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Improved metabolic functions and gut microbiota rebalance: the therapeutic potential of *Ficus carica* polysaccharides for type 2 diabetes mellitus

Weilan Wang[1,*], Guirong Song[1], Fujun Liu[1], Chenxin Zhang, Wei Jiang, Yiwen Gao, Xiang Zhang, Lixue Wang, Xinran Xu, Qingxian Zhao, Yan Yang, Kexin Liu, Jie Lü, Jinyao Li*

Xinjiang Key Laboratory of Biological Resources and Genetic Engineering, College of Life Science and Technology, Xinjiang University, Urumqi 830046, China

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ABSTRACT

The present study investigated the potential therapeutic potential of *Ficus carica* polysaccharides (FCPS) in type 2 diabetic mellitus (T2DM) mice, focusing on elucidating the underlying molecular mechanisms. Network pharmacology analysis identified 37 shared targets between FCPS and T2DM, including perixisome proliferator activated receptor alpha (PPARα), highlighting the significance of PPAR signaling pathways in FCPS-mediated T2DM treatment. The results demonstrated that FCPS treatment significantly reduced markers of glucose and lipid metabolism (fasting blood glucose (FBG), nonestesterified fatty acid (NEFA), triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C)), inflammatory cytokines (tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1beta (IL-1β), monocyte chemoattractant protein-1 (MCP-1)), and liver damage (glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT)) in T2DM mice. Additionally, FCPS ameliorated hepatic lipid droplet accumulation, fatty degeneration, and hepatocyte structural abnormalities. Western blot analysis confirmed FCPS-induced upregulation of key proteins in the IRS-1/AKT/PPARα signaling pathway, (insulin receptor substrate 1 (IRS-1), phosphatidyqinositol-3 kinase (PI3K), phospho-protein kinase B (p-AKT), glucose transporter 2 (GLUT2), phospho-glycogen synthase kinase 3 beta (p-GSK-3β), phospho-adenosine 5′-monophosphate-activated protein kinase alpha (p-AMPKα), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), PPARα, peroxisome proliferator-activated receptor gamma (PPARγ)) and downregulation of GSK-3β, sterol regulatory element binding protein 1c (SREBP-1c), fatty acid synthase (FAS), and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). 16S rRNA sequencing results revealed FCPS's ability to modulate gut microbiota dysbiosis in T2DM mice by promoting beneficial bacteria (e.g., *Lactobacillus_reuteri*, *Candidatus_Saccharimonas*) and suppressing opportunistic pathogens (e.g., *Proteobacteria*, *Gammaproteobacteria*, *Escherichia-Shigella*). These findings collectively suggest that FCPS has a marked effectiveness in improving glucose and lipid metabolism, decreasing inflammatory responses, as well as modulating the gut microbiota in T2DM mice *via* the gut-hepatic axis, demonstrating its potential as a functional food for diabetes prevention and management.

1. Introduction

Type 2 diabetes mellitus (T2DM) constitutes the predominant form of diabetes, comprising for 90%–95% of cases[1]. Characterized by hyperglycemia and insulin insufficiency due to insulin resistance, T2DM can lead to diverse health complications affecting multiple organ systems, thus diminishing quality of life[2]. Although various

[1] The authors contribute equally.

* Corresponding authors at: College of Life Science and Technology, Xinjiang University, Urumqi 830046, China.
E-mail addresses: wwl@xju.edu.cn (W.L. Wang); ljyxju@xju.edu.cn (J.Y. Li.)

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treatments exist, certain interventions may result in adverse outcomes, including organ damage, hypoglycemia, or lactic acidosis[3]. Consequently, the development of antidiabetic medications that are highly efficacious, safe, and possess low-toxicity is imperative to enhance the well-being of individuals with diabetes and mitigate complications.

Natural products derived from food sources, such as *Ficus carica*, hold significant value in drug discovery due to their diverse bioactive constituents, multifaceted therapeutic effects, and reduced incidence of adverse reactions. *F. carica*, a plant with a long history of cultivation in China, includes the native early-maturing cultiva "Xinjiang Zaohuang", renowned for its medicinal and social significance and rich in nutrients and bioactive compounds. Previous studies have demonstrated that glycosides from *F. carica* possess antioxidant, hypolipidemic, and antidiabetic properties, effectively reducing blood glucose and weight gain in diabetic rats[4]. Additionally, *F. carica* also contains polysaccharides and fiber that promote gut health and increase probiotic populations[5-7], making it a promising natural food source with potential medicinal benefits for managing diabetes and related conditions. However, further investigation into the antidiabetic activity and underlying mechanism of *F. carica* polysaccharides (FCPS) is warranted.

Network pharmacology, a rapidly evolving field at the intersection of bioinformatics and pharmacology, focuses on elucidating the therapeutic effects and consequences of drugs on diseases at a network level. The ultimate goal is to identify multi-target drugs with optimal efficacy and that minimal toxicity[8]. This study utilized network pharmacology to construct a component-target network diagram for monosaccharides derived from FCPS in the management of T2DM. Importantly, we sought to predict the potential targets and mechanisms of FCPS monosaccharides for T2DM treatment, followed by validation through molecular docking technology.

The gut microbiota, a diverse and abundant community of microorganisms inhabiting the human gastrointestinal tract, comprises approximately 500−1 000 bacterial species as identified through analysis of bacterial 16S rRNA sequences[9]. Extensive research has implicated the gut microbiota and their metabolites in the development of metabolic disorders, including T2DM[10-13]. However, the precise relationship between dysbiosis of the gut bacteria and T2DM remains to be fully elucidated[14]. This study investigated the effects of FCPS on glucose and lipid homeostasis, insulin resistance, and inflammation in mice with T2DM. Specifically, we aimed to understand the mechanisms through which FCPS regulate the gut microbiota.

2. Materials and methods

2.1 Materials

F. carica L. fruits were procured from Urumqi (Xinjiang, China). FCPS were prepared at the Xinjiang Key Laboratory of Biological Resources and Genetic Engineering, Xinjiang University. Ultrasound-assisted extraction was employed to extract FCPS, resulting in a yield of 81.2%. Subsequent monosaccharide composition analysis using high-performance liquid chromatography (HPLC) revealed that FCPS primarily consisted of glucose and galacturonic acid, with additional arabinose, galactose, and trace amounts of rhamnose, mannose, xylose, glucuronic acid, fucose, and ribose[15].

2.2 Network pharmacology on mechanism of FCPS against T2DM

2.2.1 Screening of FCPS and T2DM-related targets

The characterization of FCPS involved several bioinformatics tools: the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) for structure determination aided by ChemDraw software for analysis (Table 1), the SwissTarget Prediction platform (http://www.swisstargetprediction.ch/) for predicting potential targets of FCPS monosaccharides, and GeneCards (https://www.genecards.org/) and DisGeNET (https://www.disgenet.org/search) databases to identify for T2DM-related targets using "T2DM" as the keyword. Finally, a bioinformatics platform (https://www.bioinformatics.com.cn/) was used to identify the common targets between FCPS and T2DM, which were then visualized using a Venn diagram.

Table 1
Monosaccharides of FCPS.

Name	PubChem CID	SMILE
D-Mannose	18950	C(C1C(C(C(C(O1)O)O)O)O)O
D-Ribose	10975657	C1C(C(C(C(O1)O)O)O)O
L-Rhamnose	25310	CC1C(C(C(C(O1)O)O)O)O
Aldehydo-*D*-glucuronic acid	65041	C(=O)C(C(C(C(C(=O)O)O)O)O)O
D-Galacturonic acid	439215	C1(C(C(OC(C1O)O)C(=O)O)O)O
D-Glucose	5793	C(C1C(C(C(C(O1)O)O)O)O)O
D-Galactose	6036	C(C1C(C(C(C(O1)O)O)O)O)O
DL-Xylose	644160	C(C(C(C(C=O)O)O)O)O
L-Ribose	90428	C(C(C(C(C=O)O)O)O)O
Aldehydo-*D*-fucose	94270	CC(C(C(C(C=O)O)O)O)O

2.2.2 Establishment of the protein-protein interaction (PPI) network for FCPS monosaccharides and T2DM targets

To identify interacting proteins potentially involved in FCPS's antidiabetic effects, the predicted target proteins were uploaded to the STRING database (https://STRING-db.org/) to retrieve a PPI network. A medium confidence interaction score threshold of 0.400 was applied to ensure network reliability[16]. The resulting network data from STRING was then imported into Cytoscape software (version 3.9.1) for visualization and further analysis.

2.2.3 The functional annotation and pathway enrichment of target genes

The identified overlapping genes were uploaded to the Metascape database (https://metascape.org/gp/index.html#/main/step1) for functional enrichment analysis. Here, "*Homo sapiens*" was selected as the species of interest. Metascape then performed Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation analysis to identify biological processes (BP) and pathways significantly associated with FCPS treatment in T2DM. Finally, the top enriched clusters were visualized and further analyzed using visualization tools and online data analysis platforms.

2.2.4 Molecular docking

Utilizing the PPI network, key targets with high degree values were identified, and their corresponding protein structures were retrieved from the Protein Data Bank (PDB) and UniProt databases. The chemical structure of FCPS monosaccharides was obtained from the PubChem database. Subsequently, molecular docking experiments were conducted using AutoDockTools-1.5.7 software. A lower AutoDockTools docking score indicates a stronger binding affinity between the docking compound and the target with a score below −5.0 suggesting a robust interaction. The Pymol software was employed for visual inspection and analysis of the docking results generated by AutoDock Tools.

2.3 Animals and ethics statement

Six-week-old male Kunming male mice, the average weight (27 ± 2.013) g, were obtained from the Animal Laboratory Center at Xinjiang Medical University in Urumqi, China (certificate No. SYXK(Xin)2018-0003). The mice were housed in a temperature-controlled and light-cycled animal facility at Xinjiang University. Animals had *ad libitum* access to standard chow and water, and were maintained in a controlled environment at (23 ± 2) ℃, with a relative humidity of (50 ± 10)%, and a 12-h light/dark cycle. All experiments were approved by the Committee on the Ethics of Animal Experiments of the Xinjiang Key Laboratory of Biological Resources and Genetic Engineering (BRGE-AE001).

2.4 Establishment of T2DM mouse model

Mice were acclimatized for 1 week, and then divided into a normal group (n = 6, standard diet) and a model group (n = 24, high-sugar and high-fat feed). Body weight was recorded every 4 days. During the 5th week, FBG were measured after a 12-h fast without water. Mice in the high-sugar and high-fat diet group received an intraperitoneal injection of 80 mg/kg streptozotocin (STZ) on an empty stomach, based on their weight. After 7 days, mice were fasted for 12 h without water, and tail blood was collected to measure FBG and fasting serum insulin (FINS) levels. A FBG value greater than 11.1 mmol/L was considered indicative of T2DM modeling. The homeostatic model assessment for insulin resistance index (HOMA-IR) was calculated to evaluate β-cell function using the following equation:

$$HOMA\text{-}IR = (FBG \times FINS)/22.5$$

2.5 Grouping and treatment of experimental animals

T2DM mice were randomly assigned to 2 groups: the T2DM group and the FCPS group (low and high dose). Mice in the low-dose group (L-FCPS) were administered 200 mg/kg FCPS per body mass *via* gavage, while those in the high dose group (H-FCPS) received 800 mg/kg. The normal and T2DM groups received equal volumes of distilled water by gavage. For 5 weeks post-gavage, mice in each group were monitored for food intake, water intake, and bedding moisture levels.

