

Huangjing (*Polygonatum kingianum* Coll. et Hems) and Tiandong (*Asparagus cochinchinensis* (Lour.) Merr.) Combination relieves glycolipid metabolism disorder via both Glycolipid key Proteins and gut microbiota

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Abstract

Background

Ancient traditional Chinese medicine (TCM) books and modern studies have suggested that the Huangjing and Tiandong combination (HTC), which consists of *Polygonatum kingianum* (PK) and *Asparagus cochinchinensis* (AC) with the mass ratio 10:3, has the effect of regulating glycolipid metabolism disorder. However, its efficacy and mechanism are yet to be elucidated. This research evaluates the effect of HTC on glycolipid metabolism and explores the underlying mechanism.

Methods

GLMD was induced by HFSD diet in rats, and Huangjing and Tiandong combination (HTC) was given orally every day for 12 weeks. Then the body weight, tissue weight, blood sugar, blood lipid and liver lipid levels were measured, and lipogenesis was evaluated by Oil-Red O staining. In addition, the role of potential key signaling pathway was investigated through a network pharmacology-guided ELISA, while the gut microbiota was studied via Metagenome and 16S rDNA sequencing.

Results

Oil red O staining showed that HFSD diet led to a significant accumulation of lipid droplets in the liver. After HTC extract treatment, lipid droplets decreased, and normal morphology of the liver was gradually restored. Besides, the results showed that HTC could improve blood glucose, blood lipids, adipokines and liver lipids. Then, we found that HTC could increase the expression of INSR, IRS1, IRS2, PI3K, Akt, JAK2, and STAT3 in liver, and abdominal white adipose tissue. Subsequently, we reported that HTC exhibited beneficial effects against GLMD via the alternation of the abundance and diversity of gut microbiota.

Conclusion

In a word, the above results demonstrate that HTC is a useful drug/nutrient to relieve GLMD via both Glycolipid key Proteins and gut microbiota.

1 Introduction

Glycolipid metabolism disorder (GLMD) is a metabolic disorder characterized by insulin resistance, chronic inflammation and gut microbiota imbalance, which has been considered the primary contributing factor to hyperglycemia, hyperlipidemia, type II Diabetes mellitus (T2DM), cardiovascular disease and hypertension, *etc.*[1–3]. GLMD complicated than single factor metabolic abnormalities, because of multi-factorial interaction. Based on the complexity of the metabolic regulatory network, simultaneous

regulation of different pathways can be more potent than regulation of a single pathway [1]. Therefore, it is crucial for effective drugs against GLMD.

In traditional Chinese medicine (TCM) theory, GLMD corresponds to the clinical manifestations of "Internal heat and wasting-thirst" with basic pathogenesis of "dryness-heat due to deficiency of yin". Huangjing and Tiandong combination (HTC), a traditional Chinese prescription composed of the rhizome of *Polygonatum kingianum* Coll. et Hemsl (PK) and the tuberous root of *Asparagus cochinchinensis* (Lour.) Merr. (AC). Both PK and AC have effects of nourishing yin and reducing fire, thus are potentially suitable for GLMD prevention and treatment through improving "dryness-heat due to deficiency of yin" [4, 5].

Modern research has found that *Polygonatum sibiricum* and asparagus plants are rich in saponins, polysaccharides and flavonoids. These compounds have immunomodulatory, anti-inflammatory, antibacterial, antiviral, hypoglycemic and lipid-lowering effects[6]. As for the representative components of AC, diosgenin could regulate blood glucose metabolism and reduce lipid accumulation [7], while sarsasapogenin showed remarkable treating effects on obesity and diabetic nephropathy [8]. Moreover, by reducing miR-122 levels, the PK polysaccharide fraction was effective in reducing insulin resistance and improving gut microbiota in rats with abnormal lipid metabolism [9]. As well as inhibiting LPS entry into the circulation, PK polysaccharides also alleviate inflammation and prevent glucose and lipid metabolic imbalances [10]. Additionally, the water extracts of PK had a significant regulatory effect on the endogenous metabolites in lipid metabolism disorders rats [11]. Oral administration of PK total saponins and PK polysaccharides prevented the increase of FBG. Additionally, PK total saponins and PK polysaccharides enhanced the abundances of Bacteroidetes and Proteobacteria and increased that of Firmicutes to improve intestinal microecology [12].

Our previous network pharmacology analysis preliminary indicated that HTC may alleviate GLMD mainly via Glycolipid key Proteins [13]. However, the exact activity and the underlying mechanism are still unknown. In this study, we first conducted in-depth research on the preliminary conclusion of network pharmacology. Moreover, we analyzed the relationship between HTC, gut microbiome, and glycolipid metabolism through both 16S rDNA and metagenomic sequencing analysis. We confirmed that HTC not only regulated the Glycolipid key Proteins, but also improved gut microbial homeostasis of the GLMD model. This study provided evidence that the clinical effects of HTC alleviating GLMD were fulfilled through multiple pathways.

