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Protective mechanism of Ramulus Mori (Sangzhi) Alkaloids on T2DM combined with MASLD by hepatic lipid metabolism and gut microbiota analyses

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Abstract

Background and objective: Both type 2 diabetes mellitus (T2DM) and metabolic dysfunction-associated steatotic liver disease (MASLD) are known to be influenced by environmental and lifestyle factors. *Ramulus Mori* (Sangzhi) alkaloids (SZ-A) are effective hypoglycemic agents. Recent studies suggest that SZ-A may improve T2DM, MASLD, and metabolic syndrome, but the underlying mechanisms remain unclear. This study aimed to investigate whether SZ-A can modulate hepatic lipid metabolism and gut microbiota in a mouse model of T2DM combined with MASLD. **Methods:** A combined T2DM-MASLD mouse model was established using a high-fat diet and streptozotocin injection. Liver morphology and histology were assessed using a portable small-animal ultrasound imaging system, hematoxylin and eosin (H&E) staining, and Oil Red O staining. Serum levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using standard assay kits. Gut microbiota composition was analyzed by 16S rRNA sequencing, and hepatic lipid metabolites were profiled using liquid chromatography-mass spectrometry (LC-MS)MS. **Results:** SZ-A improved liver function by ameliorating morphological and structural abnormalities, reducing lipid droplet accumulation, and lowering serum levels of TG, TC, LDL, ALT, and AST. It also led to decreased hepatic ultrasound echo intensity compared to the kidney. Additionally, SZ-A helped restore gut microbiota balance, including a partial reversal of the Firmicutes/Bacteroidetes ratio. Lipidomic analysis revealed that SZ-A downregulated most TG and diglycerides (DG), while upregulating phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the model group. **Conclusions:** SZ-A partially alleviates liver injury in T2DM-MASLD mice by modulating hepatic lipid metabolism and gut microbiota composition.

Keywords

Ramulus Mori (Sangzhi) Alkaloids; gut microbiota; lipidomics analysis

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1 Introduction

Type 2 diabetes mellitus (T2DM) and metabolic dysfunction-associated steatotic liver disease (MASLD) are among the most common metabolic disorders worldwide, with increasing prevalence and incidence rates[1]. Growing evidence suggests a bidirectional relationship between T2DM and MASLD: MASLD contributes to liver injury in T2DM, while T2DM accelerates the progression of MASLD to hepatic steatosis. Epidemiological studies report that approximately 55%-70% of T2DM patients also suffer from MASLD, and vice versa[2-4]. Therefore, identifying effective therapeutic agents for the prevention and treatment of T2DM combined with MASLD is urgently needed.

Cold-region cities refer to urban areas characterized by heavy snowfall and prolonged low temperatures, creating challenging living environments. In China, these regions are primarily located in the northeast, northwest, north, and parts of the west. The prevalence of T2DM in these areas has reached 11.53%, higher than both the non-cold regions (10.16%) and the national average (10.37%). This discrepancy is likely influenced by climate, dietary patterns, lifestyle habits, and genetic predispositions. Similarly,

the obesity and overweight rate exceeds 35.91% in cold regions, compared to 31.72% in non-cold areas and 32.37% nationally. Furthermore, insulin resistance is more pronounced in cold regions, leading to increased comorbidity of T2DM and MASLD.

T2DM and MASLD share several common pathophysiological features, notably dysfunction in hepatic lipid metabolism and alterations in gut microbiota[5]. It is well established that hepatic lipid dysregulation contributes to lipotoxicity and inflammation, which are central to the development of hepatic steatosis in both T2DM and MASLD[6-7]. Lipidomics, a subfield of metabolomics, has emerged as a powerful tool for identifying lipid species and elucidating metabolic functions in disease processes, including T2DM and MASLD[8].

The gut microbiota, another key player, is increasingly recognized as a crucial environmental factor in metabolic disease regulation[9]. Alterations in the gut microbiota have been implicated in MASLD progression through their influence on bacterial metabolites, short-chain fatty acids, and bile acid synthesis[10]. Therefore, targeting hepatic lipid metabolism and gut microbiota using lipidomics and 16S rRNA sequencing may help elucidate the therapeutic potential of *Ramulus Mori* (Sangzhi) Alkaloids (SZ-A).

SZ-A, derived from the mulberry branch—a traditional Chinese medicine—has been approved for the treatment of T2DM due to its hypoglycemic efficacy[11]. It mainly contains 1-deoxynojirimycin, fagomine, and 1,4-dideoxy-1,4-imino-D-arabinitol. Previous studies have shown that SZ-A possesses multiple pharmacological activities, including anti-inflammatory effects and improvements in β-cell function and adipose tissue metabolism[12]. In addition, SZ-A has been reported to modulate the gut microbiota during the development of T2DM or MASLD, suggesting it may be a promising therapeutic candidate for these conditions. However, it remains unclear whether SZ-A can ameliorate T2DM combined with MASLD by targeting hepatic lipid metabolism and gut microbiota.

In this study, we aimed to investigate the effects of SZ-A on hepatic lipid metabolism and gut microbiota in a T2DM-MASLD mouse model using lipidomics and 16S rRNA sequencing. Our findings may provide new insights into the therapeutic potential of SZ-A for treating T2DM combined with MASLD.

2 Method and Materials

2.1 Mice model establishment and drug treatment

Male C57BL/6 mice ([20 ± 2] g) were housed under specific pathogen-free (SPF) conditions with free access to food and water. After one week of acclimatization, the mice were randomly assigned to three groups: control group, model group (T2DM combined with MASLD), and SZ-A treatment group. To establish the T2DM-MASLD model, mice were fed a high-fat diet for 4 weeks, followed by daily intraperitoneal injections of streptozotocin (50 mg/kg) for 5 consecutive days. Upon successful model establishment, SZ-A (100 mg/kg/day) was administered orally for 8 weeks. Control group mice were fed a standard diet and received saline by oral gavage. The study was approved by the Institutional Review Board (or Ethics Committee) of the Second Affiliated Hospital of Harbin Medical University (KY2021-298) and followed Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines.

