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Research Article

Regulation of Glucolipid Metabolism in Mice Using *Dendrobium huoshanense* Extract and PI3k/Akt Signaling Pathway Activation

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The present research is carried out to study the hypoglycemic and hypolipidemic effects of the *Dendrobium huoshanense* C. Z. Tang et S. J. Cheng extract on type 2 diabetic mice. *D. huoshanense* extract was prepared using the condensation reflux method. The chemical components were separated by UPLC Triple-TOF MS/MS to further identify the components that mainly exerted hypoglycemic and hypolipidemic effects. Type 2 diabetic C57BL/6 mice were induced by a high-fat diet (HFD) followed by intraperitoneal streptozotocin (STZ) and were daily gavaged with different doses of *D. huoshanense* extract for 5 weeks. Various methods such as HE staining, ELISA, and Western blot were used to evaluate the hypoglycemic and hypolipidemic effects of *D. huoshanense* extract on mice with type 2 diabetes mellitus model. The experimental results revealed that the main compounds of *D. huoshanense* extract were polysaccharides, flavonoids, alkaloids, amino acids, and many other chemical components. Oral administration of 200 mg/kg of the extract of *D. huoshanense* significantly reduced blood glucose and insulin levels of high-dose group mice and lowered total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) levels, but increased high-density lipoprotein cholesterol (HDL-C) levels and liver glycogen content. In addition, the protein expression levels of insulin receptor (Ins R), phosphatidylinositol-3 kinase (PI3K) and p-protein kinase B (Akt) were upregulated. These results imply that the *D. huoshanense* aqueous extract can improve insulin resistance and lipid metabolism in type 2 diabetic mice, and it may regulate the function through the PI3K/AKT pathway.

1. Introduction

"*Dendrobium huoshanense* C. Z. Tang et S. J. Cheng" is a fresh or dried stem of a plant in the family Orchidaceae. It is originally recorded in the earliest existing Pharmacopeia "Shennong Herbal Scripture" [1] and believes to be able to remove paralysis, lower "Qi," and protect the stomach and intestines. *D. huoshanense* has been officially included in the Chinese Pharmacopoeia. The main active compounds of *D. huoshanense* are polysaccharides that are mostly composed of monosaccharose such as galactose, glucose, and mannose. At the same time, it contains amino acids, polyphenols, alkaloids, flavonoids, and other active ingredients [2–6]. The modern pharmacological research results prove that *D. huoshanense* has the functions of improving body immunity and preventing and treating diabetes and has antioxidant, antivirus, antitumor, antibacterial, and anti-inflammatory properties [7–9].

Diabetes (DM), the most prevalent disease worldwide, has become a serious public health threat, which affects large numbers of people, as a metabolic disease with a global epidemic [10]. The prevalence of diabetes mellitus in China is about 10% [11], and the number of diabetic patients has reached 114 million, accounting for 1/3 of the total number of diabetic patients all over the world. Type 2 diabetes mellitus (T2DM) accounts for more than 90 percent of DM patients [12]. High-carbohydrate diets are predominant in China, which results in an increase in postprandial blood glucose in T2DM and a severe injury to islet β-cells [13]. The pathophysiology of T2DM is characterized by decreased insulin secretion due to degeneration of insulin's capacity to regulate glucose metabolism and defects in islet β-cell functions [14], which leads to a high level of blood glucose and insulin resistance in T2DM. Therefore, it is important to control blood glucose or reduce insulin resistance.

Currently, most injectable insulin or oral metformin drugs are used, but they have many side effects and can cause problems such as obesity and lipid metabolism [15, 16]. As a result, it is especially important to find suitable alternative therapeutic drugs. The purpose of this study is to investigate compositions of *D. huoshanense* extract with a high-pressure water extraction method and the effects of *D. huoshanense* extract on the glucolipid metabolism disorders and its molecular mechanism. The graphical abstract of this study is shown in Supplementary Material, Figure S1.

2. Materials and Methods

2.1. Materials. D. huoshanense was purchased from Huoshan County, Anhui Province, as identified by one of the authors (N. J. Yu). Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) kits, insulin (INS) kits, and glycogen kits were purchased from the Nanjing Jiancheng Institute of Biological Engineering; streptozotocin (STZ) was purchased from Shanghai Qiaoxing Trading Co. Antibodies insulin receptor (Ins R), phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt), β-actin, and p-Akt were purchased from Chengdu Zhengneng Biological Co.

2.2. D. huoshanense Water Extract Preparation. Two hundred grams of *D. huoshanense* was dried at 60°C and then crushed and the powder was passed through a 60 mesh sieve and then defatted by immersing the powder in 95% ethanol for 12 h. The solution was centrifuged at 4000 rpm for 10 min, the supernatant liquid was discarded, and the powder was dried. In a Soxhlet extractor, the dried powder was extracted 3 times in 300 mL water for 1.5 hours each time, and the filtrate was combined and inspissated to one-fifth of the previous volume. The whole extractive ingredients were fully mixed and freeze-dried.

