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Rosa Roxburghii Fruit Pomace Polyphenol Extract Affects Plasma Metabolome and Gut Microbiota in Type 2 Diabetic Mice

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Abstract Rosa roxburghii fruit pomace, rich in polyphenols, is an underutilized by-product in food processing. Polyphenols have been reported to have anti-diabetic properties. In this study, LC-MS metabolomics and 16S rRNA gene sequencing were used to study the effect of Rosa roxburghii fruit pomace polyphenols extract (RPPE) on plasma metabolites and gut microbiota in type 2 diabetic mice. RPPE was fed to diabetic mice at a daily dose of 400 mg/kg body weight for 8 weeks. Feeding RPPE decreased plasma glucose and proinflammatory cytokines and improved insulin sensitivity and plasma lipid profile. Oxidative stress biomarkers and inflammatory cytokines in colon were decreased by RPPE. For plasma metabolites, RPPE decreased p-cresol sulfate level and increased myristoleic acid, myristic acid, and palmitoleic acid levels, suggesting improved glucose and lipid metabolism as well as insulin resistance. Furthermore, RPPE upregulated abundance of beneficial microbes Lachnospiraceae and Erysipelotrichaceae and downregulate levels of detrimental microbes Faecalibaculum, Romboutsia, and Coriobacteriaceae. These results suggest that RPPE delays the development of type 2 diabetes via modulation of the inflammation, oxidative stress, plasma metabolites and gut microbiota.

Keywords: *type 2 diabetes, Rosa roxburghii fruit pomace, metabolomics*

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1. Introduction

Diabetes compromises the life quality of patients and imposes a significant economic burden on the society. Approximately 90% of the diabetic patients have type 2 diabetes (T2D) characterized by peripheral insulin resistance and a decrease in pancreatic β-cells activity [1] T2D is strongly affected by genetic background and environment factors [1]. Diet containing high fat, typically in T2D patients, affects the gut microbiota homeostasis and disrupts the gut epithelial integrity [2]. A leaky gut allows the exogenous compounds, such as lipopolysaccharide (LPS), to enter circulation system from the gut lumen and causes endotoxemia, which lower insulin sensitivity and induce the inflammation [2]. Meanwhile, inflammation can also be induced by the oxidative stress in T2D, for example, reactive oxygen species (ROS) can trigger NF-κB pathway and induce the production of pro-inflammatory cytokines such as IL-1 (interleukin 1) [3].

Healthy food choices have been recommended as an important approach to reducing the risk of T2D. Polyphenols, organic compounds found abundantly in plants, are known to have anti-diabetic properties, including anti-oxidation, anti-inflammation, modulation of energy metabolism, and enhancement of beta-cell activity [4,5]. Due to the low absorption and bioavailability of polyphenols, significant number of dietary polyphenols enters the colon and is utilized by gut bacteria [6,7]. Rosa roxburghii fruit has long been used in traditional Chinese medicine to treat diseases such as hyperlipemia and diarrhea [5]. Rosa roxburghii fruit is abundant in

functional compounds, for example, polyphenols, vitamin C, and polysaccharides [5]. Recently, the exploitation and consumption of Rosa roxburghii fruit have increasingly drawn a lot of attention due to its nutritious properties [5]. By 2020, the planting area of Rosa roxburghii fruit has been extended to 133.3 thousand hectares in Guizhou province, China, and the yield of the fruit was around 100 thousand tons. Most of the commercial products are produced using whole fruit or juice. While Rosa roxburghii fruit pomace as an industrial by-product of juice production will be produced, which is mostly discarded as residues. However, around 50% of the polyphenols is left in Rosa roxburghii fruit pomace [8,9]. Polyphenols compounds extracted from Rosa roxburghii fruit pomace have shown strong antioxidant activity *in vitro* [9]. Furthermore, phenolic acids and flavonoids, such as ellagic acid and isoquercitrin, are the primary polyphenols constituents in Rosa roxburghii fruit [5,8], those polyphenols compounds have been found to have anti-diabetic effects by affecting lipid metabolisms, oxidative status, and insulin resistance [10,11]. We have recently demonstrated the anti-diabetic effect of polyphenols extract from entire Rosa roxburghii fruit [12], feeding 400 mg/kg body weight polyphenols extract from the entire fruit for 8 weeks to type 2 diabetic mice have shown hypoglycemic, anti-inflammatory, and hypolipidemic effects as well as regulated gut microbiota such as downregulating *Rikenellaceae* and *Lachnospiraceae* and upregulating *Faecalibaculum* and *Erysipelotrichaceae* [12]. Thus, polyphenol extract from Rosa roxburghii fruit pomace may also have anti-diabetic properties.

The impact of polyphenols extracts from Rosa roxburghii fruit pomace being a natural source of antioxidants has not been investigated in T2D. Thus, this is the first study to investigate their effect on metabolic status and gut microbiota in type 2 diabetes. Understanding how the polyphenols extracts affect metabolic homeostasis in type 2 diabetes is important for understanding their role in the management of the disease.

2. Materials and Methods

Rosa Roxbunghii Fruit Pomace Polyphenols Extraction

Rosa roxbunghii pomace was provided by Guizhou Heng Li Yuan Co. Ltd. Rosa roxbunghii pomace was washed and dried at 55℃ for 10 hours. The pomace was then ground using a laboratory mortar. Rosa roxbunghii fruit pomace polyphenols (RPPE) were extracted by using the method reported previously [12]. Briefly, Rosa roxbunghii pomace powder was suspended in 80% v/v aqueous ethanol with a liquid–solid ratio of 10:1 (v/w) and centrifugated at 8000 × *g* for 10 min. The supernatant was collected and the extraction procedure was repeated three times. The three supernatants were collected and purified with AB-8 macrospores resin.

