide. Parsnip (*Pastinaca sativa*) has been utilized in food and medicine for centuries, owing to its high content of dietary fiber and various pharmacological properties. Although the health benefits of this root vegetable have been reported, its anti- MASLD effects remain largely understudied. Therefore, this study aimed to evaluate the prebiotic effects of a parsnip root water-soluble extract (PRE) and its alleviatory effects against MASLD and metabolic endotoxemia in a mouse model. Mice were fed a high-fat diet supplemented with 50 and 100 mg/kg of PRE for eight weeks. Mice administered with PRE exhibited reduced fat accumulation and serum metabolic changes that were associated with liver injury. Furthermore, PRE treatment reduced the hepatic lipogenic protein levels that were elevated by the high-fat diet. This extract improved intestinal barrier function by modulating endotoxin, intestinal permeability, and tight junction protein expression. This confirms that PRE is associated with improved gut health. These findings suggest that oral administration of PRE may prevent MASLD and improve metabolic health, which can facilitate the use of this extract as a dietary supplement.

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common cause of chronic liver disease worldwide. A simulation analysis utilizing data from the United States projected a 21% rise in the population affected by MASLD, increasing from 83.1 million individuals in 2015 to an estimated 100.9 million by 2030. This trend is anticipated to result in a prevalence rate of 33.5% by 2030[1]. Similarly, mortality rates associated with MASLD continue to increase, highlighting the significance of this condition as a pressing global health concern[2]. MASLD represents a spectrum of liver conditions that range from NAFL to NASH, exhibiting diverse clinical trajectories that may culminate in cirrhosis and hepatocellular carcinoma[3].

The pathophysiology of MASLD encompasses various overlapping mechanisms that contribute to the onset of lobular inflammation, which can drive disease progression. Animal studies have highlighted the role of endotoxin translocation in lobular inflammation, likely due to gut dysbiosis and metagenomic richness[4]. Gram-negative Bacilli within the gut microbiota are the predominant source of enduring endotoxins. Lipopolysaccharides (LPS) are the main endotoxins generated by these microorganisms. Anaerobic gram-negative rods experience a surge in population as intestinal conditions degrade, leading to increased LPS production by these organisms[5]. This process is significantly impacted by dietary habits. A high-fat diet can result in metabolic endotoxemia, leading to liver inflammation and lipid accumulation, ultimately contributing to the development of MASLD over time (Cremonini et al., 2022).

The parsnip (*Pastinaca sativa L.*) is a root vegetable native to Europe and Asia, belonging to the Apiaceae family. Parsnip is used medicinally in most parts of the world, not only for their use in foods such as soups, cakes, and muffins, but also due to their rich active components, including furanocoumarins, polysaccharides, and organic acids. Furthermore, these edible roots possess many

pharmacological properties, including anti-inflammatory, antispasmodic, vasodilatory, antifungal, antibacterial and antidepressant properties[6,7]. Parsnip root is mostly made up of neutral detergent fiber (18.4%), pectin (10.10%), and lignin (1.92%), and its high content of dietary fiber, which includes both soluble and insoluble components, provides its potential health benefits[8]. Parsnip is also known to have beneficial effects on dysuria, anemia, diabetes, and obesity[9,10]. The fiber content of parsnip may play an important role in regulating gut health and metabolic disorders such as MASLD, such as improving digestion, preventing constipation and gastrointestinal disorders, but the anti-MASLD effect of parsnip has not been reported. Therefore, this study aimed to investigate whether a parsnip root water-soluble extract (PRE) exerts a protective effect against MASLD by reducing metabolic endotoxemia.

2. Methods

2.1. Preparation of PRE

Fresh parsnip roots were purchased from a local market in Yecheon (Gyeongsangbuk-do, Korea). The parsnip roots were sliced to 20-mm sections and dried in an oven for 24–48 h at 50°C. They were then pulverized to a size of ≤ 2.0 mm using a grinder. Water equivalent to 20 times the volume of 5 g of the sample was then added. Next, reflux extraction was carried out at 80°C for 3 h to obtain a hot water extract. The liquid extract was filtered through a Whatman No. 41 filter and concentrated using a vacuum rotary evaporator (R-114; Buchi Labortechnik, Flawil, Switzerland). The parsnip extract obtained through this process was referred to as a PRE.

2.2. HPLC analysis of PRE

The furanocoumarins bergapten and xanthotoxin, which are known parsnip components, were purchased from ChemFaces (Wuhan, China). Chromatographic analyses were carried out using a Waters Alliance 2695 separation module (Waters Corp., Milford, MA, USA) equipped with a quaternary pump (Waters 1515) and a photodiode array detector (Waters 2996). The separation was performed on a Waters XBridge BEH C18 column (150 × 4.6 mm, 3.5 μm) at a temperature of 35°C. The mobile phase comprised 0.1% formic acid (Merck, Darmstadt, Germany) in water (A) and 0.1% formic acid in acetonitrile (B). The flow rate was 1.0 mL/min, and the detection wavelength was set at 310 nm according to the following linear gradient: 0–5 min, 30–30% B; 5–15 min, 30–35% B. The injection volume was 5 mL. The PRE was dissolved in 50% ethanol (30 mg/mL), and the ethanol solution was filtered through a 0.2-μm PTFE syringe filter. Furanocoumarin content was then analyzed using HPLC. The standard stock solutions of two marker components (bergapten, and xanthotoxin) were prepared by dissolving these components at a concentration of 1 mg/mL. Each of the six working standard solutions was made by diluting the standard solution with 50% ethanol.

2.3. Culture, viability, carbohydrate content, and MUC-2 levels of LS 174T cells

The human colorectal adenocarcinoma cell line LS 174T was obtained from the American Type Culture Collection (Manassas, VA, USA). LS 174T cells were cultured at 37°C in 5% CO_2 in minimum essential medium (Gibco, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 1% penicillin-streptomycin, and 1% glutamine. The cells were seeded in 96-well plates at a density of 5×10^4 cells per well for 24 h, followed by treatment with different concentrations (10, 50, 100, 200, 500, and 1000 μg/mL) of PRE in the medium at 37°C for another 24 h. Cell viability was assessed using the Cell Counting Kit-8 test (Dojindo, Tokyo, Japan) according to the manufacturer's instructions. Absorbance was then measured at 450 nm.

2.4. Prebiotic activity assay

The prebiotic activity score was measured to evaluate the efficacy of parsnip extract on the proliferation of probiotic bacteria. *Lactobacillus delbrueckii* subsp. *bulgaricus* MG5167, *Lactobacillus gasseri* MG4247, *Bifidobacterium longum* KCTC3421, and *Bifidobacterium bifidum* KCTC3440 strains isolated from the human intestine were used as probiotics. These strains were kindly provided by Mediogen (Jecheon, Republic of Korea). *Escherichia coli* KCTC2441 (the Korean Collection for Type Cultures, Jeongeup, Republic of Korea) was used as the corresponding strain.

Lactobacillus strains were cultured using MRS agar, which was incubated at pH 6.5 ± 0.2 and 37°C for 24 h. The oxygen demand was facultatively anaerobic, and strains were preserved through lyophilization or freezing of cell suspensions. *Bifidobacterium* strains were grown in TSA agar, incubated at 37°C for 48–72 h, and cultured under anaerobic conditions. The *E. coli* strain, which is an anaerobe, was cultured in TSA agar and incubated at 37°C for 24 h.

The culture broth for the prebiotic activity score analysis used M9 minimal broth supplemented with 2 g/L glucose, 0.015 g/L $CaCl_2$, and 0.5 g/L $MgSO_4$.