2.3. Determination of the Polysaccharide Content of the D. huoshanense Extract. D. huoshanense extract was configured into a 1 mg/mL solution, and the total polysaccharide concentration was determined by the phenol-sulfuric acid colorimetric method [17].

2.4. Detection of the Chemical Composition of the D. huoshanense Extract. The chemical composition of the extracts was identified by UPLC Triple-TOF MS/MS. In brief, *D. huoshanense* extract was dispersed in 50% methanol (10 mg/mL) and then filtered through a 0.2 μm nylon syringe filter. The filtrate (5 μL) was loaded into an UPLC Triple-TOF MS/MS equipped with an ACQUITY UPLC HSS T3 (100*2.1 mm, 1.8 μm) and a diode array detector. The mobile phases were 0.1% formic acid aqueous solution (A) and acetonitrile (B). The gradient elution program was as follows: 0–1.5 min, 95% A; 1.5–2.5 min, 95%–90% A; 2.5–14 min, 90%–60% A; 14–23 min, 60%–5% A; and 23–25 min, 5%–95% A. The column temperature was 40°C, and the flow rate was 0.4 mL/min.

2.5. Animals and Treatment. The animals required for experimental use, C57BL/6 male mice (5 weeks old) of 18–20 g weight, were provided by Hangzhou Ziyuan Laboratory Animal Technology Co., Ltd. The animals were kept at a constant temperature and in a 12-hour light/dark cycle. Mice were adaptively fed for 1 week prior to the start of the experiment. Experiments were carried out in accordance with institutional and national guidelines and regulations and approved by the Laboratory Animal Ethics Committee of Anhui University of Chinese Medicine (license number: AHUCM-mouse-2022048).

2.5.1. Induction of T2DM Mice. By referring to a previously described method [18], the normal control mice received normal feed, while the remaining mice received high-fat feed for 4 weeks. At the end of 4th week, the normal mice were injected intraperitoneally with citrate buffer solution (0.1 M, pH 4.5), while other mice were injected intraperitoneally consecutively with streptozotocin (STZ) at a dose of 50 mg/kg body weight (BW) in citrate buffer solution (0.1 M, pH 4.5) for 5 days. Blood glucose levels were assessed 3 days after STZ injection. Mice showed fasting blood glucose levels (FBG) ≥11.1 mmol/L were considered successful models of diabetes [19].

2.5.2. Animal Experimental Design. After the T2DM model was successful, mice were randomly divided into six groups (each group consisted of eight animals). The grouping was as follows: (1) normal group, gavaged with 0.2 mL normal saline; (2) model group, gavaged with 0.2 ml normal saline; (3) metformin group, gavaged with 10 mg/kg of metformin;

(4) low-dose group (i.e., DH-L), gavaged with 50 mg/kg of *D. huoshanense* extract; (5) medium-dose group (i.e., DH-M), gavaged with 100 mg/kg of *D. huoshanense* extract; and (6) high-dose group (i.e., DH-H), gavaged with 200 mg/kg of *D. huoshanense* extract. All groups were administered once a day for 5 weeks. The body weight of each mouse was measured weekly (Figure 1). For complete table, see Supplementary Material, Table S1.

2.6. Blood Glucose Test and Oral Glucose Tolerance Test (OGTT). The fasting blood glucose (FBG) of mice was recorded once a week by a blood glucose meter. The blood sample was collected from the tail vein of mice. On the last day of administration, mice were given gavage with 1 g/kg glucose solution for OGTT testing. The blood glucose levels were measured at 0, 30, 60, 90, and 120 minutes (min) after administration. The blood glucose-time curve of each dose group was plotted. The blood glucose levels were used to calculate the area under the curve (AUC) to quantify the results of OGTT.

2.7. Sample Collection. After OGTT measurement, mice were fasted for 12 hours starting at 8 : 00 pm. The mice were anaesthetized through intraperitoneal injection of 100 mg/kg BW ketamine. The blood sample was collected from the eye socket and placed at room temperature for half an hour. The sample was centrifuged at 4000 rpm/min, 4°C for 10 min. Then, the supernatant was collected carefully and frozen at −80°C. Liver tissues were collected and weighed. The liver weight index was calculated as follows: liver weight index = liver weight/body weight × 100%. A portion of the liver tissue was stored in a 4% paraformaldehyde solution for pathological investigation, and the remaining liver samples were stored at −80°C for further analysis.

2.8. Serum Test Indicators. Serum samples were retrieved from the −80°C fridge and thawed on ice. The levels of glucagon (GC), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and fasting insulin (FINS) in serum were measured using an enzyme microplate analyzer in accordance with the instructions provided in the kit. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = fasting glucose level (mmol/L) × fasting insulin level (mU/L)/22.5.

2.9. Liver Tissue Homogenate Assay Index. Removed the liver, rinsed with saline, absorbed dry on filter paper, and weighed 0.04 g precisely. According to the kit instructions, liver glycogen was detected and recorded.

2.10. Histologic Analysis. The liver was fixed in 4% (v/v) paraformaldehyde for 24 h and then placed in 70% (v/v) ethanol. For histological analysis, a portion of the tissue was fixed in paraffin wax and stained with hematoxylin and eosin (HE). Tissue morphology was examined under a microscope.