2.2 Morphological examination

After an 8-hour fast, mice were anesthetized, and the abdominal region was exposed. A portable small animal ultrasound imaging system (VINNO 6 LAB, VINNO Technology Co., Ltd, China) was used to capture images of the liver and kidney by placing the ultrasound probe on the abdomen.

2.3 Serum biochemistry analysis

Serum levels of triglycerides (TG, Cat#A110-1-1), total cholesterol (TC, Cat#A111-1-1), low-density lipoprotein (LDL, Cat#A113-1-1), alanine aminotransferase (ALT, Cat#C009), and aspartate aminotransferase (AST, Cat#C010) were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's protocols.

2.4 Histological analysis

Liver tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm thickness. Hematoxylin and eosin (H&E) staining (Cat#GP1031, Servicebio, China) and Oil Red O staining (Cat#G1260, Servicebio, China) were performed according to the manufacturer's instructions. Stained sections were visualized using a Nikon NI-U microscope.

2.5 16S rRNA sequencing analysis

Genomic DNA was extracted from fecal samples collected from each group. Polymerase chain reaction (PCR) amplification was conducted using specific primers. Following quantification and quality control, libraries were prepared using the Universal DNA Purification Kit and sequenced on the Illumina NovaSeq 6000 platform (Novogene, China). Bioinformatics analysis was conducted using QIIME2 software.

2.6 Liquid chromatography-mass spectrometry (LC-MS)/MS analysis

Lipids were extracted from liver tissues using a sequential solvent mixture of methanol, MTBE, and isopropanol.

UHPLC-MS/MS analysis was performed using the Vanquish UHPLC system (Novogene, China). Raw data were processed with LipidSearch software for peak alignment, peak picking, and metabolite quantification. Data processing followed protocols reported in previous studies[13-14].

2.7 Data analysis

Statistical analyses were performed using one-way ANOVA followed by Tukey's multiple comparison test. A *P*-value of less than 0.05 was considered statistically significant.

3 Results

3.1 SZ-A ameliorates liver dysfunction in T2DM combined with MASLD mice

We first assessed the effects of SZ-A on liver function in a mouse model of T2DM combined with MASLD. As shown in Fig. 1A, ultrasound imaging revealed that liver echogenicity exceeded that

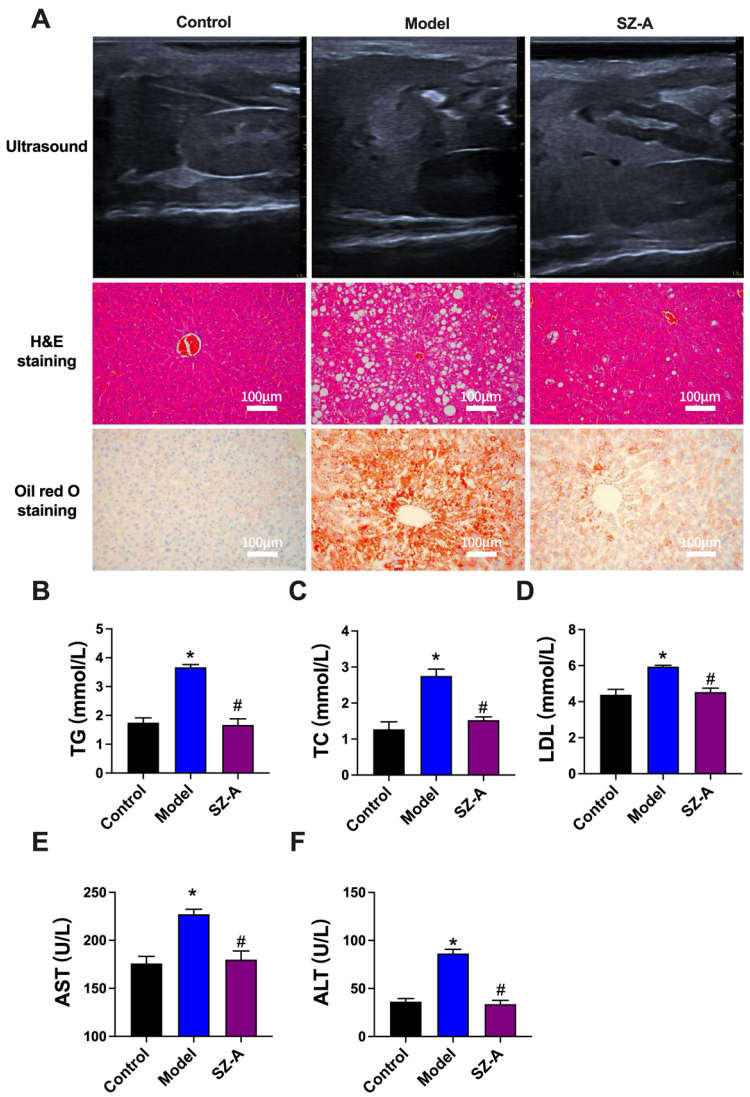

Fig. 1 Effect of SZ-A on liver function in type 2 diabetes mellitus (T2DM) combined with metabolic dysfunction-associated steatotic liver disease (MASLD) mice
The function of liver was detected by Ultrasound, H&E staining, and Oil red O staining assays. (B-D) The changes of TG (B), TC (C) and LDL (D) in serum among different groups. (E and F) Effect of SZ-A on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in different groups. *vs.* Control, $^{*}P < 0.05$. *vs.* Model $^{\#}P < 0.05$. T2DM, type 2 diabetes mellitus; MASLD, metabolic dysfunction-associated steatotic liver disease; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

of the kidney, indicative of fatty liver in model mice. SZ-A treatment reduced the liver echo intensity compared to the model group.