The culture was performed by streaking colonies of each strain onto the corresponding solid agar, followed by incubation at 37°C for 24–48 h and inoculation into 10 mL of liquid broth for a second incubation at 37°C for 24–48 h. The M9 broth was mixed with 5% (v/v) of the bacterial culture and 5 mg/ml of glucose or parsnip treatment (1 and 5 mg/ml). Absorbance was measured at 600 nm using a microplate reader immediately (0 h) and 24–48 h later. The obtained values were substituted into the equation below to obtain the prebiotic activity score:

Prebiotic activity score

$$\frac{\text{Probiotic log CFUmL}^{-1}\text{ on the prebiotic at 24 h} - \text{Probiotic log CFUmL}^{-1}\text{ on the prebiotic at 0 h}}{\text{Probiotic log CFUmL}^{-1}\text{ on glucose at 24 h} - \text{Probiotic log CFUmL}^{-1}\text{ on glucose at 0 h}}$$

$$- \frac{\text{Enteric log CFUmL}^{-1}\text{ on the prebiotic at 24 h} - \text{Enteric log CFUmL}^{-1}\text{ on the prebiotic at 0 h}}{\text{Enteric log CFUmL}^{-1}\text{ on glucose at 24 h} - \text{Enteric log CFUmL}^{-1}\text{ on glucose at 0 h}}$$

Inulin (5 mg/ml), a prebiotic reported to improve the gut flora, was used as a positive control.

The pH change of the broth before and after incubation was measured twice using a pH meter (Orion star A211; Thermo Fisher Scientific, Waltham, MA, USA), and the average value was used.

2.5. Establishment of the mouse model

Seven-week-old male C57BL/6N mice (G-bio, Gwangju, Republic of Korea) were kept under controlled conditions (60 ± 5% humidity, 12 h light/dark cycle, and 23 ± 2°C temperature), provided sterilized water, and fed the AIN-93G diet (Dyets, Bethlehem, PA, USA). All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the Korea Food Research Institute (approval number: KFRI-M- 23035).

After one week of acclimation, mice were separated into five groups: ND, Ain-93G diet; HF, 60% kcal fat diet; GG, HF with *Garcinia gummi-gutta* 50 mg/kg mouse; PRE 50, HF with PRE 50 mg/kg; and PRE 100, HF with PRE 100 mg/kg. HF and GG were used as negative and positive controls, respectively. PRE and GG were dissolved in sterilized water and administrated orally daily. Food intake and body weight were recorded weekly for eight weeks. At the end of the treatment period, 12-h fasted mice were anesthetized through exposure to isoflurane, and blood was collected into microfuge tubes. The serum was separated through centrifugation and frozen at -80°C. Finally, body composition scans were performed (Medikors Inc., Seongnam, Republic of Korea).

2.6. Oral glucose tolerance test (OGTT) and intestinal permeability

Mice were fasted for 12 h and orally administered a glucose solution (2 g/kg body weight) at Week 8. Blood glucose levels in the tail were measured at 0, 30, 60, 90, and 120 min post-administration using a glucometer (AccuChek; Roche Diagnostics, Indianapolis, IN, USA). Similarly, the intestinal permeability of the mice was evaluated at eight weeks using fluorescein isothiocyanate (FITC)-dextran. The mice were fasted for 6 h before 500 mg/kg of FITC-dextran was administered orally. Blood samples were collected from the tail vein at 2 and 5 h after administration, and plasma was separated using centrifugation. The fluorescence of FITC-dextran was quantified using a microplate reader (Molecular Devices) with excitation and emission wavelengths of 485 and 535 nm, respectively. The concentration of FITC-dextran was determined by constructing standard curves using untreated plasma samples.

2.7. Blood biochemical analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total cholesterol, triglyceride, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, endotoxin, and insulin levels were measured. Moreover, liver triglyceride (TG) and lipid peroxidation (MDA) were measured. The liver TG and MDA levels were estimated using ELISA kits from Abcam (Cambridge, MA, USA) whereas the triglyceride levels were estimated using a kit from Cayman (Ann Arbor, MI, USA). Serum ALT; AST; and total, HDL-, and LDL-cholesterol levels were estimated using biochemical analysis equipment (AU-480, Beckman) from KP &T. Furthermore, insulin and endotoxin levels were estimated using a kit from Thermo Fisher Scientific. All data were quantified according to the

manufacturers' instructions, and the homeostasis model determination of insulin resistance (HOMA-IR) index was calculated as shown below:

HOMA-IR = (fasting insulin (μU/mL) × fasting glucose (mg/dL))/405.

2.8. Western blotting

Lysis buffer (PRO-PREP, iNtRON Biotechnology, Seongnam, Republic of Korea) was used to extract total protein from the liver and colon tissues of mice. Proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. The gels were then transferred onto PVDF membranes, blocked with 5% skim milk, and incubated with primary antibodies (for ACC, FAS, SREBP1, ChREBP, CEBPα, G6PD, ZO-1, and β-actin) overnight at 4°C. Next, the membranes were incubated with secondary antibodies. Bands were subsequently quantified using Image Lab Software (Bio-Rad) and an EZ Western Lumi Femto Kit (DoGenBio Co. Ltd., Seoul, Republic of Korea). Finally, chemiluminescence was detected using a ChemiDoc XRS + imaging system.

2.9. Histological analysis Histological analysis was performed using hematoxylin and eosin (H&E) and Oil red O (ORO) staining. Frozen liver tissue slices were stained with H&E to measure liver damage and adipocyte size, as well as ORO to assess hepatic steatosis. All sections were scanned with CaseViewer software (3DHISTECH Ltd.) at 20 × magnification. The H&E staining data in liver tissues were used to construct the MASLD activity score. The ORO-stained area of the liver tissues was also quantified using Image J software (NIH, Bethesda, MD, USA).

2.10. Gut microbiota analysis

At Week 8, fresh fecal samples were collected and stored at -80°C until use. Fecal DNA was isolated utilizing a DNeasy PowerSoil kit (12888-50; QIAGEN, Hilden, Germany), and the hypervariable V3–V4 region of 16S rRNA amplicons was synthesized using a MiSeq system (Illumina, San Diego, CA, USA) from Macrogen (Seoul, Korea) according to the manufacturer's instructions to examine the composition of the gut microbiota. FLASH was used to construct paired-end readings, whereas the QIIME 2 program was used for microbial community analysis using unweighted UniFrac distance matrices[11].

2.11. Quantification of fecal metabolites

Fecal samples were measured in 50 mg aliquots before they were extracted with 980 μL of 50% MeOH and 20 μL of the internal standard. Centrifugation under identical conditions to the serum preparation was performed after 10-min sonication. The supernatants were subsequently filtered using a PTFE filter before analysis. Spectrometry was then conducted as previously described[12].

2.12. Statistical analysis

Data are expressed as the mean ± standard error. All analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and one-way analysis of variance with Duncan's multiple range test. $P < 0.05$ was considered statistically significant. Finally, correlation analysis was conducted using Pearson's

correlation in R Studio (version 2023.12.1 + 402) and visualized using the corrplot package (version 0.92).

3. Results

3.1. Effects of PRE mucin production in LS 174T cells

Cell viability in the untreated control group was 100%, indicating no cytotoxicity. As shown in Fig. 2A, treatment of LS 174T cells with PRE at 10, 50, 100, 200, 500, and 1000 μg/mL did not decrease their viability; thus, it promoted proliferation. This indicates that PRE is not cytotoxic at these concentrations. Mucin production in the control group was 100%. However, treatment of LS 174T cells with PRE at 10, 50, 100, 200, 500, and 1000 μg/mL affected mucin production (Fig. 2B). These results demonstrated that PRE stimulates MUC-2 at concentrations ≤ 1000 μg/mL (Fig. 2C). Therefore, PRE was used for subsequent experiments.

3.2. Effects of PRE probiotic growth

The prebiotic activity score of four strains (two types each of *Lactobacilli* and *Bifidobacteria*) obtained using 0.5% parsnip material (Fig. 3) revealed a higher bacterial growth than that obtained using inulin. The prebiotic activity of *L. bulgaricus* and *L. gasseri* strains was approximately 1.2 and 1.6 times higher than that of the Inulin group at a parsnip material concentration of 0.5%. Furthermore, *B. longum* and *B. bifidum* strains were 1.3 and 2 times more active than those in the Inulin group, respectively, and 2.0 and 4.2 times more active than those in the 0.1% parsnip group.