2.11. Western Blot Analysis. After the sample, mice's livers were pulverized and the total protein of the liver tissue was extracted by lysate and centrifugation and the total protein was measured by the BCA kit. The proteins were isolated in 10% SDS-PAGE gel and transferred to a PVDF membrane (400 mA 30 min). The PVDF membrane was sealed with BSA for 2 hours at room temperature and then rinsed with TBST (20 mM Tris, 140 mM NaCl, 0.1% Tween (pH 7.6)) three times every 5 min. The primary antibodies (β-actin, Ins R, PI3k, Akt, and p-Akt) were incubated with membrane overnight at 4°C and rinsed thrice in next morning. After incubation with appropriate secondary antibodies, proteins were visualized using enhanced chemiluminescence (ECL, Amersham Biosciences).

2.12. Statistical Analysis. The acquired data were imported into MassHunter software for analysis of retention time correction, peak identification, peak filtering, mass-to-nucleus ratio, etc. The collected data were subjected to substance identification using MassBank, GNPS, RIKEN PlaSMA, and other databases. Data were expressed as the mean ± standard deviation. Statistical analysis was performed using SPSS 21.0 and GraphPad Prism 7.0 (GraphPad Software). One-way ANOVA and two-way comparison between the groups using the LSD-t test were used. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Quantification of Major Components in D. huoshanense Extract. The total polysaccharide content of *D. huoshanense* extract was 56.18% ± 0.22% and the paste yield was 27.18% ± 0.22%.

The total ion flow (TIC) plots for individual small molecule are displayed in Figure 2. The separated substances were identified using mass spectrometric data after extracting the characteristic peaks with Compound Discoverer 3.0 software. A total of 32 compounds were obtained, including alkaloids, amino acids, terpenoids, flavonoids, and many other small molecules (Table 1).

3.2. Effects of D. huoshanense Extract on Body and Liver Index Weight of Mice. The change in body weight is represented in Figure 3(a). In the 5th week, the weight of T2DM-modeled mice showed a declining trend as compared to the normal group ($p < 0.05$). At the 10th week of treatment, the metformin group exhibited a 14.54% increase in body weight in comparison with the model group. The high-, medium-, and low-dose groups of *D. huoshanense* extract exhibited an increase of 15.38%, 8.65%, and 4.9%, respectively, as compared to the model group. Among the *D. huoshanense* extract treatments, the administration of 200 mg/kg (i.e., DH-H) slowed down the decline in body weight as compared to the other treatments ($p < 0.05$).

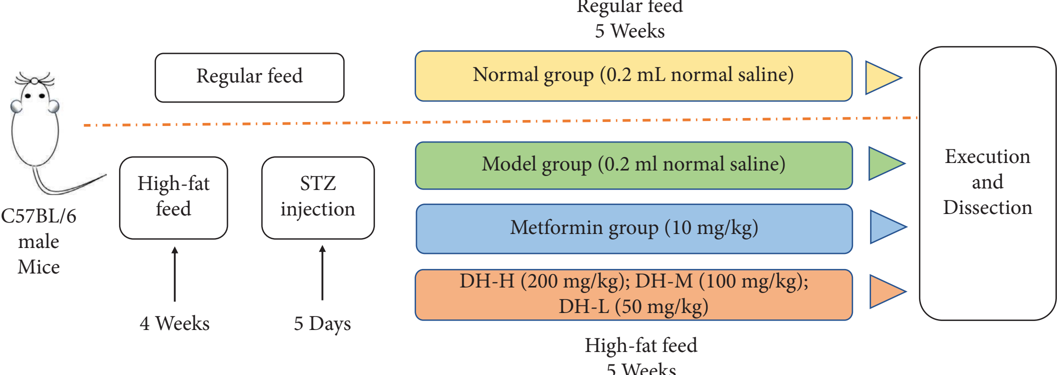


Figure 1: Animal group and administration.

Compared with the normal group, the liver index of modeled normal mice was significantly higher ($p < 0.05$). The modeled mice treated with *D. huoshanense* extract at (50, 100, and 200 mg/kg concentration) significant reduction in the liver index ($p < 0.05$). No difference was found between the metformin and DH-H (200 mg/kg) groups. It was shown that the T2DM-modeled mice developed significant liver swelling, and *D. huoshanense* extract and metformin inhibited the organomegaly in T2DM mice (Figure 3(b)).

3.3. Effect of D. huoshanense Extract on Blood Glucose Level and Glucose Tolerance. The FBG of the model group was 11.1 mmol/L higher than that of the normal group. When compared with the normal group, the type 2 diabetic mice had higher FBG, indicating that the T2DM model was successfully established (Figure 4(a)). The DH-H group (200 mg/kg) of *D. huoshanense* extract showed a better hypoglycemic effect on diabetic mice. The DH-H group exhibited the lowest glucose level, which was closest to the normal mice.

