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Water soluble fraction from ethanolic extract of *Clausena lansium* seeds alleviates obesity and insulin resistance, and changes the composition of gut microbiota in high-fat diet-fed mice

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ABSTRACT

The seeds of *Clausena lansium* have been used to treat acute and chronic gastro-intestinal disorders. To further explore its therapeutic value on metabolic diseases, a water soluble fraction from ethanolic extract of *C. lansium* seeds (HP) was evaluated on a high-fat diet (HFD) induced obesity mice model. The mice were fed with HFD for 8 weeks to induce obesity, and then orally administrated with HP for 9 weeks. The results showed HP treatment retarded body weight and fat mass gain of mice under HFD feeding. Moreover, HP significantly improved lipid profile and insulin sensitivity in HFD fed mice. 16S rRNA sequencing indicated that HP mildly reversed HFD feeding induced imbalance of Firmicutes/Bacteroidetes ratio in gut. Taken together, HP protects mice from HFD induced metabolic disorders and restores gut microbiota balance, which might be well developed as novel functional food for anti-obesity and improvement of insulin sensitivity.

1. Introduction

Obesity has been increasing in an alarming rate worldwide, which often results in higher risks of metabolic diseases, cardiovascular diseases and cancer (Kahn, Hull, & Utzschneider, 2006). Over nutrition and/or reduced physical activity lead to deposit of excess energy in adipose tissue as triglycerides, eventually causing obesity. The US Food and Drug Administration only approved a few anti-obesity drugs, either appetite suppressors or pancreatic lipase inhibitors (Daneschvar, Aronson, & Smetana, 2016). The use of certain anti-obesity drugs, such as orlistat (Xenical) and sibutramine (Reductil), has been limited by unpleasant side effects such as mood changes and cardiovascular complications. Alternatively, natural products have been proposed to provide excellent alternative therapies for this medical challenge due to their potential effectiveness and safety (Fu, Jiang, Guo, & Su, 2016; Liu, Wang, & Lin, 2015; Rayalam, Della-Fera, & Baile, 2008).

Gut microbiota is the most important internal environment factor, which has been verified to highly associate with metabolic disorders (Miglioranza Scavuzzi et al., 2015; Ridaura et al., 2013). Firmicutes and Bacteroidetes bacteria are dominant in human gut microbiota; and Proteobacteria, Verrucomicrobia, Fusobacteria, Actinobacteria, and Cyanobacteria are existed in minor proportions (Arumugam et al., 2011; Ley, Turnbaugh, Klein, & Gordon, 2006). The gut microbiota participates in energy harvest and utilization, appetite regulation, and systemic low-grade inflammation, thus serving as a regulator to sense and integrate multiple factors leading to obesity (LeBlanc, et al., 2013; Tremaroli & Backhed, 2012). Obesity has been associated with a specific profile of gut microbiota, such as a decrease in the Bacteroidetes/Firmicutes ratio in rodents and humans (Ley, et al., 2005; Million, Lagier, Yahav, & Paul, 2013). Using germ-free recipient animals and the feces transplantation technique, it was found dysregulation of the gut microbiota led to obesity (Million, et al., 2013). Thus, manipulations of gut microbiota function and composition have become promising strategies for anti-obesity interventions.

Abbreviations: F/B, Firmicutes/Bacteroidetes ratio; GTT, glucose tolerance tests; HDL-C, high density lipoprotein cholesterol; HFD, high-fat diet; HP, a water soluble fraction from ethanolic extract of *Clausena lansium* seeds; ITT, insulin tolerance tests; LDL-C, low density lipoprotein cholesterol; OUT, operational taxonomic unit; RD, regular chow diet; PBS, phosphate-buffered saline; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1-α; SD, standard deviation; TNF-α, tumor necrosis factor-α; UPGMA, unweighted pair group method with arithmetic mean

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Clausena lansium Skeels is a flowering plant from the family Rutaceae; it is widely cultivated in southern China and Southeast Asia. The edible fruit, called wampee, is highly aromatic and pleasantly sweet. In ethnic medicine, the seeds of *C. lansium* have been chewed and swallowed by patients to treat acute and chronic gastro-intestinal inflammation and ulcers (Adebajo, et al., 2009). Previous phytochemical and pharmacological studies on this plant have revealed the presences of amide alkaloids, carbazole alkaloids, and coumarins with anti-inflammation, anti-oxidation, and anti-cancer properties (Adebajo, et al., 2009; Huang, Feng, Wang, & Lin, 2017). In our previous study, lansiumamide B, a major amide alkaloid from the seeds of *C. lansium*, was found to prevent fat mass gain and insulin resistance on high-fat diet (HFD) fed mice (Huang, et al., 2017). The polyphenol extracts from the leaves of *C. lansium* were reported to protect against hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats (Kong, Su, Guo, Zeng, & Bi, 2018). The current study aimed to identify a water soluble fraction from ethanolic extract of *C. lansium* seeds (HP), which could attenuate fat mass gain and insulin resistance, and modulate gut microbiota composition in HFD fed mice.