The pH of each strain on the parsnip material was measured as shown in Table 1. The pH of the *L. gasseri* strain culture medium at 0.5% parsnip material decreased by approximately 0.6 (6.80 to 6.20 before and after the reaction, respectively). Moreover, the pH of the 0.5% parsnip material in the *L. gasseri* and *B. longum* strains decreased by approximately 1.8 to 2.3 (6.62 and 6.81 to 4.35 and 5.05 before and after the reaction, respectively). This is comparable to the pH of the inulin group before and after the reaction. The results demonstrated a decline in pH compared to the control. Consequently, it was established that the four strains (*L. bulgaricus, L. gasseri, B. longum*, and *B. bifidum*) reduced the pH of parsnip material to a slightly acidic level, which was less than that observed with inulin.

Table 1
Changes of pH on various bacteria growth with 0.1% and 0.5% parsnip (*Pastinaca sativa*) root extract (PRE) and 0.5% inulin after incubation for 24 h.

	Normal		Inulin		PRE			
			0.5%		0.1%		0.5%	
	0h	24h	0h	24h	0h	24h	0h	24h
Lactobacillus delbrueckii ssp. *bulgaricus*	6.76 ± 0.00	6.68 ± 0.00^{d}	6.80 ± 0.00	6.34 ± 0.01^{b}	6.81 ± 0.01	6.44 ± 0.00^{c}	6.81 ± 0.01	6.27 ± 0.01^{a}
Lactobacillus gasseri	6.80 ± 0.01	6.68 ± 0.00^{c}	6.83 ± 0.01	6.44 ± 0.01^{b}	6.81 ± 0.01	6.68 ± 0.01^{c}	6.80 ± 0.00	6.20 ± 0.01^{a}
Bifidobacterium longum	6.64 ± 0.01	6.25 ± 0.06^{b}	6.62 ± 0.01	4.53 ± 0.04^{a}	6.55 ± 0.05	5.91 ± 0.04^{b}	6.62 ± 0.01	4.35 ± 0.30^{a}
Bifidobacterium bifidum	6.84 ± 0.01	6.68 ± 0.00^{d}	6.86 ± 0.01	6.22 ± 0.01^{c}	6.78 ± 0.00	5.59 ± 0.11^{b}	6.81 ± 0.01	5.05 ± 0.01^{a}

Notes: Data are expressed as the final pH of medium after 24 h incubation, and presented as mean ± standard deviation ($n = 3$); The different letters (a–c) indicate significant difference ($p < 0.05$) between columns determined by Duncan's multiple range test.

3.3. Effects of PRE on body and liver weight

The body weight of mice in the HF group was significantly higher than that of those in the ND group (Fig. 4A). The assessment of body composition revealed a significant increase in fat contents in overweight mice compared to ND mic (Fig. 4B and C). Moreover, we observed a marked increase in liver weight, hepatic triglyceride levels, and MDA levels in the HF group (Fig. 4D-F). In contrast, mice in both the PRE50 and PRE100 groups exhibited significantly reduced body weight and fat. These results indicate that PRE not only reduced the HF-induced increase in liver weight but also improved metabolic disorder parameters.

3.4. Effects of PRE on serum lipid profiles

We measured serum levels of ALT, AST, TG, TC, HDL, and LDL to evaluate the effects of PRE on serum lipid profiles. HF-treated mice showed significantly higher serum ALT, AST, TG, TC, HDL, and LDL levels than ND mice ($P < 0.05$) (Fig. 5A–F). The increased activity of AST and ALT in the HF group indicated liver damage. Although PRE treatment improved ALT, AST, TG, TC, and HDL cholesterol levels, LDL cholesterol levels showed no significant differences HF the group.

3.5. Effects of PRE on glucose tolerance and insulin resistance

Glucose tolerance and insulin resistance were evaluated to investigate the relationship between PRE administration and fatty liver disease in mice induced with a high-fat diet. The OGTT and insulin

resistance are significant pathophysiological elements of MASLD. HF administration significantly increased glucose intolerance, fasting blood glucose levels, serum insulin levels, HOMA-IR, and leptin ($P < 0.05$) (Fig. 6A–E). However, PRE administration decreased these biomarkers when combined with HF. Furthermore, the PRE 50, and100 mg/kg groups showed 8.89%, and 6.42% lower OGTT AUC values than the HF group, respectively. However, this decrease was not significantly different in the HF group (Fig. 6A).

3.6. Effects of PRE on lipid metabolism in liver tissue

The ORO-positive staining areas of HF-fed mice were significantly increased (Fig. 7A). Similarly, H&E staining showed a significant increase in fat deposition and steatosis scores in the liver (Fig. 7B). However, both high and low doses of PRE significantly reduced the liver ORO-positive staining area and fat deposition. The expression levels of proteins associated with lipid metabolism were evaluated to determine the potential efficacy of PRE treatment against hepatic fat accumulation. HF consumption significantly upregulated the expression of lipid metabolism-related proteins, such as ACC, CHREBP, CEBPα, G6PD, SREBP1, and FAS compared with that in the ND group (Fig. 7C).

3.7. Effects of PRE on gut barrier function

Gut permeability and endotoxin levels were significantly higher and ZO-1 expression was significantly lower in the HF group than those in the ND group. Conversely, mice in the PRE group did not exhibit improved gut permeability. In addition, PRE-treated mice showed significantly increased protein expression (Fig. 8A–C). The PRE group inhibited the infiltration responses of inflammatory cells whereas the HF group showed remarkable histological changes in the colon tissues (Fig. 8D).

3.8. Effects of PRE on the gut microbial composition

The phylum and genus level fecal microbiome is summarized in Fig. 9A. At the genus level, the HF group showed a significantly altered gut microbial composition and had a lower proportion of *Ligilactobacillus, Mediterraneibacter, Ruminiclostridium*, and *Feifania*. However, this group had a markedly higher proportion of *Mammaliicoccus, Enterococcus, Harryflintia*, and *Phocaeicola* than the ND group (Fig. 9B).

3.9. Correlation between fecal metabolites and metabolic parameters

Differences in the metabolites between the ND, HF, and PRE-treated groups were verified by comparing heatmaps (Supplementary Fig. 1A). Amino acid metabolites such as ornithine, proline, and 3-indole propionic acid were significantly increased in the HF group. However, deoxycholic acid exhibited a significant decrease in the HF group and a significant increase in the PRE-treated group ($P < 0.05$). The association between fecal metabolites and metabolic parameters linked to metabolic disorders was assessed through Pearson's correlation analysis (Supplementary Fig. 1B). Most metabolites related to amino acid pathways, except for lysin and cystine, were positively correlated in the weight gain and OGTT category. Conversely, most amino acid- and indole-related metabolites were negatively correlated with ZO-1. In particular, a positive correlation was observed between gut permeability, HOMA-IR, G6PD,

SPREBP1, and ACC associated with 3-indole propionic acid. In contrast, deoxycholic acid was predominantly negatively correlated with various metabolic disorder parameters.

4. Discussion

Metabolic endotoxemia, defined as an elevation of serum endotoxin levels in response to a high-fat Western diet, has recently gained increasing recognition[5]. This increase in LPS was associated with inflammation in the liver and adipose tissue, which ultimately contribute to the onset of MASLD and insulin resistance[13]. A high-fat diet disrupts the balance of the gut microbiota, resulting in the generation of harmful compounds such as LPS. Furthermore, the proliferation of detrimental gut bacteria and toxic compounds in response to a high-fat diet is associated with heightened gut permeability through inflammatory processes and the impairment of tight junctions, consequently resulting in metabolic endotoxemia[14]. In addition, several studies have shown a clear correlation between the inflammatory and metabolic disorders induced by high-fat diets and the LPS mechanisms[15].