2 Methodology

2.1 Reagents and Materials

The following test kit instructions were followed for measuring total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C), blood sugar meter and blood sugar test paper (Roche Diagnostics, Shanghai, China). The rat Elisa kits of leptin

(LEP), adiponectin (ADPN), low-density lipoprotein (LDL), high-density lipoprotein cholesterol (HDL), phosphoinositide 3-kinase (PI3K), insulin receptor (INSR), protein kinase B (PKB/Akt), janus kinase 2 (JAK2), bicinchoninic acid (BCA), insulin receptor substrate 1 (IRS1), signal transducer and activator of transcription 3 (STAT3), insulin receptor substrate 2 (IRS2) protein were provided by Meimian Industrial Co., Ltd (Jiangsu, China). The rat ELISA kits of insulin (FINS) were supplied by Wuhan (Huamei Biotech Co., Ltd. Wuhan, China). The basic diet was supplied by Suzhou Shuangshi Experimental Animal Feed Technology Co., Ltd. (Suzhou, China). Metformin tablets were provided by Sino-American Shanghai Squibb Pharmaceuticals Ltd (Shanghai, China). Sigma-Aldrich (St. Louis, MO, USA) supplied Oil Red O.

2.2 Plant materials, Preparation and quality control of HTC water extract and dioscin

The PK and AC were bought from Wenshan Shengnong Trueborn Medicinal Materials Cultivation Cooperation Society in Wenshan, China, and have been verified by Professor Jie Yu. An AC voucher specimen (No.9609) and a PK voucher specimen (No.9608) were prepared and deposited in the Key Laboratory of Preventing Metabolic Diseases of Traditional Chinese Medicine, Yunnan University of Chinese Medicine (Kunming, China).

Fresh PK and AC were dried and processed according to our previous published method [14]. PK and AC (mass ratio 10:3) were crushed and refluxed with distilled water (sample: solvent, 1:10, weight/volume ratio) for 2 hours. After leaching, filtrates were collected. A ten-fold volume of water was then added to the filter residue to continue the decoction process for another two hours. The two successive filtrates were then combined and condensed using a RE-6000A rotatory evaporator (Shanghai Yarong Biochemical Instrument Factory, Shanghai, China) under reduced pressure at -50°C. Finally, the concentrates were *frozen* into lyophilized powder with an FD5-3 freeze dryer (SIM International Group Co. Ltd., Newark, DE, USA) and stored at -20 °C until utilization. The two consecutive filtrates were merged and concentrated with a RE-6000A rotary evaporator from Shanghai Yarong Biochemical Instrument Factory in Shanghai, China, operating at -50°C under reduced pressure. The concentrates were ultimately transformed into lyophilized powder using an FD5-3 freeze dryer from SIM International Group Co. Ltd. in Newark, DE, USA, and were then kept at -20 °C until needed.

Five grams of HTC water extract powder was dissolved in 15 mL of distilled water by previously conducted approach from the authors' research. Prepare total saponins according to the corresponding method in reference and weigh them. Repeat this operation for 3 times. The corresponding method descriptions can be found in the literature and are not repeated here [15].

A reference substance of Dioscin (1.00 mg) was dissolved in 1 mL of methanol to create a solution with a concentration of 1 mg/mL. This solution was then diluted with methanol to obtain concentrations of 0.0125 mg/mL, 0.025 mg/mL, 0.04 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.125 mg/mL, 0.25 mg/mL, and 0.5 mg/mL. With the analyte concentration (x-axis) and response value (y-axis) of the reference substance, the calibration curves were established. The UPLC setup included a communications bus module (LC-30A, Shimadzu, CBM-20A), a DAD detector (SPD-M20A, Shimadzu), and a column oven (CTO-

30A, Shimadzu). The Shimadzu C8 analytical column had dimensions of 2.0 mm inner diameter and 50 mm length, with Shim-pack XR-ODS III particles measuring 1.6 μm . A mixture of acetonitrile and water in a ratio of 40:60 was utilized as the solvent with a flow rate of 0.20 mL per minute. 203 nm was selected as the detection wavelength. The amount of liquid injected was 10 microliters. There was a 30°C column temperature and a 45-minute run time[15].

Another 2.50 g of HTC water extract powder was dissolved in 10 milliliters of distilled water to extract crude polysaccharides. The *crude polysaccharide* of HTC water extract was prepared using previous methods. Our prior research indicated that the purity of raw polysaccharides was assessed through the phenol-sulfuric acid technique with glucose as the reference standard[16].

2.3 Animal experiments

Male SD rats (weighing 200 ± 20 g and 8 weeks old) were obtained from Dashuo Biotech Co., Ltd. in Chengdu, China. The rats were kept in a regulated setting with a temperature of 22 ± 1 °C, humidity of $60 \pm 10\%$, and a light/dark cycle of 12 hours each, while also freely access to food and water. Approval for the animal studies was granted by the Laboratory Animal Ethics Committee at the Institute of Basic Theory of Yunnan University of Chinese Medicine (R-06201967).