2.6 Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

An OGTT was conducted after a 12-h overnight fast. Mice were administered a 50% glucose solution (2 g/kg bw) *via* gavage, and blood samples were collected at 0, 15, 30, 60, 90, and 120 min following tail vein injection. Blood glucose levels were measured using a glucose monitoring device, and glucose tolerance was assessed by calculating the area under the curve (AUC). After 5 days, an ITT was performed after a 6-h fasting period. Mice were injected with insulin (0.5 units/kg bw) obtained from Novo Nordisk China Pharmaceutical, Beijing, China[17]. Blood samples were collected 0 min before and 15, 30, 60, 90, and 120 min after injection to measure blood glucose levels.

2.7 Serum and liver biochemical analysis

Following a 5-week administration period, livers were harvested from the mice. Serum was isolated by centrifuging blood samples at 3 000 r/min for 20 min at 4 ℃. Total triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), free fatty acid (NEFA), glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT) were quantified using commercial kits (Nanjing Jiancheng, China)[17]. Tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), interleukin-1β (IL-1β), and monocyte chemotactic protein-1 (MCP-1) were measured using ELISA kits (Elabscience, Shanghai, China).

2.8 Histology analysis of liver tissue

Liver specimens were fixed in 4% paraformaldehyde for 24 h and embedded in embedding in paraffin. The embedded samples were then sectioned at a thickness of 3 μm and stained with hematoxylin-eosin (HE). The stained sections were examined under an optical microscope (Nikon, Shanghai, China) at a magnification level of 400×, and photomicrographs were captured.

2.9 Western blot analysis

Antibodies targeting insulin receptor substrate 1 (IRS-1), phosphatidylinositide 3-kinases (PI3K), protein kinase B (AKT), phospho-protein kinase B (p-AKT), glycogen synthase kinase 3 beta (GSK3β), phospho-glycogen synthase kinase 3 beta (p-GSK3β), adenosine 5′-monophosphate-activated protein kinase alpha (AMPKα), phospho-adenosine 5′-monophosphate-activated protein kinase alpha (p-AMPKα), sterol regulatory element binding protein 1c (SREBP-1c), fatty acid synthase (FAS), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), peroxisome proliferator activated receptor delta (PPAR-δ), peroxisome proliferator-activated receptor gamma (PPAR-γ), peroxisome proliferator activated receptor alpha (PPAR-α), and β-actin, as well as the phosphorylation antibodies for AKT, IRS-1, and AMPKα, were obtained from Beyotime Biotechnology, China. Liver proteins were extracted using RIPA lysis buffer (Beyotime Biotechnology, China) and quantified using a BCA Kit (Thermo Fisher Scientific, USA). Equal amounts of protein from each sample were separated by 10%

SDS-PAGE and transferred onto PVDF membranes (Biosharp, China). After blocking with 5% nonfat milk, the membranes were incubated with the appropriate primary antibodies and secondary antibodies conjugated to horseradish peroxidase. Proteins were detected using the EasySee Western Kit (Transgene, Beijing) following washing with PBS containing 0.05% Tween 20, and the resulting blots were analyzed with ImageJ[17].

2.10 Gut microbiota analysis

Following euthanasia intestinal content samples were collected from each group, immediately frozen in liquid nitrogen, and subsequently stored at −80 ℃. Genomic DNA extraction from the samples was performed using the cetyltrimethylammonium Bromide (CTAB) method. Polymerase chain reaction (PCR) was employed to amplify the V3—V4 hypervariable region of the bacterial 16S rRNA gene with the forward primer 5′-CCTAYGGGRBGCASCAG-3′ and the reverse primer 5′-GGACTACNNGGGTATCTAAT-3′. Novogene Bioinformatics Technology Co. (Beijing, China) performed the metagenomic sequencing following established protocols. Based on the amplified 16S rRNA region characteristics, a short-fragment library was constructed based on the characteristics of the amplified 16S rRNA region, and then sequenced using the Illumina NovaSeq platform. Raw sequencing data for the normal, T2DM, and H-FCPS groups were analyzed for quality control and microbiome composition using the Novo Cloud Platform (https://magic.novogene.com).

2.11 Statistical analysis

Data were presented as the mean ± standard deviation (SD). Bartlett's test was used to assess the homogeneity of variances. Statistical analyses were performed using a one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test at a 5% significance level to compare to the control. A *P*-value less than 0.05 was considered to be statistically significant.

3. Result

3.1 Analysis of potential targets associated with FCPS against T2DM

A total of 70 FCPS-associated targets were identified using the SwissTarget Prediction database (Fig. 1B). Meanwhile, T2DM-associated targets were retrieved from the GeneCards and DisGeNET

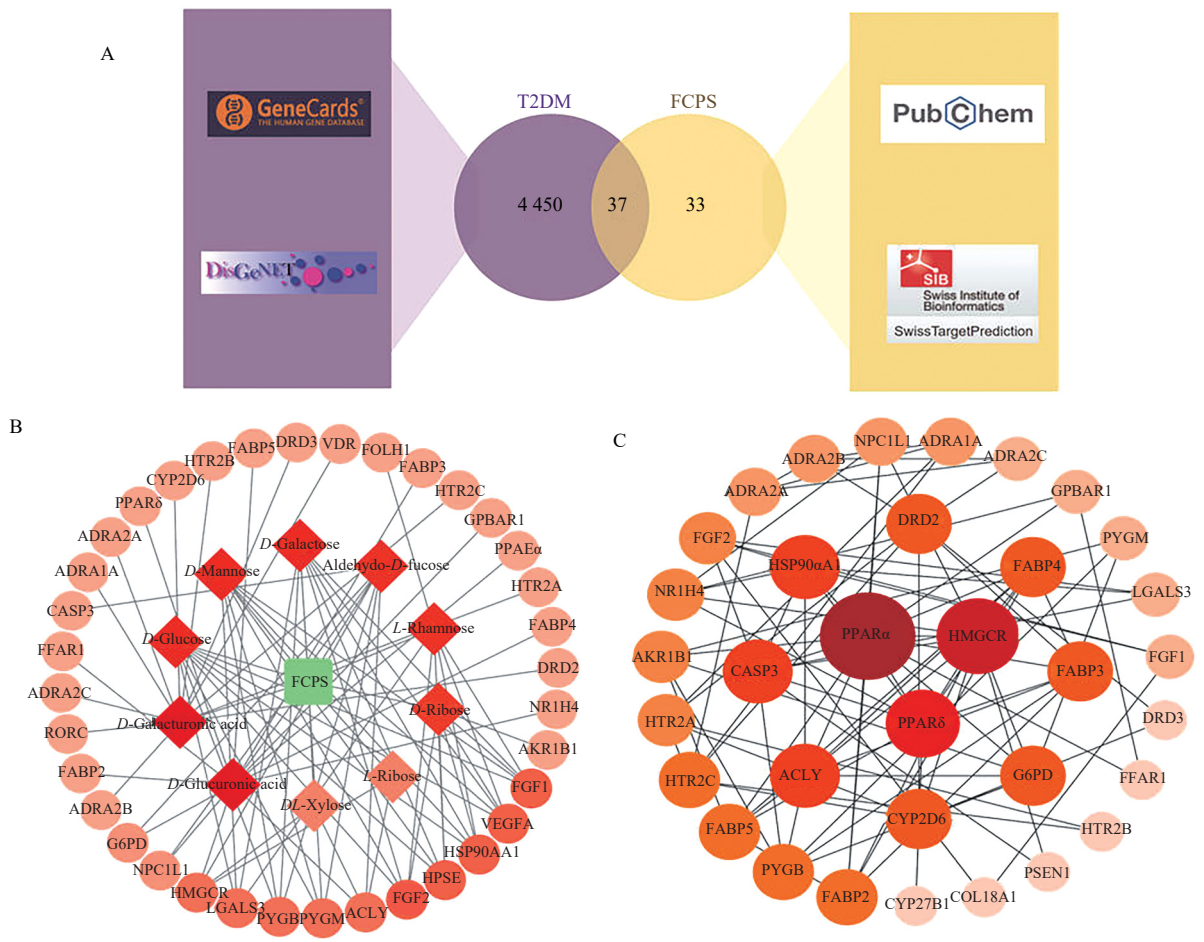

Fig. 1 Network pharmacology and molecular docking to analyze the molecular mechanism of FCPS against T2DM. (A) The screening of FCPS monosaccharides and T2DM-related targets. (B) FCPS-associated targets. (C) The PPI network for FCPS monosaccharides and T2DM targets. (D) KEGG pathway annotation analysis. (E) GO enrichment analysis. (F) The molecular docking of PPARα and *D*-galacturonic acid. (G) The molecular docking of PPARα and *L*-rhamnose. (H) The molecular docking of PPARα and *D*-galactose. (I) The molecular docking of PPARα and *D*-glucose.

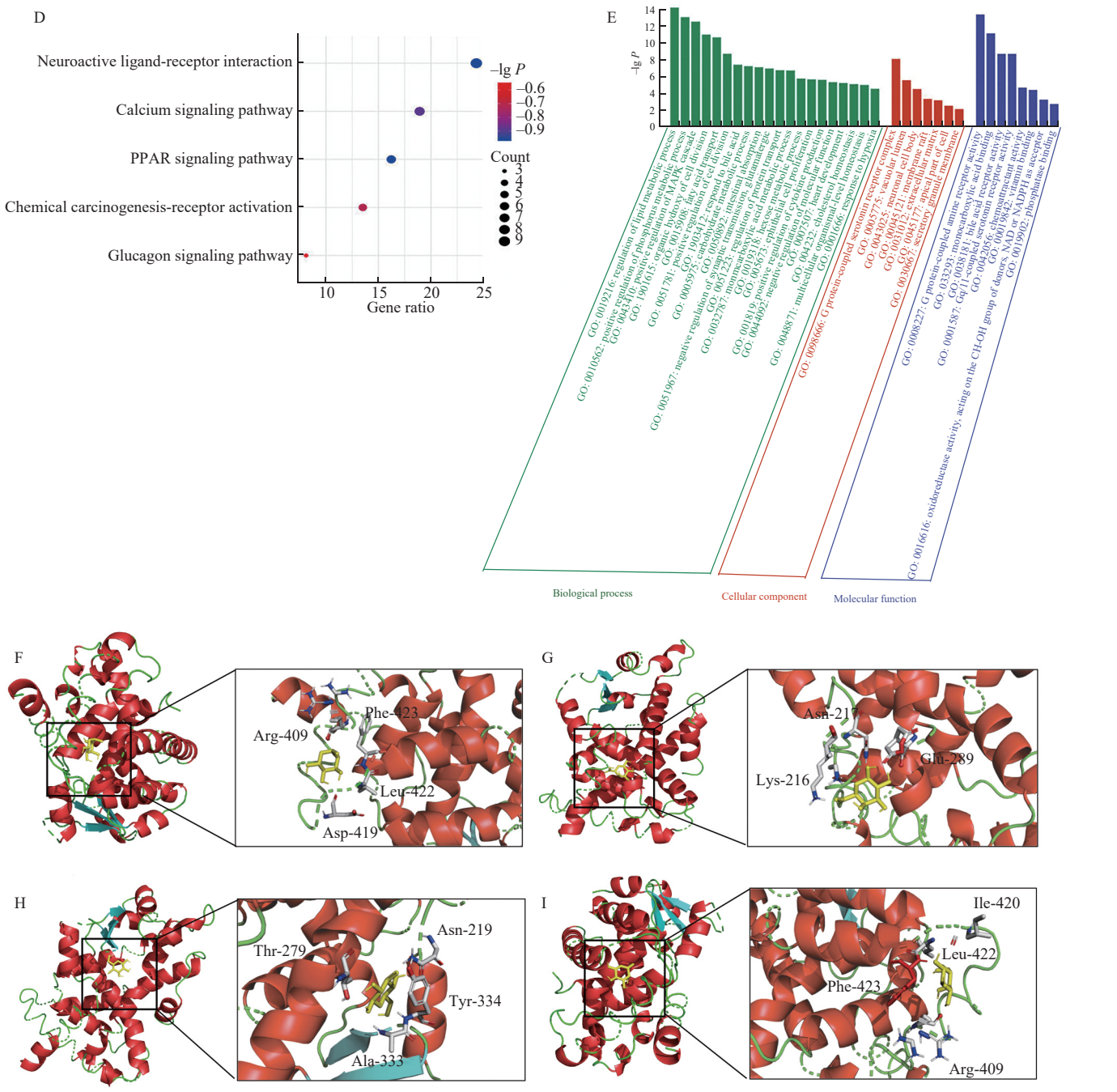

Fig. 1 (*Continued*)

