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Hawthorn pectin plays a protective role in myocardial ischaemia by regulating intestinal flora and short chain fatty acids

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ABSTRACT

Studies have shown that there is a close relationship between acute myocardial ischaemia (AMI) and intestinal flora imbalance. And pectin has a protective effect on AMI and regulates intestinal flora. Raw hawthorn pectin from hawthorn (RHP) is high methoxyl pectin, which is able to protect injury induced by AMI. After stir-frying of hawthorn, pectin from stir-fried hawthorn (FHP) transformed to low methoxyl pectin, the protective mechanisms against AMI is not well-understood. In this study, the protective effects of RHP and FHP against AMI rats were explored. The results revealed that FHP regulated myocardial enzymes including CK, CK-MB and CTn-1, oxidative stress-related indicator SOD more significantly than RHP. According to the determination of proportion of different kinds of short-chain fatty acids (SCFAs) and abundance of microbiota producing SCFAs, it was speculated that RHP and FHP were fermented by these microbiota. RHP increased the proportion of acetic acid and butyric acid, while FHP increased the proportion of acetic acid in feces. Pretreatment with RHP and FHP enriched the beneficial microbiota and maintained the levels of SCFAs, which significantly increased after modeling. These results revealed that RHP and FHP played a protective role in myocardial ischaemia by regulating intestinal flora and SCFAs.

1. Introduction

Cardiovascular diseases (CVDs) is the leading cause of morbidity and mortality worldwide disease which endangers human health recently with increasing trend seriously(Goldsborough et al., 2022; Jin et al., 2019). Acute myocardial ischaemia (AMI), as a representative pathological process of CVDs, the molecular mechanisms of which is complex and involves multiple pathways, including energy deficit, inflammation reaction, oxidative stress, and so on(Yang et al., 2022; Zheng et al., 2022). Over recent years, the imbalance between myocardial blood-oxygen supply and demand associated with oxidative stress has been recognized as the primary driver of the pathological changes of myocardial ischaemia causing cardiac metabolic disorders, arrhythmias, myocardial infarction, and sudden death seriously(Liu et al., 2023; Zhang et al., 2023b).

Chinese medicine was applied to the prevention and treatment of myocardial ischaemia. Hawthorn has been used as a natural medicine for a long time in treating CVDs(Lu et al., 2023). Hawthorn ethanol extract reduced the level of malondialdehyde (MDA), and increased in the activity of antioxidant enzymes and thus protected blood vessel in CVD(Jing et al., 2023). The ethanolic extract of hawthorn also increased the activity of glutathione peroxidase (GSH-Px), reduced the content of MDA, and inhibited the oxidative stress on chronic heart failure rats caused by doxorubicin(Cheng et al., 2020). The previous study of our group shown the ethanolic extract of hawthorn and hawthorn processed with honey protected AMI induced by isoproterenol hydrochloride (ISO) through regulating the levels of creatine kinase isozyme (CK-MB), α-hydroxybutyrate dehydrogenase (α-HBDH), and creatine phosphokinase (CPK), as well as lactate dehydrogenase (LDH) (Ao et al., 2020).

A study from the Global Burden of Diseases (GBD) concluded that suboptimal diet was responsible for more deaths than any other risk factor globally (Collaborators, 2019). Promoting the intake of dietary components was considered an effective way to reduce the burden of disease associated with dietary risks. And mounting evidence suggested a role of dietary fibers in the prevention of CVDs (Partula et al., 2020).

Pectin, as a kind of dietary fiber, was comprised of as many as 17 different monosaccharides and more than 20 types of interlinkages. The backbone of pectin was composed of α (1 → 4) linked D-galacturonic

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acid with different degree of methyl esterification. Different regions consisting of homogalacturonan (HG), rhamnose-galacturonic acid I (RG-I), and rhamnose-galacturonic acid II (RG-II) were formed(Elshahed et al., 2021). As a kind of dietary fiber, pectin had a variety of bio-activities (Edirisinghe et al., 2019; McClements and Decker, 2018; Nie et al., 2023). Hawthorn pectin is effective in preventing and treating cardiovascular disease and has potential as a dietary supplement (Zhu et al., 2015). The structure of pectin affected the distribution and composition of gut microbiota(Zhou et al., 2023). The functional properties of pectin were closely related to their monosaccharide composition, molecular weight (MW), degree of esterification (DE) etc (Jiang et al., 2020).

Although indigestible in the oral cavity, stomach and small intestine (Cao et al., 2023), pectin was fermented by gut bacteria to produce SFCAs with acetate, propionate and butyrate being the main metabolites after reaching the colon (Hu et al., 2022). Hawthorn pectin was able to restore the imbalance of gut microbiota and produce SCFAs in hyperlipidaemia rats (Wang et al., 2023). And SCFAs were known to improve energy homeostasis and metabolism (Lednovich et al., 2024). Butyric acid can serve as an energy source for the heart, thus improving energy homeostasis and metabolism in the body (Fu et al., 2019). An *in vitro* study showed that MW and DE of pectin affected their modulation on microbial community as well as on the contents of SFCAs (Firrman et al., 2022). Differences in the contents of acetic acid and propionic acid confirmed that the methylation degree of pectin was a key factor in regulating SCFAs composition (Liu et al., 2023).

Raw hawthorn (RH) and its stir-fried product (FH) were recorded in the 2020 edition of Chinese Pharmacopoeia with different effects (Commission, 2020). Our previous research on the structural characteristics of pectins from RH and FH shown the DE decreased after stir-frying. Besides, the content of galacturonic acid and the proportion of neutral saccharides also changed after stir-frying. These changes caused the different binding abilities to cholesterol micelles and cholate *in vitro*(Zhang et al., 2023a).

The traditional use of hawthorn in China included promoting blood circulation and removing blood stasis, that was concerned with the protection from myocardial ischaemia. The present study evaluated the protective effects of pectins form RH (RHP) and FH (FHP) from AMI. The results of this study tried to clarify the protective effect from AMI of RHP and FHP, and to elucidate influence of structure on this effect, as well as on the modulation on gut microbiota, which might concern to the mechanism of AMI protection.

2. Materials and methods

2.1. Materials

The raw hawthorn slices (batch no. 1220630) were purchased from Shaohuatang Sinopharm co., Ltd (Bozhou, Anhui, China) and were identified by Prof. Tianmin Wang, from College of Pharmacy, Liaoning University of Traditional Chinese Medicine as the fruit of *Crataegus pinnatifida* Bge.

ISO (batch no. K1826104) was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

Compound danshen dripping pills (CDDP) (batch no. 220925) was obtained from Tasly Pharmaceutical Co., Ltd. (Tianjin, China).

LDH (batch no. 20230420) and MDA (batch no. 20230420) assay kits were purchased from Jiancheng Biological Engineering Institute, Nanjing, China.

Superoxide dismutase (SOD) assay kit (batch no.2305001) and Fluorescein (FITC) Tunel Cell Apoptosis Detection Kit (batch no. MPC2306023) were purchased from Solarbio Science&Technology Co., Ltd (Beijing, China).