Plant extracts and their derivatives have several pharmacological properties, including antibacterial, anticancer, anti-inflammatory, and antidiabetic effects. *Pastinaca sativa* contains polyacetylenes and furanocoumarins, phytochemicals that have therapeutic effects against neurological, respiratory, gastrointestinal, hepatic, dermatological, cardiovascular, and urogenital diseases[16,17]. In the present study, bergapten and xanthotoxin were identified as specific compounds of PRE. Therefore, we sought to determine whether *P. sativa* could alleviate high-fat diet-induced MASLD by reducing metabolic endotoxemia. We confirmed that PRE has anti-MASLD effects and improves gut health both *in vitro* and *in vivo*.

Prebiotics are defined as food ingredients that selectively stimulate the growth and activity of beneficial bacteria in the gut, such as probiotics. The prebiotic activity of PRE on four strains of probiotics was measured *in vitro*, and superior prebiotic activity was observed compared to inulin. This method can be used to predict the production of short-chain fatty acids (SCFAs) through culture. SCFAs act as an energy source for intestinal epithelial cells, strengthen the immune system, and regulate metabolism. They can also help suppress the growth of harmful bacteria by maintaining a slightly acidic intestinal environment. Production of the mucin protein MUC-2, a major component of the mucus layer that protects the intestinal barrier[18], was evaluated. MUC-2 production was evaluated using LS174T cells, which are widely used as an *in vitro* model for evaluating mucin synthesis and secretion effects[19,20]. PRE stimulated carbohydrate (mucin) and MUC2 production in a dose-dependent manner but did not affect cell survival rates in LS174T cells. These results demonstrate that PRE induces mucin production.

PRE treatment alleviated MASLD by changing the pathological values of common symptoms of MASLD and metabolic regulation disorders, such as high body weight, liver weight, liver triglyceride levels, and liver function enzymes induced by a high-fat diet in mice[21]. Obesity is a well-characterized risk factor for MASLD[22]. MASLD is associated with features of metabolic syndrome, including dyslipidemia and insulin

resistance, making it easy to induce liver damage[23]. Therefore, reliable biomarkers of liver damage, such as ALT, AST, and TG were measured in this study. PRE treatment significantly reduced the levels of these indicators, suggesting that PRE alleviates metabolic diseases induced by a high fat consumption. Additionally, PRE treatment reduced serum hormone levels, such as insulin and leptin, during exposure to high fat contents. These hormones are important factors in obesity management and affect metabolic homeostasis[24].

MASLD characteristic features include steatosis, inflammation, hepatocellular ballooning, and fibrosis[25]. Our histological staining results showed that PRE decreased lipid accumulation, steatosis, lobular inflammation, and hepatocellular ballooning in the liver tissues of mice subjected to a high-fat diet. Prebiotics, probiotics, and synbiotics improve intestinal permeability and reduce endotoxemia, which is a critical factor in gut-liver dysfunction[26]. Studies showed that prebiotics increase Bifidobacterium spp., enhancing gut barrier function and reducing endotoxemia in high-fat diet mice[27,28].

In the present study, the administration of PRE during a high-fat diet regimen affected the lipid pathway within the liver. High fat contents significantly increased the expression of SREBP1, ChREBP, ACC, FAS, G6PD, and CEBPα. SREBP1 and ChREBP play important roles in the development of fatty liver disease[29,30]. SREBP1 is a major transcriptional regulator of fatty acid and TG synthesis in response to insulin. ChREBP is activated by high glucose levels and plays an important role in the process and regulation of fat synthesis, which is unrelated to insulin[31]. Furthermore, FAS and ACC are important rate-limiting enzymes in fatty acid synthesis[32]. Enzymes that generate NADPH, such as G6PD, are abundantly expressed in adipose tissue and positively associated with the lipid synthesis activity of adipocytes[33]. Furthermore, C/EBPα is mainly found in tissues involved in energy metabolism, including the liver and intestinal epithelium. The increased expression of CEBPα is associated with adipocyte hypertrophy, impaired insulin signaling, and decreased glucose utilization[34,35]. However, treatment with PRE can significantly reverse lipid metabolism changes and reduce hepatic fat accumulation, improving liver damage caused by high fat consumption.

A high-fat diet can enhance LPS absorption by regulating the composition of the gut microbiota. In addition, it can affect mucosal integrity, potentially leading to metabolic endotoxemia[36]. In recent studies, high-fat administration reduced the abundance of *Mediterraneibacter*[37] and increased that of *Harryflintia*[38] and *Phocaeicola*[39]. *Ligilactobacilli* can alleviate liver damage by producing SCFAs such as butyric acid, acetic acid, and propionic acid and regulating hepatic lipid metabolism[40]. Moreover, the relative abundance of Enterococci is significantly upregulated in obese children with MASLD, indicating a positive correlation between *Enterococcus* and the MASLD phenotype[41]. In this study, high fat consumption reduced *Ligilactobacillus* and *Mediterraneibacter* and increased *Enterococcus, Harryflintia*,and *Phocaeicola* abundance. However, PRE improved the composition of intestinal microorganisms and enhanced the expression of epithelial barrier integrity-related proteins such as ZO-1. The intestinal microbiota and intestinal-derived metabolites, especially tryptophan derivatives, regulate metabolic and immune functions related to health and disease. Indolepropionic acid (IPA), a

tryptophan derivative, indicated the onset and development of metabolic disorders such as type 2 diabetes and MASLD in a previous study[42]. Consistent with this finding, our results showed a positive correlation between metabolic disorder mediators and IPA. This highlights the importance of effective management of indole propionic acid distribution, particularly in MASLD alleviation. However, further studies, including human trials, are necessary to investigate the precise mechanism linking the gut-liver axis and dietary PRE intake. Furthermore, the bacteria identified in our study constituted a minimal fraction of the gut microbiota. Therefore, additional research is required to elucidate their precise functions and significance. Overall, our study findings provide a valuable model for preventing and treating the progression of MASLD caused by excessive consumption of high-fat foods.

5. Conclusion

In this study, PRE promoted the growth of beneficial bacteria and protected against MASLD. It showed great potential as a prebiotic by promoting the growth of probiotic strains such as *Lactiplantibacilli* and *Bifidobacteria*. Furthermore, this extract reduced the fat-induced overexpression of lipid metabolism-related proteins in the liver. When administered at doses of 100 mg/kg, parsnip extract alleviated liver damage caused by a high-fat diet and improved gut health. Overall, PRE can be used as a dietary supplement to improve the gut-liver axis and reduce liver damage caused by HF.

Declarations

Author Contributions

Ji-Eun Park: Data curation, Formal analysis, Writing – original draft. Hye-Bin Lee: Formal analysis, Methodology. Yu Ra Lee: Data curation, Formal analysis. Hee-Kyoung Son: Formal analysis. Guijae Yoo: Formal analysis. Sang Yoon Choi: Resources. Miri Park: Formal analysis, Writing – review & editing. Ho-Young Park: Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

Conflict of Interest

The authors declare no conflict of interest.