Chemical composition Analysis of Rosa Roxburghii Fruit Pomace Polyphenols Extract

Metabolic profiling of RPPE was performed at Biotree Biotechnology (Shanghai, China) by using the method reported previously [12]. Briefly, 20 mg polyphenol extract was dissolved in 700 μL of 75% aqueous extract solution containing internal standard. The resultant solution was injected to the UHPLC system combined with A Sciex QTrap 6500+ (SCIEX, Redwood City, USA). Metabolite identification was based on in-house library.

Animal Experiment

4-week-old male C57BL/6 mice were purchased from Henan Skbeth Biotechnology Co., Ltd. (Zhengzhou, China). The experiment was authorized by the Guizhou University Ethics Committee (Ethical approval number: EAE-GZU-2020-P005). The mice were randomly assigned into two groups after one week of adaption: control group fed with normal diet (Con, n = 8) and another group fed with a high-fat diet. T2D was induced by injection of streptozotocin (50 mg/kg body weight i.p.) in mice fed with high-fat diet. The injection was given three times every three days. The study only included type 2 diabetic animals with high fasting plasma glucose (higher than 150 mg/dl). [8] These animals were then separated into another two groups: the diabetic group (the M group, n = 8) and the RPPE group (the RPPE group, n = 8) fed with additional RPPE at a daily dose of 400 mg/kg body weight for 8 weeks. The mice were sacrificed under isoflurane anesthesia after overnight fasting after 8 weeks of intervention. The contents of the colon and colon tissue were taken. Plasma were collected after centrifuging blood samples at 3000 × *g* for 10 minutes at 4℃. The samples were kept at -80℃ until analysis.

Plasma Parameters

Oral glucose tolerance test (OGTT) was performed at the week 7. Blood glucose level were tested at 0, 30, 60, 90, and 120 minutes after giving1.5 mg/g body weight glucose to mice. Similar to our previous study [12], total glyceride (TG), plasma glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and hemoglobin A1c (HbA1c) were determined by a biochemical analyzer (Mindray BS-480, Shenzhen, China). The IL-1β and TNF-α levels in the colon and plasma were tested by ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Plasma insulin test was performed by using ELISA kit (Alpco, Salem, USA). Levels of malondialdehyde (MDA), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were examined by kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Non-targeted Plasma Metabolomics

Nontargeted metabolomics for plasma was conducted at Biotree Biotechnology (Shanghai, China), the protocol followed the previous study [12]. Briefly, 300 μL of methanol with internal standards was mixed with plasma sample (100 μL). The supernatant was collected and filtered after centrifuging at 12000 × *g* for 15 min at 4℃. The extracts were analyzed by LC-MS. [12] Metabolite identification was based on in-house library (BiotreeDB).

Gut Microbiota Analysis

The metagenomics was conducted by Majorbio (Shanghai, China) and followed the previous protocol [12]. Briefly, after amplification of V3−V4 hypervariable

region of the bacterial 16S rRNA gene, the resulting PCR products were extracted, purified, and quantified. DNA library was constructed and sequenced on an Illumina MiSeq platform. The SSU rRNA database was used for taxonomic annotation. Microbial metabolic functions were analyzed based on KEGG pathway by using PICRUSt2 [13].

Statistical Analysis

The data were shown as the mean ± standard deviation. The Student t-test was used to compare data between two groups using GraphPad Prism 9. The significance of differences was described as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the M group, or # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ compared with the Con group. Significantly changed metabolites were determined by Student t-test p value smaller than 0,05 and VIP value greater than 1. A two-side hypergeometric test was used as the statistical test method in the KEGG enrichment analysis.

3. Results

Polyphenols Composition of RPPE Based on Nontargeted Metabolic Profiling

The polyphenols composition of the extract and their percentage were presented in Table S1. 55.93% of the extrat was polyphenols (including phenols, stilbenes, flavonoid, and lignans).

Effects of RPPE on Plasma Biomarkers and Oxidative Stress Markers

In the current study, the body weight of the mice in the M group began to be considerably greater than that of the Con group on the 14th day of the intervention. RPPE prevented diabrtic mice from gaining weight (Figure 1A). Furthermore, RPPE reduced liver weight (Figure 1B). The aberrant lipid metabolism in the M group was demonstrated by higher levels of LDL, total cholesterol, and triglyceride (Figure 1C, D, and F) as well as lower level of HDL level as compared to Con group (Figure 1E); level of fasting blood glucose, the glucose level in the OGTT test, hemoglobin A1c (HbA1c), insulin, HOMA-IR (Figure 1G-K), and the plasma TNF-α and IL-β levels were higher in the M group (Figure 1L and M). In addition to improving glucose intolerance, the administration of RPPE dramatically lowered fasting blood glucose, HbA1c, insulin, HOMA-IR, LDL, total cholesterol, triglyceride, plasma TNF-α and IL-β, and increased HDL levels.