The animals were handled in strict accordance with international regulations. All rats were adaptively fed for 1 week. Then, all rats were randomly divided into 5 groups ($n = 6$ per group): control (CON)group (normal saline); high-fat and high-sucrose diet model (MOD)group (normal saline); metformin (MET)group (90 mg/kg); low dose of HTC extraction (HTCL)group (0.9 g/kg); high dose of HTC extraction (HTCH)group (3.6 g/kg). The CON group was fed with basic food (For ten weeks in a row, the percentages of carbohydrates, proteins, and fat were 62%, 26%, and 12%, respectively, 4.06 kcal/g) while the other groups were fed with high fat and sugar diet (HFSD) diet (comprised of 15% sucrose, 10.9% refined lard, 2% cholesterol, 0.1% pig bile salt, and 72% basic diet, 5.07 kcal/g, for 10 consecutive weeks).

Daily food consumption was documented, while body weight was measured weekly throughout the study. After the completion of the 10th week, all rat was subjected to 12 hours of fasting followed by anesthesia using pentobarbital sodium. Blood sample assays were obtained from the heart. After centrifuging at 1400 g for 15 minutes at 4 degrees Celsius, the serum was separated and then stored at -80 degrees Celsius for analysis. The weight of liver, kidney, and abdominal white adipose tissue (WAT, including epididymis fat, subcutaneous fat, and mesenteric fat) were measured with an electronic balance. All organ was gathered, frozen in liquid nitrogen, and kept at -80°C until analysis.

2.4 Glucose measurement

Fasting blood glucose (FBG)concentration was measured directly using a glucose meter and test strips after the 4th, 8th, and 10th week. The level of FINS was measured by the ELISA kit with Infinite® F50 Absorbance Microplate Reader (Tecan Trading AG, Switzerland). The Homeostatic Model Assessment-Insulin Resistance (HOMA-IR)score was calculated using fasting blood glucose and insulin concentrations.

HOMA-IR score = FBG (mmol/L)*FINS (mU/L)/22.5

2.5 LEP, ADPN, TC, TG, HDL-C, LDL-C, NEFA, ALT, ISR, IRS1, PI3K, JAK2, AKT, Oil Red O, and STAT3 assays

LEP and ADPN were measured on Infinite® F50 Absorbance Microplate Reader (Tecan Trading AG, Switzerland) by the ELISA kits. The liver levels of TC, TG, HDL-C, and LDL-C were detected according to the instructions of ELISA kits, and the ISR, IRS1, PI3K, JAK2, AKT, and STAT3 contents of the liver and WAT were also tested (RT-6100 Microplate Reader, Rayto Life and Analytical Sciences Co., Ltd, Shenzhen, China). Serum TC, TG, HDL-C, and LDL-C levels were measured with the automatic biochemistry analyzer ROCHE COBAS 8000 (Roche Diagnostics, Shanghai, China). Liver tissue from the same area of the left lobe was placed in a plastic embedding box with an optimal cutting temperature compound before being frozen at 20°C for 30 minutes. The liver tissue was cut into 8-micron sections using a freezing microtome, placed on slides, and then dipped in 60% isopropanol for 5 seconds. We stained the slides with Oil Red O for 15 minutes in 60% isopropanol, washed them in PBS, and then stained them with hematoxylin for 10 seconds. Liver histopathology was evaluated with the CX31 Olympus imaging system from Olympus Corporation in Tokyo, Japan, following sealing of sections with glycerin gelatin [17].

2.6 Fecal 16S rDNA gene sequence analysis

Genomic DNA was extracted from fresh feces taking on the final day of 10th week using a Feces DNA Kit. Four samples were chosen by each group for pyrosequencing the V3-V4 regions of 16S rDNA. The 16S rDNA gene was amplified using primers 338F (5'-ACTCCTACGGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR reactions were conducted with an initial denaturation at 95°C for 3 minutes, followed by 27 cycles of 30 seconds each at 95°C, 30 seconds of annealing at 55°C, 45 seconds of elongation at 72°C, and a final extension at 72°C. Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) pooled the purified amplicons in equal numbers and sequenced them on an Illumina MiSeq platform in accordance with industry standard procedures. It offered a cloud platform for conducting bioinformatics analyses.

2.7 Fecal metagenomic sequence analysis

To extract DNA, a Feces DNA Kit was used. TBS-380 was used to measure DNA concentration, NanoDrop200 to measure DNA purity, and 1% agarose gel electrophoresis was used to measure DNA integrity. Through Covaris M220 (genomics company, China), DNA fragments of about 400 bp were screened for the construction of the PE library. In order to construct the library, NEXTflex™ Rapid DNA-SEQ (Bioo Scientific, USA) was used. Metagenomic sequencing using Illumina NovaSeq/Hiseq Xten (Illumina, USA) sequencing platform (Shanghai Meggie Biomedical Technology Co, LTD.) Raw data has been submitted to NCBI. The bioinformatics analyses of the results were operated on the cloud platform of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.8 Statistical analysis of the experiment

Multiple comparisons were conducted using SPSS 19.0 software through an analysis of variance (ANOVA). The data were presented as the average plus the standard deviation. A P-value less than 0.05

was considered to be statistically significant. The visuals were generated using Origin 6.1 software from MicroCal in the United States.