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Figures

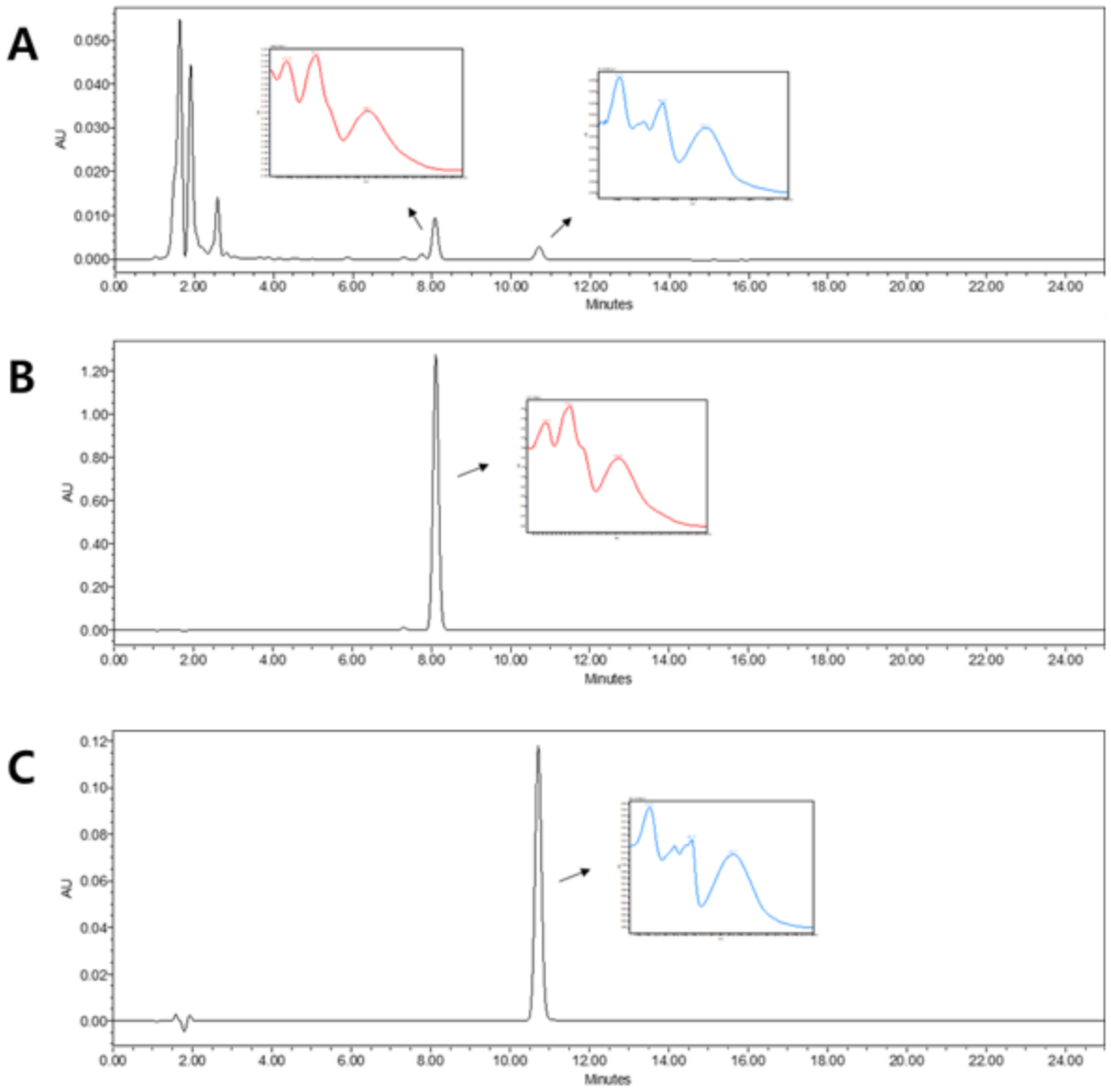

Figure 1

HPLC chromatograms of parsnip (*Pastinaca sativa*) root extract (PRE) and their two specific compounds. (A) *P. sativa* extract; (B) xanthotoxin; (C) bergapten at 310nm.

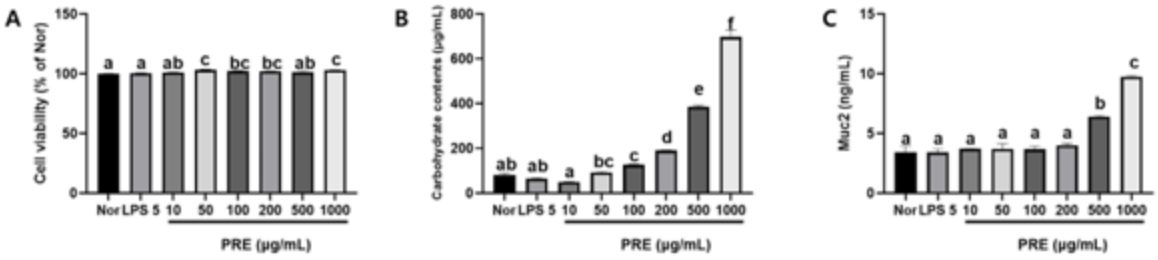

Figure 2

Parsnip (*Pastinaca sativa*) root extract (PRE) enhances mucin production in LS 174T goblet cell line. (A) Cell viability; (B) carbohydrate contents; (C) Muc2. All data are presented as mean ± standard error of the mean (n = 3). Values with different letters are significantly different at $p < 0.05$ by Duncan's multiple range tests.

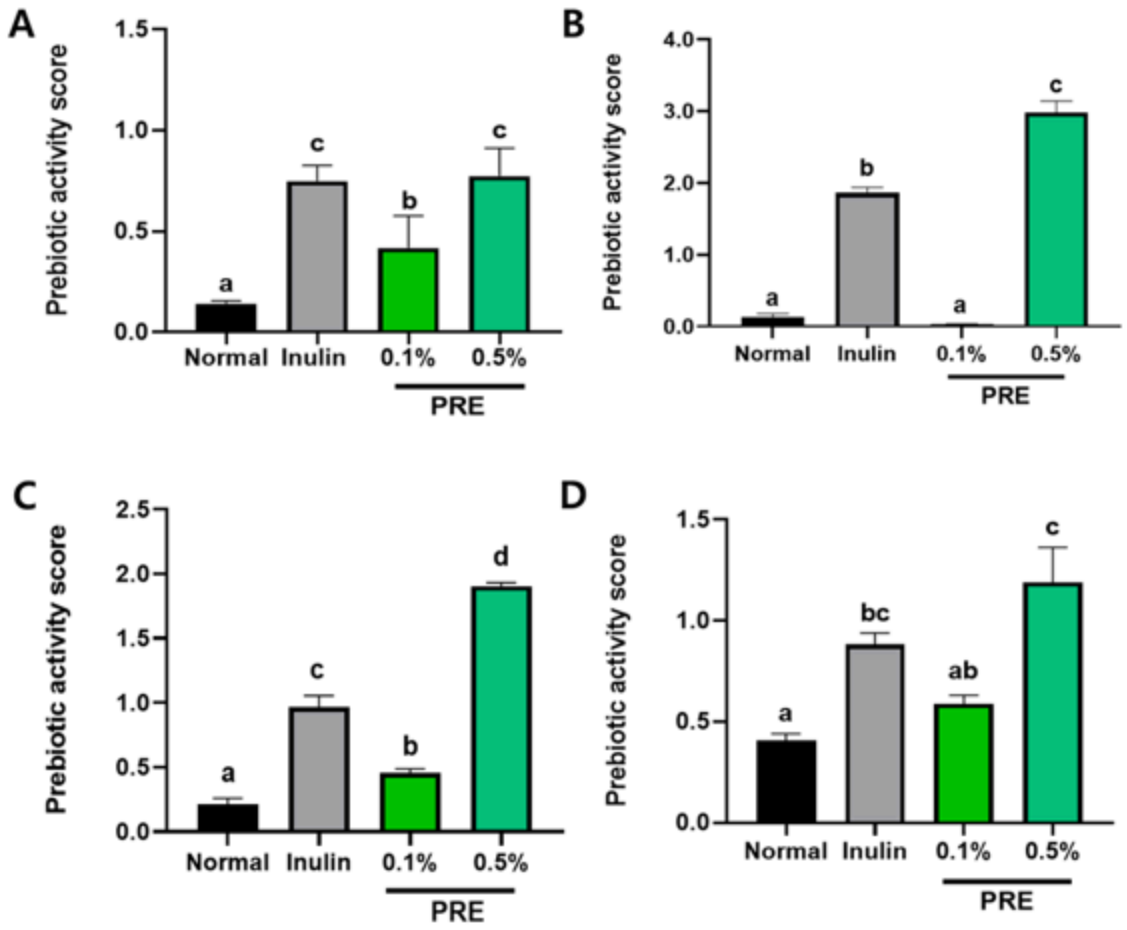

Figure 3

Parsnip (*Pastinaca sativa*) root extract (PRE) exhibits prebiotic activity and increases beneficial bacteria-derived short-chain fatty acids. (A) *Lactobacillus delbrueckii subsp. bulgaricus*; (B) *Lactobacillus gasseri*; (C) *Bifidobacterium bifidum*; (D) *Bifidobacterium longum*. All data are presented as mean ± standard error of the mean (n = 3). Values with different letters are significantly different at $p < 0.05$ by Duncan's multiple range tests.