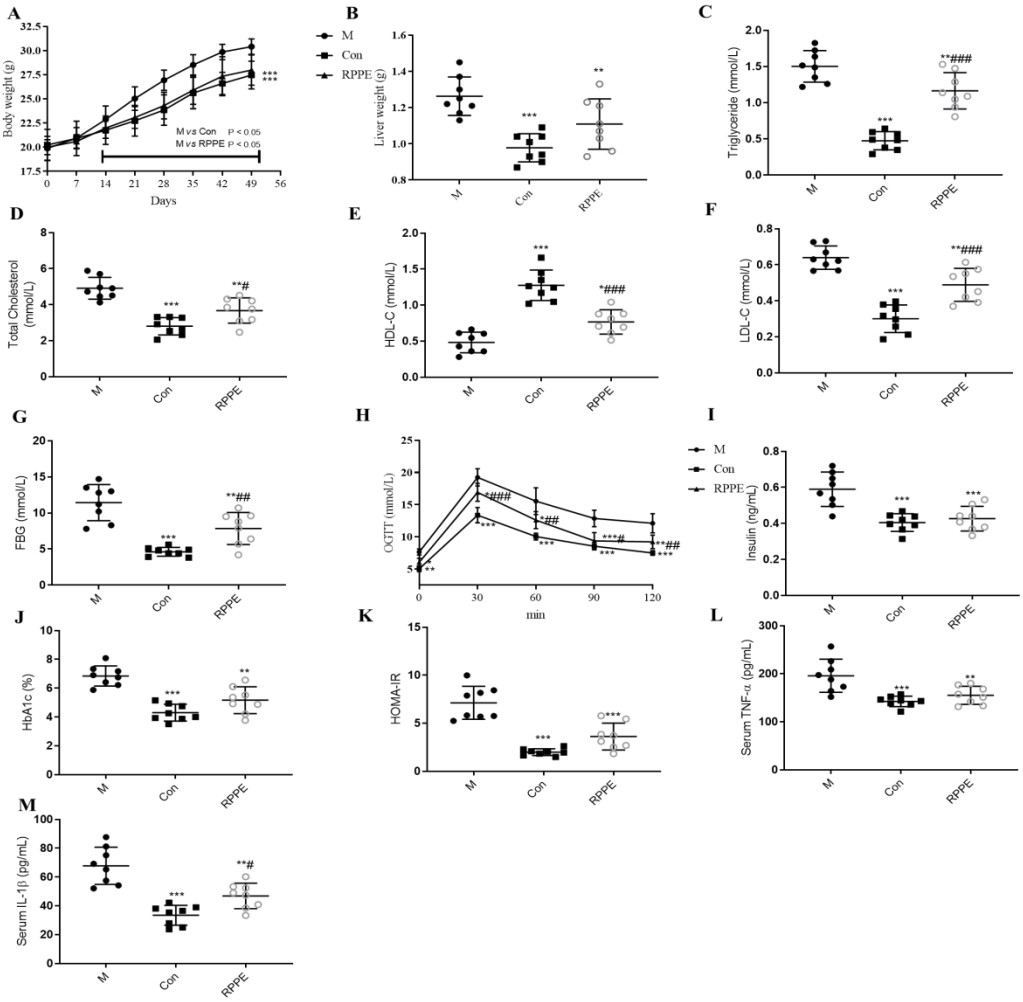

Figure 1. The impact of RPPE on the bodyweight (A), weight of liver (B), plasma triglyceride level (C), plasma cholesterol level (D), plasma HDL-C level (E), plasma LDL-C level (F), plasma glucose level (G), glucose level in oral glucose tolerance test (H), plasma insulin level (I), plasma HbA1c level (J), HOMA-IR (K), plasma TNF-α level (L), plasma IL-β level, (M) in diabetes. n = 8, each group

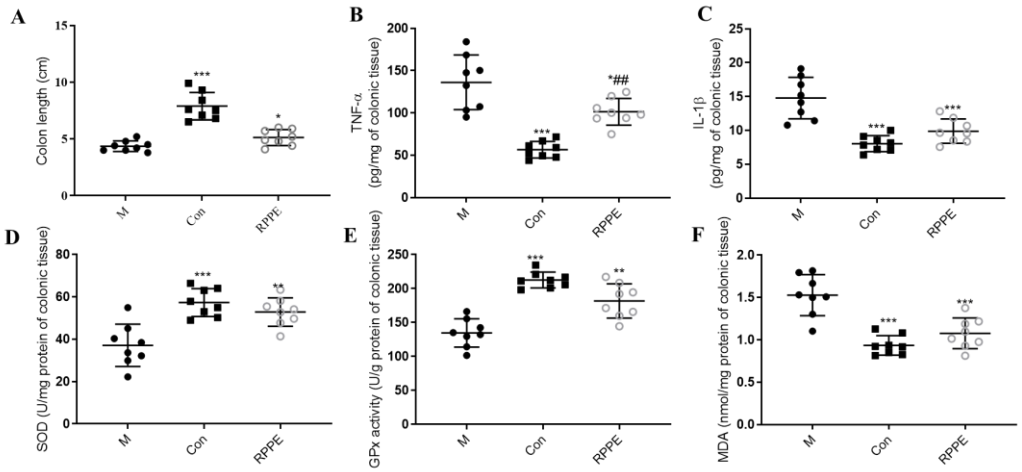

Figure 2. The impact of RPPE on the length of the colon (A), TNF-α level in the colon (B), IL-β level in the colon (C), SOD level in the colon (D), GPx level in the colon (E), MDA level in the colon (F) in diabetes. n = 8, each group