Cardiac troponin I (CTn-I) (batch no. 23042423N), creatine kinase (CK) (batch no. 23042415N), and CK-MB (batch no. 23042420N) enzyme linked immunosorbent assay (ELISA) kits were purchased from Sinovac Biotech Co., Ltd (Shanghai, China).

All other chemical reagents used in this work were commercially available and of analytical or chromatographic grade.

2.2. Sample preparation

2.2.1. Preparation of FH

According to the "Chinese Medicine Processing Science" processing method, slices of RH were fried at 120–150 °C until the color deepened to yield FH(Gong, 2019). Then slices of RH and FH was grinded and crushed, passed through a 60-mesh sieve to give powder of RH and FH, respectively.

2.2.2. Extraction of pectin

RH or FH powder were blended with pure water (1:3 w/v). The extraction was carried out at 90 °C for 3 h in a constant temperature water bath. The resulting extract was filtered through Whatman filter paper using a Buchner funnel which was connected to a vacuum pump while hot. The filtrate was cooled to room temperature (20 ± 2 °C) then added with 95% ethanol at 1:2 vol ratio, kept for 12 h at 5 °C, to reach the equilibrium state and allow pectin precipitation. The pectin was then separated by filtration through Whatman filter paper and washed twice with anhydrous ethanol to eliminate impurities. The filtrate was dried in air during 24 h and subjected to freeze drying for 30 h to yield RHP and FHP, respectively(Zhang et al., 2023a; Zhou et al., 2021). The yield of RHP and FHP were both 10% with insignificant difference.

2.2.3. Preparation of hawthorn water decoction

Powders of RH and FH were decocted with water (1:3 w/v) for 1 h. The final decoction was filtered with gauze and concentrated to 0.3 g/mL (calculated from raw material) by heating(Wang et al., 2019).

2.3. Structural characterization of RHP and FHP

Structural characterization of RHP and FHP were achieved by experimental method in reference(Zhang et al., 2023a). And the major structure characters were shown in Table S1.

2.4. Experimental animals

Male Sprague-Dawley rats (certificate NO. SCXK (Liao) 2020–0001), weighting 200 ± 20g were purchased from Changsheng Biotechnology Co., Ltd. Liaoning, China. Rats were housed in a pathogen-free room with temperature controlled at 23 °C, 12-h light/dark cycle and fed diets and water ad libitum. All studies involving animals in this study were in accordance with the ARRIVE guidelines and were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and welfare committee of Liaoning University of Traditional Chinese Medicine Research Ethics Committee, license number 210000420220209, 8\25\2022.

After one week of acclimatization, a total of 42 rats were randomly divided into seven groups including control group (CON), model group (MOD), positive group (CDDP), raw hawthorn (RH) group, stir-fried hawthorn group (FH), raw hawthorn pectin (RHP) group, and stir-fried hawthorn pectin (FHP) group. Rats in CDDP group were oral administrated CDDP at 85.05 mg/kg, which was calculated from the recommended dosage of CDDP 13.5 mg/kg per day for human. Rats in RH and FH groups were oral administrated the decoctions of RH and FH at 1260 mg/kg respectively, which was calculated from the recommended dosage of RH and FH 200 mg/kg per day for human. Rats in RHP and FHP groups were oral administrated the suspension of RHP and FHP at 126 mg/kg respectively, which was calculated from the yield of RHP and FHP from RH and FH. While rats in CON and MOD groups were oral administered with water. The oral administration lasted for three consecutive weeks. On the 19th day of oral administration, all the rats excepted for those in CON group were injected with ISO (10 mg/kg, i.p.)

for three consecutive days to cause AMI. Feces were collected at the 20th night. Then, all experimental rats fasted overnight. On the 21st day, 1 h after the oral administration and the injection of ISO, the feces were collected again. Rats were anesthetized using ethyl carbamate at a dose of 1200 mg/kg (i.p.). Blood was collected from the abdominal aorta. After that, rats were euthanized by cervical dislocation. The hearts were quickly removed and dissected immediately, washed with physiological saline, blotted dry with filter paper, and the left ventricle from cardiac tissue was taken and immersed in 10% formalin solution for pathological analysis. The blood was stored at 4 °C for 1 h, and then centrifuged at 900×*g* for 15 min to obtain serum samples for kit measurement (Tao et al., 2020).

2.5. Serum biochemical indices analysis

Cardiac marker enzymes of CK, CK-MB, CTn-I, LDH and oxidative indices SOD, MDA were measured by commercially available kits(Tao et al., 2020).

2.6. Heart histopathological examination

Dissected heart tissues were fixed overnight in 4% paraformaldehyde. Then the tissues were embedded in paraffin and sliced into 5 μm thick section. Conventional hematoxylin and eosin (H&E) staining were performed for histopathological examination. Digital images were captured with the light microscope (200 × magni-fiction, Nikon eclipse E Ts2-FL, Nanjing)(Fan et al., 2020).

2.7. Detection of myocardial apoptosis

DNA fragmentation was detected using the TUNEL assay according to the apoptosis kit instructions. After dewaxing and rehydration, the sections of myocardial tissues were incubated with proteinase K (20 μg/mL) for 15 min at room temperature. The slices were washed with PBS, and then incubated with working-strength terminal deoxynucleotidyl transferase in a wet box for 60 min at 37 °C. The slices were washed with PBS again, then stained with 4, 5- diamino-2-phenylindole (DAPI) for 8 min and dropped anti-fluorescence quencher, and observed under a fluorescence microscope immediately (Leica, Heidelberg, Germany) (Xiao et al., 2022).

2.8. High-throughput sequencing analysis of gut microbiota

Nucleic acids were extracted using the TGuide S96 Magnetic Bead Method Fecal Genomic DNA Extraction Kit (TiangenDP812, Beijing, China). Nucleic acids were tested for concentration using an enzyme marker, amplified according to the test, and the amplified PCR products were tested for integrity by electrophoresis using agarose at a concentration of 1.8%. The amplified products were tested for concentration (Qubit) and banding (agarose gel electrophoresis), and the eligible samples were mixed. The conditions of electrophoresis were as follows: 1.8% detector gel, voltage 120 V, 40–45 min, 250 bp Marker. For the constructed gene bank, damage repair, end repair, and ligation junction of the mixed products were carried out using the gene bank construction kit (SMRTbell Template Prep Kit), and the reaction process was carried out on the PCR instrument, and finally purified and recovered by using the AMpure PB beads were purified and recovered to obtain the uploaded gene bank. The final gene bank was tested by concentration (Qubit) and size (Agilent 2100) to determine whether it met the uploading requirements. The uploaded gene bank was bound using the PacBio Binding kit so that the gene bank could be combined with the Primer and Polymerase to carry out the final reaction product. AMpure PB Beads were purified and placed on the Sequel II sequencer for on-line sequencing and analyzed on the BMK cloud platform(He et al., 2022).