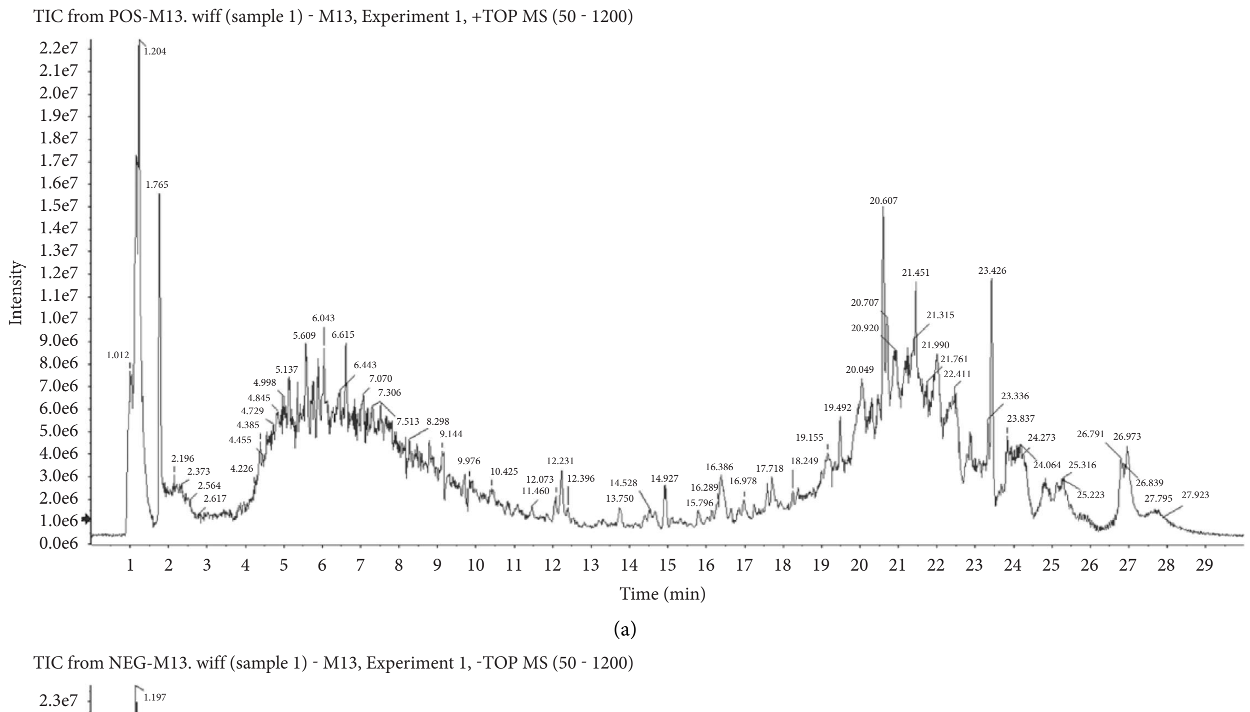
The diabetic mice in the model group had higher blood glucose levels than the normal group during 0–120 min, indicating impaired glucose tolerance in the model group (Figures 4(b) and 4(c)). The AUC was two times higher as compared to the normal group. In comparison with the model group, the AUC of *D. huoshanense* extract was considerably lower, particularly in the DH-H group (200 mg/kg).

3.4. Effects of D. huoshanense Extract on Insulin Secretion. Fasting insulin levels were increased in the model group compared to the normal group ($p < 0.05$), and the HOMA-IR index was substantially five times higher (Figure 5(a)). In comparison with the model group, the modeled mice administered with *D. huoshanense* extract (200 mg/kg) resulted in reduction of insulin levels and the HOMA-IR index. Furthermore, fasting insulin levels in the DH-H group were significantly lower than those in the medium- and low-dose groups, ranging from 6.68 to 3.88 mIU/L. In addition, the HOMA-IR index decreased more in the high-dose group from 5.68 to 1.62 than in the medium- and low-dose groups ($p < 0.05$, Figure 5(b)). The metformin and DH-H groups did not differ significantly.