Histological examinations using H&E staining showed morphological and structural abnormalities in the livers of model mice, which were significantly reversed following SZ-A administration. Moreover, Oil Red O staining revealed abundant hepatic lipid droplets in model mice, while SZ-A treatment markedly reduced their accumulation.

Serum biochemical analyses demonstrated elevated levels of TG, TC, and low-density lipoprotein cholesterol (LDL-C) in

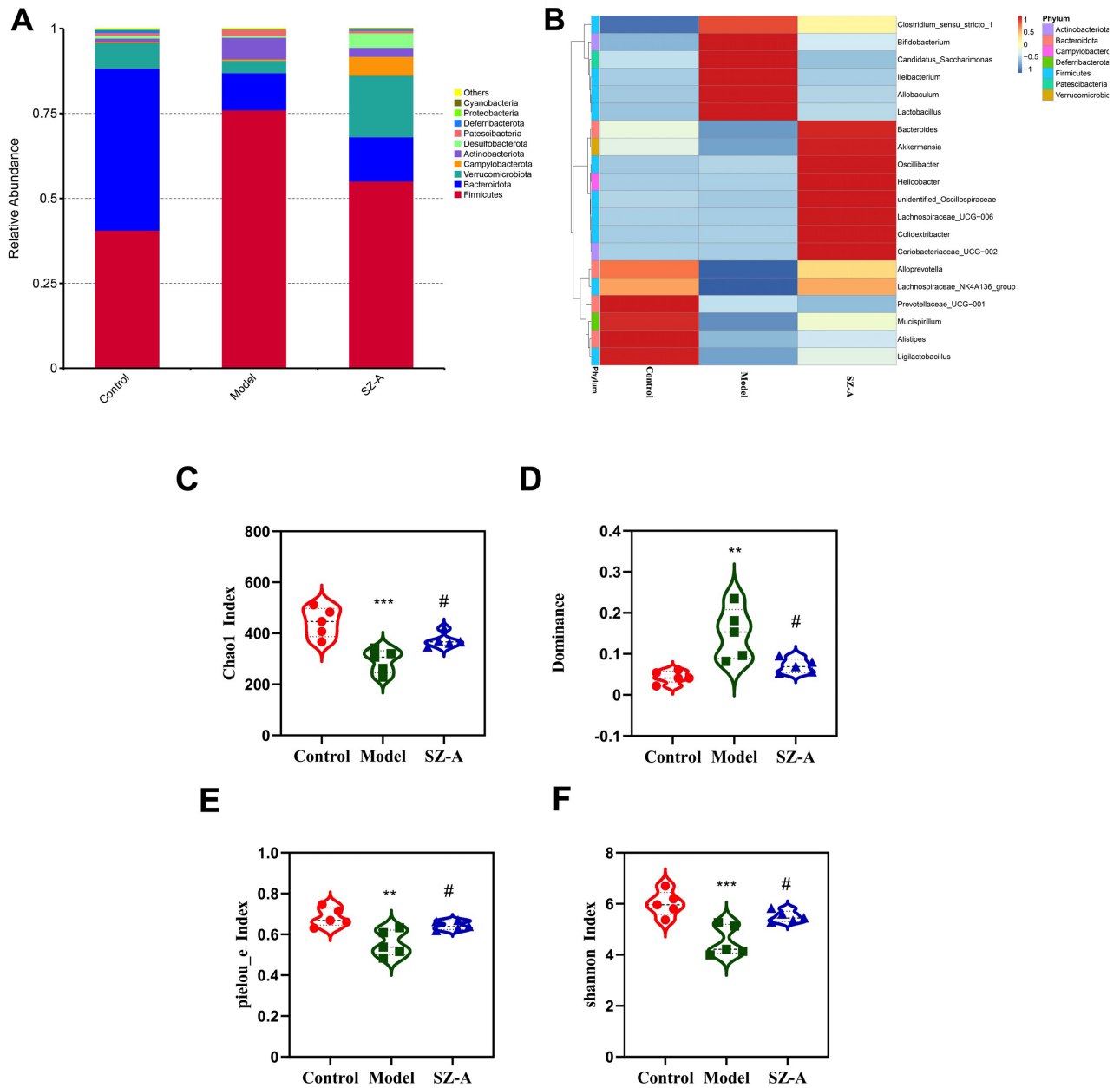

Fig. 2 Effect of SZ-A on gut microbiota by alpha diversity analysis in type 2 diabetes mellitus (T2DM) combined with metabolic dysfunction-associated steatotic liver disease (MASLD) mice

(A and B) Top 10 species at the phylum level (A) and at the genus level (B). (C-F) Alpha diversity indices were analyzed, including Chao1 (C), Dominance (D), pielou_e (E) and Shannon (F) in different groups. $N = 5$ in each group. *vs.* Control, $^{*}P < 0.05$. *vs.* Model $^{\#}P < 0.05$. $^{**}P < 0.01$; $^{***}P < 0.001$.

the model group, all of which were significantly decreased by SZ-A (Fig. 1B-1D). Liver enzyme levels (AST and ALT) were also reduced in the SZ-A group compared to model mice (Fig. 1E-1F). These findings indicate that SZ-A effectively ameliorates liver dysfunction in T2DM combined with MASLD.

3.2 Effect of SZ-A on gut microbiota composition in T2DM combined with MASLD mice

To evaluate SZ-A's impact on gut microbiota, 16S rRNA gene sequencing was performed using Illumina NovaSeq. The top 10 most abundant species at the phylum and genus levels are presented in Fig. 2A and 2B, indicating that SZ-A alters microbial composition.