2.9. Analysis of SCFAs

The feces of rat were weighted precisely and added distilled water at the ratio of 3 : 10 (m: v). After vortex to mix thoroughly, the suspension was centrifugated at 14520×*g* for 10 min to yield the supernatant. And then it was added concentrated hydrochloric acid at the ratio of 10 : 1 (v: v). The mixture was mixed thoroughly, and added of ether at the ratio of 2 : 1 (v: v) to extract at room temperature for 20 min, then centrifugated at 900×*g* for 10 min. The upper organic phase transferred to another tube and added sodium hydroxide at a concentration of 1 mol/L at the ratio of 10 : 1 (v: v) and mixed well, extracted at room temperature for 20 min, then centrifugated at 900×*g* for 10 min. The lower aqueous phase was collected, and added concentrated hydrochloric acid at the ratio of 55 : 1 (v: v), After vortex thoroughly, and the sample was passed through 0.22 μm microporous filter membrane for measurement.

The conditions of quantitative analysis were as follows: Column: Welchrom C_{18} (250 mm × 4.6 mm, 5 μm). Mobile phase: acetonitrile (A)-0.025% phosphoric acid (B). Elution mode: gradient elution: 0–11 min, 3%A; 11–20 min, 3%Ã10%A; 20–25 min, 3%A. Flow rate: 1 mL/min. Column temperature: 30 °C. Injecting volume: 20 μL. Detective wavelength: 210 nm(Wang et al., 2017).

2.10. Statistical analysis

2.10.1. Statistical analysis for biochemical indices

The experimental data were expressed as mean ± standard deviation, and SPSS 26.0 statistical software was used for data analysis. The differences among groups were analyzed using one-way analysis of variance (one-way ANOVA). If the variances were equal, LSD tests were applied for post hoc test. And if the variances were not equal, Tamhane's T2 tests were applied for post hoc test. If the data did not satisfy normal distribution Mann–Whitney U tests were applied for post hoc test. $P < 0.05$ was considered statistically significant.

2.10.2. Statistical analysis for gut microbiota

Alpha diversity analysis was used to study the species richness, evenness in specific environments. Beta diversity analysis was used to study the species diversity among different environmental communities. The Metastats test was applied to detect features that were significantly different between assigned taxa. Linear discriminant analysis (LDA) and effect size (LEfSe) analysis were performed to characterize the microbial differences between different treatment groups. The LDA was used to quantify the effect size of each feature. A significance alpha value of less than 0.05 and an effect size threshold of 3.5 were used for this analysis.

3. Results

3.1. Effects of RHP and FHP on body weight and cardiac weight index

CDDP, RH, FH, RHP, and FHP intervention exhibited no influence on rats weight. And after intraperitoneal injection with ISO rats of MOD, RH, FH, RHP, and FHP groups showed a significant decrease in body weight as compared with CON group, (Fig. 1A), that possibly due to a decrease in food intake associated with pathological status. The cardiac index of rats in MOD group increased significantly. And CDDP, RHP, FHP, RH, and FH did not decrease the cardiac indices of rats comparing with those of the MOD group ($P > 0.05$) (Fig. 1B).

3.2. Effects of RHP and FHP on serum indexes in AMI rats

The levels of serum biochemical indexes in each group were shown in Fig. 2A–D. It was clearly observed that serum LDH, CK and CK-MB levels significantly increased in the MOD group as compared with those in the CON group. Compared with the MOD group, the levels of LDH, CK and CTn-I of the CDDP, RHP and FHP groups significantly decreased, while the level of CK-MB of the FHP group was significantly

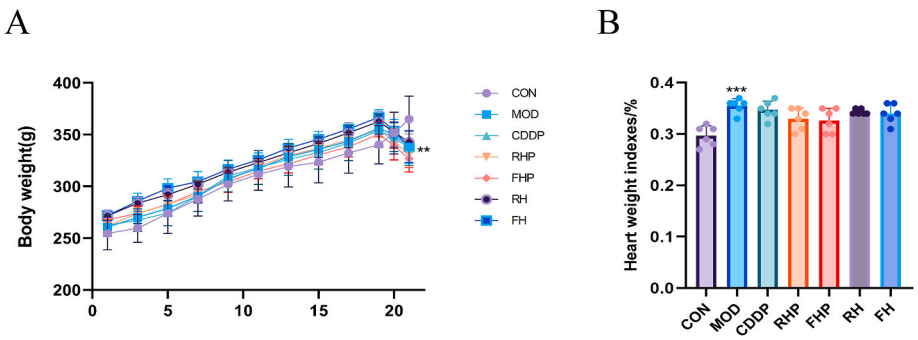

Fig. 1. Body weight growth trend (A) and heart indexes (B) Values were expressed as mean ± SD in each group ($n = 6$). MOD group was compared with the CON group, $^{**}P < 0.01$, $^{***}P < 0.001$.

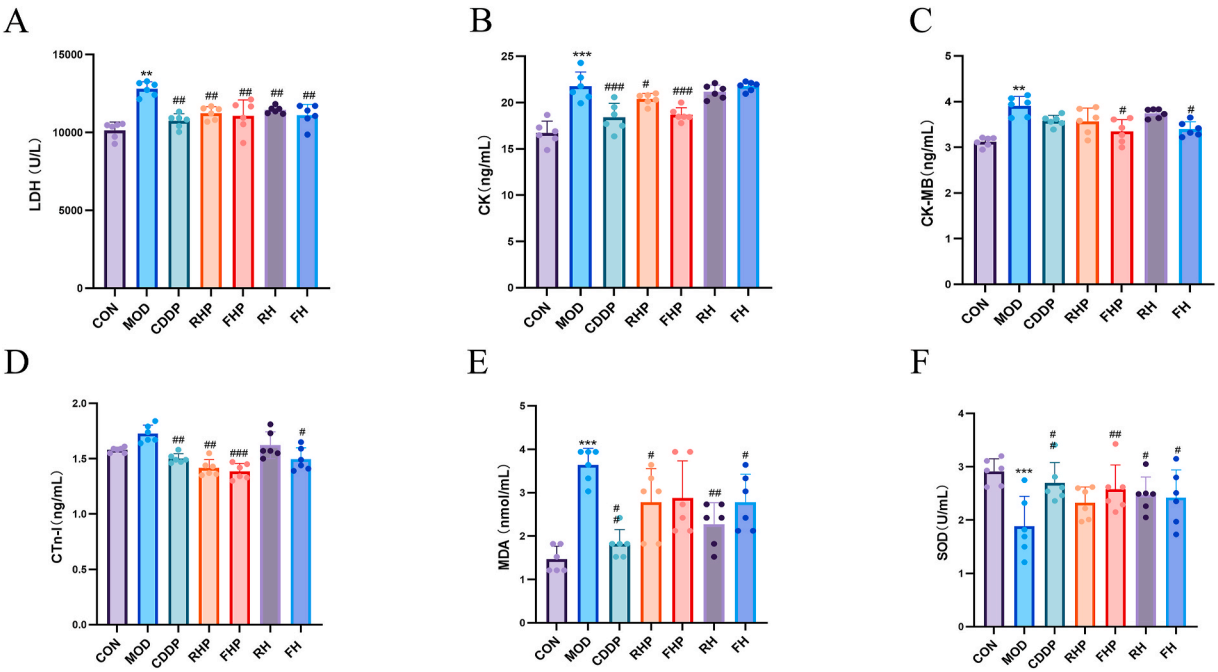

Fig. 2. Biochemical indexes in rats serum. (A) LDH; (B) CK; (C) CK-MB; (D) CTn-I; (E) MDA; (F) SOD Values were expressed as mean ± SD in each group ($n = 6$). Significantly different from CON group, $^{**}P < 0.01$, $^{***}P < 0.001$. Significantly different from MOD group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

lower than that of the MOD group. RH and FH also reduced the levels of LDH, CTn-I or CK-MB to different degrees. Compared with the CON group, the MDA level of the MOD group was significantly higher, while the SOD content was significantly lower. RH and FH were as effective in reversing the levels of MDA and SOD, whereas RHP and FHP tended to restore MDA and SOD respectively (Fig. 2E and F). Although there was