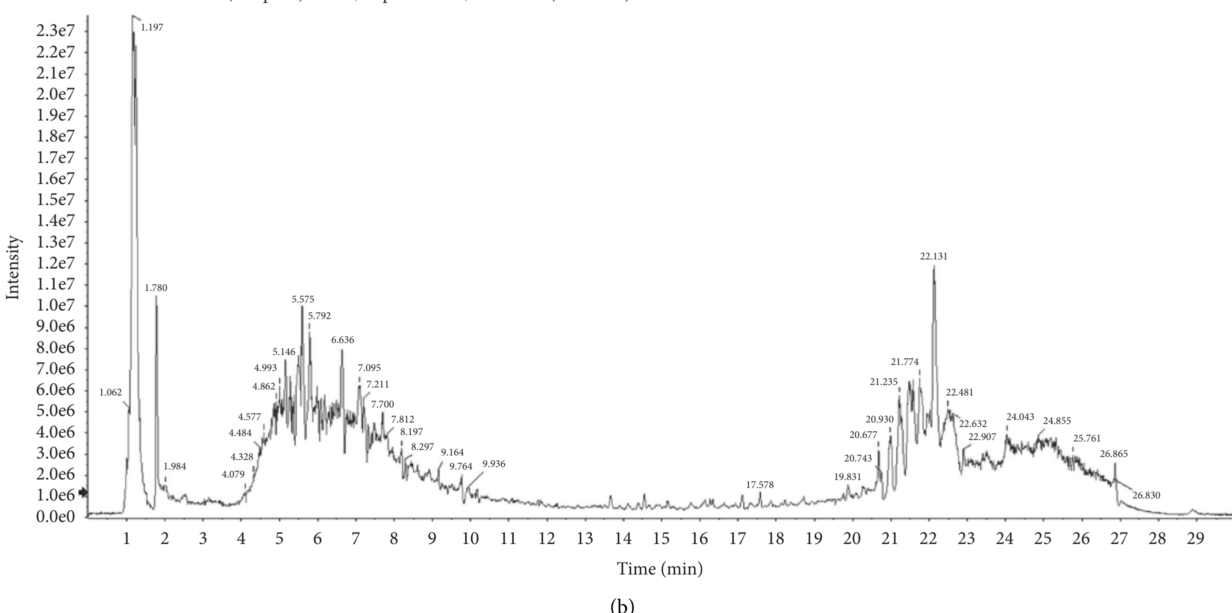
3.5. Effect of D. huoshanense Extract Supplementation on Liver Lipids and Lipid Accumulation. In the model group, TC, TG, and LDL-C levels in the model group were higher, while HDL-C levels were significantly lower (Table 2). However, in diabetic mice, the high-dose supplementation of *D. huoshanense* extract dramatically decreased TC, TG, and LDL-C concentrations and increased HDL-C. The TC, TG, and LDL-C levels reduced by 40.59%, 46.12%, and 36.03% ($p < 0.05$), respectively, while HDL-C levels increased by 42.45% ($p < 0.05$). The changes in lipid accumulation were also confirmed by histopathological findings in the liver.

Hepatocytes in the normal group were neat and wired, whereas in the model group, they were disrupted by fatty vesicles and inflammatory cell infiltration. It is clear from Figure 6 that the high dose of *D. huoshanense* extract supplementation showed slight symptoms of liver damage and steatosis as compared to other doses.

3.6. Effects of D. huoshanense Extract on Liver Glycogen and PI3K/Akt Signaling Pathway. The liver glycogen content of mice in the model group was significantly lower when compared to the normal group ($p < 0.05$) (Figure 7(b)). When modeled mice were supplemented with high dose of *D. huoshanense* extract, their liver glycogen content significantly increased in comparison with the model group. This finding suggested that *D. huoshanense* extract had the ability to increase the liver glycogen content and corrected the disorder of glucose metabolism in T2DM and showed hypoglycemic effects. These observations suggested that diabetes altered the expression of the PI3K/Akt signaling pathway in diabetic mice, and *D. huoshanense* extract (200 mg/kg) could correct this interference (Figures 7(a) and 7(c)–7(e)). The high-dose group's liver protein production of PI3K, Ins R, and phosphorylated Akt was dramatically elevated and their levels were found to be close to normal ($p < 0.05$).



(a)



(b)

Figure 2: Total ion flow (TIC) diagram of samples: positive (a) and negative (b) ion patterns of samples of *D. huoshanense* from left to right in order.

4. Discussion

In this research, a total of 32 compounds were identified by UPLC Triple-TOF MS/MS, comprising flavonoids, steroids, alkaloids, amino acids, coumarins, and organic acids (Table 1). Obviously, polysaccharide is the predominant active components that accord with Hong-Yan Wang's research [20–22]. At the same time, the flavonoid extract showed an important part in hypoglycemic and hypolipidemic effects, which had been demonstrated previously [23–25]. Nonetheless, the role of alkaloids and other ingredients in hypoglycemic and hypolipidemic are still obscure and so deeper research is needed later.

Dysglycemia and dyslipidemia are common metabolic disorders that are observed in patients with diabetes. Disturbances in glucose metabolism are currently an important basis for the development of diabetes, and OGTT and corresponding AUC values are diagnostic tests frequently used to evaluate glucose tolerance function [26, 27]. In addition to fasting insulin levels reflecting pancreatic-cell damage, the HOMA-IR index is a common indicator for assessing insulin resistance. Our results showed decreased

Table 1: Detection of small molecule compounds of *D. huoshanense* extract by UPLC Triple-TOF MS/MS.