databases, yielding a total of 4 450 targets. Subsequently, a Venn diagram analysis revealed 37 FCPS-associated targets specifically relevant for T2DM treatment (Fig. 1A).

3.2 Construction of target protein interaction network for FCPS treatment of T2DM

PPI analysis was conducted to identify key targets of FCPS for T2DM treatment. Using Cytoscape 3.9.1 software, a network comprising 34 nodes and 90 connections was constructed (Fig. 1C). The analysis revealed a median degree of 5, indicating that the most nodes have a high number of connections within the network. Additionally, the median node centrality was 0.012, suggesting weak interconnectivity, while the median node tightness was 0.50, indicating strong connectivity. Based on these findings, 6 crucial target genes were identified: PPARα, PPARδ, HMGCR, heat shock protein 90 alpha family class A member 1 (HSP90αA1), Caspase-3 (CASP3), ATP citrate lyase (ACLY) (Table 2). These genes, with their high connectivity and tightness within the network, may play significant roles in the therapeutic effects of FCPS for T2DM.

Table2
Key targets in the protein interaction network of FCPS treatment for T2DM.

Name	Degree	Closeness centrality
PPARα	14	0.515 625
HMGCR	11	0.532 258 065
PPARδ	9	0.471 428 571
CASP3	8	0.458 333 333
HSP90αA1	8	0.412 5
ACLY	8	0.464 788 732

3.3 GO annotation and KEGG analysis of functional targets of FCPS against T2DM

Functional annotation of the 37 potential targets of FCPS for T2DM was performed using the Metascape database. A total of 20 BP, 7 cell component (CC), and 8 molecular function (MF) terms were obtained for the target genes. After conducting KEGG pathway enrichment analysis, revealed 5 significant pathways. The top-ranked clusters were selected for further analysis and visualized using the online mapping tool of microbioinformatics (Figs. 1D and E). These pathways primarily involved PPAR signaling pathways associated with lipid metabolism, blood glucose uptake, and inflammation, as well as signaling pathways related to calcium signaling pathway and glucagon signaling.

3.4 Result of molecular docking

Based on PPI analysis, 3 key targets, PPARα, PPARδ, and HMGCR, were identified as significant due to their high degree values. These targets were subsequently subjected to molecular docking with 10 monosaccharide components of FCPS. Among these targets, 2 exhibited favorable docking results, which were further visualized using Pymol. Notably, PPARα demonstrated superior binding affinity towards the compounds, with galacturonic acid displaying the strongest binding interaction (Figs. 1F-I, Table 3).

Table3
Molecular docking between FCPS monosaccharides and T2DM key targets.

Gene	PDBID	Compound	Affinity (kcal/mol)
PPARα	6KAX	*D*-Galacturonic acid	−5.74
PPARα	6KAX	*L*-Rhamnose	−5.14
PPARα	6KAX	*D*-Galactose	−4.98
PPARα	6KAX	*D*-Glucose	−4.90
PPARα	6KAX	Aldehydo-*D*-glucuronic acid	−4.74
PPARα	6KAX	*D*-Ribose	−4.60
PPARα	6KAX	*D*-Mannose	−4.55

3.5 FCPS improved the physiological state and insulin resistance in T2DM mice

As depicted in Figs. 2A and B, a T2DM mouse model was successfully established by combining a high-glucose high-fat diet with STZ treatment. T2DM mice exhibited significant elevations in random blood glucose, FBG (Fig. 2B), and glycated serum protein (GSP) (Fig. 2C), indicating the development of severe hyperglycemia. Furthermore, the mice demonstrated a marked

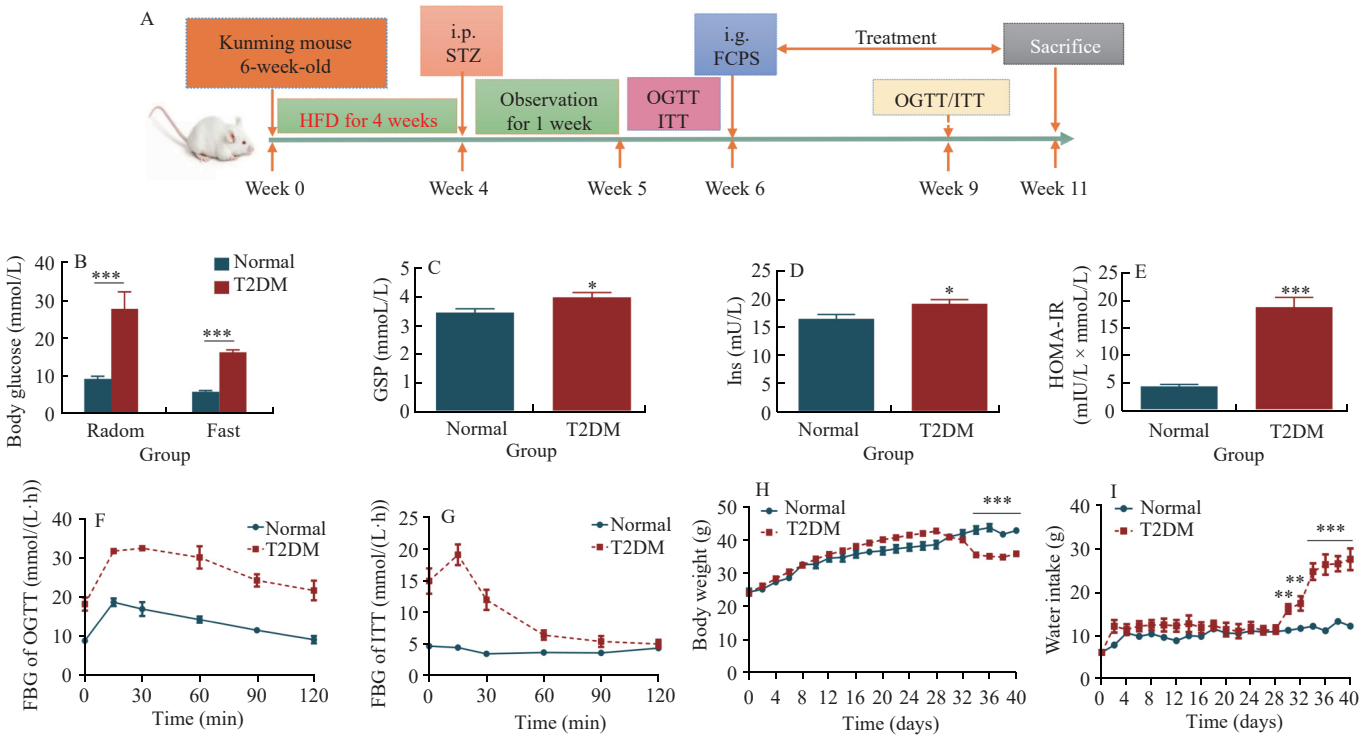

Fig. 2 Establishment of a T2DM model induced by high-fat diet and STZ. (A) Therapeutic regimen of T2DM mouse model. i.p., Intraperitoneal injection, i.g., intragastrical administration. (B) The levels of body glucose after injecting STZ for 3 days. (C-E) The levels of GSP, insulin (ins), HOMA-IR. (F-I) OGTT, ITT, body weight, water intake. The values were shown as mean ± SD. Differences were expressed by ANOVA and denote as follows: $^{*}P < 0.05$, $^{***}P < 0.001$ *vs.* Normal group.

increase in FINS (Fig. 2D), reflecting insulin resistance (Fig. 2E), accompanied by glucose and insulin intolerance (Figs. 2F and G). Concurrently, the mice exhibited typical diabetes symptoms, including polydipsia, polyuria, and lethargy, while also experiencing higher levels of water intake and urinary excretion, as well as weight loss, with a decrease in body weight from (44.75 ± 4.78) g in the normal group to (40.07 ± 6.92) g in the T2DM group (Figs. 2H-I).

Following FCPS treatment, food intake, and water intake were lower in FCPS-treated compared to T2DM mice (Figs. 3B and C), although body weight did not show any notable difference (Fig. 3A), indicating that FCPS effectively improved the physiological status of diabetic mice. Insulin resistance, the hallmark of T2DM, is characterized by decreased glucose tolerance and insulin sensitivity[18]. We observed significantly increased OGTT, ITT levels, and AUC indices in T2DM mice compared to the normal group. While L-FCPS and H-FCPS treatment decreased OGTT levels and AUC, these differences were not statistically significant (Figs. 3D and E). However, FBG, ITT levels, and their AUC were notably lower in FCPS-treated mice compared to the T2DM group ($P < 0.05$), demonstrating a dose-dependent response (Figs. 3F-H). These results suggest that FCPS improved glycemic stability and insulin sensitivity in T2DM mice.

3.6 FCPS ameliorated systemic dysfunction of the lipid metabolism in T2DM mice

T2DM is often accompanied by lipid metabolism disorders, resulting in elevated levels of lipids (e.g., cholesterol and triglycerides) in the blood[19]. As shown in Figs. 4A-G, compared with the normal group, the T2DM group exhibited a significant increase in serum TG, LDL-C, and liver TG, TC, LDL-C and NEFA levels. In comparison to T2DM mice, L-FCPS treatment led to a reduction in serum TG ($P < 0.01$) and liver TG levels, but the changes in serum LDL-C, HDL-C and liver TC, LDL-C, NEFA levels, were not statistically significant.

Conversely, the results in the H-FCPS group exhibited significant improvements. Serum TG ($P < 0.05$) and LDL-C ($P < 0.01$) levels were notably lower, while serum HDL-C levels were significantly elevated ($P < 0.05$) (Figs. 4A-C). More importantly, the H-FCPS group demonstrated a dose-dependent effect (Figs. 4D-G), effectively reducing TG, TC, LDL-C, and NEFA levels in the liver. These findings suggest that FCPS treatment ultimately ameliorated systemic lipid metabolism disorders by mitigating diabetes-induced hepatic lipid metabolism accumulation, particularly at high doses. This may reduce cardiovascular risk and improve overall health in individuals with T2DM.