2. Materials and methods

2.1. Preparation of water soluble fraction from ethanolic extract of C. lansium seeds

The seeds of *C. lansium* were collected at Lincheng, Qinzhou City, Guangxi Zhuang Autonomous Region, People's Republic of China, in August 2015, and identified by Professor Jingui Shen from Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher was deposited at the herbarium of the Institute of Chinese Medical Sciences, University of Macau (LL-20151002). The preparation procedure of HP was shown in Fig. 1. The air-dried seeds (2.5 kg) were ground into powder and defatted with petroleum ether (1.5 L) for three times. The residues were then extracted with 95% ethanol under reflux for 3 h (2 L × 3 times). After evaporation of the collected percolate, the crude extract (120.2 g) was suspended in 400 mL H_2O and extracted with *n*-butanol (250 mL × 3 times). The water soluble fraction was concentrated under vacuum to afford HP (70.5 g).

2.2. Animals experiments

All animal experiments were approved by the Animal Ethical and Welfare Committee of University of Macau (No. ICMS-AEC-2014-06). All procedures involved in the animal experiments were carried out in accordance with the approved guidelines and regulations. Male C57BL/6J mice were purchased from the animal facility of Faculty of Health Sciences, University of Macau, and housed at 22 ± 1 °C under a 12 h light, 12 h dark cycle with a 50% humidity, and fed with a regular chow diet (Guangdong Medical Lab Animal Center, Guangzhou, Guangdong, China) and water *ad libitum* under standard conditions (specific-pathogen-free) with air filtration.

The procedure of animal experiments was shown in Fig. 2. Eight-week-old male mice were randomly distributed into four groups, each six mice. One group of mice (RD) were fed with a regular chow diet (calorie, 2.35 kcal/g). The other three groups of mice were fed with a 45% HFD (calorie, 4.5 kcal/g, Trophic Animal Feed High-Tech Co., Nantong, Jiangsu, People's Republic of China) for eight weeks to induce obesity. Then, the first group of the HFD fed mice were treated with 400 mg/kg HP (dissolved in PBS, HP.L); and the second group of the HFD fed mice were treated with 800 mg/kg HP (HP.H). The last group of the HFD fed mice (HFD) and the RD group of mice were treated with the same volume of PBS. All the mice were orally administrated once a day for nine weeks. Body weight and food intake were monitored every week. Tissues and serum were collected after 16 h fasting and stored at −80 °C for further studies. The wet weights of epididymal fat, inguinal fat and liver were recorded; and the tissue weight to body weight ratios were calculated as following: tissue weight/body weight × 100%.

2.3. Analysis of serum lipids

Triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) levels in serum were analyzed using commercially available kits (Nanjing Jiancheng, Nanjing, Jiangsu, China), following the manufacturer's instruction.

2.4. Glucose tolerance tests and insulin tolerance tests

The glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed at six and seven weeks post HP treatment, respectively, as described previously (Lee, et al., 2016; Lin, et al., 2011). After 18 h fasting, the tail blood glucose was measured using OneTouch Ultra blood glucose meter and LifeScan test strips. Then, the mice were received an intraperitoneal injection of glucose solution (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 2.0 g/kg body weight. The tail blood glucose was measured at 15, 30, 60, 90 and 120 min after injections. Regarding to ITT, the mice tail blood glucose concentration was measured after 6 h fasting. The mice were then received an intraperitoneal injection of human insulin (Eli Lilly, Indianapolis, IN, USA) at a dose of 1.0 U/kg body weight. Tail blood glucose concentration was measured at 15, 30, 60, 90 and 120 min after injections.

2.5. Bacterial DNA extraction, PCR amplification, and Illumina sequencing

Gut microbiota was analyzed as described previously (Lv, Peng, Liu, Xu, & Su, 2015). Mice were housed in chambers individually for 24 h without food. The feces samples were collected and immediately snap frozen in liquid nitrogen, and then stored in −80 °C freezer. A piece of feces from one mouse was used for DNA extraction, PCR amplification, amplicon quantification, pooling, and sequencing (Meyer & Kircher, 2010). Bacterial genomic DNA was extracted from samples using the QIAamp DNA stool mini kit (Qiagen, Germantown, MD USA), according to the manufacturer's instructions. Consensus primers 338F (5′-ACTCC TACGGGAGGCAGCA-3′) and 806R (5′-GGACTACCAGGGTATCT AAT-3′) were used to amplify the V3 and V4 regions of bacterial 16S rRNA. A bar code and adapter were incorporated between the adapter and the forward primers. The PCR amplification was carried out in triplicate using a 20 μL reaction mixture with 5 μM of each primer, 10 ng of template DNA, 2.5 mM dNTPs, 4 μL of the FastPfu buffer, and 0.4 μL of FastPfu polymerase. The PCR was run on an ABI GeneAmp 9700 PCR machine. The amplification program consisted of an initial denaturation step at 95 °C for 3 min, followed by 25 cycles, where 1 cycle consisted of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s. A final extension of 72 °C was performed for 10 min, and the mixture was held at 10 °C until the reaction was halted by the user. Negative controls were used to verify the lack of Taq performance without the DNA template. Three replicate PCR products of the same sample were pooled in a PCR tube. They were then visualized on agarose gels (2% in Tris-borate-EDTA buffer) containing ethidium bromide and purified with an Axygene DNA gel extraction kit (Corning, NY, USA). Prior to sequencing, the DNA concentration of each PCR product was determined using a QuantiFluor TM-ST handheld fluorometer with a UV/blue channel (Promega, Madison, MI, USA). The purified amplicons were sequenced using the paired-end method on an Illumina Miseq platform following the PE300 sequencing protocol (Major BioPharm Technology, Shanghai, China).