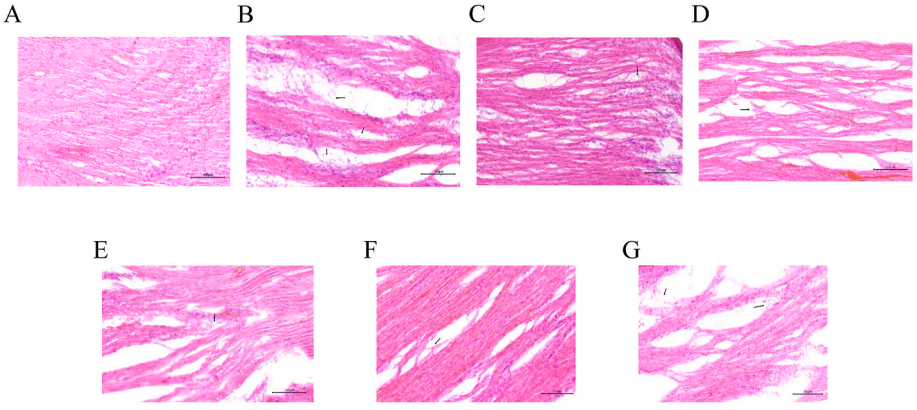

Fig. 3. Histology observation of myocardial tissue Ã G were CON group, MOD group, CDDP group, RHP group, FHP group, RH group, FH group, respectively (magnification, × 20). Myocardial tissue damage was marked with a black arrow.

no significant difference in CTn-I level between the CON and MOD groups, there was a significant decrease in the RHP, FHP and FH groups on CTn-I level.

3.3. Effects of RHP and FHP on the histopathology of heart

To further investigate the protective effect of RHP, FHP, RH and FH on the myocardial tissues, the histopathology of rat's heart was examined. As shown in Fig. 3, photomicrograph of CON group revealed a normal myofibrillar architecture with striations and branched appearance arranged neatly, and the morphology of myocardial cells were uniform (Fig. 3A). However, rats in MOD group showed widespread myocardial structure disorder, characterized by myocardial fiber fracture, interstitium widening, local wavy cytoplasm dissolution, and membrane rupture (Fig. 3B), which displayed that the myocardium was injured after ISO treatment. Pretreatment with RHP, RH, and FHP, the tissue sections showed some neutrophil infiltration, interstitial edema and some discontinuity with adjacent myofibrils, but the morphology of cardiac muscle fiber was relatively well preserved with no evidence of necrosis and less cellular infiltration when compared to MOD group (Fig. 3D, F and 3E), indicating that RHP, RH and FHP had significant cardioprotective effects. Photomicrograph of CDDP group also showed well preserved myocardial structure with less cellular infiltration and necrosis (Fig. 3C). In contrast, myocardial tissue in FH group had disorganized fiber arrangement, ruptured myocyte membranes and interstitial adhesions with severe damage (Fig. 3G).

3.4. Effects of RHP and FHP on cardiomyocyte apoptosis

As shown in Fig. 4A and B, the number of TUNEL-positive cells increased considerably in the MOD group compared with that in the CON group. And CDDP, RHP, FHP, RH, and FH pretreatment significantly reduced the number of TUNEL-positive cells compared with that in the MOD group. The above results suggested that CDDP, RHP, FHP, RH, and FH inhibited ISO-mediated apoptosis.

3.5. Effects of RHP and FHP on gut microbiota and SCFAs

3.5.1. Effects of RHP and FHP on gut microbiota

Shannon and Simpson indexes were used to evaluate the bacterial diversity of gut microbiota. Chao 1 and abundance coverage-based estimator (ACE) indexes were used to evaluate the bacterial richness of gut microbiota (Liu et al., 2023). There were no significant difference between CON and MOD groups indicating that ISO i.p. for three continuous day did not affect the richness and evenness of gut microbiota. And long-term pretreatment with CDDP, RH, and FH significantly decreased Shannon or Simpson indices. While the pretreatment with RHP and FHP had insignificant influence on the alpha diversity of gut microbiota.

The ACE and Chao 1 indexes (Fig. 5A and B) of FHP were higher than those in the FH groups, and similar trends were also observed between RHP and RH groups, indicating a higher microbial richness and diversity in the FHP and RHP groups. Principal component analysis (PCA) revealed evident differences in the intestinal flora between the CON group and the other groups (Fig. 5E). A Venn diagram (Fig. 5F) was used to better characterize the shared richness among the seven groups simultaneously, verifying that FHP and RHP intervention could change the structure of gut microbiota, as compared with corresponding FH and RH.