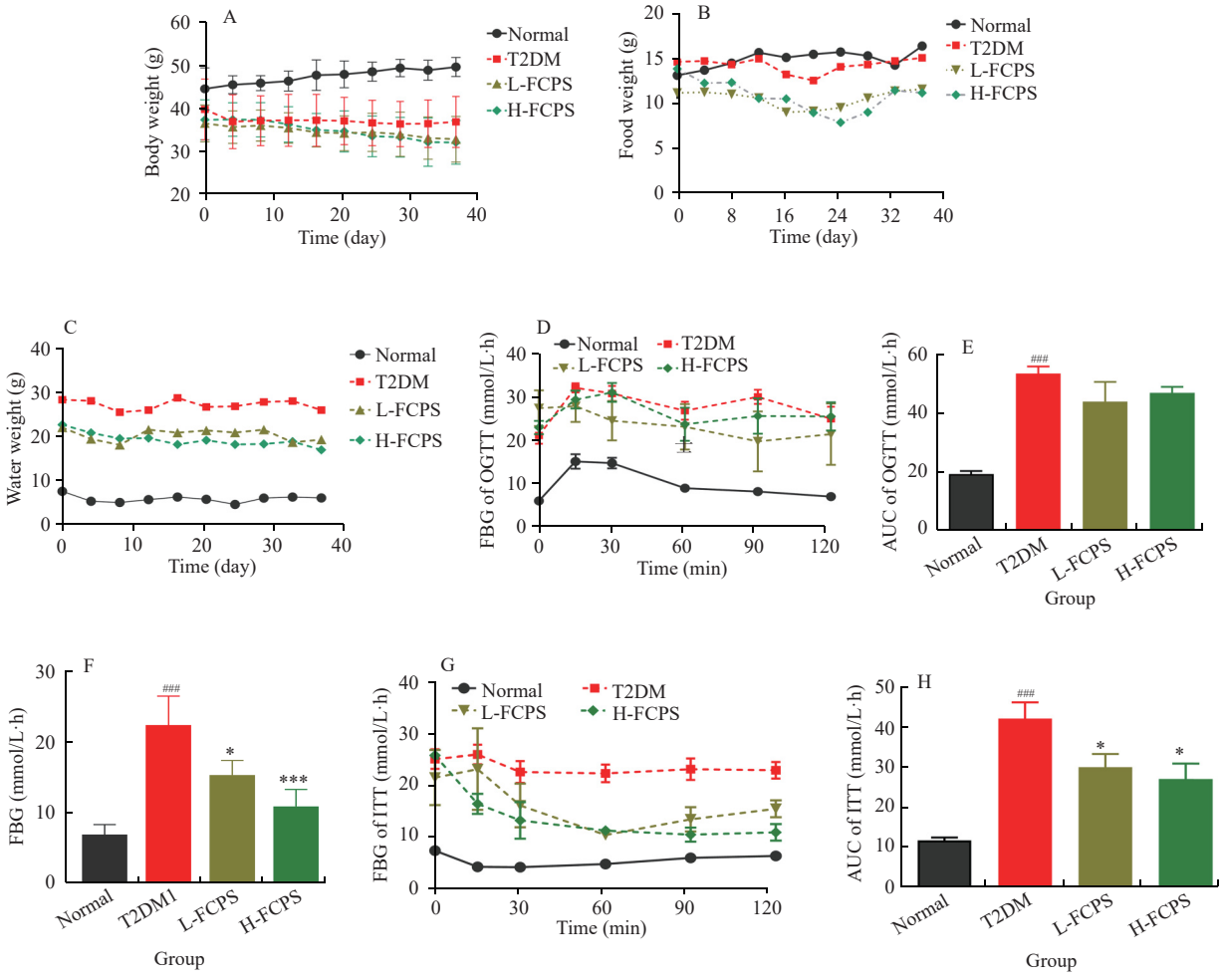

Fig. 3 Effect of FCPS treatment on physiological status, OGTT and ITT in T2DM mice. (A) Body weight of T2DM mice after FCPS treatment. (B) Food intake of mice after FCPS treatment. (C) Water intake of mice after FCPS treatment. (D) OGTT was measured after FCPS treatment. (E) AUC of OGTT. (F) FBG after FCPS treatment for 5 weeks. (G) ITT was measured after FCPS treatment. (H) AUC of ITT. The values were shown as mean ± SD. Differences were expressed by ANOVA and denote as follows: $^{###}P < 0.001$, T2DM group *vs*. Normal group. $^{*}P < 0.05$, $^{***}P < 0.001$, FCPS group *vs*. T2DM group.

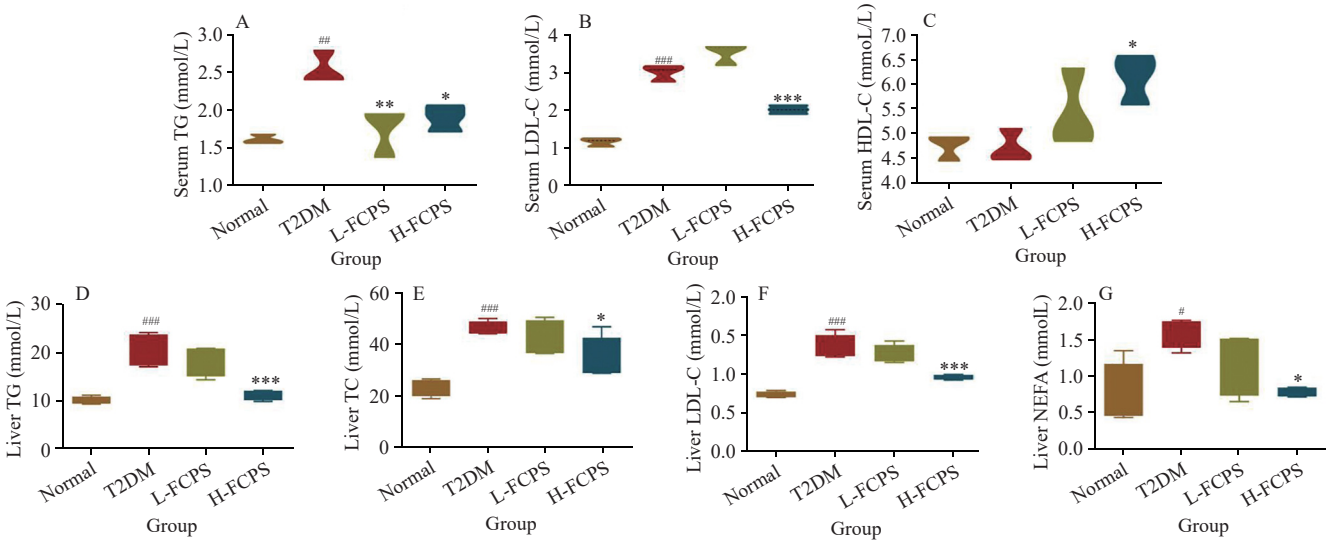

Fig. 4 Effect of FCPS treatment on the lipid metabolism in T2DM mice. (A-C) TG, LDL-C and HDL-C levels in serum. (D-G) TG, TC, LDL-C and HDL-C levels in liver. The values were shown as mean ± SD. Differences were expressed by ANOVA and denote as follows: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, T2DM group vs. Normal group. $^{*}P < 0.05$, $^{***}P < 0.001$, FCPS group vs. T2DM group.

3.7 FCPS suppressed inflammatory response in T2DM mice

T2DM is often characterized as a chronic, low-grade inflammatory condition associated with excessive production of inflammatory cytokines, which disrupt normal insulin signaling, leading to insulin resistance, reduced glucose uptake and utilization by body cells, and abnormalities in lipid metabolism[20]. Our study observed that FCPS treatment significantly suppressed the levels of various inflammatory factors in the serum and liver of T2DM mice. Specifically, FCPS resulted in notable reductions in IL-6, IL-1β, and MCP-1 levels in both serum (Figs. 5B-D) and liver (Figs. 5F-H) of T2DM mice. Interestingly, H-FCPS treatment also led to a significant decrease in TNF-α level in the liver (Fig. 5E), although a similar effect was not observed in serum (Fig. 5A). These findings suggest that FCPS may be beneficial in suppressing diabetes-induced inflammatory responses, particularly in modulating inflammatory factor levels within the liver.

3.8 FCPS repaired the liver damage in T2DM mice

The liver plays a pivotal role in regulating glucose and lipid metabolism. Hepatic metabolic dysfunction is often associated with disorders in glucose and lipids metabolism[21]. To evaluate the restorative effects of FCPS on liver damage, we analyzed the concentrations of GPT and GOT in both serum and liver tissue. Our findings revealed a significant increase in GPT and GOT levels in the serum and liver of T2DM mice compared to the normal group, indicating that the liver damage in diabetic mice was induced by hyperglycaemia and hyperlipidaemia. Both L-FCPS and H-FCPS significantly reduced GPT and GOT levels in serum and liver, respectively, in a dose-dependent manner (Figs. 6A-D). Moreover, the T2DM group exhibited enlarged livers compared to the normal group, while the L-FCPS and H-FCPS groups had slightly lower liver weights. HE staining further confirmed that diabetes resulted in increased hepatocyte size, severe steatosis, and

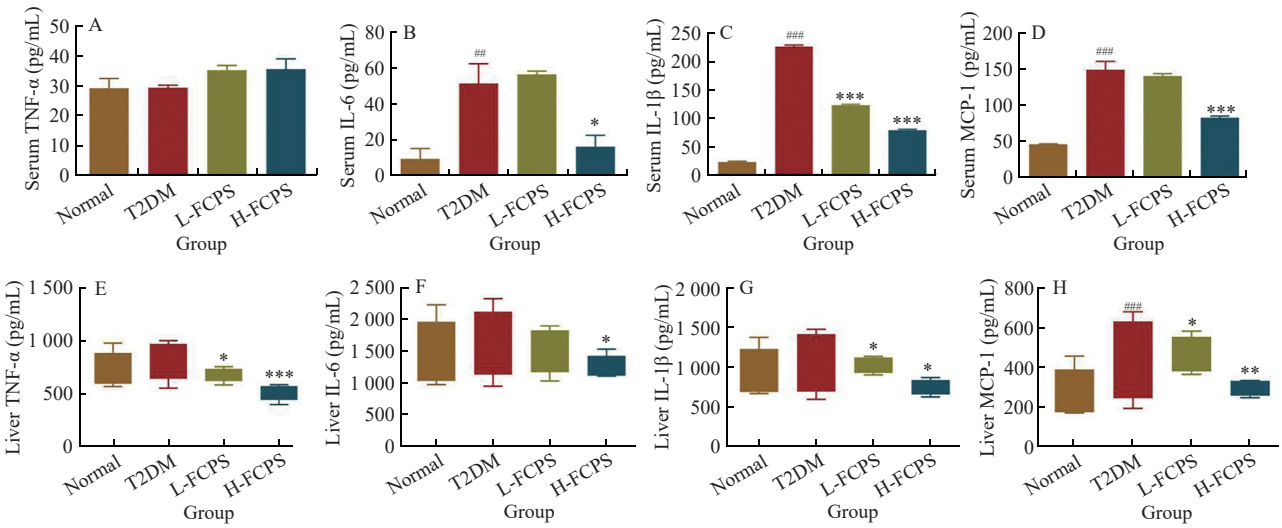

Fig. 5 Effect of FCPS on the inflammatory in T2DM mice. (A-D) TNF-α, IL-6, IL-1β and MCP-1 levels in serum. (E-H) TNF-α, IL-6, IL-1β and MCP-1 levels in liver. The values were shown as mean ± SD. Differences were expressed by ANOVA and denote as follows: $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, T2DM group *vs*. Normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, FCPS group *vs*. T2DM group.