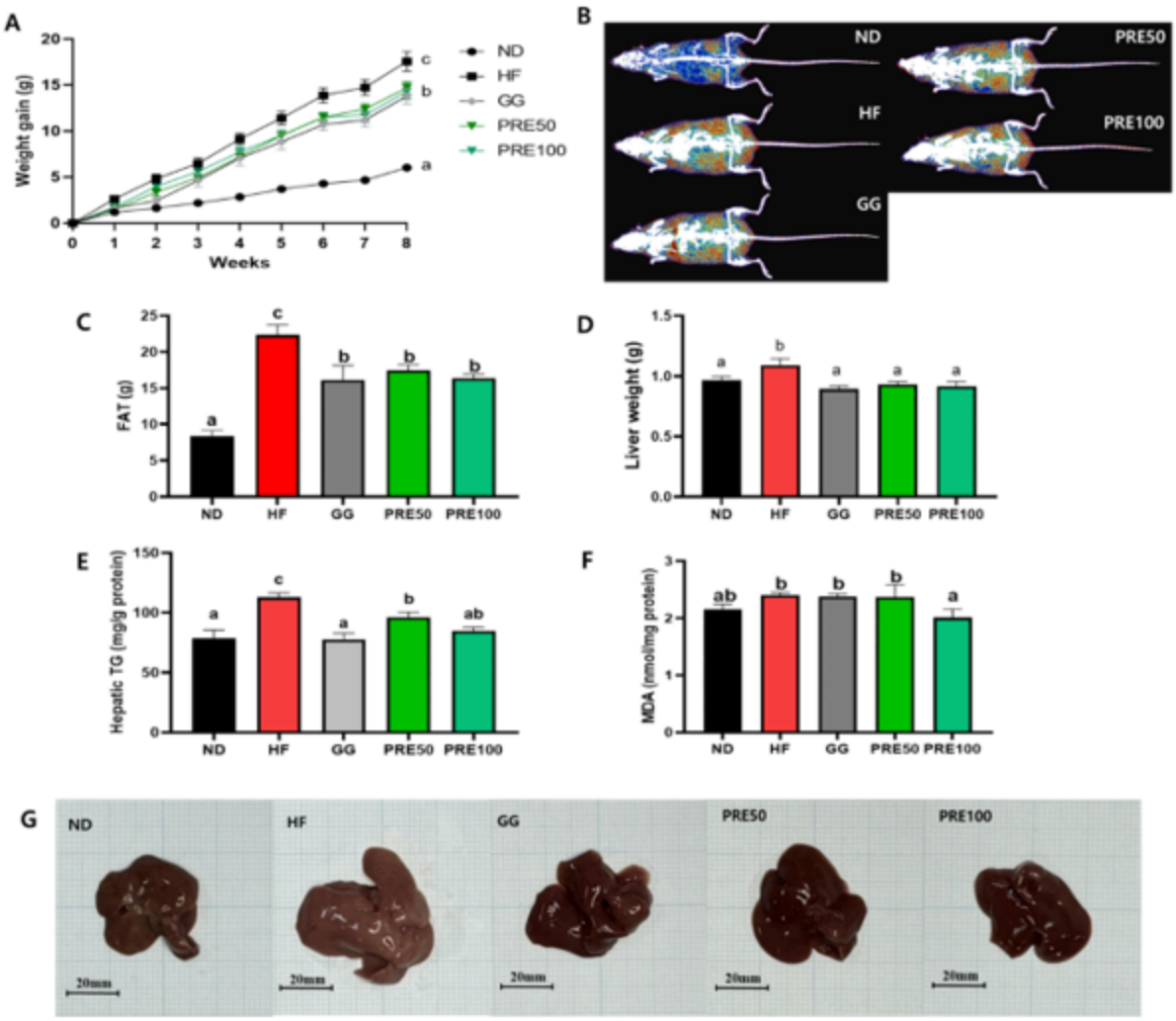

Figure 4

Parsnip (*Pastinaca sativa*) root extract (PRE) improves high-fat-diet-induced metabolic disorder parameters. (A) Changes of weight gain during the experimental period; (B) Representative body composition images (red = fat tissue, blue = lean tissue); (C) A fat gram of fat tissues; (D) Liver weight; (E) Hepatic triglyceride (TG); (F) Malondialdehyde (MDA); (G) Photos of the liver. All data are presented as mean ± standard error of the mean (n = 9). Values with different letters are significantly different at p < 0.05 by Duncan's multiple range tests. ND, normal diet; HF, high-fat diet; GG, *Garcinia gummi-gutta*.

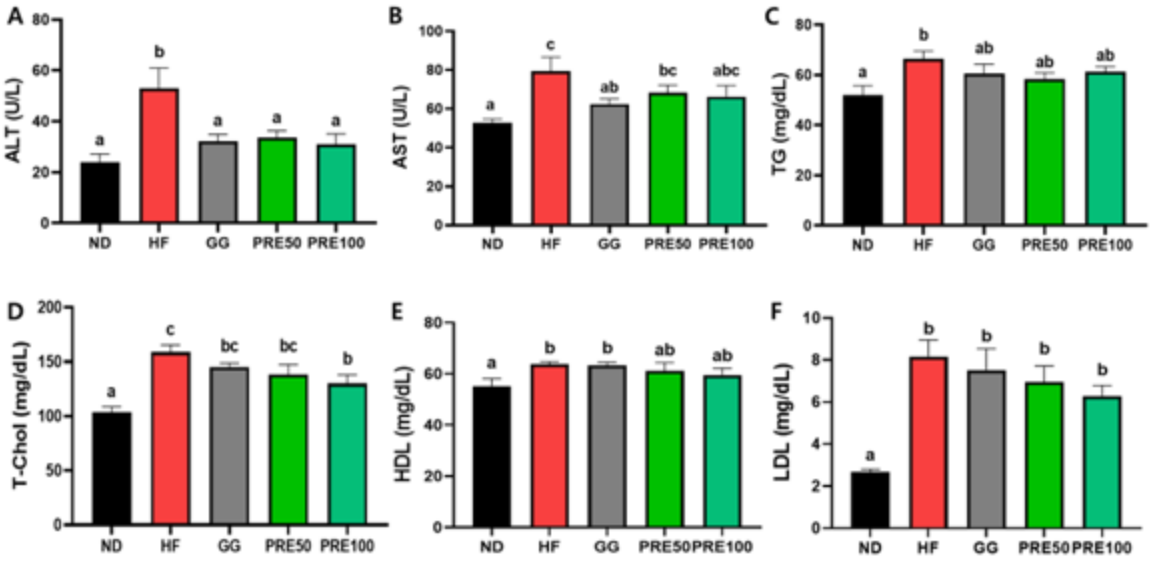

Figure 5

Parsnip (*Pastinaca sativa*) root extract (PRE) improves high-fat-diet-induced serum lipid profiles. (A) Serum alanine aminotransferase (ALT); (B) Serum aspartate aminotransferase (AST); (C) Serum triglyceride (TG); (D) Serum total cholesterol; (E) Serum HDL cholesterol; (F) LDL cholesterol; All data are presented as mean ± standard error of the mean (n = 9). Values with different letters are significantly different at $p < 0.05$ by Duncan's multiple range tests. ND, normal diet; HF, high-fat diet; GG, *Garcinia gummi-gutta.*