3. Results

3.1 Contents of total saponins, total polysaccharides, and dioscin

The yield of HTC water extract is 56.33%. The proportion of total saponins in HTC water extract is 3.9%, which means the total saponins account for $2.20 \pm 0.29\%$ of the raw weight of HTC ($n = 3$). The dioscin content in total saponins was $0.023 \pm 0.003\%$ ($n=3$, standard curve of reference substance was $y=10^7x + 3193.7$, $R^2=0.9990$) (supplementary fig 1 and supplementary table 3).

On the other hand, the proportion of total polysaccharides in HTC water extract is 3.2%, which means the total polysaccharides account for 1.80% of the raw weight of HTC ($n = 3$). Besides, the purity of crude polysaccharide was 52.60% ($n=3$, standard curve of reference substance was $y = 3.1277x+0.072$, $R^2=0.9994$).

3.2 HTC can effectively improve the pharmacological indexes of GLMD

At the end of the experiment, the average calorie intake of each group of rats per week showed no significant difference. However, in contrast to the CON group, the MOD group experienced a notable increase in body weight. While the body weight of HTCL ($P<0.05$) was significantly lower than MOD (Fig 1 A-B). As shown in Fig 1C, after 12 weeks of HFSD-feeding, rat livers showed severe lipid accumulation. Following administration of the HTC extract, there was a reduction in the quantity of lipid droplets in the liver of rats, thereby mitigating the lipid buildup caused by HFSD.

The heart, lung, and kidney coefficients were not significantly different among the groups (see Supplementary Table 1), suggesting that the experimental method did not result in notable visceral alterations in these organs. In comparison to the CON group, the MOD group showed a significant increase of 29.3% in liver and 11.20% in WAT coefficient, suggesting that feeding HFSD led to liver enlargement and deposition of abdominal fat. After HTC administration, the liver coefficient decreased slightly, while the WAT coefficient decreased by 19.45% and 22.42% in HTCL and HTCH groups, respectively.

Ten weeks of HFSD-feeding significantly increased the FBG ($P<0.05$, Supplementary table 1), HOME-IR, and FINS ($P<0.05$, Fig 1D-E) relative to CON. However, after treatment, all groups showed a marked decrease in FBG ($P<0.05$, Supplementary table 1), while HOME-IR significantly decreased only in MET group.

In comparison to the CON group (shown in Fig 1 H-O), the MOD group had significantly higher levels of serum TC, TG, and LDL-C, while HDL-C levels were significantly lower. All these variables were improved after HTC administration. The serum TG ($P<0.01$), HDL-C ($P<0.05$), and LDL-C ($P<0.05$) had a significant

decline, and the liver LDL-C was significantly elevated in HTC group. Ten weeks of HFSD feeding caused a significant increase in LEP ($P < 0.001$) and a decrease in ADPN (Fig 1F-G). The LEP level in both MET and HTC groups was significantly decreased ($P < 0.01$, $P < 0.05$). The ADPN level got a certain degree of increase in the administration group as well.

3.3 HTC effectively regulates the expression of glycolipid metabolism-related proteins in the liver and WAT

The MOD group showed lower expression levels of all investigated proteins in liver tissues compared to the CON group (Fig 2A-G). IRS2, JAK2, and STAT3 showed significant decreases ($P < 0.05$, $P < 0.001$, $P < 0.01$). In comparison to MOD, the protein expression in each administration group showed an increase, with INSR, IRS1, PI3K, and Akt/PKB all showing an increase in the HTCH group, while IRS2 exhibited a significant increase ($P < 0.01$).

The protein expression levels in the MOD group were significantly lower than those in the CON group in the WAT tissue (all with $P < 0.001$) (Fig 2 H-N). In comparison to MOD, the protein expression in every administration group showed an increase. Among them, the protein levels of INSR, IRS1, PI3K, Akt, STAT3, and IRS2 were significantly increased in the HTCH and HTCL groups.

3.4 HTC effectively regulates intestinal flora imbalance

At the *OUT* level, microbial richness and diversity were estimated using *Chao* and *Shannon* index (Supplementary table 2). Compared with CON, the two above indicators of MOD fecal microorganisms decreased significantly. After HTC administration, they were recovered to a certain extent in each group.

The phyla Bacteroidetes and Firmicutes were the most prevalent, making up over 90% of the total abundance (Fig.3A). Rats fed a HFSD diet showed a significant increase in Firmicutes abundance, with a corresponding decrease in Bacteroidetes abundance. The HTC administration reversed the ratio of Firmicutes to Proteobacteria compared to the HFSD group. HTC's involvement effectively restored the levels of four Firmicutes families (*Lactobacillus*, *Blautia*, *Faecalibacterium*, and *Lactobacillaceae*) to a stable state.(Fig.3B).

According to Beta Diversity, the PCoA analysis showed that the drug groups (HTCL and HTCH) were gradually separated from the HFD group, confirming that HTC could significantly alter the intestinal microbiota structure of hyperlipidemic rat and that the effect of low-dose HTC was similar to that of high-dose HTC (Figs. 3C). ANOSIM (Analysis of similarities) is a nonparametric test used to detect whether the differences among groups are significantly greater than those within groups. ANOSIM indicated the gut microbiota differed significantly among the different experimental groups ($P = 0.01$; Figs. 3D).

Collectively, these data suggest that HTC can significantly change the structure of the intestinal microbial community of HFSD rat.