At the phylum level, the composition of the gut microbiota was

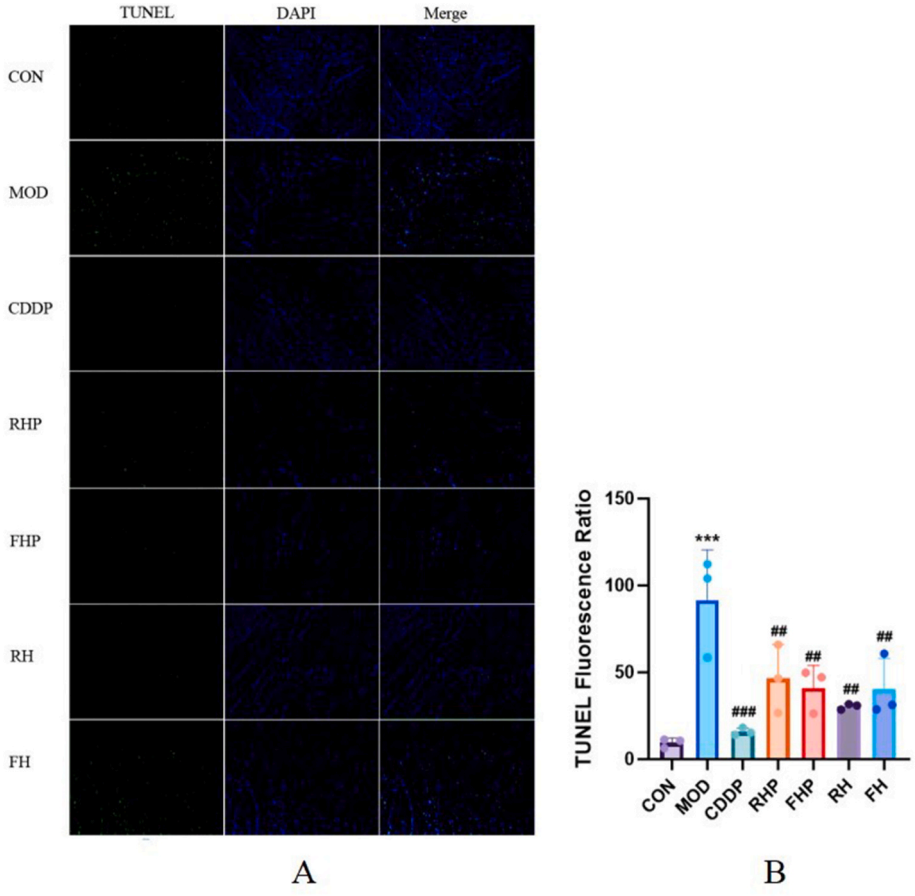

Fig. 4. TUNEL staining results (A), (magnification, × 40); statistics result of TUNEL fluorescence ratio in each group (B). Values were expressed as mean ± SD in each group ($n = 3$). Significantly different from CON group, $^{***}P < 0.001$. Significantly different from MOD group, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

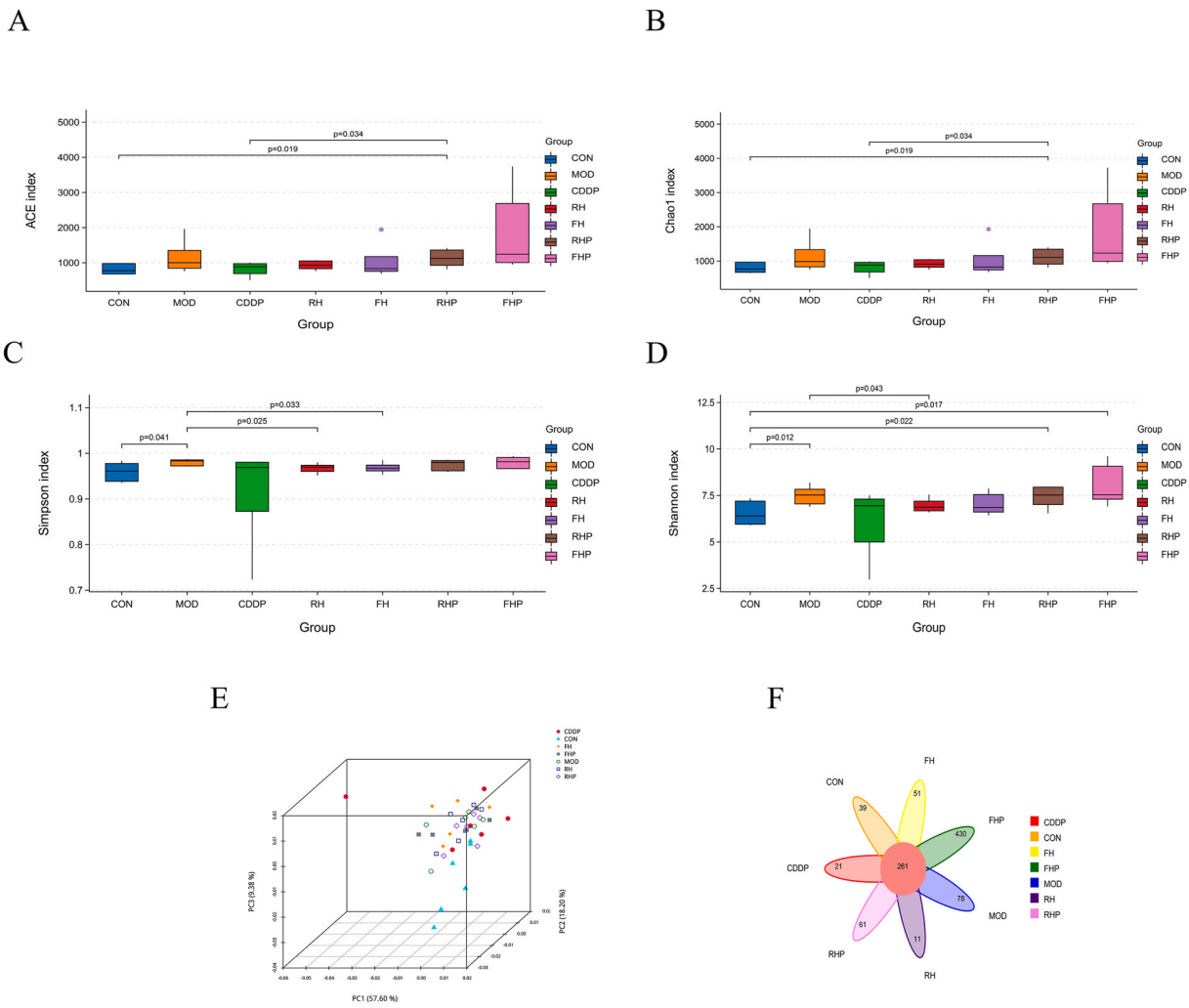

Fig. 5. Diversity analysis of intestinal microflora in rat. Alpha diversity index ACE index (A), Chao 1 index (B), Simpson index(C), and Shannon index (D) of intestinal flora of rats in each group. Beta diversity index. (E) principal component analysis (PCA). (F) Venn diagram.

dominated by the *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteriota*. At the genus level, *Akkermannia* was absent in MOD. And the drugs affected gut microbiota selectively. The RHP and RH intervention were characterized by an increase in the abundance of *Ruminococcus*. Besides, RH treatment resulted in an increase in the abundance of *Blautia*. RH and FH decreased the abundance of *Rosburia*. CDDP significantly promoted the growth of *Lactobacillus* ($P < 0.05$), inhibited that of *Rosburia*. In addition, the abundance of *Escherichia_Shigella* increased in the MOD and FH groups as compared with other groups, though insignificantly. Genera with higher abundance or significant differences or a tendency of difference in each group were listed in Fig. 6C–H.

Next, LEfSe analysis was used to detect significant differences in the relative abundance of dominant microbiota (from phylum to genus level) in the gut microbiota between groups (Fig. 7A). The results of LDA showed the different genera enriched in each group (Fig. 7B). At the phylum level, FHP enriched *Proteobacteria*. At the genus level, *Akkermansia*, *Cetobacterium* were enriched in the CON group. *prevotellaceae_UCG_003*, *Lachnospiraceae_NK4A136_group* were enriched in the MOD group. *Lactobacillus* was enriched in the CDDP group, and *unclassified_Oscillospiraceae* was enriched in the RHP group.