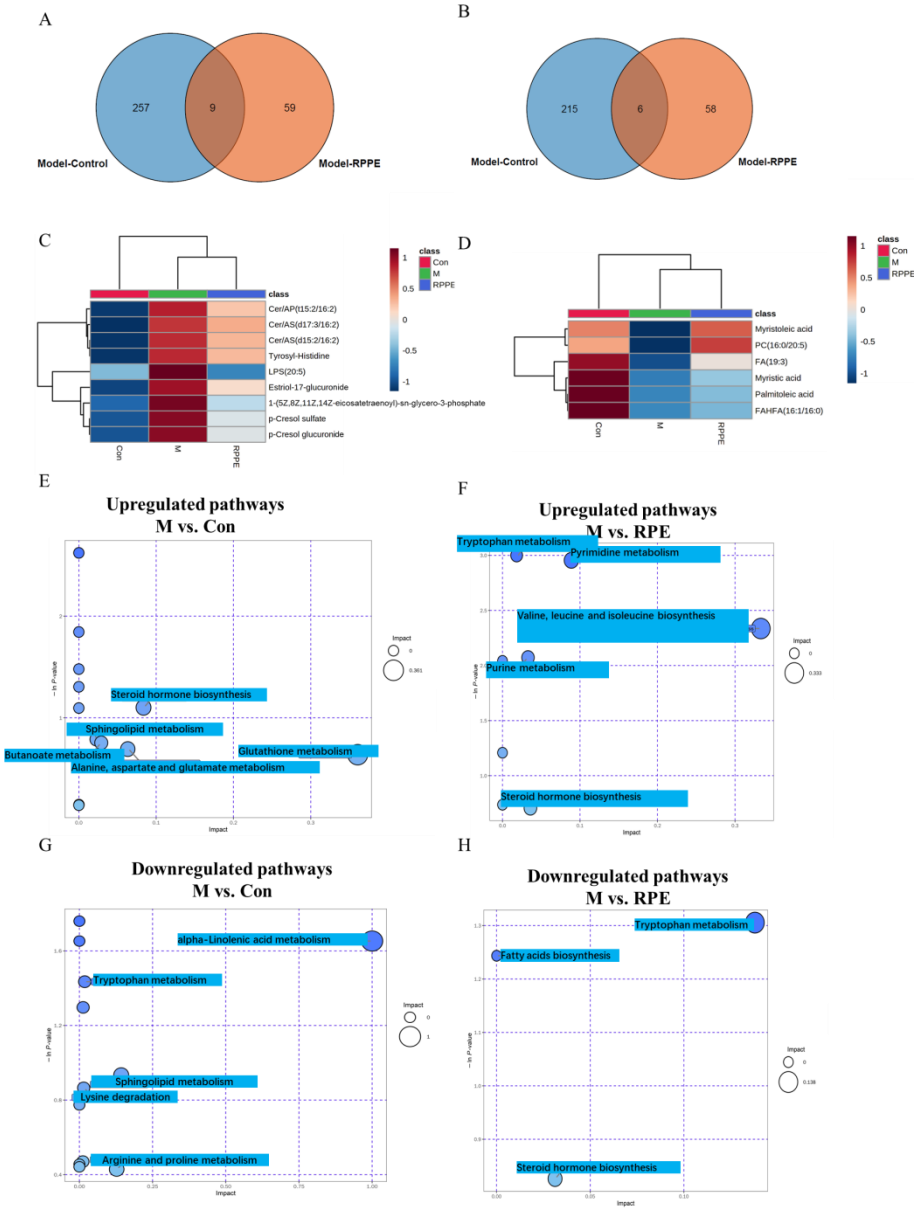

Figure 3. Overlaps of upregulated (A) and downregulated (B) plasma metabolites between comparisons M/Con and M/RPPE. Upregulated plasma metabolites in diabetes that were downregulated by RPPE (C). Downregulated plasma metabolites in diabetic mice that were upregulated by RPPE (D). KEGG pathway enrichment analysis based on upregulated metabolites in M compared to the Con group (E) and RPPE group (F). KEGG pathway enrichment analysis based on downregulated metabolites in M compared to the Con group (G) and RPPE group (H). n = 8, each group

RPPE increased the colon length which was shorter in diabetes compared to the healthy mice (Figure 2A). RPPE also decreased colonic level of TNF-α and IL-β (Figure 2B and C). Oxidative stress markers in the colon were tested, SOD, GPx, and MDA were changed in the diabetic group in comparison to the healthy Con group and feeding RPPE reversed changes of oxidative stress markers (Figure 2D-E).

Effects of RPPE on the plasma metabolic profile

Overlaps of significantly changed metabolites between M/Con comparison and M/RPPE comparison were shown in Venn plots. 257 metabolites were upregulated in the diabetic M group in comparison to the healthy Con group, and 9 of them were downregulated by RPPE (Figure 3A and C). When compared to the Con group, 215 metabolites were downregulated in the M group and 6 of which were upregulated by RPPE (Figure 3B and D). Metaboanalyst database was used to conduct pathway enrichment analyses. Upregulated metabolites in M/Con comparison were enriched in the Glutathione metabolism, Steroid hormone biosynthesis, Alanine, aspartate and glutamate metabolism, and Sphingolipid metabolism (Figure 3E). Upregulated metabolites in M/RPPE comparison were enriched in Valine, leucine and isoleucine biosynthesis, Pyrimidine metabolism, Purine metabolism, Tryptophan metabolism, and Steroid hormone biosynthesis (Figure 3F). Downregulated metabolites in M/Con comparison were enriched in the Tryptophan metabolism, alpha-Linolenic acid metabolism, Arginine and proline metabolism, Lysine degradation, and Sphingolipid metabolism (Figure 3G). Downregulated metabolites in M/RPPE comparison were enriched in Tryptophan metabolism and Steroid hormone biosynthesis (Figure 3H).