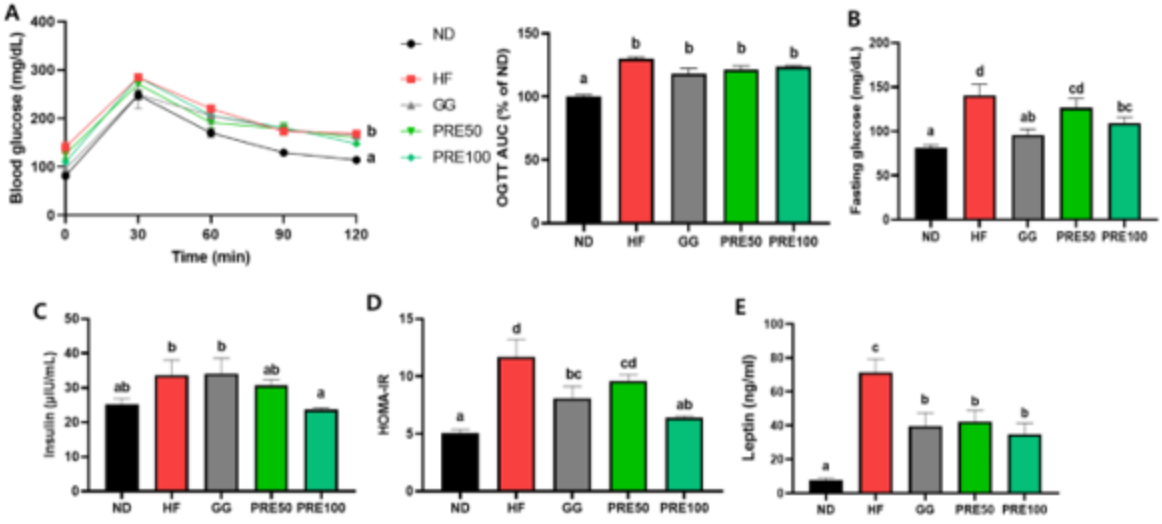

Figure 6

Parsnip (*Pastinaca sativa*) root extract (PRE) improves high-fat-diet-induced serum liver function in non-alcoholic fatty liver disease. (A) Blood glucose levels during oral glucose tolerance tests and area under

the curve of blood glucose levels; (B) Fasting blood glucose levels; (C) Serum insulin levels; (D) Homeostatic model assessment for insulin resistance (HOMA-IR) index; (E) Leptin. All data are presented as mean ± standard error of the mean (n = 9). Values with different letters are significantly different at $p < 0.05$ by Duncan's multiple range tests. ND, normal diet; HF, high-fat diet; GG, *Garcinia gummi-gutta.*

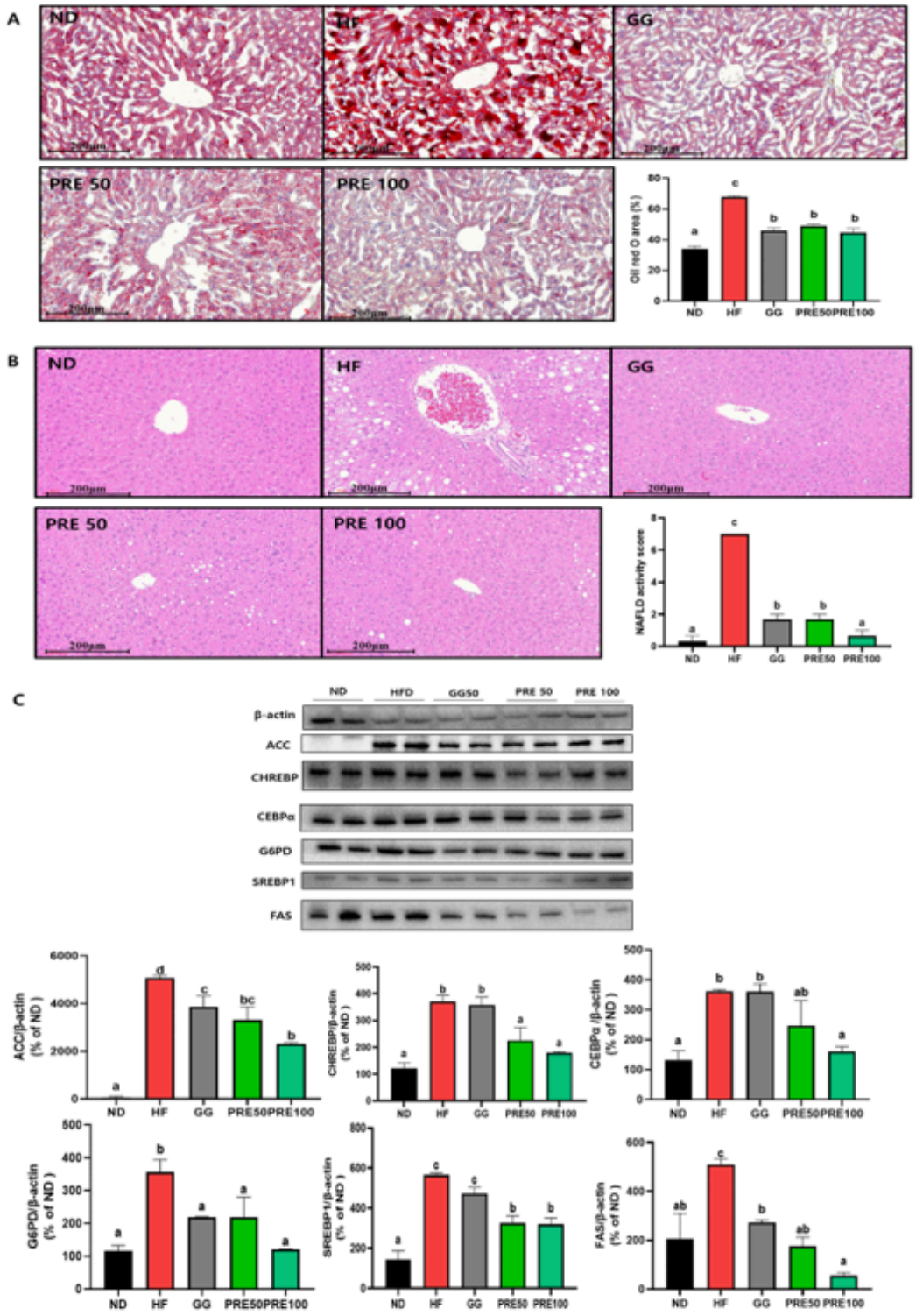

Figure 7

Parsnip (*Pastinaca sativa*) root extract (PRE) reduceshigh-fat-diet-induced lipid metabolism in liver tissue. (A) Representative histological results using oil red O staining; (B) representative histological results of H&E staining and non-alcoholic fatty liver disease (NAFLD) activity score; (C) Representative western blots of acetyl-CoA carboxylase (ACC), carbohydrate response element-binding protein (ChREBP), CCAAT/enhancer-binding protein α (C/EBPα), Glucose-6-phosphate dehydrogenase (G6PD), sterol regulatory element-binding protein-1 (SREBP1), and fatty acid synthase (FAS). Band intensities are normalized with β-actin and data are expressed as the mean ± SEM. Values with different letters are significantly different at $p < 0.05$ by Duncan's multiple range tests. ND, normal diet; HF, high-fat diet; GG, *Garcinia gummi-gutta.*