As is shown in Fig. 8, the correlation of biochemical indices with the gut microbiota of rats in the AMI model was analyzed at the genus level based on Spearman's analysis. MDA was positively correlated with *Roseburia*, *Lachnospiraceae_NK4A136_group*, *unclassified_ Osciliospiraceae*, *unclassified Lachnospiraceae*, *Bacteroides*, *NK4A214_group*, and *Monoglobus*. SOD was negatively correlated with *unclassified_Osciliospiraceae*. CK was positively correlated with *Roseburia*, *Incertae_Sedis*, *unclassified_RF39*, *unclassified_ Osciliospiraceae*, *Lachnospiraceae_NK4A136_group*. CK-MB was positively correlated with *Roseburia*. LDH was positively correlated with *Roseburia*, *Lachnospiraceae_NK4A136_group*, *unclassified_Osciliospiraceae*, and negatively correlated with *Bacillus*. CTn-I was positively correlated with *Prevotellaceae_UCG_003* and negatively correlated with *Lunclassified_Osciliospiraceae*, *unclassified Lachnospiraceae*, *Prevotellaceae_Ga6A1_group*, and *uncultured_rumen_bacterium*.

3.5.2. *Effects of RHP and FHP on SCFAs levels*

As shown in Fig. 9 and Table 1, after modeling, the levels of all kinds of SFCAs increased. While pretreatment with RHP and FHP maintained the levels of SFCAs. According to the molar proportion of SFCAs, RHP and FHP supplementation obviously increased in the proportion of acetic acid. The proportion of butyric acid increased significantly in the CDDP, RH and RHP groups.

4. Discussion

Pectins and polysaccharides had protective effects against myocardial ischaemia. And our previous research revealed that hawthorn has a protective effect against AMI(Ao et al., 2020). As the main component of hawthorn, the protective effect of hawthorn pectin on myocardial

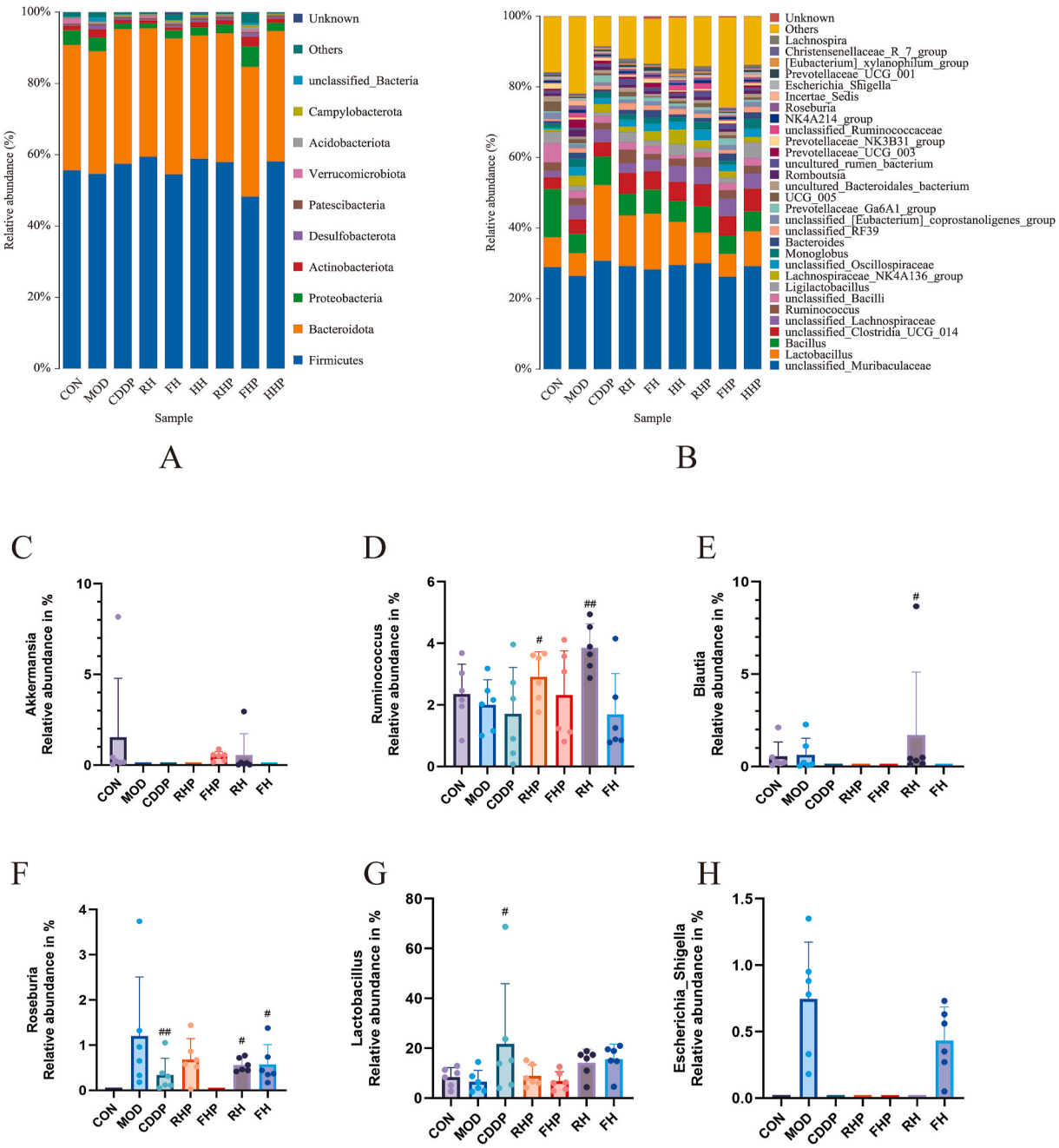

Fig. 6. Stacked column of species abundance of intestinal microflora of rats in each group at (A) phylum (B) genus levels. C ~ H were bar charts of abundance in *Akkermansia*, *Ruminococcus*, *Blautia*, *Roseburia*, *Lactobacillus*, and *Escherichia_Shigella*. Values were expressed as mean ± SD in each group ($n = 6$). Significantly different from MOD group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

ischaemia had not been clarified. Therefore, the present experiment was conducted to investigate the protective effect of pectin from RH and FH on ISO-induced AMI on rats.

When AMI occurred, the myocardial cells were damaged, and therefore a large amount of myocardial enzymes entered the bloodstream. Thus LDH, CK, CK-MB, and CTn-I were used as indicators for evaluating myocardial ischemic injury (Qu et al., 2020; Yossa Nzeuwa et al., 2020). The results of this study demonstrated that RHP and FHP had a protective effect against AMI, and the effects of FHP on the indexes of CK, CK-MB and CTn-1 showed a better tendency than those of RHP, indicating the structural changes of FHP during the processing of FH might lead to the enhancement of its ischemic protective effect. The effects of RHP and FHP in regulating LDH, CK-MB and CTn-1 were similar to those of RH and FH, suggesting that pectin was the material basis of hawthorn's myocardial protective effect.