Effects of RPPE on the Gut Microbiota

Different gut microbiota profiles were observed among three groups in the PCoA plots, the Con group was separated from the RPPE and M groups by the first component; the RPPE group was separated from the M group by the second component (Figure 4A). There was little difference in α-diversity indexes (Figure 4B–D) between the M and Con groups, however, RPPE significantly increased the Shannon index compared to other groups. Figure 4E–F showed microbial composition at phylum level. The proportion of Firmicutes was higher and the proportion of Bacteroidota was lower in the M group in comparison to the Con group, feeding RPPE to the diabetic mice reversed these changes. The abundance of Desulfobacterota was not altered in M/Con comparison, while RPPE increased its abundance. Firmicutes / Bacteroidota ratio was higher in the diabetes and feeding RPPE lowered this ratio (Figure 4G).

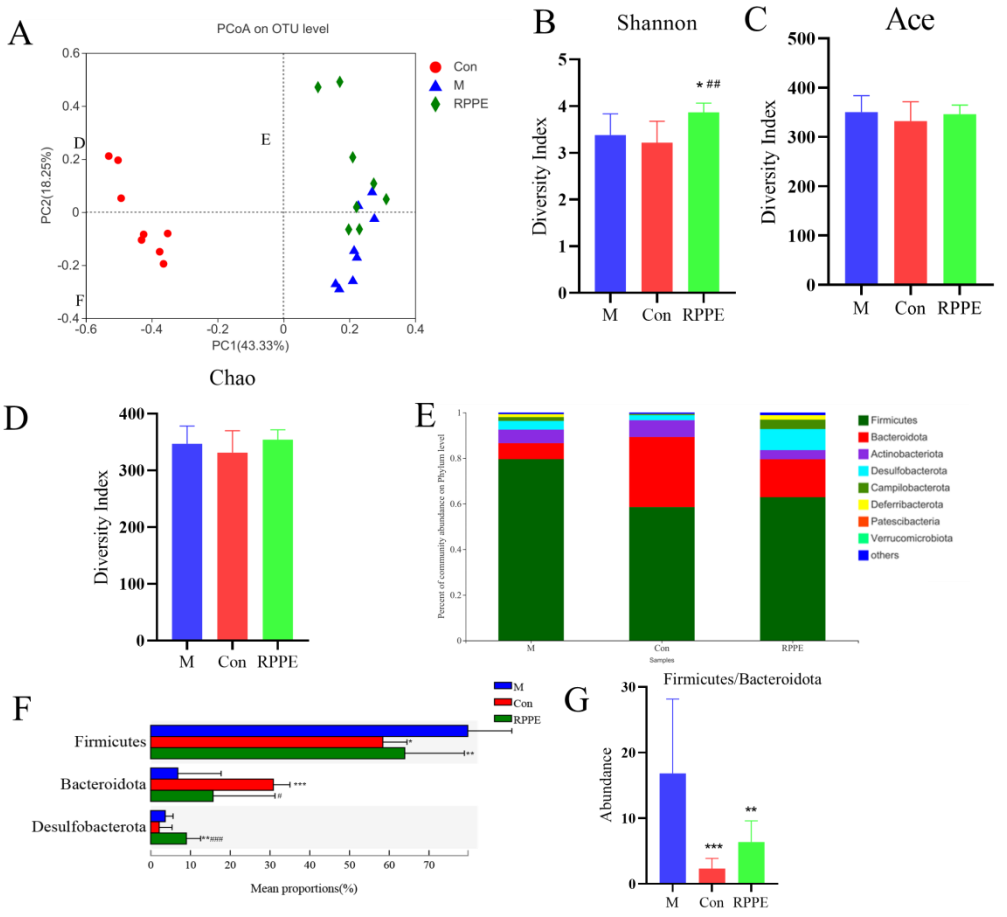

Figure 4. PCoA plot on the OTU (A). Shannon index (B). Ace index (C). Chao index (D). The composition (E) and comparisons (F) of phyla in gut. Firmicutes/Bacteroidota ratio (G). n = 8, each group

Figure 5A and 5B show the fifteen most abundant microbes at the family level. The proportion of *Erysipelotrichaceae*, *Peptostretococcaceae*, *Atopobioceae*, and *Clostridiaceae* was higher and the proportion of *Lactobacillaceae*, *Muribaculaceae*, *Bifidobacteriaceae*, and *Clostridium* lower in the M group in comparison to the Con group. RPPE decreased abundances of *Atopobioceae* which was higher in diabetes. Feeding RPPE also enriched the abundance of *Desulfovibrionaceae* and *Ruminococcaceae* in diabetic M group. The fifteen most abundant genera and the six identified species from the top fifteen most abundant species are shown in Figure 5C and Figure 5D. The abundance of *Faecalibaculum* (predominantly *F. rodentium*), *Clostridium*, *Romboutsia* (predominantly *R. ilealis*), *Lachnoclostridium*, *Coriobacteriaceae* and *Streptococcus* were higher and the proportion of *Lactobacillus* (including *L. murinus*) and *Bifidobacterium* (predominantly *B. pseudolongum*) were lower in the M group in comparison to the Con group. RPPE diet reversed the increased proprtion of *Faecalibaculum* (including *F. rodentium*), *Coriobacteriaceae*, and *Romboutsia* (including *R. ilealis*) in the M group in comparison to the Con group. In addition, RPPE lowered the proportion of *Lactobacillus reuteri* which was higher in the M group in comparison to the Con group. Moreover, RPPE also enriched the proportion of *Blaudia*, *Colidextribacter*, *Roseburia*, *Oscillibacter*, and *Rikenella* (predominantly *R. microfusus*) in the diabetic mice.