2.6. Real-time RT-PCR

Total RNA was isolated from epididymal adipose tissue using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The cDNA was synthesized from 1 μg RNA using the SuperScript III First-Strand Synthesis System (Invitrogen). The qPCR

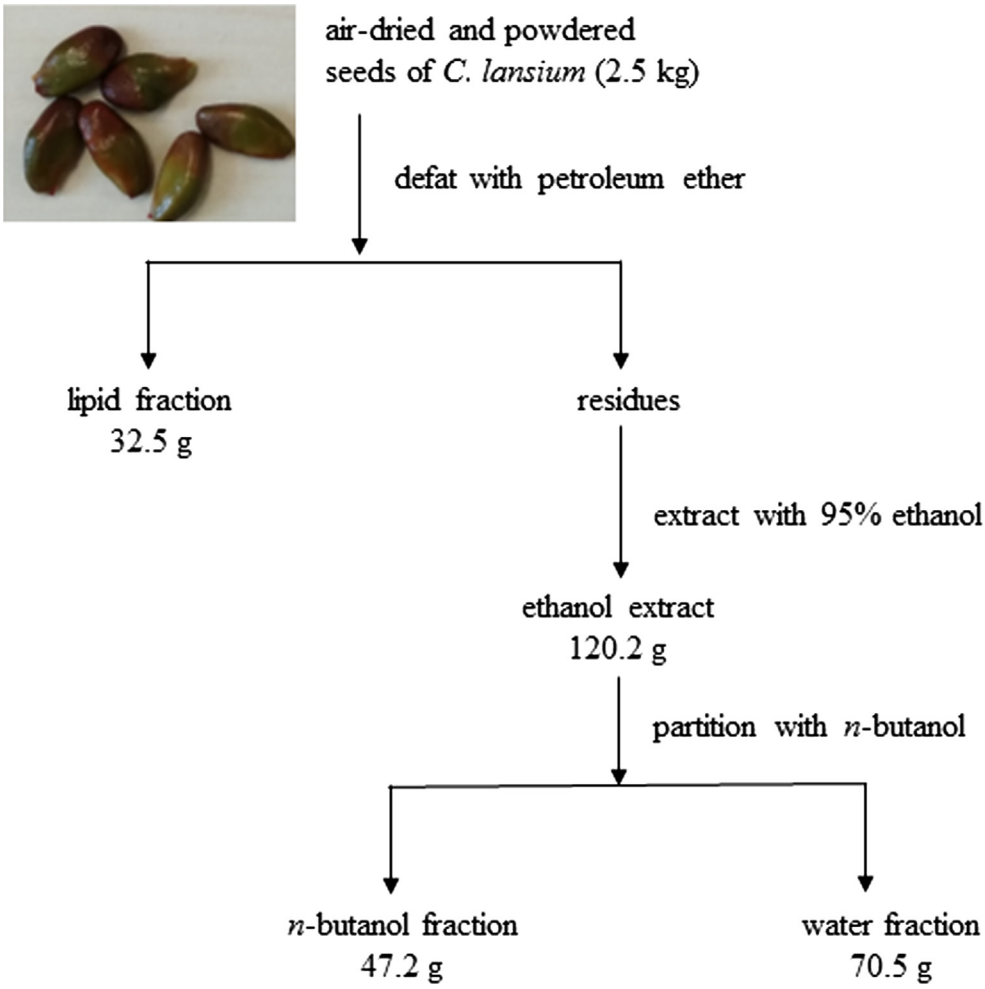

Fig. 1. Scheme of preparation of water soluble fraction from ethanolic extract of *C. lansium* seeds.

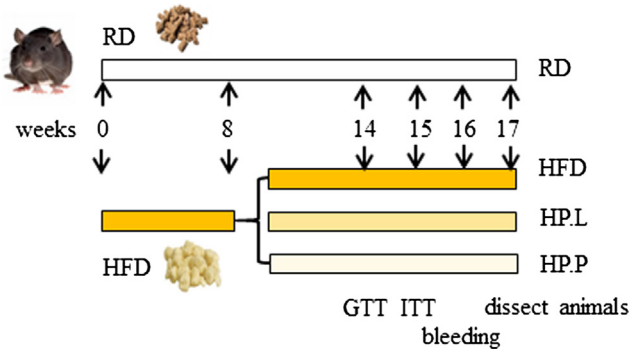

Fig. 2. The experimental procedure of HFD induced obese mice model.

Table 1
Primers used for qPCR assay.