Alpha diversity indices (Chao1, Dominance, Pielou_e, and Shannon) demonstrated increased microbial richness and diversity following SZ-A treatment compared to the model group (Fig. 2C-2F).

Beta diversity analysis revealed distinct clustering patterns. Principal Coordinates Analysis (PCoA) showed consistent microbial profiles after SZ-A treatment (Fig. 3A), and UPGMA clustering revealed group-specific abundance differences (Fig. 3B). SZ-A markedly altered the gut microbiota compared to model mice (Fig. 3C-3D). Fig. 3E highlights commonly altered microbial communities across groups.

At the phylum level, SZ-A increased the abundance of Firmicutes

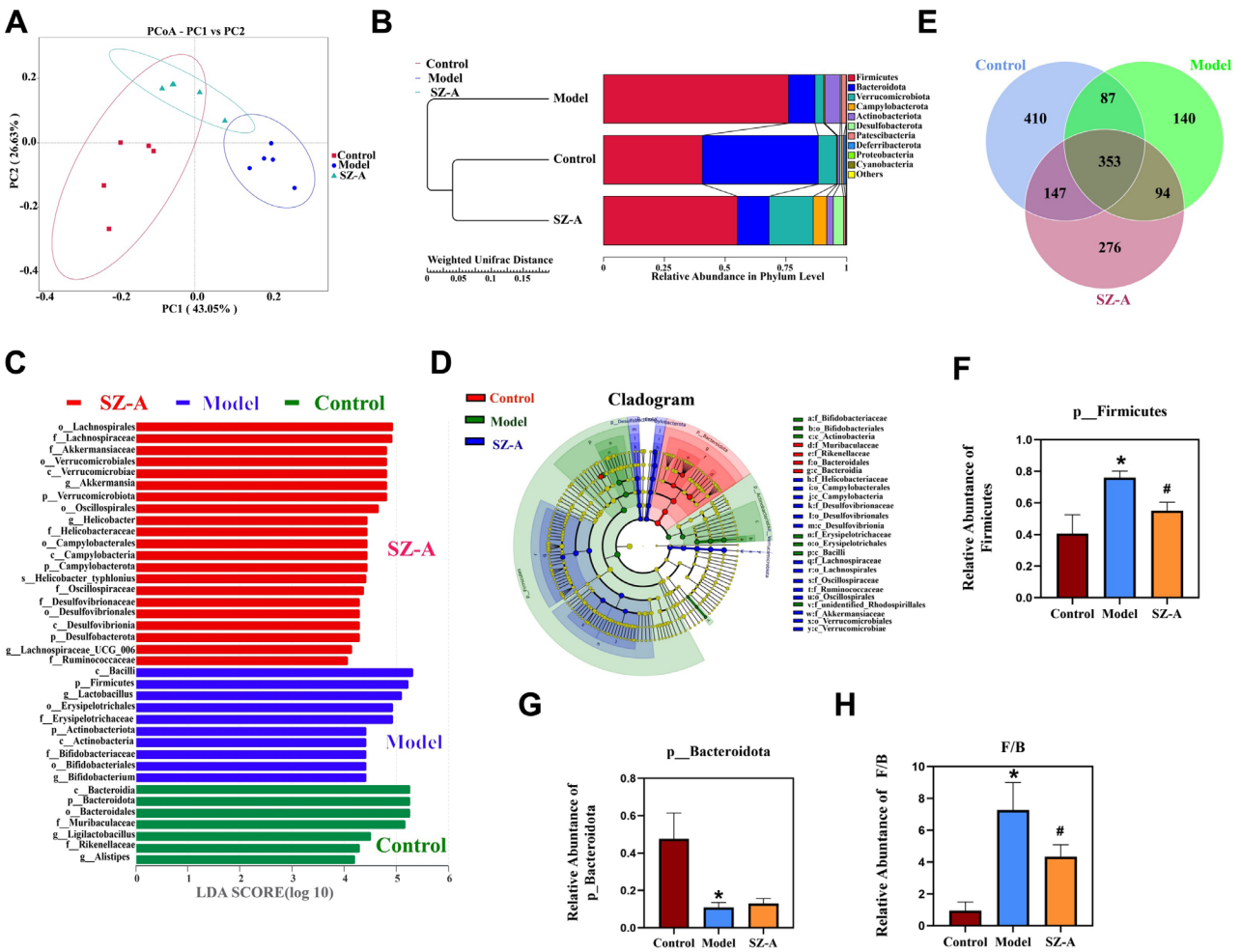

Fig. 3 Effect of SZ-A on gut microbiota by beta diversity analysis in type 2 diabetes mellitus (T2DM) combined with metabolic dysfunction-associated steatotic liver disease (MASLD) mice

(A) Principal co-ordinates analysis (PCoA) analysis. (B) Unweighted pair-group method with arithmetic means (UPGMA) cluster tress analysis. (C and D) Linear discriminant analysis (LDA) Effect Size (C) and branching diagram (D). (E) Venn diagram showed the overlap or unique microflora among different groups. (F-H) Effect of SZ-A on Firmicutes (F), Bacteroidetes (G) and ratio of Firmicutes to Bacteroidetes (H). $N = 5$ in each group. *vs.* Control, $^{*}P < 0.05$. *vs.* Model $^{\#}P < 0.05$.

and decreased Bacteroidetes, thereby restoring the Firmicutes/Bacteroidetes ratio disrupted in the model group (Fig. 3F-3H).

3.3 SZ-A modulates hepatic lipid metabolites in T2DM combined with MASLD mice

To investigate hepatic lipid metabolism, lipidomic profiling of liver tissue was performed across control, model, and SZ-A groups. Fig. 4A-4B show improved intra-group cohesion between control and model groups and a distinguishable SZ-A cluster. PLS-DA confirmed group stability and separation (Fig. 4C-4D).