The thirty most altered KEGG pathways in M/RPPE and M/Con comparisons were shown in Figure 6 A and B. The top three most enriched pathways are Transporters, ABC transporters, and Bacteria motility proteins and the top 3 most depleted pathways are Ribosome, Purine metabolism, and Membrane and intracellular structure molecules in the M group in comparison to the Con group. Bacterial mobility proteins, Flagellar assembly, and Two-component system were the top 3 most enriched pathways by RPPE compared to the M group. And RPPE depleted the Phosphotransferase system, DNA repair and recombination proteins, and Fucose and mannose metabolism in diabetes.

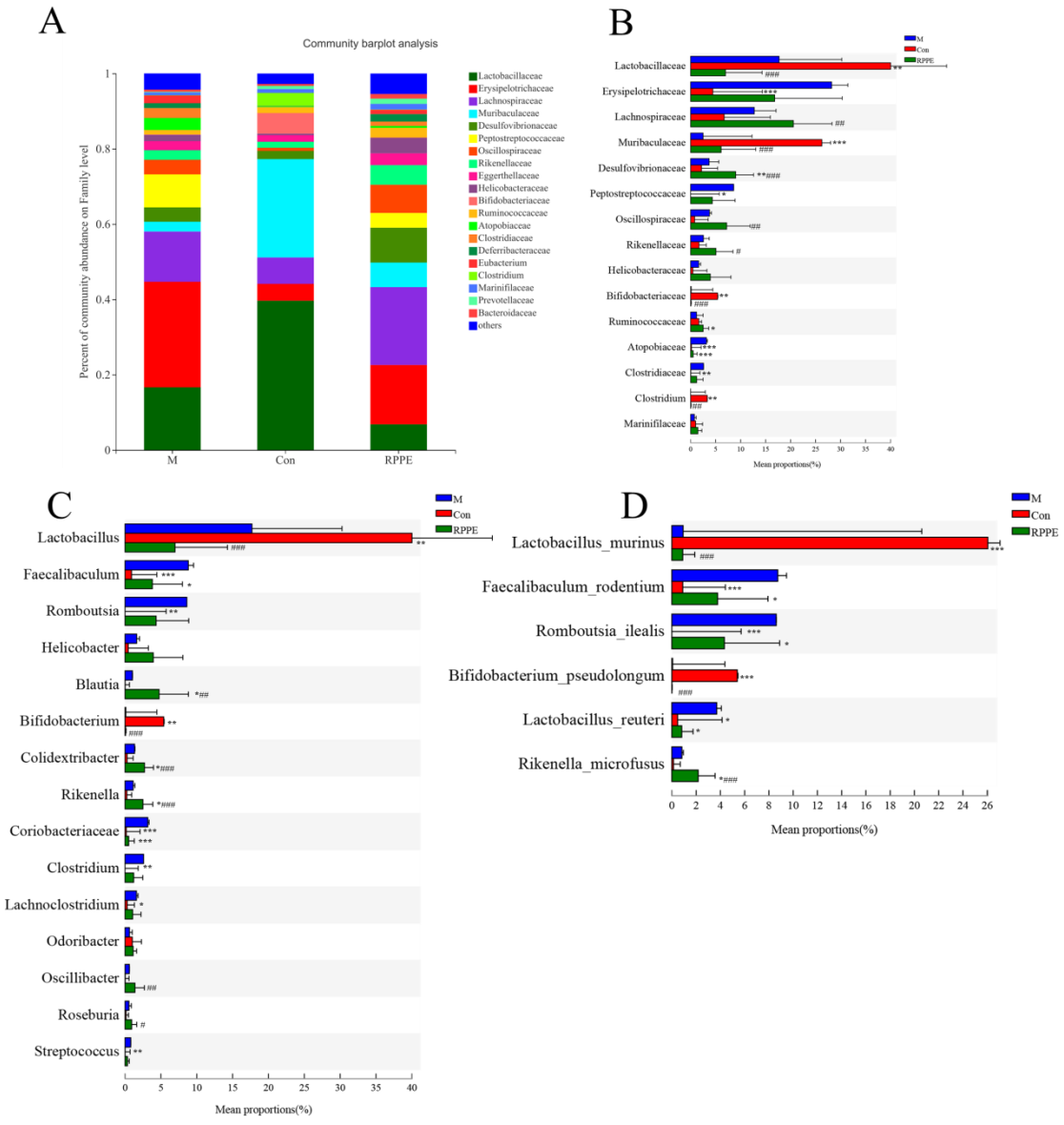

Figure 5. The impact of RPPE on gut microbiota at family (A-B), genus (C), and species (D) level. n = 8, each group