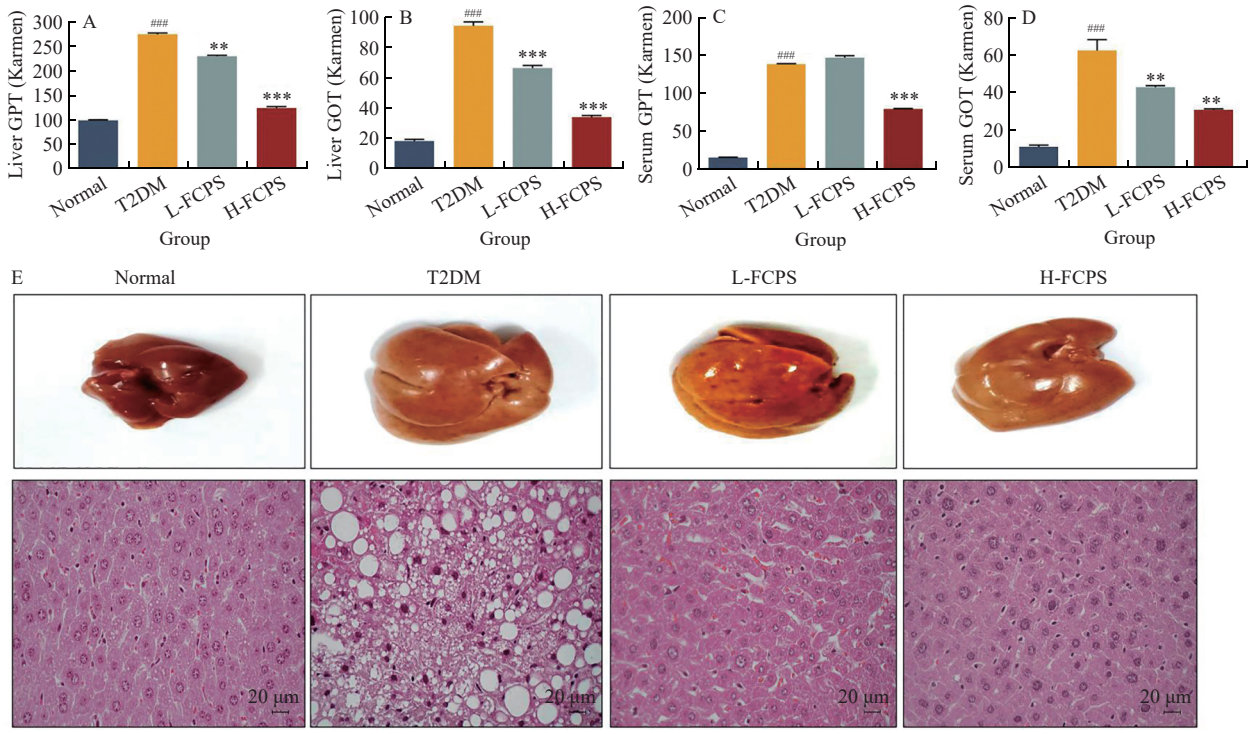

Fig. 6 Effects of FCPS treatment on liver injury and histomorphology in T2DM mice. (A-B) GPT and GOT levels in liver. (C-D) GPT and GOT levels in serum. (E) HE staining of liver (original magnification, 400×). The values were shown as mean ± SD. Differences were expressed by ANOVA and denote as follows: $^{###}P < 0.001$, T2DM group *vs*. Normal group. $^{**}P < 0.01$, $^{***}P < 0.001$ FCPS group *vs*. T2DM group.

disorganized hepatocyte morphology and structure. Conversely, FCPS significantly reduced lipid droplet infiltration and hepatic steatosis (Fig. 6E), suggesting its effectiveness in repairing the structural damage of hepatocytes induced by T2DM. This provides strong evidence for the potential of FCPS to ameliorate liver damage.

3.9 FCPS regulated T2DM related glycolipid metabolic pathways and key targets

To further elucidate the potential mechanisms underlying FCPS's anti-diabetic effects, we conducted Western blot analysis to confirm the predicted glycolipid metabolic pathways and key targets. Compared to the T2DM group, FCPS treatment significantly upregulated the protein expression levels of IRS-1, PI3K, p-AKT, GLUT2, p-GSK-3β, p-AMPKα, PGC-1α, PPARγ, and PPARα, suggesting enhanced glucose utilization, suppressed glycogenolysis, and improved mitochondrial function and lipid metabolism. However, no significant difference was observed in PPARδ expression (Fig. S1). Conversely, the expression levels of GSK-3β, SREBP-1c, FAS and HMGCR exhibited a downward trend, indicating reduced hepatic glucose output, and lipid and cholesterol synthesis. These findings suggest that FCPS may potentially ameliorate metabolic disorders associated with diabetes by enhancing insulin signaling pathways and improving related energy metabolism pathways.

3.10 FCPS altered the gut microbiota diversity and composition of T2DM mice

Emerging evidence suggests that the gut microbiota plays a role in the pathogenesis of T2DM and its associated metabolic complications[22]. Individuals with T2DM often exhibit gut microbiotas characterized by diminished community stability and functional dysregulation. To investigate the impact of FCPS on the gut microbiota, cecal contents from mice in the normal, T2DM, and H-FCPS groups were analyzed using the MetaStat method to assess species abundance and the prevalence of 10 primary taxa.

Bacteroidetes and Firmicutes were the most dominant phyla, accounting for over 60% of the total abundance. Compared to the control group, Proteobacteria increased and Actinobacteria decreased in the T2DM group compared to the normal group (Fig. 8A). In the H-FCPS group, Proteobacteria exhibited a significant decrease in abundance compared to the T2DM group (Fig. 8G), whereas Actinobacteriota displayed a non-significant. At the class level, the H-FCPS group demonstrated higher relative abundances of Bacilli and Saccharimonadia than the T2DM group (Fig. 8B), particularly a notable decrease in Gammaproteobacteria (Fig. 8H). The H-FCPS group also exhibited a shift in intestinal flora composition at the order level, characterized by elevated Lactobacillales and Saccharimonadales and reduced Peptostreptococcales-Tissierellales and Enterobacterales (Fig. 8C). Notably, Enterobacterales abundances decreased significantly (Fig. 8I). At the family level, the H-FCPS group exhibited increased relative abundances of Enterococcaceae, Muribaculaceae, Lactobacillaceae, and Saccharinomandaceae compared to the T2DM group, while Rikenellaceae, Peptostreptococcaceae, and Enterobacteriaceae decreased (Fig. 8D). Notably, Enterobacteriaceae and Muribaculaceae experienced significant alterations (Fig. 8J). Genus-level analysis indicated that H-FCPS treatment modulated the gut microbiota composition of T2DM mice, with increased levels of *Lactobacillus*, Lachnospiraceae NK4A136 group, and *Candidatus_Saccharimonas*. Conversely, *Bacteroides*, *Escherichia-Shigella*, *Alistipes*, and *Romboutsia*

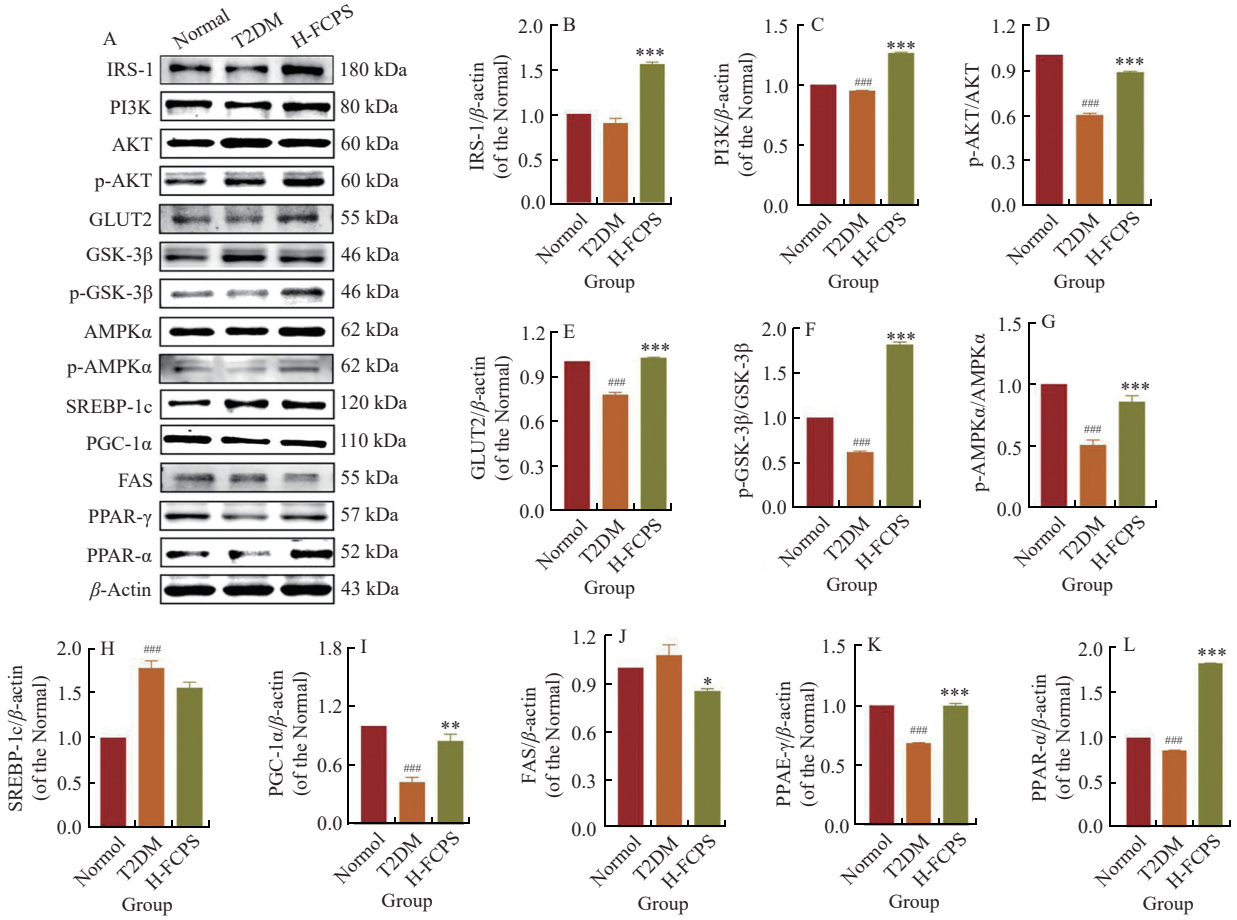

Fig. 7 Effects of FCPS treatment on glycolipid metabolic pathways and key targets in T2DM mice. (A) Western blot analysis. (B) IRS-1 level. (C) PI3K level. (D) p-AKT and AKT levels. (E) GLUT2 level. (F) p-GKS-3β and GKS-3β levels.(G) p-AMPKα and AMPKα levels. (H) SREBP-1c level. (I) PGC-1α level. (J) FAS level. (K) PPARγ level. (L) PPARα level. The values were shown as mean ± SD. Differences were expressed by ANOVA and denote as follows: $^{###}P < 0.001$, T2DM group *vs*. Normal group. $^{**}P < 0.01$, $^{***}P < 0.001$ FCPS group *vs*. T2DM group.

significantly decreased compared to the T2DM group (Figs. 8E and K). At the species level, the H-FCPS group exhibited a shift in gut microbiota composition with increased *Enterococcus faecium*, *Lactobacillus reuteri* and *Lactobacillus johnsonii*, as well as a decrease of *Romboutsia ilealis*, *Mucispirillum schaedleri*, *Escherichia coli*, and *Proteus mirabilis* (Figs. 8F and L).