RT	*m/z*	Adducts	Formula	Area	Compound name	Ontology
1.1871	138.0552	$[M+H]^+$	$C_7H_7NO_2$	1352611	Trigonelline	Alkaloids and derivatives
4.6562	167.071	$[M+H]^+$	$C_9H_{10}O_3$	272814.4	Paeonol	Alkyl-phenylketones
26.8526	118.087	$[M+H]^+$	$C_5H_{11}NO_2$	112264.9	Glycine-betaine	Alpha amino acids
1.7618	130.0501	$[M+H]^+$	$C_5H_7NO_3$	781259.7	L-Pyroglutamic acid	Alpha amino acids and derivatives
5.5697	193.0862	$[M+H]^+$	$C_{11}H_{12}O_3$	625210.6	4-Methoxy-6-prop-2-enyl-1,3-benzodioxole	Benzodioxoles
6.1046	147.0441	$[M+H-H_2O]^+$	$C_9H_8O_3$	129547.1	Coumaric acid	Coumaric acid and derivatives
16.0111	357.2811	$[M+H-2H_2O]^+$	$C_{24}H_{40}O_4$	119571.4	Chenodeoxycholic acid	Dihydroxy bile acids, alcohols, and derivatives
2.5215	413.1466	$[M+Na]^+$	$C_{21}H_{26}O_7$	314987.3	Pseudolaric acid C	Diterpene lactones
13.5363	333.2061	$[M+H]^+$	$C_{20}H_{28}O_4$	278003.4	14-Deoxy-11,12-dehydroandrographolide	Diterpene lactones
20.1508	307.265	$[M-H_2O+H]^+$	$C_{20}H_{36}O_3$	113260.3	1-Naphthalene pentanoic acid	Diterpenoids
6.5406	235.1708	$[M-H_2O+H]^+$	$C_{15}H_{24}O_3$	425795.6	2-[(2R,4aR,8R,8aR)-8-Hydroxy-4a,8-dimethyldecahydro-2-naphthalenyl]acrylic acid	Eudesmane isoeudesmane or cycloeudesmane sesquiterpenoids
6.9254	235.1708	$[M-H_2O+H]^+$	$C_{15}H_{24}O_3$	242001.3	2-[(2R,4aR,8R,8aR)-8-Hydroxy-4a,8-dimethyldecahydro-2-naphthalenyl]acrylic acid	Eudesmane isoeudesmane or cycloeudesmane sesquiterpenoids
5.4473	321.0649	$[M+H]^+$	$C_{15}H_{12}O_8$	199906.6	Flavanone base + 6O	Flavanone O-glycosides
9.7335	273.0764	$[M+H]^+$	$C_{15}H_{12}O_5$	1362279	Naringenin	Flavanones
4.9552	581.1531	$[M+H]^+$	$C_{26}H_{28}O_{15}$	224823.8	Flavone base + 4O	Flavone C, C-glycosides
3.6106	273.0954	$[M+H]^+$	$C_{19}H_{12}O_2$	175441	Alpha-naphthoflavone	Flavones
5.6304	535.1486	$[M+H]^+$	$C_{25}H_{26}O_{13}$	1587861	5,7-Dihydroxy-2-(4-hydroxyphenyl)-6,8-bis(3,4,5-trihydroxyoxan-2-yl)chromen-4-one	Flavonoid 8-C-glycosides
8.1981	683.2321	$[M+Na]^+$	$C_{33}H_{40}O_{14}$	153366.3	2″-O-Rhamnosyl icariside II	Flavonoid-3-O-glycosides
5.4473	233.1546	$[M+H]^+$	$C_{15}H_{20}O_2$	343760.2	Cyclodeca[b]furan-2(3H)-one	Germacranolides and derivatives
7.3119	233.1534	$[M+H]^+$	$C_{15}H_{20}O_2$	119390.2	Cyclodeca[b]furan-2(3H)-one	Germacranolides and derivatives
16.6456	537.3073	$[M+Na]^+$	$C_{27}H_{46}O_9$	284167.9	2-Hydroxy-3-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}propyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Glycosylmonoacylglycerols
19.4844	441.1914	$[M+Na]^+$	$C_{23}H_{30}O_7$	101586.6	Gomisin H	Hydrolyzable tannins
9.0676	243.1017	$[M+H]^+$	$C_{15}H_{14}O_3$	218506.8	7-Methoxy-9,10-dihydrophenanthrene-2,5-diol	Hydrophenanthrenes
13.37	243.1036	$[M+H]^+$	$C_{15}H_{14}O_3$	155382.3	7-Methoxy-9,10-dihydrophenanthrene-2,5-diol	Hydrophenanthrenes
26.7682	247.1685	$[M-H_2O+H]^+$	$C_{16}H_{24}O_3$	925038.5	NCGC00385962-01_C16H24O3_	Macrolides and analogues
5.1421	267.1607	$[M+H]^+$	$C_{15}H_{22}O_4$	347243.3	Naphtho[1,2-c] furan-3(1H)-one	Naphthofurans
6.3661	219.1765	$[M-H_2O+H]^+$	$C_{15}H_{24}O_2$	204709.5	2(1H)-Naphthalenone	Sesquiterpenoids
23.6514	413.3827	$[M+H]^+$	$C_{29}H_{48}O$	1408917	(3beta, 22E)-Stigmasta-5,22-dien-3-ol	Stigmastanes and derivatives
11.0998	245.1205	$[M+H]^+$	$C_{15}H_{1}6O_3$	201509.9	3-[2-(3-Hydroxyphenyl)ethyl]-5-methoxyphenol	Stilbenes
11.0876	275.1279	$[M+H]^+$	$C_{16}H_{18}O_4$	489588.5	4-[2-(3-Hydroxy-5-methoxyphenyl) ethyl] -2-methoxyphenol	Stilbenes
7.3119	453.2136	$[M+Na]^+$	$C_{21}H_{34}O_9$	935641.9	2(1H)-Azulenone	Terpene glycosides
6.8632	249.1492	$[M-H_2O+H]^+$	$C_{15}H_{22}O_4$	436808.9	Naphtho[2,3-b]furan-2(4H)-one	Terpene lactones