Based on Beta Diversity, the PCoA analysis indicated a gradual separation of the drug groups (HTCL and HTCH) from the HFD group, providing evidence that HTC could effectively change the composition of the

gut microbiota in hyperlipidemic rats. Furthermore, the impact of HTCL was comparable to that of HTCH (Figs. 3C). ANOSIM is a nonparametric statistical test that determines if the dissimilarities between groups are significantly larger than the dissimilarities within groups. ANOSIM analysis revealed significant differences in gut microbiota composition among the various experimental groups ($P < 0.01$; Figs. 3D). All of these findings point to the possibility that HTC may dramatically alter the composition of the HFSD rat's gut microbial community.

An analysis using Spearman's correlation indicated a significant relationship between the levels of the mentioned bacteria (*Collinsella*, *Norank-f-Muribaculaceae*, *Blautia*, *Facealibacterium*, *Shuttleworthia*, *Ruminococcus*, *Anaerostipes*, and *Oscillospiraceae*) and alterations in the lipid levels of the host. Among them, *Collinsella*, *Blautia*, *Facealibacterium*, *Shuttleworthia*, and *Anaerostipes* were negatively associated with HDLC, meanwhile, they were positively associated with TG and LDLC. While the *Norank-f-Muribaculaceae*, *Ruminococcus* and *Oscillospiraceae* presented opposite correlations in each of these areas (Fig. 3E).

3.5 Mining metagenomic data reveals functional differences after HTC administration

50 species experienced significant changes in abundance due to either HTCL or HTCH treatment. Four separate clusters were formed by grouping the 50 species (Fig. 4A). Cluster 1 included species that had higher numbers following HTCL and HTCH treatment; Cluster 2 included species that had lower numbers mainly after HTCL and HTCH treatment; Cluster 3 included species that had increased numbers after either HTCL or HTCH treatment; and Cluster 4 included species that had decreased numbers after either HTCL or HTCH treatment.

Six lipid characteristics evaluated in the study were associated with changes in the relative abundance of each sample, to determine the association between these species and corresponding changes in the lipid index (Fig. 4A).

Blautia, *Clostridium*, and other species dominated Cluster 1 (22 species), which was linked to gains in FBG, TC, TG, and LDLC. Cluster 2 (7 species) comprised *Ruminococcus*, *Collinsella*, *Adlercreutzia*, *Lachnospira* and so on. They are significantly positive correlated to TC, TG, LDLC following HTCL and HTCH treatment. Cluster 3 (11 species) comprised *Prevotella*, *Butyricicoccus*, *Bacteroides*, *Roseburia*, *Marvinbryantia*, *Coprobacillus* that were negatively associated with TC, TG, and LDLC. Cluster 4 (10 species) comprised *Mediterraneibacter* as well as *Methanosphaera*, *Frisingicoccus*, *Ileibacterium*, and *Anaerobutyricum* and correlated to FINS. The rat gut microbiome genes were annotated with KEGG (Kyoto Encyclopedia of Genes and Genomes) in order to assess the microbial metabolic pathways linked to the compositional alterations in the gut microbiome. 49 of them underwent significant alteration following HTCL or HTCH therapy. Abundance-shifted KEGG modules and related functional pathways are shown in Figure 4B. The metabolic pathways most prominently regulated were those for amino acid metabolism, energy metabolism, carbohydrate metabolism, lipid metabolism, and cofactor and vitamin metabolism.

Changes in the gut microbiome between HTCL and HTCH were mainly linked to impacts on the processing of carbohydrates (particularly amino sugar and nucleotide sugar metabolism), energy production (such as carbon fixation in photosynthetic organisms, and methane metabolism), lipid processing (glycerophospholipid metabolism), and the breakdown of cofactors and vitamins (nicotinate and nicotinamide metabolism).

4. Discussion

GLMD silently poses a threat to human life and health by causing persistent anomalies in lipid and blood glucose levels, which can harm various organs and lead to their gradual decline in function.[1]. For example, T2DM, hyperlipidemia and intestinal imbalance are typical complications of GLMD [18–20]. HTC water extract could significantly improve the abnormal blood sugar, lipid accumulation, and gut microbiome in GLMD rats. It could exert such efficacy because HTC not only could regulate related key proteins, but also improve the gut microbiome homeostasis of GLMD rat's model.

GLMD has clinicopathological characteristics of the increase of TC, TG, LDL-C, FBG, FINS, and HOME-IR in serum and/or liver, and the decrease of HDL-C [21]. This study indicated that after HTC treatment, the above indicators were obviously reversed. Furthermore, staining with oil red O revealed a substantial buildup of lipid droplets in the liver due to the HFSD diet. Following the HTC extraction process, there was a reduction in lipid droplets and the liver's normal morphology was reinstated. Therefore, without influencing food intake, HTC extract therapy considerably controls the alterations brought on by HFSD feeding, suggesting that HTC might effectively regulate the GLMD and lessen its influence on GLMD rats.