3.11 FCPS modulation of gut flora abundance improved hepatic glycolipid metabolism and inflammation in T2DM mice

Spearman correlation analyses were conducted to investigate the associations between altered intestinal bacterial taxa and various parameters related to glucose metabolism (FBG), hepatic lipid metabolism (TC, TG, LDL-C), hepatic inflammation level (MCP-1) and liver damage (GPT, GOT), aiming to elucidate the potential link between FCPS-modulated gut microbiota and T2DM. Results revealed significant positive correlations between specific taxonomic categories of Proteobacteria, Gammaproteobacteria, Peptostreptococcales-Tissierellales, Enterobacterales, Peptostreptococcaceae, Enterobacteriaceae, *Romboutsia*, *Escherichia-Shigella*, *Bacteroides*, *R. ilealis*, *E. coli*, and *P. mirabilis* with the diabetes-related indicators. However, Peptostreptococcales-Tissierellales, Peptostreptococcaceae, *Romboutsia*, and *R. ilealis* were not significantly associated with MCP-1. Meanwhile, a significant negative correlation was observed between Actinobacteriota, *L. reuteri* and the aforementioned factors. Additionally, *Bacilli* exhibited a negative correlation with GOT, while Lactobacillales displayed negative correlations with FBG, GOT and GPT. Furthermore, Muribaculaceae showed a negative correlation with TC and LDL-C. Conversely, Lactobacillaceae, *Lactobacillus* exhibited negative correlations with the indicators, except for FBG. Finally, Saccharimonadia, Saccharimonadales, Saccharimonadaceae, and *Candidatus_Saccharimonas* were significantly negatively associated with MCP-1 and TC (Fig. 9). These results suggest that FCPS can modulate hepatic glycolipid metabolism and inflammation by restoring dysregulated gut microbiota abundance in mice with T2DM mice.

3.12 FCPS regulated metabolic functions of intestinal flora in T2DM mice

Cluster analysis of the top 35 functions in the database revealed that FCPS regulated gut microbiota in T2DM mice, impacting metabolism and various BP, including enzyme families, glycan biosynthesis, cellular processes, and metabolism of amino acids, lipids, and carbohydrates. Conversely, gut microbiota was associated with genetic information processing, translation, environmental adaptation, nervous system function, immune system function,

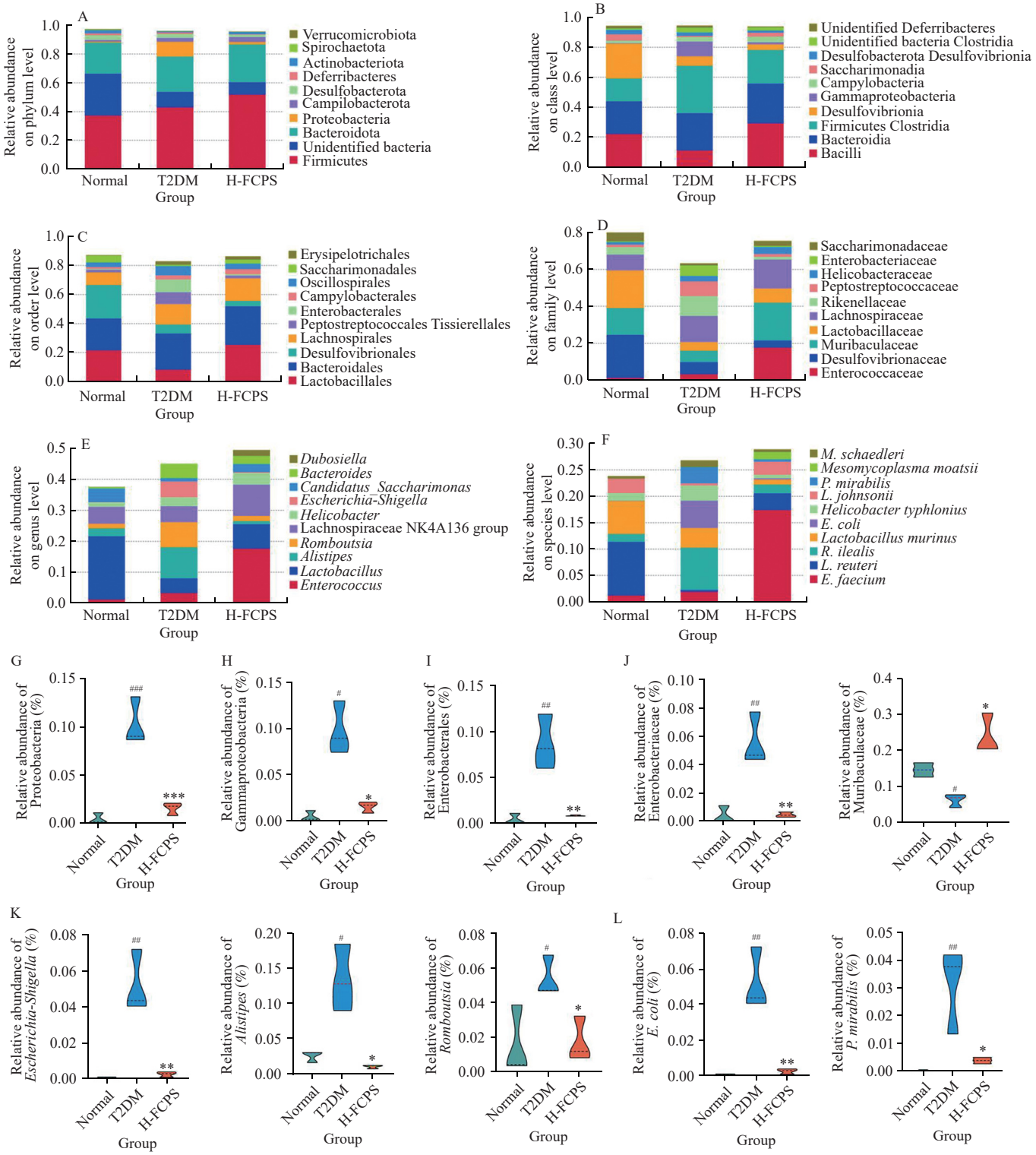

Fig. 8 Effect of FCPS treatment on the abundance of intestinal flora species in T2DM mice. Bacterial taxonomic profiling at the (A) phylum, (B) class, (C) order, (D) family, (E) genus, (F) species level of gut bacteria. The relative abundance of (G) Proteobacteria, (H) Gammaproteobacteria, (I) Enterobacterales, (J)Enterococcaceae and Muribaculaceae, (K) *Escherichia-Shigella*, *Alistipes*, and *Romboutsia*, (L) *E. coli*, and *P. mirabilis* in T2DM mice after FCPS treatment. Differences were expressed by ANOVA and denote as follows: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, T2DM group *vs*. Normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ FCPS group *vs*. T2DM group.

nucleotide metabolism, cofactor and vitamin metabolism, and membrane transport, and other functions (Fig. 10A).

PICRUSt analysis revealed that FCPS significantly influenced the metabolic pathways of the gut microbiota in T2DM mice. Notably, FCPS reduced the activity of several pathways, including the two-component system, bacterial motility proteins, quorum sensing, and porphyrin and chlorophyll metabolism. Additionally, FCPS significantly increased the activity of numerous metabolic pathways,

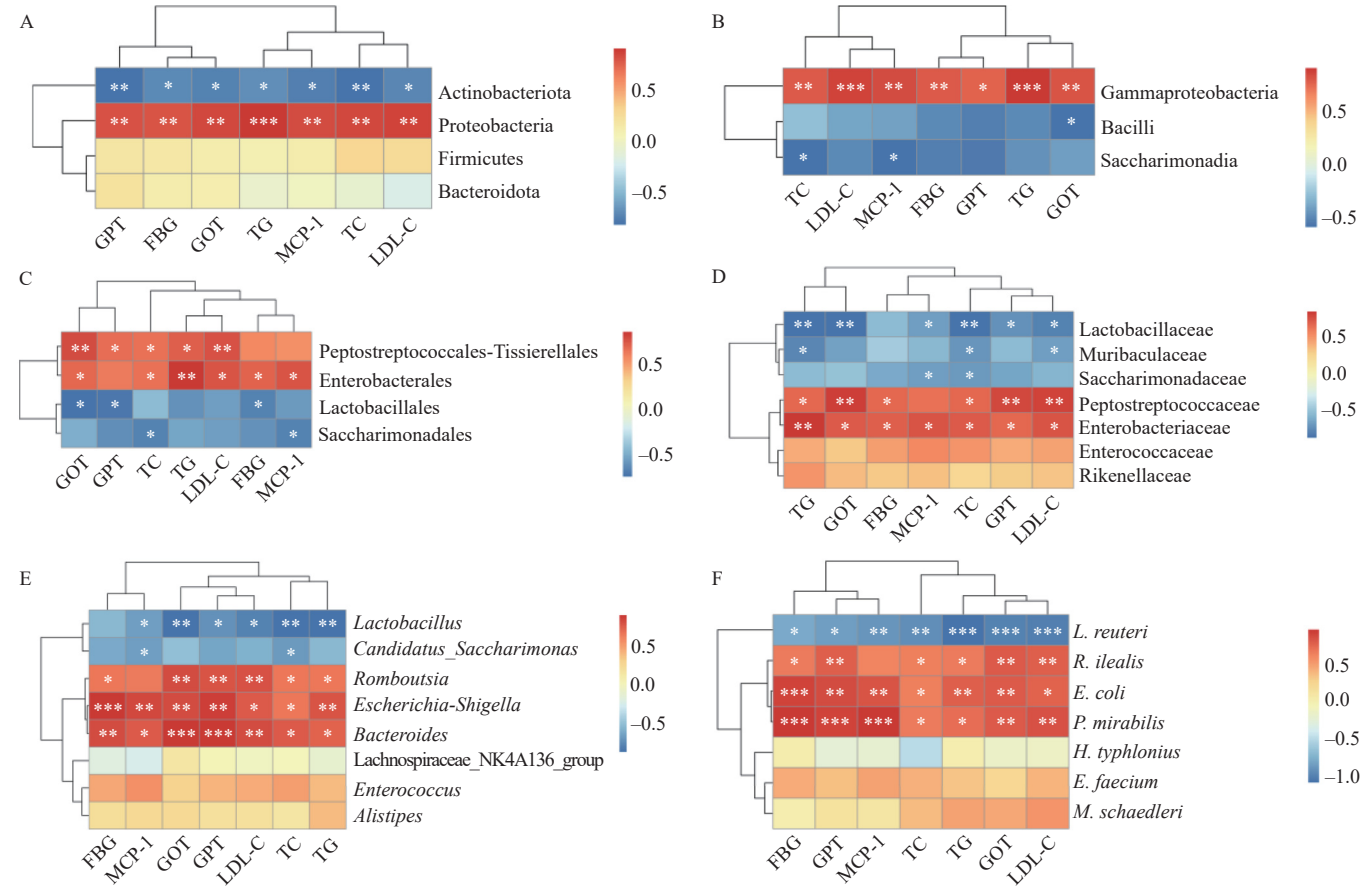

Fig. 9 Correlation analysis of higher abundance of intestinal flora in each level with FBG, TC, TG, LDL-C, MCP-1, GPT, and GOT. Heatmap for correlation analysis of key (A) phylum, (B) class, (C) order, (D) family, (E) genus, (F) species and the T2DM indicators. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