Hawthorn exhibited protective effect against myocardial ischaemia through anti-oxidative stress (Shatoor et al., 2019). Polyphenolic compounds were the main substances that played antioxidant effects in hawthorn (Cheng et al., 2020; Zhang et al., 2022; S. B. Du et al., 2022; Han et al., 2016). In this study, RH and FH those contained polyphenolic compounds were more effective than RHP and FHP in modulating oxidative stress indexes due to AMI. However, RHP and FHP also possessed certain antioxidant activities, this result suggested that pectin in hawthorn was also associated with its antioxidant activity.

Hawthorn attenuated the degree of apoptosis in rats with ISO-induced AMI (Vijayan et al., 2012). The HE staining and TUNEL staining further confirmed that RHP and FHP had a protective effect on AMI induced by ISO. At the dosages set in our experiments, RHP, FHP, RH

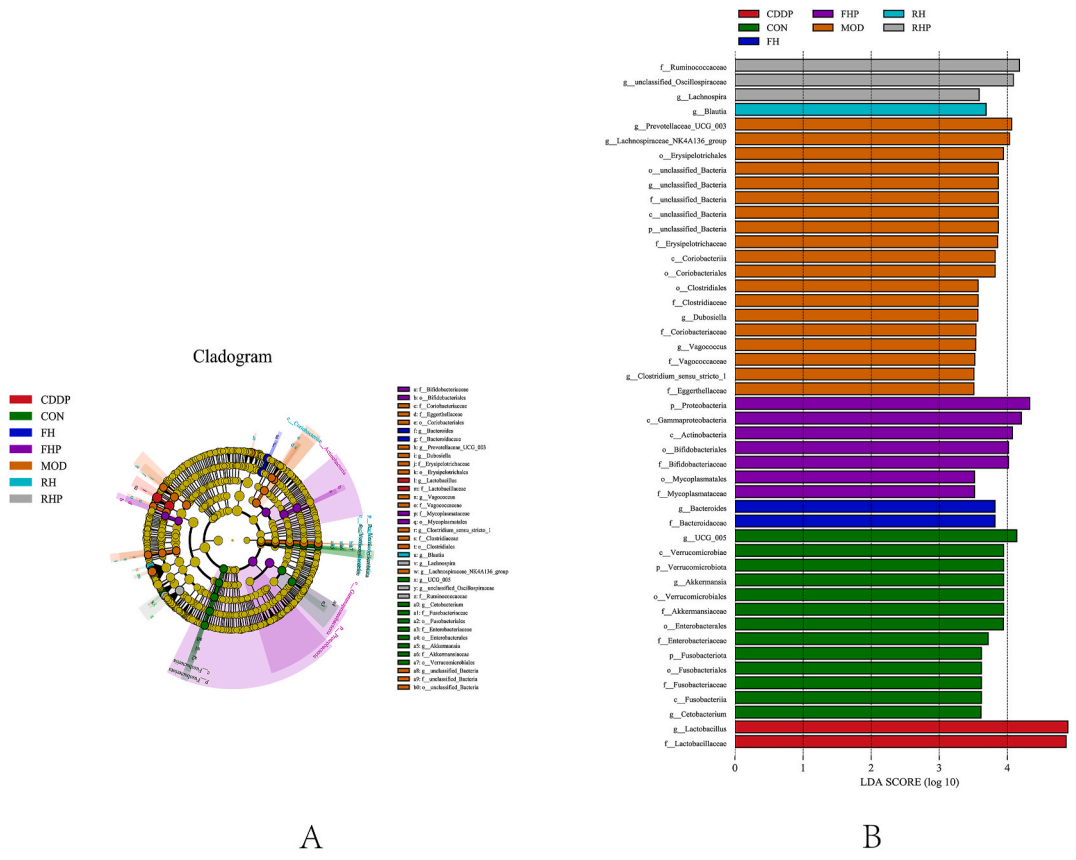

Fig. 7. LEfSe comparison of gut microbiota (A) and Linear discriminant analysis (LDA) of gut microbiota (B).

and FH attenuated myocardial cell apoptosis to similar extent, which once again proved that pectin was one of the material basis of the cardioprotective effect of hawthorn.

The function of the microbial community in the gut determined the energy balance of the host. Maintaining intestinal homeostasis was essential for host health. Gut microbiota was closely associated with the development of AMI (W. Du et al., 2022), and indicators such as the diversity and abundance of gut microbiota changed when AMI induced by arterial ligation (Wu et al., 2017). Compared to the arterial ligation method, AMI induced with ISO was more convenient and caused less harm to the animals, served as an excellent alternate model to the surgical models of AMI (Nanda et al., 2023). But the results of the gut microbiota of rats with ISO-induced AMI showed that the difference in alpha diversity between CON and MOD was not significant. This situation might be caused by different methods and duration of modeling. However, there was a significant difference of the beta diversity between CON and MOD as shown in the PCA scatter plots indicating that AMI led to the changes of intestinal micro-environment.

It has been reported that glycolysis is an important pathway for ATP production in ischemic or hypoxic conditions. Acetic acid and butyric acid were produced from pyruvic acid, and propionic acid was produced from succinic acid in the process of glycolysis(Markowiak-Kopeć and Śliżewska, 2020). Acetic acid, propionic acid and butyric acid were the main kinds of SCFAs, and their proportion was relative constant in the normal state(Fu et al., 2019).

Lachnospiraceae_NK4A136_group was enriched in the MOD group, which was associated with lipid metabolism and facilitated the production of MDA. Additionally, *Lachnospiraceae_NK4A136_group* was producer of SCFAs (Ma et al., 2020). Thus large amounts of acetic acid, propionic acid, and butyric acid were produced in the MOD group. This was due to the body's oxidative stress in response to the sudden situation in myocardial ischaemia(Lopaschuk, 1997). The low abundance of *Roseburia* played a protective role in acute ischemic stroke(Gu et al., 2021). *Lactobacillus* increases the antioxidant properties of the body(Li et al., 2023). And it could produce butyric acid(Thananimit et al., 2022). In the present study, CDDP down-regulated *Roseburia* and enriched *Lactobacillus*. Combined with the SCFAs results of this experiment, it was speculated that the protective effect of CDDP against AMI was related to gut microbiota and SCFAs.

Ruminococcus fermented carbohydrates to produce acetic acid(Crost et al., 2023; Hung et al., 2022). *Unclassified_Oscillospiraceae*, which fermented and degraded pectin, was considered to be a producer of butyric acid (Lawson and Finegold, 2015; Rang et al., 2023). The abundance of *Ruminococcus* was higher in the RHP group, and *unclassified_Osciliospiraceae* was enriched in this group. The results of SCFAs showed that the proportions of acetic acid and butyric acid were elevated in the RHP group, which indicated that RHP was able to be fermented into SCFAs by intestinal microorganism and maintain the levels of SCFAs in the intestines during AMI. Similar result was observed in the FHP group. The difference was that FHP produced a higher proportion of acetic acid, whereas RHP produced higher proportion of both acetic acid and butyric acid, which might due to their different DE (Zhang et al., 2023a; Liu et al., 2023).