It is known that under normal circumstances, insulin binds to INSR and acts on substrates IRS1 and IRS2, which makes the substrates phosphorylate and bind to PI3K to obtain 3,4,5-phosphatidylinositol triphosphate, and then activates Akt, which activates glycogen synthase kinase, mammalian rapamycin target protein and other targets respectively, affecting the utilization of nutrients by metabolic organs[22]. If the expression of liver INSR, substrates IRS1 and IRS2, PI3K or Akt decreases, the process of gluconeogenesis will be accelerated and the blood sugar will increase. The ability of liver to absorb and transform FFA, TG and other lipids is weakened, and lipid deposition also occurs [23]. A decrease in the strength of the signal proteins mentioned above will result in a lower rate of glucose and FFA absorption by white fat cells, causing an increase in FFA levels in the body and disrupting glucose and lipid metabolism in peripheral tissues like the liver and pancreas [24]. Signal weakening will also lead to abnormal secretion of adiponectin and leptin, resulting in imbalance of white fat synthesis and decomposition [24]. We found that HTC therapeutic efficacy was related to the activity of many important targets in GLMD. The activity of INSR, IRS1, IRS2, PI3K, Akt, JAK, and STAT3 of glucolipid metabolism were reduced in liver and WAT of MOD. HTC intervention can significantly revert the expression levels of the above key proteins, and improve the disorder of blood glucose, blood lipid, adipokines, and liver fat caused by HFSD. Besides, it is reported that PI3K, IRS1, and IRS2 are energy metabolite markers of liver and fat, which can also improve insulin resistance in obese mice. Our experiment also showed similar results.

Moreover, 16S rDNA and metagenomics were usually applied to investigate gut microbiome changes. According to 16S rDNA sequencing results, HFSD feeding altered gut microbiota community richness compared to CON feeding. HFSD rats exhibited notably reduced levels of the Firmicutes phylum while displaying elevated levels of the Actinobacteria phylum. Long-term treatment with HTCH prompted gut microbiome of HFSD feeding closer to the CON group. According to our findings, HTCH lowers the F/B ratio. Reduced F/B ratios have lipid-lowering and anti-obesity benefits because they cause obese mice to lose weight [25]. In particular, Rats treated with HTCL and HTCH demonstrated significant similarities in the alterations to their gut microbiome, mostly due to an increase in the number of species within Firmicutes (*Blautia*, *Clostridium*), and a comparable decrease in the abundance of Actinobacteriota (*Adlercreutzia*) and Firmicutes (*Ruminococcus*), which are known to be associated with obesity. Visceral fat area, which is thought to be an obesity biomarker of cardiovascular and metabolic illness risk. *Blautia* has a negative correlation with Visceral fat area. [26]. *Blautia wexlerae* can alter the composition of intestinal bacteria, which can reduce obesity and diabetes brought on by HFSD [27]. Furthermore, the prevalence of *Clostridium* has been comparatively reduced in obese individuals and animal subjects compared to those in healthy populations [28]. *Clostridium butyricum* through its metabolite butyrate and then enhances the production of Treg cells, which inhibits fat deposition [29]. In human studies, obesity was found to be positively associated with high levels of *Adlercreutzia* [30, 31]. Studies have shown a direct relationship between the abundance of *Adlercreutzia* and the rise in overall fat content in db/db mice fed a high-fat diet. However, after drug administration, the abundance of *Adlercreutzia* decreased [32]. Our findings suggest that consuming HTC can reduce obesity by changing the levels of bacteria linked to obesity.

Many studies have used metagenome method to explore the function of microbial community. Analysis of KEGG functions indicates that HTC can mitigate NAFLD primarily by modulating amino acid, carbohydrate, and lipid metabolism, as well as cofactor and vitamin metabolism. This includes pathways like glycine, serine, and threonine metabolism, Lysine biosynthesis, glycerophosphate metabolism, and pantothenic acid and coenzyme A biosynthesis. The metabolism of glycine, serine, and threonine may play a role in longevity and its associated molecular pathways [33], which decline in individuals with metabolic syndrome but can be improved by consuming the right amount of glycine [34]. Serine has been shown to alleviate symptoms of metabolic syndrome. Research has indicated that L-cysteine can lower the lipid levels in the blood and liver of rats according to a study [35]. Therefore, we speculate that HTC may prevent GLMD by regulating the metabolic pathway, which is also consistent with the partial prediction of intestinal microflora. All these mentioned pharmacodynamic indexes were remarkably restored in the HTC treated groups, indicating it is closely related to the changes of gut microbiome and the restoration of various metabolic pathways.

The limitation of this study is that the active compounds in HTC are unknown. In addition, microbial transplantation is needed to verify HTC's intestinal microbial dependence on improving GLMD. In a word, HTC may improve GLMD by regulating key proteins related to glucose and lipid and gut microbiota.

5. Conclusion

On one hand, HTC can alleviate HFSD induced GLMD by regulating Glycolipid key Proteins. On the other hand, HTC can alleviate GLMD by regulating the abundance and diversity of gut microbiota and improving metabolic pathways. Therefore, HTC is a useful drug/nutrient to relieve GLMD and maintain gut microbiota homeostasis.