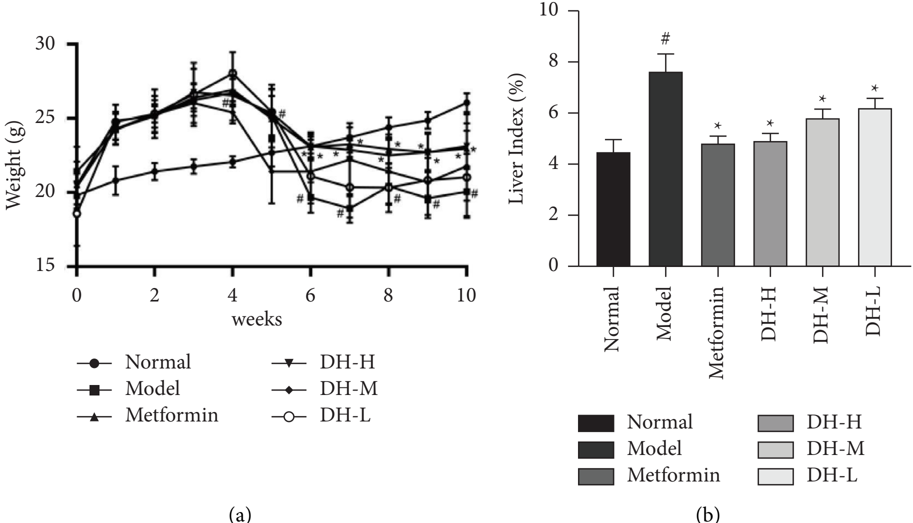


FIGURE 3: (a) Effect of *D. huoshanense* extract on the body weight of mice with type 2 diabetes. (a) Body weight of mice during ten weeks. (b) Effect of *D. huoshanense* extract on the liver index of mice. Data were shown as the mean ± SD ($n = 8$ for each group). $^{\#}p < 0.05$, compared to the normal group. $^{*}p < 0.05$, compared to the model group.