Gene	Forward sequence (5′-3′)	Reverse sequence (5′-3′)
18S	AGCCTGCGGCTTAATTTGAC	CAACTAAGAACGGCCATGCA
TNF-α	GAGAAAGTCAACCTCCTCTCTG	GAAGACTCCTCCCAGGTATATG
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTC
PGC-1α	GACAATCCCGAAGACACTACAG	AGAGAGGAGAGAGAGAGAGAGA

experiments were conducted on Step-One plus real-time PCR System using SYBR green PCR Master Mix (Thermo Scientific, Grand Island, NY, USA) with gene specific primers (Table 1). 18S RNA was used as an internal control.

2.7. Bioinformatics and multivariate statistics

Clean data were obtained after using Trimmomatic and FLASH programs for demultiplexing and quality-filtering the raw fastq files. Sequences were clustered into operational taxonomic units (OTUs) at 97% identity. The clean data were normalized by the OTU matrices by rarefying. Rarefying samples for normalization is now the standard in microbial ecology and is present in all major data analysis toolkits for this field (Caporaso, et al., 2010; Dixon, 2003; McMurdie & Holmes, 2013; Schloss, et al., 2009). Taxonomy assignment was determined using RDP classifier (version 2.2) (Wang, Garrity, Tiedje, & Cole, 2007), which is a is a naive Bayesian classifier, against SILVA bacterial 16S rRNA database (release 128), at an 70% bootstrap cut-off. The Shannon-Wiener index, Simpson's diversity index, and rarefaction estimates were calculated using QIIME (version 1.17). Then principal coordinates analysis (PCoA) analysis was performed using R software (version 3.2.3) (R Core Team (2013)). Then, PICRUSt software (Langille, et al., 2013) was used to normalize the abundance of OTUs and aligned against COG and KEGG database.

2.8. Statistical analysis

Data were expressed as mean ± SD and analyzed on Graphpad

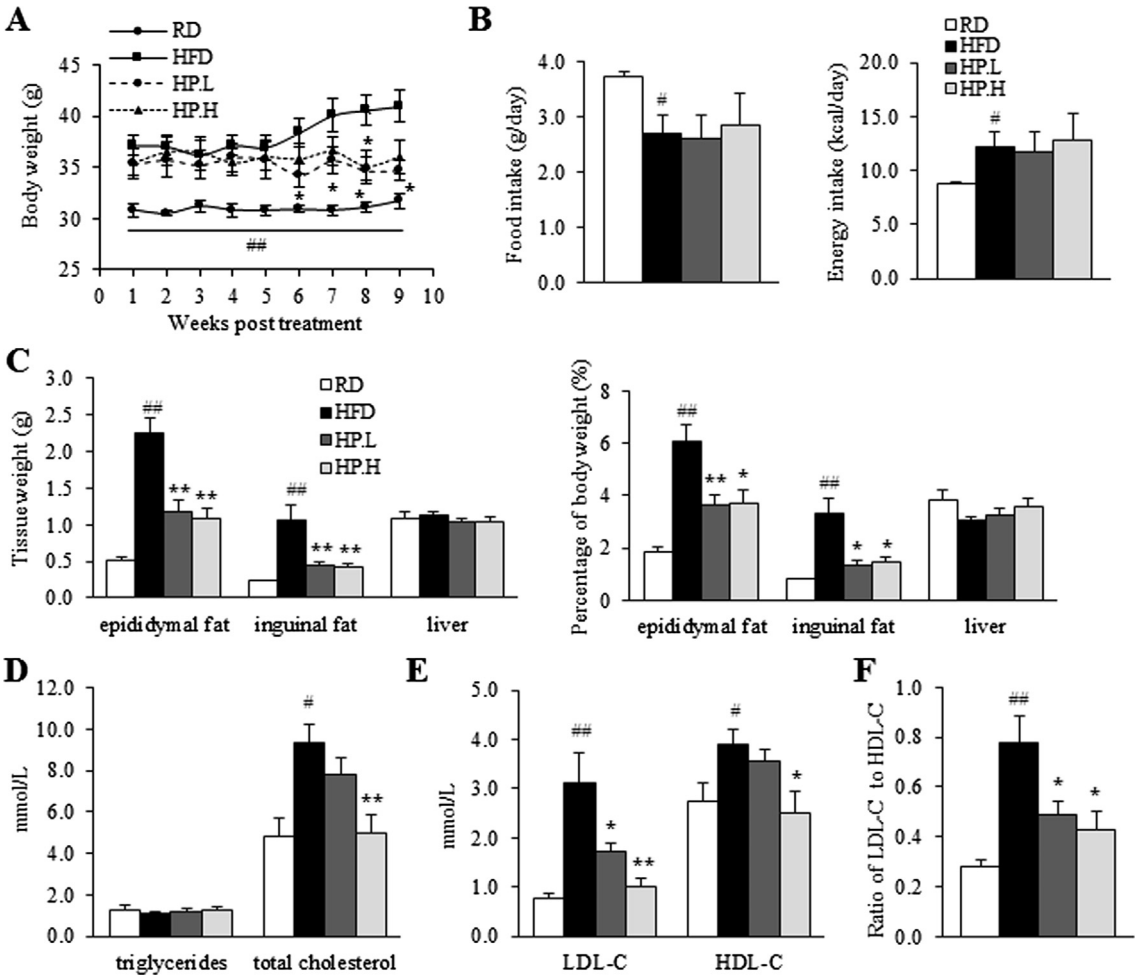

Fig. 3. Effects of HP on HFD induced obesity and hyperlipidemia in mice. (A) Body weight change of mice. (B) Daily food intake and energy intake of mice. (C) Raw weight and percentage of body weight for epidicymal fat, inguinal fat and liver. (D) Triglycerides and total cholesterol in serum. (E) LDL-C and HDL-C in serum. (F) The ratio of LDL-C to HDL-C. Data are shown as mean ± S.D. (n = 6), # and ##, $P < 0.05$ and 0.01, RD *vs* HFD; * and **, $P < 0.05$ and 0.01, HFD *vs* HP treated group.