Differential metabolite analysis identified 521 altered metabolites between control and model groups, and 209 between model and SZ-A groups, with 100 overlapping metabolites (Fig. 4F). Heatmaps (Fig. 5A-5D) revealed that TG and diglycerides (DG) were upregulated in the model group and reversed by SZ-A. Conversely, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which were downregulated in the model group, were upregulated with SZ-A.

Hierarchical clustering and Spearman correlation analyses (Fig. 5E-5F) further characterized these changes. Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis showed enrichment in lipid-related pathways such as glycerophospholipid metabolism, linoleic and arachidonic acid metabolism, and autophagy. These data indicate that SZ-A modulates hepatic lipid metabolism, particularly TG, DG, PC, and PE.

3.4 Correlation between hepatic lipid metabolites and gut microbiota induced by SZ-A

To assess the relationship between gut microbiota and liver metabolites, correlation analyses were performed. As shown in Fig. 6A-6B, positive correlations were observed between PC and

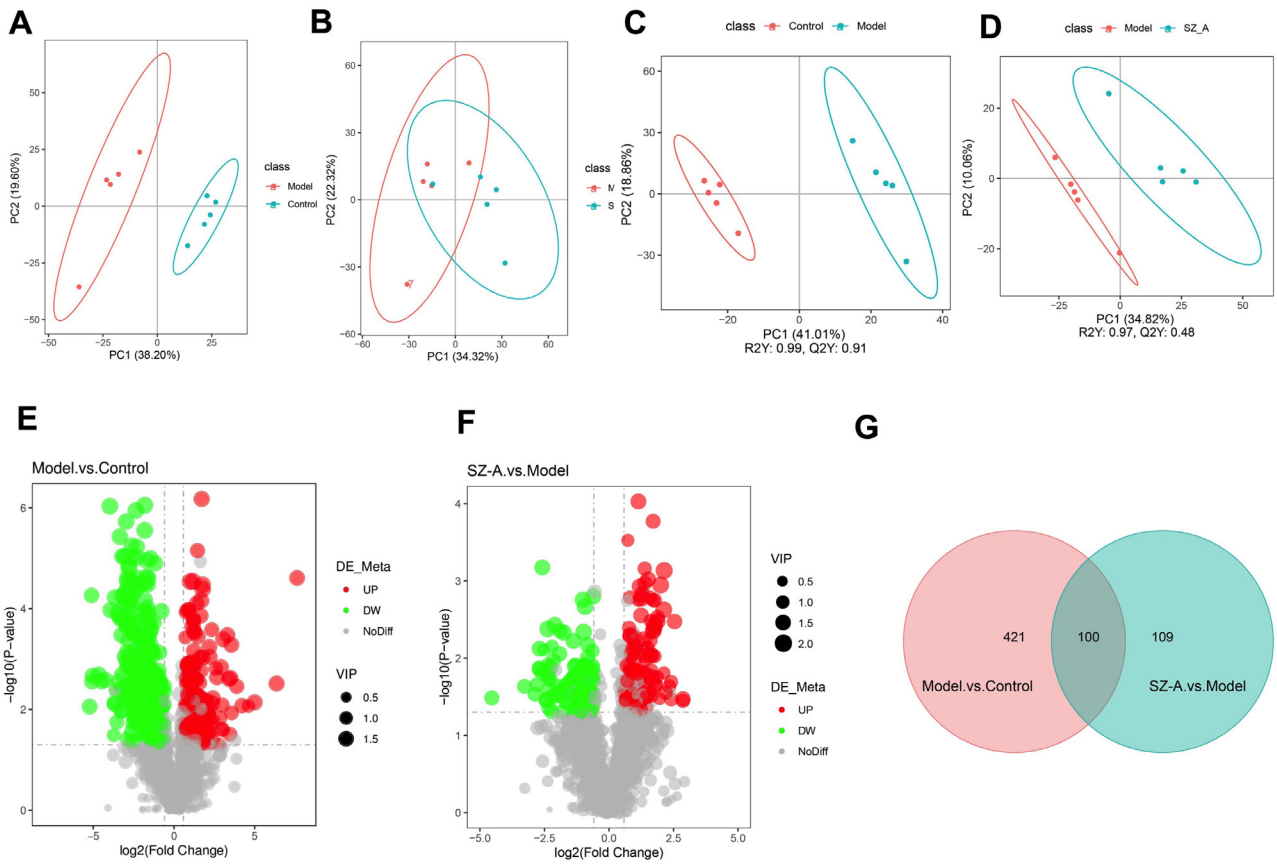

Fig. 4 Effect of SZ-A on hepatic lipid metabolites by multivariate statistical analysis in type 2 diabetes mellitus (T2DM) combined with metabolic dysfunction-associated steatotic liver disease (MASLD) mice

(A) Principal co-ordinates analysis (PCoA) analysis between control and model group. (B) PCA analysis between model and SZ-A group. (C) Partial least squares discrimination analysis (PLSDA) analysis between control and model group. (D) PLSDA analysis between model and SZ-A group. (E) Volcanic map between control and model group. (F) Volcanic map between model and SZ-A group. (G) Venn diagram among different groups.