Declarations

Author contributions: Jie Li: Conceptualization, Data curation, Formal analysis, Writing—original draft. Yating Tao: Resources, Writing—review & editing. Wenbo Wang: Resources, Writing—review & editing. Lianli Zhou: Resources, Writing—review & editing. Xingxin Yang: Resources, Writing—review & editing. Jinfeng Xia: Resources, Writing—review & editing. Ruidan Tang: Resources, Writing—review & editing. Wen Gu: Resources, Writing—review & editing. Zhen Chen: Resources, Writing—review & editing. Fan Zhang: Project administration, Writing—review & editing. Jie Yu: Project administration, Writing—review & editing.

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Competing interests

All authors declare no competing interests.

Data and materials availability

Data is available upon request.

Declarations of ethical approval and participation consent

The animal experiments were approved by the Laboratory Animal Ethics Committee of the Institute of Basic Theory of Yunnan University of Chinese Medicine(Kunming, China)(R-06201967).

Consent for publication

Not applicable.

References

1. Fang X, Miao R, Wei J, Wu H, Tian J. Advances in multi-omics study of biomarkers of glycolipid metabolism disorder. *Comput Struct Biotechnol J*. 2022;20:5935–51.
2. Wu Z, Ma Q, Cai S, Sun Y, Zhang Y, Yi J. *Rhus chinensis* Mill. Fruits Ameliorate Hepatic Glycolipid Metabolism Disorder in Rats Induced by High Fat/High Sugar Diet. *Nutrients* 2021, 13.

3. Yang H, Li Y, Huang L, Fang M, Xu S. The Epigenetic Regulation of RNA N6-Methyladenosine Methylation in Glycolipid Metabolism. *Biomolecules* 2023, 13.
4. Zhao GDS, Chen R. The dictionary of Chinese herbal medicine. Shanghai, China. Shanghai Scientific&Technical; 2006.
5. Committee NP. Pharmacopoeia of the People's Republic of China. Beijing: China Medical Science; 2020.
6. Xu C, Xia B, Zhang Z, Lin Y, Li C, Lin L. Research progress in steroidal saponins from the genus *Polygonatum*: Chemical components, biosynthetic pathways and pharmacological effects. *Phytochemistry*. 2023;213:113731.
7. Zhang C, Liu J, He X, Sheng Y, Yang C, Li H, Xu J, Xu W, Huang K. *Caulis Spatholobi* Ameliorates Obesity through Activating Brown Adipose Tissue and Modulating the Composition of Gut Microbiota. *Int J Mol Sci* 2019, 20.
8. Liu Y, Hao Y, Chen Y, Yin S, Zhang M, Kong L, Wang TJPP. Protective effects of sarsasapogenin against early stage of diabetic nephropathy in rats. 2018, 32:1574–82.
9. Dong J, Gu W, Yang X, Zeng L, Wang X, Mu J, Wang Y, Li F, Yang M, Yu J. Crosstalk Between *Polygonatum kingianum*, the miRNA, and Gut Microbiota in the Regulation of Lipid Metabolism. *Front Pharmacol*. 2021;12:740528.
10. Gu W, Yang M, Bi Q, Zeng LX, Wang X, Dong JC, Li FJ, Yang XX, Li JP, Yu J. Water extract from processed *Polygonum multiflorum* modulate gut microbiota and glucose metabolism on insulin resistant rats. *BMC Complement Med Ther*. 2020;20:107.
11. Yang X, Wei J, Mu J, Liu X, Dong J, Zeng L, Gu W, Li J. Yu JJWjog: *Polygonatum kingianum* Integrated metabolomic profiling for analysis of antilipidemic effects of extract on dyslipidemia in rats. 2018, 24:5505–24.
12. Yan H, Lu J, Wang Y, Gu W, Yang X, Yu J. Intake of total saponins and polysaccharides from *Polygonatum kingianum* affects the gut microbiota in diabetic rats. *Phytomedicine*. 2017;26:45–54.
13. Tao Y, Li F, Min Y, Zhang Z, Qin P, Mu J, Xingxin Y, Wen G, Chen, Jie Y. Mechanism of Huangjing Pill on improving disorder of glycolipid metabolism based on network pharmacology. *Acad J Shanghai Univ Traditional Chin Med* 2021:83–92.
14. Wang Y, Shi X, Dong TT, Li JC, Zeng FJ, Yang LX, Gu M, Li W, Yu JP. Mitochondrial dysfunction in high-fat diet-induced nonalcoholic fatty liver disease: The alleviating effect and its mechanism of *Polygonatum kingianum*. *Biomed Pharmacother*. 2019;117:109083.
15. Lu JM, Wang YF, Yan HL, Lin P, Gu W, Yu J. Antidiabetic effect of total saponins from *Polygonatum kingianum* in streptozotocin-induced daibetic rats. *J Ethnopharmacol*. 2016;179:291–300.
16. Gu W, Wang Y, Zeng L, Dong J, Bi Q, Yang X, Che Y, He S, Yu J. Polysaccharides from *Polygonatum kingianum* improve glucose and lipid metabolism in rats fed a high fat diet. *Biomed Pharmacother*. 2020;125:109910.
17. Mu JK, Zi L, Li YQ, Yu LP, Cui ZG, Shi TT, Zhang F, Gu W, Hao JJ, Yu J, Yang XX. *Jiuzhuan Huangjing* Pills relieve mitochondrial dysfunction and attenuate high-fat diet-induced metabolic dysfunction-