Prism 6 (GraphPad Software, San Diego, CA, USA). The significance of differences between groups was assessed by one-way analyses of variance (ANOVA) and Dunnett's test. Between-group comparison was evaluated by one-way ANOVA test using SPSS software 16.0 (Chicago, IL, USA). $P < 0.05$ indicated the presence of a statistically significant difference, and $P < 0.01$ was considered highly significant.

3. Results and discussion

3.1. HP retards fat mass gain and improves hyperlipidemia in HFD fed mice

Eight-week of HFD feeding induced higher body weight gain than RD feeding (10.32 ± 0.41 *vs*. 5.86 ± 0.23, p < 0.001). After regrouping, 400 or 800 mg/kg body weight of HP, or the same volume of PBS was orally administered to HP.L, HP.H or HFD groups of mice, respectively, for nine weeks. As shown in Fig. 3A, HFD-fed mice had increased body weight compared to the RD-fed mice. In contrast, either low dose or high dose of HP treatment obviously decreased the body weight of the HFD-fed mice, when compared with HFD group (Fig. 3A). The food intake was reduced and the energy intake was increased in the HFD group compared with RD group; while, food intake or energy intake didn't alter by HP administration (Fig. 3B). The body weight gain of HFD-fed mice was accompanied by a notable fat accumulation, including visceral (epididymal) and subcutaneous (inguinal) fat, but not liver, when compared to RD group (Fig. 3C). In contrast, HP administration significantly decreased the fat mass and the ratios of fat mass to body weight, compared to mice in HFD-fed group (Fig. 3C). The above data indicated that HP treatment contributed to the reduced body weight gain, and less fat mass, but didn't alter food intake in HFD-fed mice.

HFD feeding is associated with hyperlipidemia. Next, the lipid profile in serum was evaluated. HFD feeding increased serum levels of total cholesterol, LDL-C and HDL-C, but not triglycerides; either high or low dose of HP treatment greatly improved lipid profile (Fig. 3D and E). The ratio between detrimental LDL-C and beneficial HDL-C was also increased in HFD group, and decreased by HP treatment (Fig. 3F). Thus, HP alleviated HFD induced hyperlipidemia.

3.2. HP improves insulin sensitivity

HFD induced obesity is accompanied with insulin resistance. To figure out whether HP treatment could improve insulin sensitivity, glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed. During GTT, the glucose clearance rate was greatly interrupted in HFD mice, and either high or low dose HP treatment obviously improved glucose disposal rate, similar to that of RD mice (Fig. 4A). In ITT experiments, the glucose levels of HP treated mice were significantly reduced under insulin stimulation, as low as those of RD groups of mice, which indicated HP greatly enhanced insulin sensitivity (Fig. 4B) in mice. Collectively, HP treatment effectively