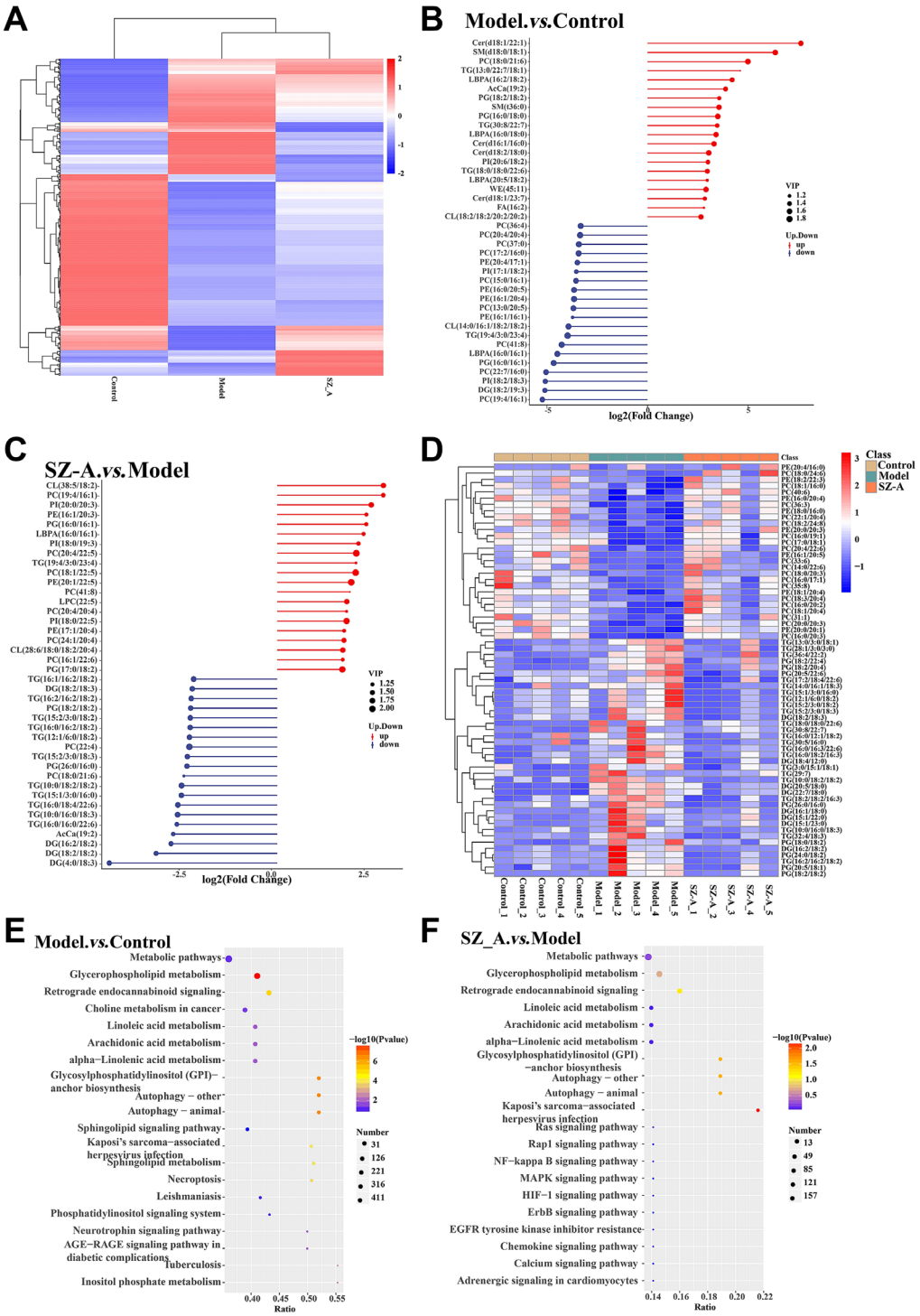

Fig. 5 Effect of SZ-A on hepatic lipid metabolism pathway in T2DM combined with MASLD mice

(A) Heap maps among different groups. (B) Top changed metabolites between control and model group. (C) Top changed metabolites between model and SZ-A group. (D) The changes of TG, DG, PC and PE among different groups. (E) KEGG analysis between control and model group. (F) KEGG analysis between model and SZ-A group. T2DM, type 2 diabetes mellitus; MASLD, metabolic dysfunction-associated steatotic liver disease; TG, triglycerides; DG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; KEGG, Kyoto encyclopedia of genes and genomes.