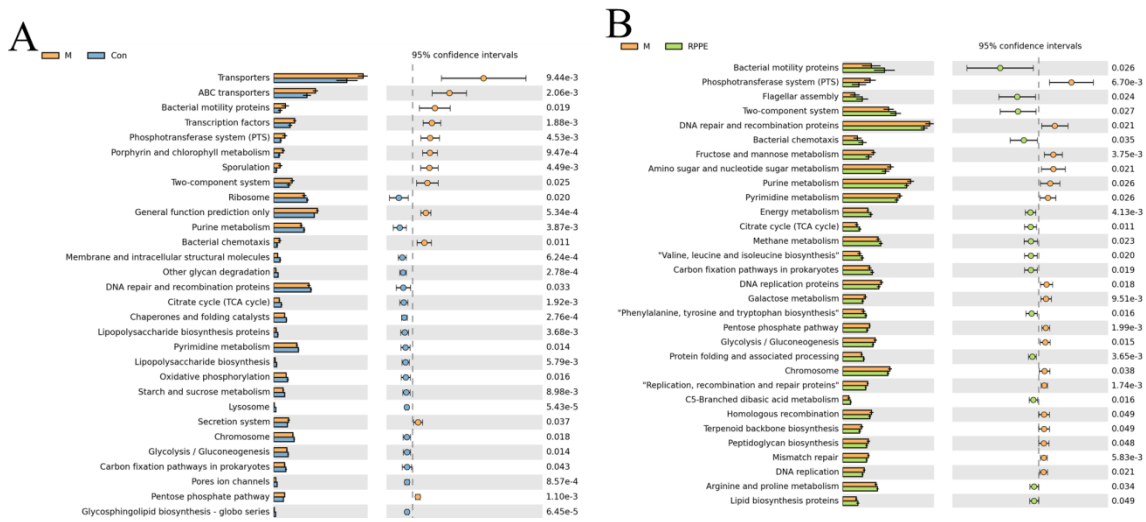

Figure 6. Altered KEGG pathways in M/Con (A) M/RPPE (B) comparisons

4. Discussion

Rosa roxburghii fruits contain functional components, such as polyphenols, polysaccharides, and vitamins [14]. Polyphenols extracted from Rosa roxburghii fruit pomace have shown strong antioxidant properties *in vitro* [9]. We have previously revealed the anti-diabetic effect of polyphenols extract from whole entire Rosa roxburghii fruit [12] and found the polyphenols extract has shown hypoglycemic, hypolipidemic, and anti-inflammatory effects as well as regulated gut microbiota such as depleted *Lachnospiraceae* and *Rikenellaceae* and enriched *Erysipelotrichaceae* and *Faecalibaculum* [12]. However, the effect of Rosa roxburghii fruit pomace polyphenols extract on diabetes has not been investigated. In this study, feeding polyphenols extract from Rosa roxburghii fruit pomace to high-fat diet- and STZ-induced type 2 diabetic mice at a daily dose of 400 mg/kg body weight for 8 weeks modulated plasma parameters and metabolic and gut microbiota profiles.

Abnormalities in the metabolisms of lipid and carbohydrate as well as inflammation are major contributing factors in T2D [17]. Therefore, the improvement of lipid and carbohydrate metabolisms and inflammation is important for treatment of T2D. Similar to our previous study investigating the anti-diabetic effect of polyphenols extracted from entire Rosa roxburghii fruit [12], in this study, feeding polyphenols extracted from Rosa roxburghii fruit pomace to diabetic mice also prevented the increase in the body weight and liver weight; modulated plasma levels of LDL, HDL, and glucose; improved insulin resistance. The RPPE decreased TNF-α and IL-β levels in plasma and colon, those pro-inflammatory cytokines have been acknowledged to contribute to the development of type 2 diabetes [15]. Other polyphenol extracts such as purple sweet potato polyphenol extract [16] and goji berry polyphenol extract [17] have also improved the inflammatory status of diabetes. Diabetes has been linked to gut oxidative stress and gut inflammation [18,19,20]. An *in vitro* study has demonstrated polyphenols extracted from entire Rosa roxburghii fruit showed a strong antioxidant activity by decreasing ROS level and increasing the activities of SOD and catalase CAT. Oxidative stress arises when the production of free radicals exceeds the antioxidant capacity. Excessive free radicals can cause indiscriminate damage to biological molecules, for example, free radicals can convert cellular lipoproteins to substances that can activate pro-inflammatory signaling pathways [21]. MDA is a product of polyunsaturated fatty acids peroxidation induced by free radicals, a lower level of malondialdehyde indicates an improved antioxidant capacity [22]. SOD and GPx which are antioxidant enzymes have free radical scavenging properties [22]. In the current study, the RPPE lowered the high level of MDA in the colon of diabetic mice, meanwhile, the RPPE also increased GPx and SOD in colon. These results demonstrated inflammation and oxidative stress in diabetic mice were improved by RPPE.

Altered KEGG pathways between the high-fat diet- and STZ-induced type 2 diabetic group and healthy mice were similar to our previous study [12], such as altered Glutathione metabolism, Steroid metabolism, and alpha-Linolenic acid metabolism. It is worth to be noted that RPPE altered Tryptophan metabolism (Indoleacetaldehyde and N-acetylserotonin decreased by RPPE and 5-hydroxy-tryptophan increased by RPPE). Accumulating evidence has suggested that altered metabolism of tryptophan and its downstream metabolites are linked with pathogenesis of type 2 diabetes [23]. For example, intake of 5-hydroxy-tryptophan decreased carbohydrate intake and caused weight loss in type 2 diabetes patients [24]. Increased 5-hydroxy-tryptophan level by RPPE might contribute to the lower of bodyweight in diabetic mice.

As reviewed, lower level of ceramides has been shown to be associated with improved insulin sensitivity in obesity [25], the mechanism is due to the ability of ceramides to deactivate leptin [26]. Besides, ceramides could increase the risk of cardiovascular diseases since they promote the formation of atherosclerotic plaques [27,28]. In this study, RPPE decreased three ceramides, Cer/AS(d15:2/16:2), Cer/AS(d17:3/16:2), and Cer/AP(t15:2/16:2) which were higher in the diabetic group, suggesting the RPPE possibly improved the activity of leptin and insulin resistance in those diabetic mice. p-Cresol sulfate is a major metabolite with uremic toxin generated from p-cresol by gut microbes, which has been reported to be related to insulin resistance [29].