- associated fatty liver disease. *Biomed Pharmacother.* 2021;142:112092.
18. Du H, Zhao Y, Yin Z, Wang DW, Chen C. The role of miR-320 in glucose and lipid metabolism disorder-associated diseases. *Int J Biol Sci.* 2021;17:402–16.
 19. Yuan X, Zheng J, Ren L, Jiao S, Feng C, Du Y, Liu H. Glucosamine Ameliorates Symptoms of High-Fat Diet-Fed Mice by Reversing Imbalanced Gut Microbiota. *Front Pharmacol.* 2021;12:694107.
 20. Luo R, Chen L, Song X, Zhang X, Xu W, Han D, Zuo J, Hu W, Shi Y, Cao Y et al. Possible Role of GnIH as a Novel Link between Hyperphagia-Induced Obesity-Related Metabolic Derangements and Hypogonadism in Male Mice. *Int J Mol Sci* 2022, 23.
 21. Girard D, Petrovsky N. Alström syndrome: insights into the pathogenesis of metabolic disorders. *Nat Rev Endocrinol.* 2011;7:77–88.
 22. Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. *Int J Biol Sci.* 2018;14:1483–96.
 23. Zaulkffali AS, Md Razip NN, Syed Alwi SS, Abd Jalil A, Abd Mutalib MS, Gopalsamy B, Chang SK, Zainal Z, Ibrahim NN, Zakaria ZA. Khaza'ai H: Vitamins D and E Stimulate the PI3K-AKT Signalling Pathway in Insulin-Resistant SK-N-SH Neuronal Cells. *Nutrients* 2019, 11.
 24. Hodson L, Gunn PJ. Publisher Correction: The regulation of hepatic fatty acid synthesis and partitioning: the effect of nutritional state. *Nat Rev Endocrinol.* 2020;16:340.
 25. Baek GH, Yoo KM, Kim SY, Lee DH, Chung H, Jung SC, Park SK, Kim JS. Collagen Peptide Exerts an Anti-Obesity Effect by Influencing the Firmicutes/Bacteroidetes Ratio in the Gut. *Nutrients* 2023, 15.
 26. Ozato N, Yamaguchi T, Mori K, Katashima M, Kumagai M, Murashita K, Katsuragi Y, Tamada Y, Kakuta M, Imoto S et al. Two *Blautia* Species Associated with Visceral Fat Accumulation: A One-Year Longitudinal Study. *Biology (Basel)* 2022, 11.
 27. Hosomi K, Saito M, Park J, Murakami H, Shibata N, Ando M, Nagatake T, Konishi K, Ohno H, Tanisawa K, et al. Oral administration of *Blautia wexlerae* ameliorates obesity and type 2 diabetes via metabolic remodeling of the gut microbiota. *Nat Commun.* 2022;13:4477.
 28. Kong C, Gao R, Yan X, Huang L, Qin H. Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. *Nutrition.* 2019;60:175–84.
 29. Li H, Jia Y, Weng D, Ju Z, Zhao Y, Liu S, Liu Y, Song M, Cui L, Sun S, Lin H. *Clostridium butyricum* Inhibits Fat Deposition via Increasing the Frequency of Adipose Tissue-Resident Regulatory T Cells. *Mol Nutr Food Res.* 2022;66:e2100884.
 30. Yun Y, Kim HN, Kim SE, Heo SG, Chang Y, Ryu S, Shin H, Kim HL. Comparative analysis of gut microbiota associated with body mass index in a large Korean cohort. *BMC Microbiol.* 2017;17:151.
 31. Del Chierico F, Abbatini F, Russo A, Quagliariello A, Reddel S, Capoccia D, Caccamo R, Ginanni Corradini S, Nobili V, De Peppo F, et al. Gut Microbiota Markers in Obese Adolescent and Adult Patients: Age-Dependent Differential Patterns. *Front Microbiol.* 2018;9:1210.
 32. Oh TJ, Sul WJ, Oh HN, Lee YK, Lim HL, Choi SH, Park KS, Jang HC. Butyrate attenuated fat gain through gut microbiota modulation in db/db mice following dapagliflozin treatment. *Sci Rep.*

2019;9:20300.

33. Aon MA, Bernier M, Mitchell SJ, Di Germanio C, Mattison JA, Ehrlich MR, Colman RJ, Anderson RM, de Cabo R. Untangling Determinants of Enhanced Health and Lifespan through a Multi-omics Approach in Mice. *Cell Metab.* 2020;32:100–e116104.
34. Handzlik MK, Gengatharan JM, Frizzi KE, McGregor GH, Martino C, Rahman G, Gonzalez A, Moreno AM, Green CR, Guernsey LS, et al. Insulin-regulated serine and lipid metabolism drive peripheral neuropathy. *Nature.* 2023;614:118–24.
35. Lee S, Han KH, Nakamura Y, Kawakami S, Shimada K, Hayakawa T, Onoue H, Fukushima M. Dietary L-cysteine improves the antioxidative potential and lipid metabolism in rats fed a normal diet. *Biosci Biotechnol Biochem.* 2013;77:1430–4.