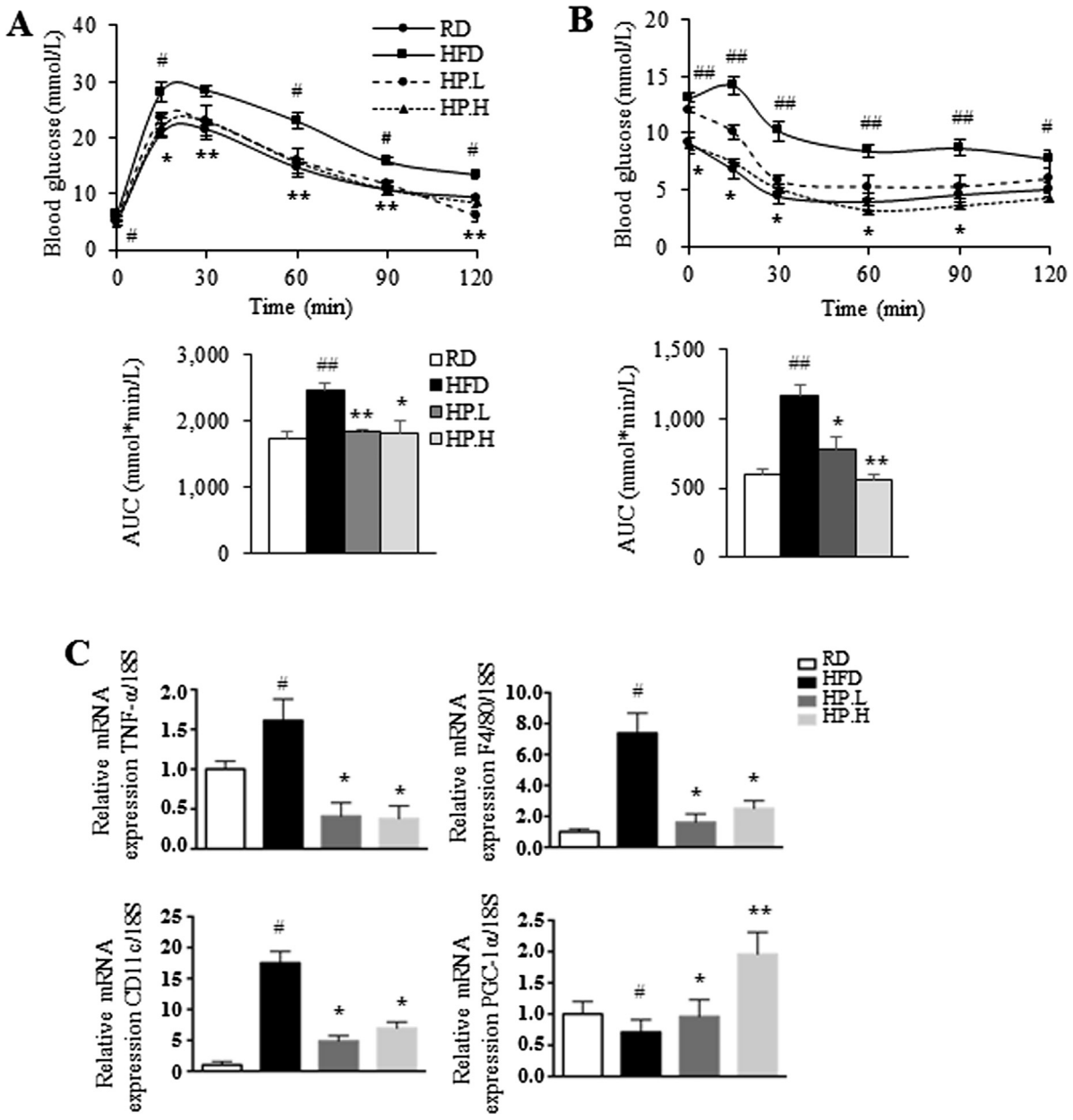

Fig. 4. HP improved HFD induced insulin resistance in mice. (A) Glucose tolerance test after six weeks HP treatment. Area under curve (AUC) of each group was calculated. (B) Insulin tolerance test after seven weeks HP treatment. Area under curve (AUC) of each group was calculated. (C) mRNA expressions of inflammatory cytokines (TNF-α, F4/80 and CD11c) and thermogenesis marker (PGC-1α) in epididymal adipose tissue. Data are shown as mean ± S.D. (n = 6), # and ##, $P < 0.05$ and 0.01, RD *vs* HFD; * and **, $P < 0.05$ and 0.01, HFD *vs* HP treated group.

improved HFD induced insulin resistance. Furthermore, qPCR analysis revealed that HFD feeding increased the expressions of inflammatory cytokines, including tumor necrosis factor-α (TNF-α,) F4/80 and CD11c, and decreased the expression of thermogenic marker, such as peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α) in epididymal adipose tissues; while HP treated almost reversed the changes (Fig. 4C). Taken together, HP prevented HFD-induced insulin resistance by suppressing the pro-inflammatory state.

3.3. HP restores gut microbiota in HFD-induced obese mice

On average, a total of 938,279 good quality pair reads were generated and assigned to SILVA database. The sequencing depth was deemed to be sufficient with the use of rarefraction curves (Supplementary Fig. S1). The HFD group had the lowest diversity, while samples from RD group had the highest phylogenetic diversity and Shannon diversity index values (Supplementary Fig. S2). As shown in Fig. 5A, the PCoA analysis showed that the microbiome diversity is significant different between different extract dosage treatment.

UPGMA (unweighted pair group method with arithmetic mean) analysis indicated a significant separation in the microbiota between RD- and HFD-fed mice, and between HFD and HP treatment groups, suggesting that HP treatment shifted the overall structure of the gut microbiota of HFD-fed mice toward that of RD-fed mice (Fig. 5B).

In RD group, gut microbial community was dominated by members of the phyla Bacteroidetes (67.67%) and Firmicutes (21.98%), with lower proportion of Proteobacteria (2.50%) (Fig. 5C). However, after HFD feeding, the respective proportions of the three major phyla were altered significantly, with a notable reduction in the proportion of Bacteroidetes (24.62%) and increases in the proportions of Firmicutes (60.15%) and Proteobacteria (11.55%) (Fig. 5C). HP administration didn't restore the diversity of the gut microbiota, but shifted the