Intraperitoneal injection of p-cresol sulfate has promoted insulin resistance in skeletal muscle and the mechanism might be involved in activating ERK1/2 [29]. In the current study, the administration of RPPE decreased the high circulating level of p-cresol sulfate in T2D mice compared to the healthy controls, suggesting the intake of RPPE promoted insulin sensitivity partially by decreasing the circulating concentration of p-cresol sulfate. p-Cresol glucuronide as another metabolite of p-cresol also showed a high level in the M and was decreased by the RPPE, however, p-cresol glucuronide has not been shown to have an effect on insulin sensitivity [30]. Both high levels of p-cresol glucuronide and p-cresol sulfate in T2D mice were also lowered by whole Rosa roxburghii fruit polyphenol extract [12]. These results suggested Rosa roxburghii fruit has a potential anti-diabetic effect through regulating p-cresol metabolism. Lysophosphatidylserine 20:5 (LPS 20:5) has been reported to be upregulated by intake of synbiotic yogurt fortified with monk fruit extract in the liver of T2D rats [31], however, in this study, the circulating LPS 20:5 level was decreased by RPPE, which deserve further study to explain this controversial result.

Increased myristoleic acid level induced by ginseng extract containing polyphenols has been shown to be associated with the reduction of adiposity by brown adipose tissue activation and beige fat formation in *db/db* mice [32]. In the current study, the RPPE increased myristoleic acid level in high-fat diet- and STZ-induced diabetic mice. Palmitoleic acid has hormone-like properties and can improve insulin sensitivity that is impaired in obesity and T2D [33]. In the current study, RPPE increased palmitoleic acid, suggesting RPPE improved insulin resistance partially by increasing palmitoleic acid level. A study has revealed that intake of myristic acid could decrease blood glucose and glucose uptake in skeletal muscle of T2D rats [34]. The mechanism might be associated with the activation of diacylglycerol kinase δ which is able to prevent high glucose-induced insulin resistance [35]. In this study, the RPPE increased circulating myristic acid which was lower in the M group in comparison to the Con group.

The modulatory effect of RPPE on the gut microbiota was investigated. The RPPE increased the Shannon index, a gut microbiota diversity index. Firmicutes/Bacteroidota ratio is considered an indicator for type 2 diabetes and obesity [36]. In this study, a higher Firmicutes / Bacteroidota ratio was observed in the M group in comparison to the Con and RPPE reversed this ratio to the normal level, suggesting a beneficial modulatory effect on gut microbial composition. A higher abundance of family *Erysipelotrichaceae* has been shown in gastrointestinal diseases and obesity [37]. In this study, the RPPE group showed a decreased trend in the abundance of *Erysipelotrichaceae* compared to the M group. Furthermore, RPPE upregulated the abundance of family *Lachnospiraceae* which is considered a major propionate producer in human gut [38]. In addition, other phenolic extracts from purple potatoes and bilberries also have been shown to increase the abundance of family *Lachnospiraceae* [39]. Genus *Faecalibaculum* has been reported to be a lactate-producing bacteria, and a high level of fecal lactate is a characteristic of T2D and obesity [40]. In this study, the M group displayed a higher level of *Faecalibaculum* (predominantly *F. rodentium*) in comparison to the Con group and RPPE lowered its abundance. Obesity patients with various metabolic disorders have shown higher abundances of *Romboutsia* and *Coriobacteriaceae* which were associated with body weight and serum lipids in obese patients [41] [42], in this study, RPPE significantly decreased these genera. The diabetic group showed a lower abundance of *Bifidobacterium* which was in accordance with the previous T2D research [43], whereas RPPE did not affect it. KEGG pathway analysis based on the sequence data showed RPPE decreased the activity of phosphotransferase system (PTS) which was more active in the M group than Con group. Fecal microbiota in patients with diabetes [44] and obese children [45] have shown more active PTS which affects phosphorylation of carbohydrates and carbohydrate uptake [45,46]. Moreover, due to PTS being more active in Actinobacteria and Firmicutes [45], RPPE decreased the PTS activity possibly by decreasing the abundance of these microbes.

5. Conclusion

We found RPPE affected plasma metabolome and gut microbiota in STZ- and high-fat diet-induced diabetes. The extract lowered plasma glucose and proinflammatory cytokines, and improved insulin sensitivity and plasma lipid profile. It downregulated colonic proinflammatory cytokines and oxidative stress markers. The extract also affected plasma metabolites. Myristoleic acid, myristic acid, and palmitoleic acid were increased and p-cresol sulfate was decreased by the extract, possibly suggesting improved lipid and glucose metabolism as well as insulin resistance. The extract also affected gut microbiota, such as *Erysipelotrichaceae* and *Lachnospiraceae* with positive effects were upregulated; *Faecalibaculum*, *Romboutsia*, and *Coriobacteriaceae* with detrimental effects in diabetes were downregulated by the extract. Although the positive effect of RPPE was observed in high-fat diet- and STZ-induced diabetes, other diabetic models are needed to verify the results. All in all, we first time report the anti-diabetic effects of Rosa roxburghii fruit pomace polyphenols extract, which is conducive to understanding the nutritional and medicinal value of the extract, promoting its potential use as a new resource of natural antioxidants.

Supplementary Material

The polyphenols composition of the extract and their percentage (Table S1).

Author Contributions

Methodology, H.W., K.C.; data curation, Z.C., M.W, S.H., J.L., M.L., T.R., J.L., and X.Z.; writing—original draft preparation, H.W.; writing—review and editing, K.C, H.W.; supervision, S.T.; funding acquisition, S.T. All authors have read and agreed to the published version of the manuscript.

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Statement of Competing Interests

The authors declare no conflict of interest.

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