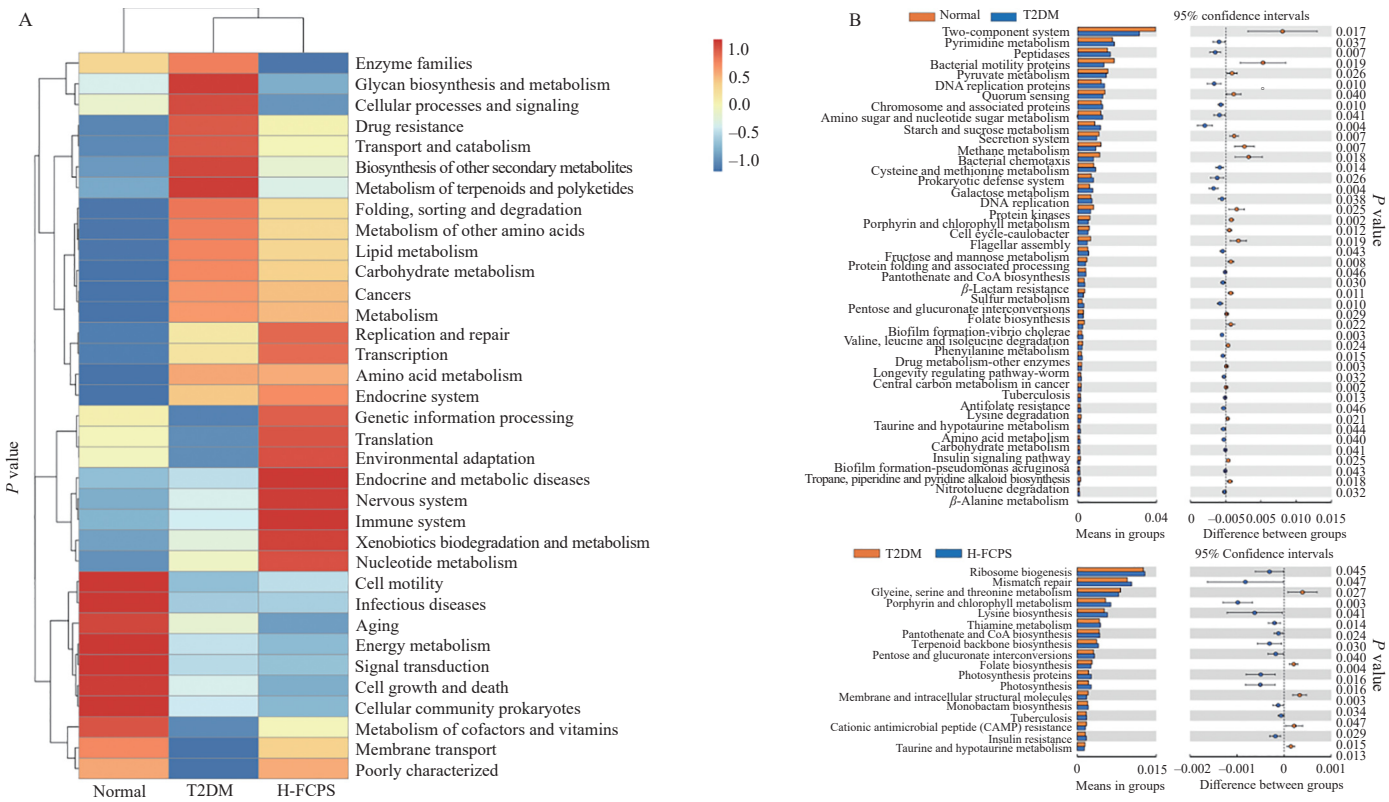

Fig. 10 Related function prediction, clustering heat map, and correlation analysis of tax4fun functional annotation. (A) Tax4Fun functional annotation clustering heatmap. (B) Significant difference analysis. (C) Correlation analysis of KEGG metabolic pathways with FBG, TC, TG, LDL-C, MCP-1, GPT, and GOT.

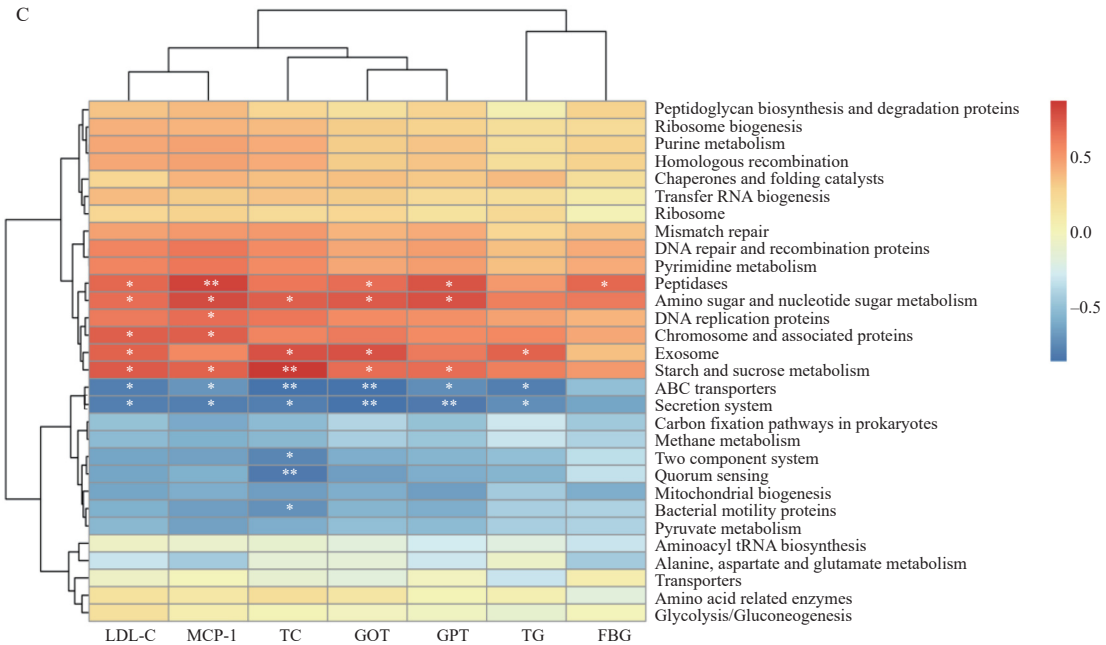

Fig. 10 (*Continued*)

such as ribosome biogenesis, mismatch repair, lysine biosynthesis, thiamine metabolism, pantothenate and CoA biosynthesis, terpenoid backbone biosynthesis, and others (Fig. 10B).

Spearman correlation analysis revealed a positive association between FBG and the peptidase-related metabolic pathways in response to FCPS treatment (Fig. 10C). Hepatic TG levels were positively correlated with the exosome pathway. Other hepatic markers, including GPT, GOT, TC, MCP-1, and LDL-C were also positively associated with various metabolic pathways, such as peptidases, amino sugar and nucleotide sugar metabolism, exosome, DNA replication proteins, and starch and sucrose metabolism. In comparison, diabetes-related markers displayed negative correlations with metabolic pathways like ABC-transports, and secretion system. These results indicate that FCPS can modulate multiple functional pathways within the gut microbiota, encompassing energy metabolism, carbohydrate metabolism, amino acid metabolism, and the synthesis of secondary metabolites, thereby contributing to improved diabetes outcomes.

4. Discussion

Insulin resistance in patients with diabetes can lead to persistent hyperglycemia, which in turn triggers lipid metabolism disorders, such as elevated cholesterol and triglyceride levels[23]. Concurrently, diabetes is often accompanied by chronic low-grade inflammation, increasing the risk of complications. These interrelated factors can exacerbate the severity of diabetes and its associated complications[24-25]. Effective diabetes management requires proper regulation of metabolic functions. This study aimed to evaluate the impact of FCPS treatment on improving hyperglycemia and hyperlipidemia in a T2DM mouse model while also investigating its potential for enhancing and remediating diabetes.

This study successfully established a T2DM mouse model using a high-sugar, high-fat diet combined with STZ injection. Although the model mice exhibited typical T2DM symptoms such as hyperglycemia and insulin resistance, their food intake did not increase as expected, consistent with previous report[26]. This phenomenon might be attributed to several factors. First, the high-sugar, high-fat diet, and STZ injection may have affected the appetite-regulating mechanisms in the mice, leading to impaired appetite regulation without noticeable weight loss or other overt disease signs. High-fat diets can disrupt hypothalamic regulation of hunger and satiety, and STZ-induced diabetes may exacerbate these effects[27-28], resulting in no significant increase in food intake. Besides, alterations in the gut microbiota may also significantly influence food intake. Research suggests a close association between imbalances in the gut microbiota and T2DM[29-30], which may alter food intake by affecting the host's energy metabolism and appetite regulation. Following FCPS treatment, blood glucose levels and insulin resistance in T2DM mice improved significantly, and their food intake decreased. This reduction may be related to FCPS-mediated modulation of gut microbiota composition and its effects on gut hormones such as leptin and ghrelin, which play a crucial role in appetite and satiety regulation[31-32]. Furthermore, FCPS treatment improved glucose tolerance, which may indirectly reduce food intake by normalizing blood glucose levels and reducing hunger. These findings suggest that FCPS not only improves the condition of T2DM mice by lowering blood glucose levels, but also potentially influences their food intake behavior, such as reducing food consumption, further aiding in blood sugar control. The ability of FCPS to affect food intake may be an important mechanism of FCPS in T2DM treatment, warranting further research to understand its underlying mechanisms and potential clinical application value.

Network pharmacology, utilizing publicly accessible databases, identified potential drug targets for FCPS treatment of T2DM[33-35]. Through the PPI interactions, and GO pathways, a total of 37 common targets were identified, with PPARα, PPARδ, and HMGCR recognized as central targets. Additionally, the KEGG pathway analysis revealed enrichment in the PPAR signaling pathway. PPARs, a subset of ligand-activated receptors belonging to the nuclear

hormone receptor family, comprise 3 unique isoforms: PPARα, PPARδ, and PPARγ. These isoforms play crucial roles in modulating various intracellular metabolic pathways[36]. Specifically, PPARα is predominantly expressed in hepatic tissues, where it is primarily activated by fatty acids released during triglyceride catabolism. This activation leads to increased fatty acid absorption and biosynthesis, ultimately aiding in the mitigation of lipid deposition and hepatocyte lipid accumulation in the liver[37-38]. Molecular docking results indicated a strong affinity between PPARα and monosaccharide components in FCPS. Subsequent Western blot analysis confirmed a significant upregulation of hepatic PPARα and PPARγ in T2DM mice treated with FCPS, suggesting the involvement of the PPAR signaling pathway in the mechanisms by which FCPS ameliorates T2DM.