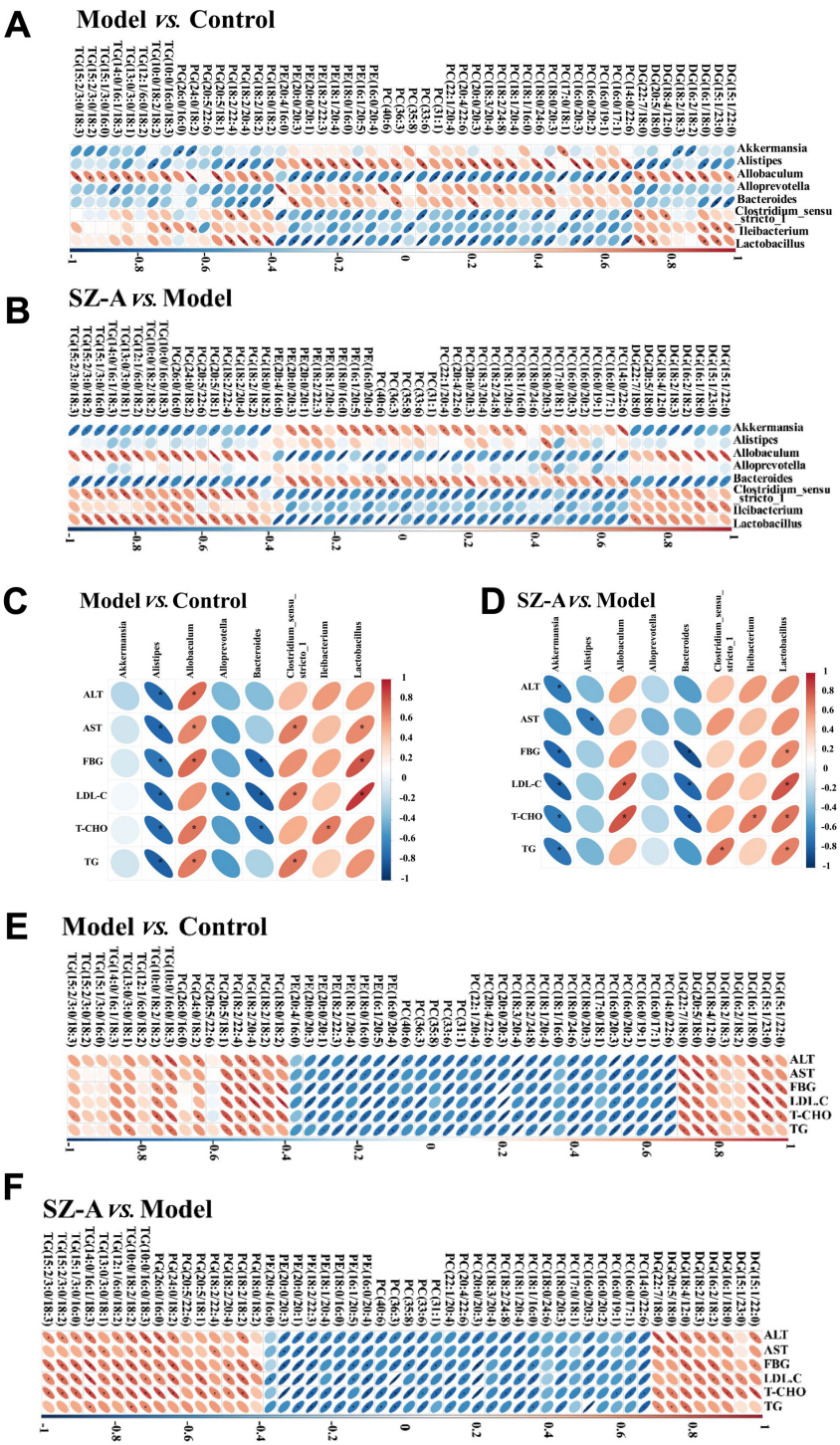

Fig. 6 Effect of SZ-A on the correlation of hepatic lipid metabolites and gut microbiota

(A) The correlation of hepatic lipid metabolites and gut microbiota between control and model group. (B) The correlation of hepatic lipid metabolites and gut microbiota between model and SZ-A group. (C) The correlation of gut microbiota serum and biochemistry analysis between control and model group. (D) The correlation of gut microbiota serum and biochemistry analysis between model and SZ-A group. (E) The correlation of hepatic lipid metabolites and serum biochemistry analysis between control and model group. (F) The correlation of hepatic lipid metabolites and serum biochemistry analysis between model and SZ-A group.

Bacteroides, and between PE and *Akkermansia* in the SZ-A group. Conversely, DG and *Bacteroides*, PG and *Akkermansia*, and TG and *Alloprevotella* showed negative correlations.

Further analysis revealed that *Clostridium_sensu_stricto_1* correlated positively with ALT, *Ileibacterium* with AST, *Allobaculum* with LDL-C, and *Lactobacillus* with TC in SZ-A-treated mice (Fig. 6C-6D). In contrast, *Bacteroides*, *Akkermansia*, *Alloprevotella*, and *Alistipes* were negatively correlated with these serum markers.

Correlations between lipid metabolites and serum biochemistry were also noted (Fig. 6E-6F): DG correlated positively with ALT, PG with AST, and TG with LDL-C, while PC and PE showed negative correlations with ALT and AST, respectively.

These findings suggest that SZ-A-induced changes in gut microbiota are associated with improvements in lipid metabolism and serum biochemical markers, highlighting a gut-liver axis of action.

4 Discussion

T2DM and MASLD are increasingly recognized as major global health burdens. Notably, a high proportion of T2DM patients also present with MASLD. Regional differences in climate and lifestyle contribute to the varying prevalence of metabolic diseases. For instance, in the cold northern regions of China, high-fat and high-salt diets are commonly consumed, leading to a higher incidence of T2DM and MASLD. These conditions are closely associated with atherosclerotic cardiovascular disease, ultimately reducing quality of life and life expectancy. Therefore, there is an urgent need to develop new therapeutic strategies for the prevention and treatment of T2DM coexisting with MASLD, particularly in populations in colder climates.

SZ-A is widely recognized as an effective hypoglycemic agent for T2DM patients. Previous studies have suggested that SZ-A may also alleviate liver injury associated with T2DM or obesity[15]. Consistent with these findings, our study demonstrated that SZ-A treatment reduced hepatic echogenicity on ultrasound, lowered serum AST and ALT levels, improved liver morphology and structure, decreased hepatic lipid droplet accumulation, and reduced serum levels of TG, TC, and LDH. These results indicate that SZ-A ameliorates liver dysfunction in mice with T2DM and MASLD.

Gut microbiota imbalance plays a pivotal role in the pathogenesis of metabolic syndrome. Emerging evidence supports gut microbiota modulation as a promising therapeutic strategy. Notably, SZ-A has been reported to influence gut microbiota in the context of T2DM and/or MASLD[16]. However, the impact of SZ-A on gut microbiota in the combined T2DM and MASLD model had not been fully explored. Our study revealed that SZ-A increased the richness and diversity of the gut microbiota and significantly altered microbial community composition. Importantly, SZ-A restored the Firmicutes/Bacteroidetes ratio, a hallmark often disrupted in metabolic diseases such as obesity, T2DM, and MASLD. These findings suggest that SZ-A contributes to the correction of gut microbiota imbalance, which may partially underlie its protective effects on liver function.