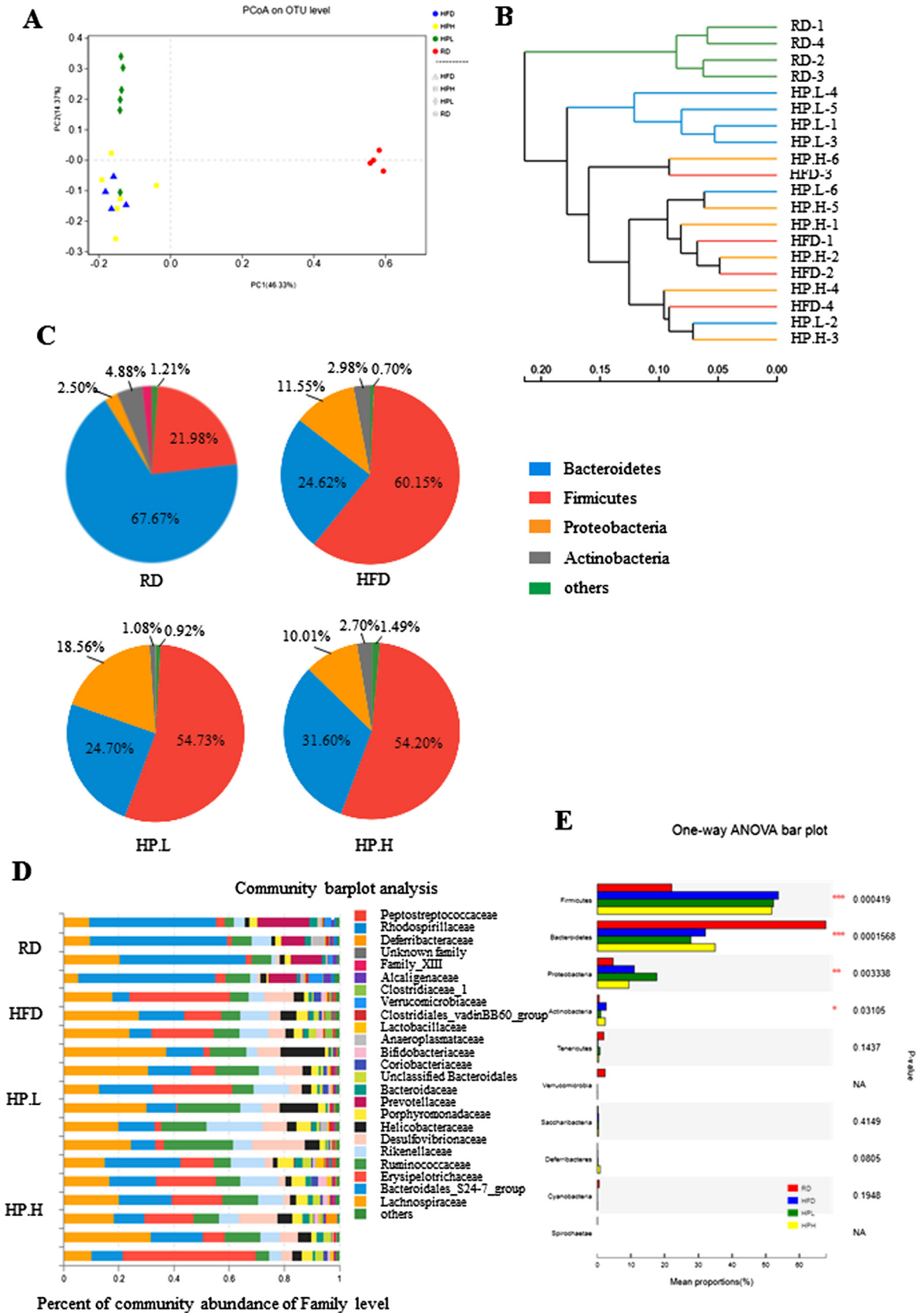

Fig. 5. HP altered gut microbiota composition in HFD fed mice. Microbiota composition in feces of RD-fed and HFD-fed mice, and HFD mice treated with 2 doses of HP were analyzed using the paired-end method on an Illumina Miseq platform. (A) PCoA of the between samples distances (beta diversity) computed using Unweighted UniFrac distance. (B) UPGMA analysis based on weighted UniFrac distance. Shifts in microbial community structure and composition associated with high-fat diet feeding and HP treatment at the class level (C) and family level (D). (E). One-way ANOVA analysis of shifts in microbial community structure and composition on phylum level. n = 4 for RD and HFD groups; n = 6 for HP.L and HP.H groups.

proportions of Bacteroidetes and Firmicutes towards a healthy balance like in RD group. Low dose of HP treatment resulted in a decrease in the proportion of the phylum Firmicutes (54.73%); while high dose of HP treatment caused a decrease proportion of Firmicutes (54.20%) and an increase proportion of Bacteroidetes (31.60%) (Fig. 5C).

At the family level, the *Lachnospiraceae* (belonging to the Clostridiales), *Prevotellaceae* (belonging to the Bacteroidales), and S24-7 (an unclassified family belonging to the Bacteroidales) were dominant in the RD group, while in the HFD feeding group, the proportions of *Lachnospiraceae*, *Erysipelotrichaceae* (belonging to the Firmicutes), *Rikenellaceae* (belonging to the Bacteroidales) and *Desulfoyibrionaceae* (belonging to the Proteobacteria) were increased, and the proportions of *Prevotellaceae* and S24-7 were decreased in the gastrointestinal tract (Fig. 5D). HP treatment inhibited the growth of *Lachnospiraceae*, *Erysipelotrichaceae*, *Rikenellaceae* and *Desulfoyibrionaceae*, and stimulated the growth of S24-7 (Fig. 5D). The microbiome which displayed statistics different has been separated into a plot using one-way ANOVA analysis as shown in Fig. 5E. Thus, HP could restore the microbiota balance in the gastrointestinal tract in HFD-fed mice. Functional annotation was performed using PICRUSt and found the abundance of pathways including fatty acid elongation in mitochondria (ko 00062) and steroid biosynthesis (ko 00100) were significant different (Table S1). Also, it showed that COG function classification was similar among the groups (Supplementary Fig. S3).