The liver, a key target organ for insulin resistance, plays a crucial role in regulating systemic glucose homeostasis. Upon insulin binding, the insulin receptor (InsR) undergoes phosphorylation, triggering downstream activation of IRS. IRS-1, a critical downstream protein of InsR in the insulin signaling cascade, is activated by phosphorylated InsR and initiates the PI3K/AKT signaling pathway[16,39]. Activated AKT phosphorylates and deactivates GSK-3β, facilitating glycogen synthesis[39-40]. GLUT2 is essential for glucose sensing and homeostasis, and its hepatic deficiency impairs insulin secretion[40-42]. Our findings demonstrated that FCPS significantly influenced various physiological markers in T2DM mice, including reductions in FBG, increases in insulin levels and glucose tolerance, and upregulation of hepatic IRS-1, PI3K, p-AKT, p-GSK3β, and GLUT2 expression. These results suggest that FCPS may modulate the IRS-1/AKT/GSK3β/GLUT2 signaling pathway to ameliorate hepatic insulin resistance and impaired glucose metabolism in T2DM mice.

The PI3K/AKT signaling pathway plays a role in glucose metabolism and suppressing lipid synthesis[43]. Phosphorylation of AKT activates AMPK, and decreased AMPK activity has been linked to insulin resistance in a hyperglycemic environment. Activated AMPK can downregulate SREBP-1c and its associated lipogenic enzymes, inhibiting lipid synthesis. Additionally, by suppressing HMGCR, the rate-limiting enzyme in cholesterol synthesis, AMPK further inhibits the synthesis of TC in the serum[44-47]. Simultaneously, AMPK upregulates PGC-1α, PPARα, and PPARγ to enhance mitochondrial biogenesis, fatty acid oxidation, and reduce inflammation, ultimately improving insulin sensitivity[48-49]. Our results indicated that FCPS led to a significant reduction in the levels of TC, TG, LDL-C, and NEFA in the liver and serum of T2DM mice. Additionally, FCPS increased HDL-C levels, with a more pronounced effect observed at higher doses. Furthermore, FCPS activated AMPK phosphorylation, decreased SREBP-1c and FAS levels, and increased PGC-1α expression. These findings suggest that FCPS may modulate hepatic and systemic lipid metabolism disorders related to T2DM through the AMPKα/SREBP-1c/PGC-1α/PPAR signaling pathway.

Persistent hyperglycemia and hyperlipidemia levels trigger the release of inflammatory mediators, such as MCP-1, IL-1β, and IL-6, exacerbating the inflammatory process in the liver, adipose tissue, and pancreas[50-51]. This disruption in glucose metabolism and cellular functions ultimately leads to insulin resistance and the onset of T2DM[52-53]. PPARγ has the ability to suppress the inflammatory response by competitively inhibiting the inflammatory signaling pathway and reducing the production of inflammatory mediators[54]. MCP-1, IL-1β, and IL-6 are cytokines that play significant roles in the pathogenesis of insulin resistance and β-cell dysfunction. Specifically, MCP-1 acts as an inflammatory chemokine that inhibits InsR tyrosine phosphorylation[55], while IL-1β regulates InsR substrates and contributes to β-cell dysfunction[56]. Additionally, IL-6 is involved in modulating gut microbiota on the insulin signaling pathway by interfering with downstream effectors[57-58]. In the present study, we found that FCPS effectively decreased the concentrations of inflammatory factors in both liver tissue and serum, as well as GPT and GOT, which serve as indicators of liver function associated with inflammation. These findings suggest that FCPS may mitigate systemic inflammation through PPARγ activation, representing a potential therapeutic approach for T2DM.

The relationship between the gut microbiota and the health of biological organisms is significant, as disruptions in the gut microbiota can lead to the development of diseases such as obesity and diabetes, along with their associated complications. The gut microbiota-intestinal-hepatic axis plays a crucial role in the body's processing of lipid absorption and metabolism[59-60]. Correlation analysis was used to investigate changes in the gut microbiota of T2DM mice before and after treatment. The human gut harbors a diverse and complex microbial community, with Actinobacteria, Proteobacteria, Bacteroidetes, and Firmicutes as the predominant constituents of the gut microbiota[61]. Proteobacteria, encompassing pathogenic microorganisms like *E. coli*, *Helicobacter pylori*, and *Salmonella*, have been the subject of extensive scientific investigation. Recent studies have suggested that elevated levels of Proteobacteria, a hallmark in the gut microbiota dysbiosis, may be linked to intestinal inflammation. The present study demonstrated a significant association between increased levels of Proteobacteria, specifically Gammaproteobacteria, *Escherichia-Shigella*, *E. coli*, and *P. mirabilis* in a T2DM mouse model and the development of inflammation, metabolic disturbances, and hepatic dysfunction[62], suggesting a complex role of Proteobacteria in the pathogenesis of T2DM. *Escherichia-Shigella*, a bacterium known to induce intestinal inflammation by invading epithelial cells and triggering macrophage death and IL-1β release, exhibits conditional pathogenicity and heightened infectivity under inflammatory conditions[63]. Following FCPS treatment, a decrease in Proteobacteria, Gammaproteobacteria, *Escherichia-Shigella*, *E. coli*, and *P. mirabilis* was observed, accompanied by a notable reduction in inflammation, improved glycolipid metabolism disorders, and amelioration of liver damage were observed. Taken together, these findings suggest that reducing these gut microbiota levels may potentially mitigate inflammation, enhance glycolipid metabolism, and support liver health.

Enterobacteriaceae have the ability to synthesize lipopolysaccharides, which can induce inflammation by activating toll-like receptor 4[64]. The presence of Enterobacterales and Enterobacteriaceae exhibited a notable positive association with FBG, inflammatory markers, and hepatic lipid accumulation, consistent with previous findings[62]. Peptostreptococcales-Tissierellales, and Peptostreptococcaceae have been identified as potential contributors to the inflammatory cascade[65]. Interestingly, our study found a noteworthy positive correlation between Peptostreptococcales-Tissierellales and Peptostreptococcaceae with liver glycolipid metabolism indexes. Although a positive correlation with inflammation was observed, it did not reach statistical significance.

L. reuteri, a lactic acid bacterium belonging to the genus *Lactobacillus* within the order *Bacilli* of the phylum Firmicutes, is a naturally occurring microorganism found in the intestinal tract of

most vertebrates and mammals. This bacterium has been reported to have beneficial properties, including preventing the growth of various microorganisms, such as Gram-positive bacteria, gram-negative bacteria, yeasts, fungi, and pathogens. Additionally, *L. reuteri*, has been shown to prevent early colonization by *H. pylori*. Research has demonstrated that *Lactobacillales*, *Lactobacillus*, and *L. reuteri* exhibit hepatoprotective effects by regulating hepatic lipid accumulation and modulating the expression of inflammatory mediators, thereby retarding the progression of diabetes[66-68]. Our findings corroborated these observations, providing additional support for this assertion.

Candidatus_Saccharimonas is a group of beneficial bacteria known for their anti-inflammatory properties and secretion of IL-10. Research indicates a positive correlation between the abundance of *Candidatus_Saccharimonas* and the concentrations of liver or serum HDL-C, GLP-1, and IL-10, while a negative correlation has been observed with MCP-1 and TC in obesity and T2DM[68-71]. These findings suggest a potential link between decreased levels of *Candidatus_Saccharimonas* and liver damage and inflammation. Muribaculaceae, a prominent carbohydrate-catabolizing bacterium in the gut microbiota, may be a potential biomarker for reducing cholesterol levels, as evidenced by its significant negative correlation with lipid metabolism-related indices such as TG, TC, and LDL-C[72]. Spearman's correlation analysis revealed significant negative associations between Saccharimonadia, Saccharimonadales, Saccharimonadaceae, and *Candidatus_Saccharimonas* with hepatic TC, as well as a significant negative correlation between MCP-1 and hepatic cholesterol metabolism. Additionally, Muribaculaceae exhibited a negative correlation with hepatic cholesterol metabolism. Following FCPS treatment, there was an increase in the abundance of Muribaculaceae and *Candidatus_Saccharimonas*, consistent with previous research[69,73]. These findings suggest that FCPS may alleviate disruptions in glucose-lipid metabolism and inflammatory reactions in T2DM mice by influencing the gut microbiota, providing evidence for the crucial role of the gut microbiota in the therapeutic benefits of FCPS in T2DM.

The preservation of glycolipid metabolism homeostasis is essential for providing energy and resources to the body[74-76]. Adequate DNA repair is necessary for cellular health and can mitigate glycolipid metabolic abnormalities. Conversely, impaired DNA repair mechanisms can result in the accumulation of DNA damage, leading to disturbances in cellular metabolism and potentially triggering inflammation, apoptosis, and oxidative stress[77]. Dysfunctional DNA repair proteins have been linked to heightened inflammation and insulin resistance, which may contribute to the development of diabetes and associated complications[77-79]. Mutations in p53 and other DNA damage repair proteins have been shown to promote lipid synthesis and elevate serum triglyceride levels, thereby intensifying oxidative stress and DNA damage[80]. Our research found an increase in mismatch repair pathways following FCPS treatment, indicating a potential role of the gut microbiota in mitigating DNA damage and modulating glucose-lipid metabolism disorders by activating these pathways. Ribosome biosynthesis plays a crucial role in determining protein synthesis rates in cells[81]. Additionally, the Raptor/mTORC1 signaling pathway has been demonstrated to directly affect pancreatic β-cell size and activity, as well as lipid synthesis and adipose tissue differentiation through SREBP and PPAR signaling[82]. The inhibition of Raptor leads to a decrease in β cell protein translation initiation and ribosome biosynthesis, impacting the synthesis of mature insulin and the maturation of secretory vesicles[83-84]. Our findings showed an upregulation of the ribosomal biosynthesis pathway in T2DM mice treated with FCPS, indicating a potential role of the gut microbiota in regulating glucose metabolism by modulating this pathway. Furthermore, there was a notable increase in pathways associated with porphyrin and chlorophyll metabolism following FCPS treatment, among other metabolic pathways, which supports previous findings[85].

5. Conclusion

In summary, FCPS demonstrated significant efficacy in treating T2DM in mice. Network pharmacology analysis identified PPARα and its associated signaling pathway as the primary targets of FCPS action. The therapeutic potential of FCPS in managing T2DM was evidenced by its ability to effectively modulate blood glucose and lipid levels, mitigate inflammation and liver damage through targeting the IRS-1/AKT/PPARα signaling pathway. Furthermore, FCPS demonstrated the capacity to rectify gut microbiota dysbiosis in T2DM mice by promoting the proliferation of beneficial microorganisms and diminishing the abundance of opportunistic pathogens. In the future, FCPS is anticipated to serve as a crucial component in the formulation of multi-targeted functional foods or health products, demonstrating promise for its application in the holistic management of T2DM.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250400.

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