Ruminococcus and *Roseburia* was enriched in the RH group, which produced butyric acid (La Reau and Suen, 2018; Vital et al., 2014). Besides, *Roseburia* was positively correlated with MDA, CK, CK-MB, and LDH. And as high abundance of *Roseburia* was detected in the MOD group, it was assigned as harmful bacteria in the process of AMI. And FH down-regulated this genus suggesting that the protective effect of FH against AMI might be related to *Roseburia*. In this study, the proportion of acetic acid in RH group was relatively high as compared with that of FH group. This may have been due to the increase in the abundance of *Ruminococcus* in RH.

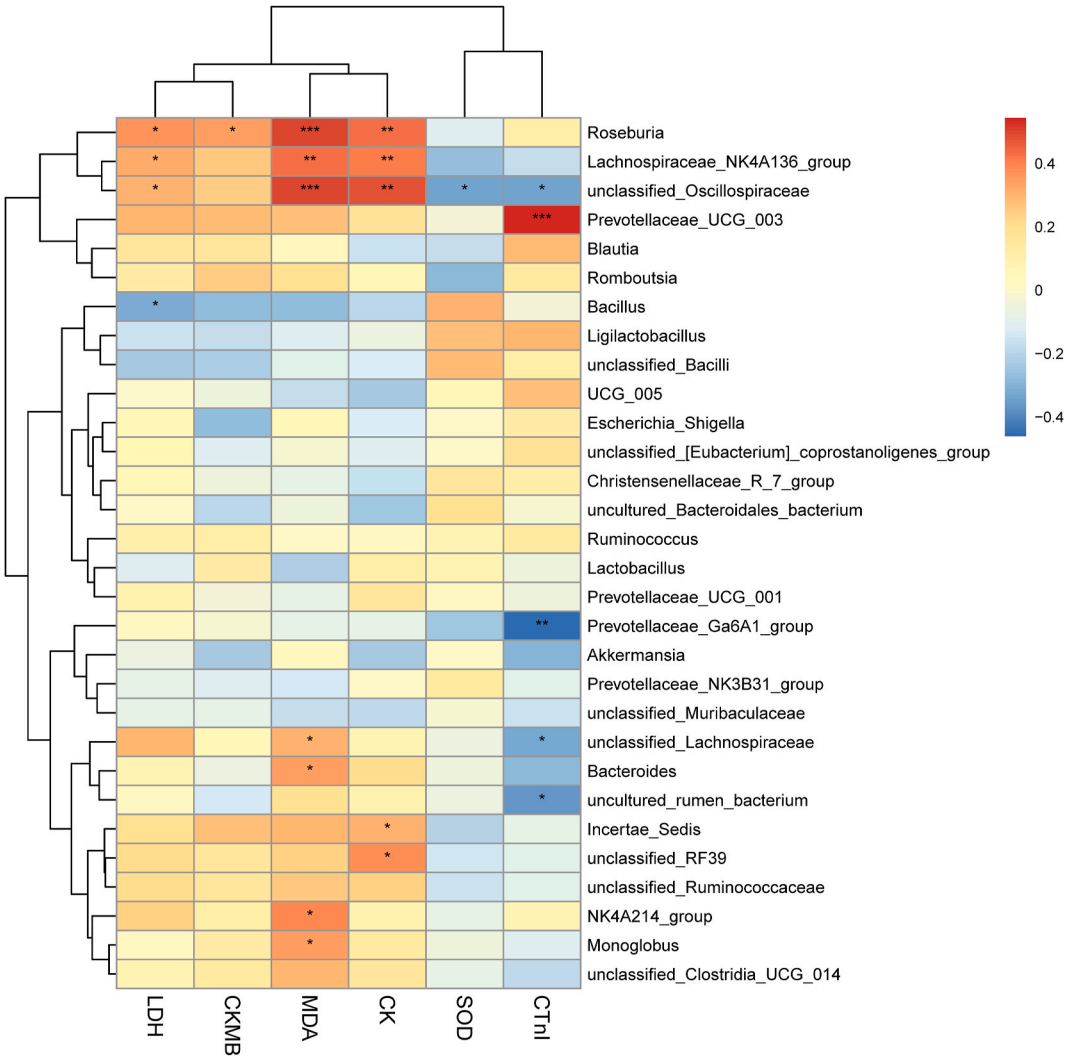

Fig. 8. Heatmap of correlation between myocardial ischaemia related indexes and gut microbiota at the genus level. Spearman correlations analysis was applied between the gut microbiota and AMI related index of each experimental groups. The depth of the color represented the degree of correlation between gut microbiota and AMI related index. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

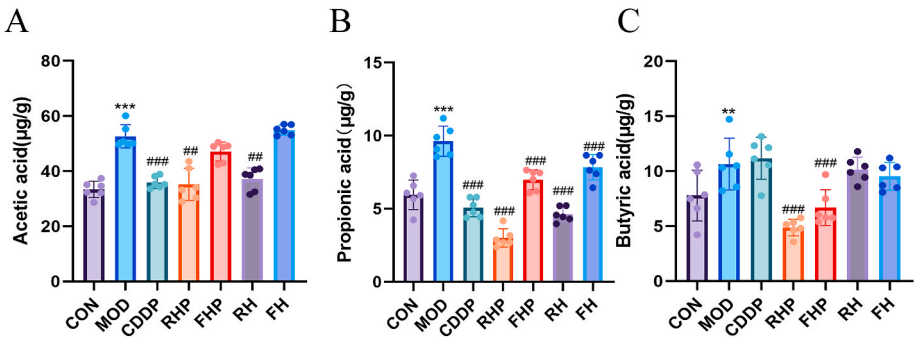

Fig. 9. Contents of acetic acid (A), propionic acid (B) and butyric acid (C) in feces of rats in each group. Values were expressed as mean ± SD in each group ($n = 6$). Significantly different from CON group, $^{**}P < 0.01$, $^{***}P < 0.001$. Significantly different from MOD group, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

5. Conclusion

The pretreatment of RHP and FHP before AMI had protect effect on AMI in regulating levels of myocardial enzymes and oxidative stress-related indicator, as well as in inhibiting apoptosis. RHP and FHP enriched beneficial bacteria and maintained the levels of SCFAs, which was one of the mechanisms of their effect. The difference of their effect on the myocardial enzymes, the bacteria enriched or down-regulated and the proportion of SCFAs produced might be due to their different structural characters, especially DE. All the findings revealed that the hawthorn and pectin of it had the potential to serve as a functional food to protect against AMI. However, the effects of pectin of different

Table 1
Molar proportion of SFCAs in feces of rats in each group ($n = 6$).

group	acetic acid: propionic acid: butyric acid
CON	6.91:1:1.10
MOD	6.75:1:0.93
CDDP	8.73:1:1.85
RHP	14.47:1:1.37
FHP	8.33:1:0.81
RH	9.92:1:1.85
FH	8.64:1:1.02

structural characters against AMI needs to be further assayed.

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CRediT authorship contribution statement

Jiayue Lou: Writing – original draft, Methodology, Data curation. **Baojie Zhang:** Writing – original draft, Formal analysis, preparation. **Yu Zheng:** Writing – review & editing. **Meiqi Liu:** Investigation. **Yang Qu:** Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100863.

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