4. Discussion

Different parts of *C. lansium* have been traditionally used for digestive disorders and chronic gastrointestinal ulcers. The methanolic extract of the barks of *C. lansium* and the major constituents, imperatorin and chalepin, were reported with anti-hyperglycemic and anti-inflammatory activities (Adebajo, et al., 2009). Additionally, the major amide alkaloid from the seeds of *C. lansium*, lansiumamide B, was found to prevent HFD induced obesity and insulin resistance (Huang, et al., 2017). Herein, HP was found to reduce visceral and subcutaneous fat, and improves lipid profile and insulin sensitivity in HFD-induced obese mice. These findings indicated that HP is a potential weight controlling and insulin sensitizing agent.

Strong evidences have been indicated that gut microbiota is an important factor related to obesity (Trigueros, et al., 2013). Gut microbiota composition varied after prolonged HFD feeding in rats (Lin, An, Hao, Wang, & Tang, 2016). Gut microbiota, particularly Firmicutes and Bacteroidetes, play important roles in the regulation of carbohydrate, lipid, and bile acid metabolism in host (Tremaroli & Backhed, 2012). More importantly, Firmicutes and Bacteroidetes bacteria share roles in regulating the response of the host to dynamic changes in diets (Jumpertz, et al., 2011). Previous studies indicated that there is a causal relationship between obesity and the relative Firmicutes and Bacteroidetes (F/B) ratio (Lv, et al., 2015; Million, et al., 2013). In obese subjects, an increased F/B ratio results in an enhanced capacity to collect energy from food and a low-grade inflammation (Clemente, Ursell, Parfrey, & Knight, 2012). Consistently, the gut microbiota of patients with distinct and sustained weight loss contains more Bacteroidetes and less Firmicutes. The anti-obesity effect of HP might be mediating through modulation of gut microbiota. Thus, we explored the composition of gut microbiota. In the HFD-fed mice, the F/B ratio was significantly increased to 7.52-fold to that of RD-fed mice, while low and high dose of HP treatment decreased the ratios to 6.82-fold and 5.28-fold to that of RD-fed mice, respectively, which coincides with previous observations in humans.

However, several lines of evidence indicated that an increased F/B ratio is not always implicated in obesity. In a human cohort study, no variations in the F/B ratio were observed between obese and control subjects (Zhao, 2013). Therefore, changes in other bacterial phyla or families need to be taken into consideration. For example, the *Lachnospiraceae* family is significantly increased in both obese nonalcoholic fatty liver disease and type 2 diabetes in humans (Zhang, et al., 2013). In our study, HP treatment caused a reduction in *Lachnospiraceae* populations, which were positively correlated with obesity in subjects fed a HFD (Kameyama & Itoh, 2014). In addition, HP treatment restored the HFD-mediated reduction in the S24-7 populations, which was observed in subjects with increased physical activity (Evans, et al., 2014). The mechanism by which HP regulates the proportion of gut microbiota remains to be resolved in future research.

Hyperlipidemia is a hallmark of obesity and insulin resistance. In the current study, HP treatment rescued HFD-induced hyperlipidemia, which might be due to inhibition of lipid absorption and/or enhanced lipid consumption. The increasingly evident causative relationship between inflammation and insulin resistance has been confirmed by many studies. Pro-inflammatory cytokines, especially TNF-α, play a crucial role in insulin resistance. Currently, HP treatment alleviated the expressions of pro-inflammatory cytokines, which might also contribute the anti-obesity and insulin sensitizing effect of HP.

In the present study, 16S rRNA sequencing was utilized to identify how the gut microbiota composition change after HP treatment in HFD mice. The limitation of the study is that metabolomics activity of the alternative microbiota is absent. In the future, metabolomic assay for analyzing gut microbiota derived short chain fatty acids will be performed combining with the 16S rRNA sequencing. Moreover, the alternative microbiota should be isolated and cultured to further identify the mechanism of fatty acids metabolism.

5. Conclusions

In conclusion, our data showed that oral administration of HP attenuates HFD-induced obesity, hyperlipidemia and insulin resistance in mice by beneficially modulating the abundance of the gut bacterial phylotypes related to metabolic parameters. Interestingly, the bacterial communities of the RD-fed group were more closely clustered, and HP didn't alter the diversity of gut microbial community in HFD-fed mice. The fruits of *C. lansium* have been used to treat digestive disorders. Therefore, translation of our findings on HP to clinical populations has a solid practical foundation. Gut microbiota–targeted HP intervention strategies may become a promising approach to attenuate obesity and insulin resistance.

6. Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jff.2018.05.057.

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