In addition to gut microbiota dysbiosis, hepatic lipid metabolism dysfunction is central to the progression of T2DM and MASLD. Prior work has shown that SZ-A modulates lipid metabolism in T2DM models[17]. To further investigate this, we conducted hepatic lipidomic analysis in mice with T2DM and MASLD. We identified 521 differentially expressed metabolites between the control and model groups, and 209 between the model and SZ-A-treated groups, with 100 metabolites overlapping across comparisons. Notably, SZ-A treatment downregulated TG and DG, while upregulating PC and PE, both of which are reduced in lipid metabolic disorders. Given that elevated TG and DG levels are implicated in lipotoxicity and fatty liver, and reduced PC and PE levels are associated with membrane dysfunction and liver pathology, these changes suggest a beneficial role for SZ-A in restoring lipid homeostasis.

Correlation analysis revealed that *Bacteroides* and *Akkermansia* were positively associated with PC and PE but negatively correlated with DG and phosphatidylglycerol (PG). These findings further support the notion that SZ-A modulates hepatic lipid metabolism in concert with gut microbiota alterations in T2DM combined with MASLD.

In conclusion, our study demonstrates that SZ-A ameliorates liver injury at least partially by regulating both hepatic lipid metabolism and gut microbiota composition in a mouse model of T2DM combined with MASLD. These results suggest SZ-A as a promising therapeutic agent for metabolic comorbidities, particularly in populations at heightened risk due to environmental and dietary factors.

Acknowledgments

Not applicable.

Research ethics

The study was approved by the Institutional Review Board (or Ethics Committee) of the Second Affiliated Hospital of Harbin Medical University (KY2021-298). Additionally, this research followed the Animal Research: Reporting *In Vivo* Experiments (ARRIVE) Guidelines.

Informed consent

Not applicable.

Author contributions

Qiao H and Fu X L were responsible for research design and manuscript writing, Meng H and Wang M T performed *in vivo* study, Zhao J was responsible for data collection and statistical analysis.

Use of large language models, AI and machine learning tools

No large language models, AI or machine learning tool was used for any part of the present study.

Conflict of interest

The authors declare no competing financial interest.

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Data availability

All relevant data are within the manuscript.

References

[1] Ferguson D, Finck B N. Emerging therapeutic approaches for the treatment of MASLD and type 2 diabetes mellitus. Nat Rev Endocrinol, 2021; 17(8): 484-495.
[2] Targher G, Corey K E, Byrne C D, *et al*. The complex link between MASLD and type 2 diabetes mellitus - mechanisms and treatments. Nat Rev Gastroenterol Hepatol, 2021; 18(9): 599-612.
[3] Stefan N, Cusi K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. Lancet Diabetes Endocrinol, 2022; 10(4): 284-296.
[4] En Li Cho E, Ang C Z, *et al*. Global prevalence of non-alcoholic fatty liver disease in type 2 diabetes mellitus: An updated systematic review and meta-analysis. Gut, 2023; 72(11): 2138-2148.
[5] Tanase D M, Gosav E M, Costea C F, *et al*. The intricate relationship between type 2 diabetes mellitus (T2DM), insulin resistance (IR), and nonalcoholic fatty liver disease (MASLD). J Diabetes Res, 2020; 2020: 3920196.
[6] Poustchi H, Alaei-Shahmiri F, Aghili R, *et al*. Hepatic steatosis and fibrosis in type 2 diabetes: A risk-based approach to targeted screening. Arch Iran Med, 2021; 24(3): 177-186.
[7] Hong S H, Choi K M. Sarcopenic obesity, insulin resistance, and their implications in cardiovascular and metabolic consequences. Int J Mol Sci, 2020; 21(2): 494.
[8] Zhou M, Liu X, Wu Y, *et al*. Liver lipidomics analysis revealed the protective mechanism of Zuogui Jiangtang Qinggan Formula in type 2 diabetes mellitus with non-alcoholic fatty liver disease. J Ethnopharmacology, 2024; 329: 118160.
[9] Alfadda A A, Almaghamsi A M, Sherbeeni S M, *et al*. Alterations in circulating lipidomic profile in patients with type 2 diabetes with or without non-alcoholic fatty liver disease. Front Mol Biosci, 2023; 10: 1030661.
[10] Schoeler M, Ellero-Simatos S, Birkner T, *et al*. The interplay between dietary fatty acids and gut microbiota influences host metabolism and hepatic steatosis. Nat Commun, 2023; 14(1): 5329.
[11] An X, Yang X, Ding X, *et al*. Ramulus Mori (Sangzhi) alkaloids tablets for diabetes mellitus: A regulatory perspective. Fitoterapia, 2023; 166: 105444.
[12] Sun Q W, Lian C F, Chen Y M, *et al*. Ramulus Mori (Sangzhi) alkaloids ameliorate obesity-linked adipose tissue metabolism and inflammation in mice. Nutrients, 2022; 14(23): 5050.
[13] Yang Y, Wu L, Lv Y, *et al*. LC-MS/MS based untargeted lipidomics uncovers lipid signatures of late-onset preeclampsia. Biochimie, 2023; 208: 46-55.
[14] Bailey L S, Huang F, Gao T, *et al*. Characterization of glycosphingolipids and their diverse lipid forms through two-stage matching of LC-MS/MS Spectra. Anal Chem, 2021; 93(6): 3154-3162.
[15] Liu D, Ye J, Yan Y, *et al*. Ramulus Mori (Sangzhi) alkaloids regulates gut microbiota disorder and its metabolism profiles in obese mice induced by a high-fat diet. Front Pharmacol, 2023; 14: 1166635.
[16] Liu W, Xu S, Zhang B, *et al*. Ramulus Mori (Sangzhi) alkaloids alleviate diabetic nephropathy through improving gut microbiota disorder. Nutrients, 2024; 16(14): 2346.
[17] Wang F, Xu S J, Ye F, *et al*. Integration of transcriptomics and lipidomics profiling to reveal the therapeutic mechanism underlying Ramulus Mori (Sangzhi) Alkaloids for the treatment of liver lipid metabolic disturbance in high-fat-diet/streptozotocin-induced Diabetic Mice. Nutrients, 2023; 15(18): 3914.