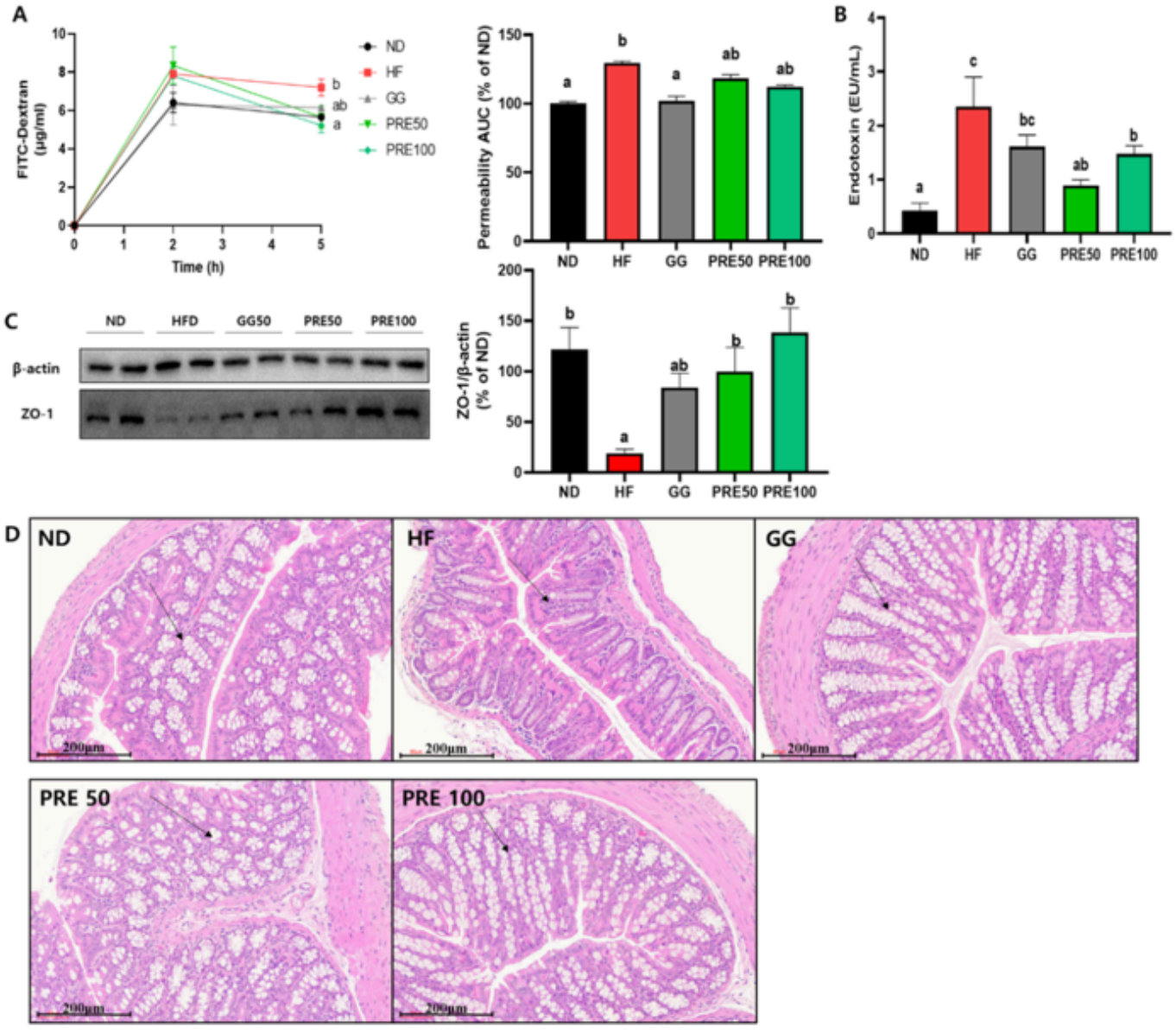

Figure 8

Parsnip (*Pastinaca sativa*) root extract (PRE) improves gut health and tight junction in high-fat-diet-induced mice. (A) Plasma FITC-dextran concentration during gut permeability test and area under the

curve of plasma FITC-dextran levels; (B) serum endotoxin; (C) protein expression of ZO-1. Band intensities are normalized with β-actin and data are expressed as the mean ± SEM; (D) representative histological results of colon tissues H&E staining. Values with different letters are significantly different at $p < 0.05$ by Duncan's multiple range tests. ND, normal diet; HF, high-fat diet; GG, *Garcinia gummi-gutta.*

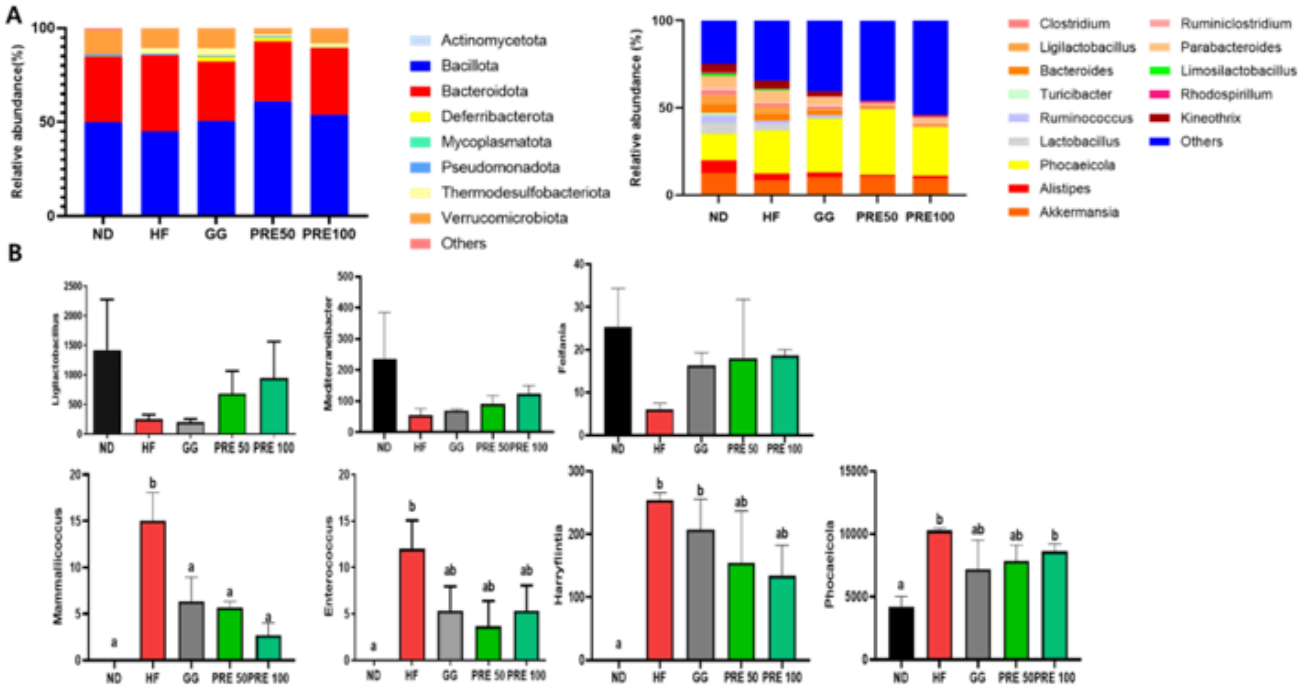

Figure 9

Parsnip (*Pastinaca sativa*) root extract (PRE) changes the microbial composition in high-fat-diet-induced mice. (A) Relative abundance plot of bacterial phyla and genera; (B) Proportion of *Ligilactobacillus, Mediterraneibacter, Ruminiclostridium, Feifania, Mammaliicoccus, Enterococcus, Harryflintia, Phocaeicola*. All data are presented as mean ± standard error of the mean ($n = 3$). Values with different letters are significantly different at $p < 0.05$ by Duncan's multiple range tests. ND, normal diet; HF, high-fat diet; GG, *Garcinia gummi-gutta.*

Supplementary Files

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