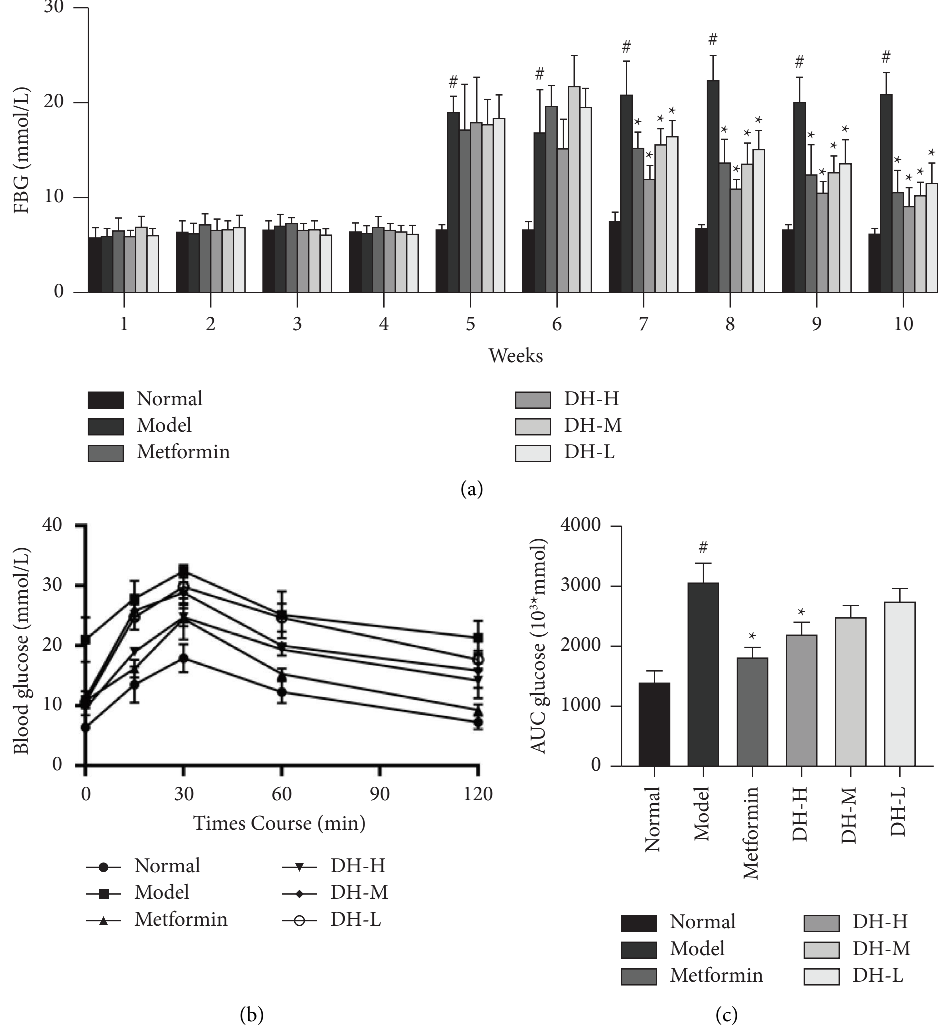


FIGURE 4: (a) Effects of *D. huoshanense* extract on fasting blood glucose levels of diabetic mice. (b) Effects of *D. huoshanense* extract on the OGTT curves of diabetic mice. (c) The AUC glucose calculated for diabetic mice. Data were shown as the mean ± SD ($n = 8$ per group). $^{\#}p < 0.05$, compared with the normal group. $^{*}p < 0.05$, compared with the model group.

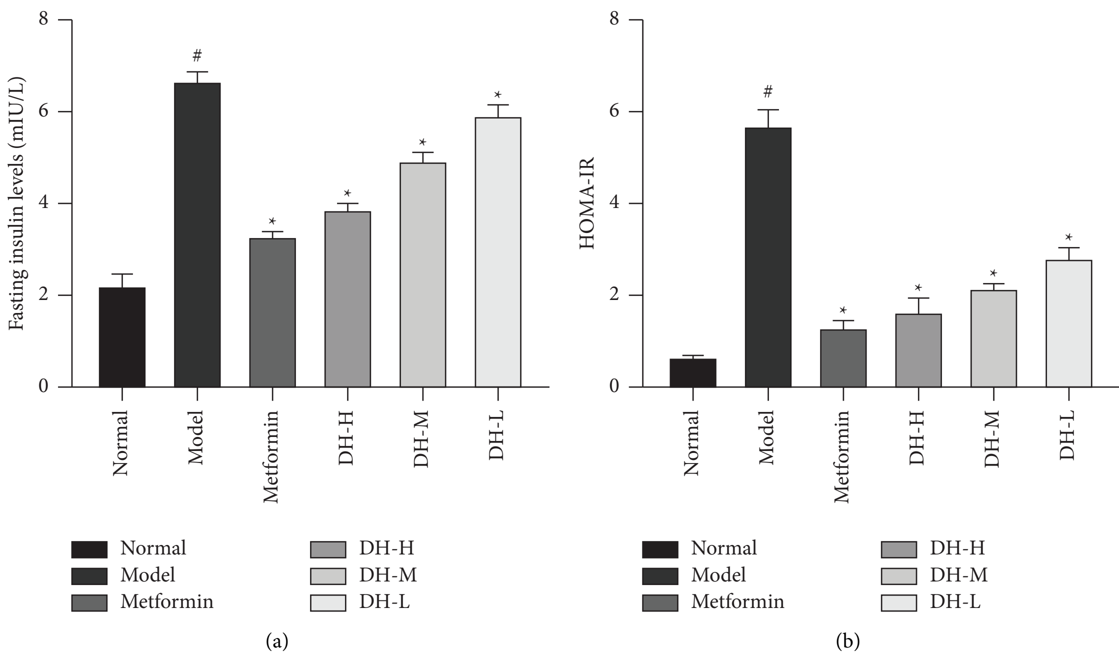


Figure 5: Effects of *D. huoshanense* extract on insulin levels and values of HOMA-IR from diabetic mice. (a) Fasting insulin levels and (b) HOMA-IR index. Data were shown as the mean ± SD ($n = 8$ per group). $^{\#}p < 0.05$, compared with the normal group. $^{*}p < 0.05$, compared with the model group.

Table 2: Effect of *D. huoshanense* extract on serum lipids of diabetic mice ($n = 4$ per group).

Group	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Normal	3.83 ± 0.21^{f}	1.16 ± 0.12^{c}	0.96 ± 0.13^{c}	1.73 ± 0.06^{a}
Model	8.83 ± 0.14^{a}	4.12 ± 0.34^{a}	1.72 ± 0.12^{a}	1.06 ± 0.07^{b}
Metformin	4.58 ± 0.12^{e}	1.30 ± 0.56^{c}	1.11 ± 0.34^{b}	1.59 ± 0.31^{a}
DH-H	5.84 ± 0.18^{d}	2.22 ± 0.32^{b}	1.10 ± 0.22^{b}	1.51 ± 0.12^{a}
DH-M	6.47 ± 0.26^{c}	2.65 ± 0.11^{b}	1.51 ± 0.29^{ab}	1.31 ± 0.09^{ab}
DH-L	8.21 ± 0.32^{b}	3.53 ± 0.14^{a}	1.61 ± 0.04^{ab}	1.28 ± 0.15^{ab}

Note. Different letters (a, b, c, d, e, and f) indicate significance between columns ($p < 0.05$). Group comparison was evaluated by Duncan's multiple comparison test.

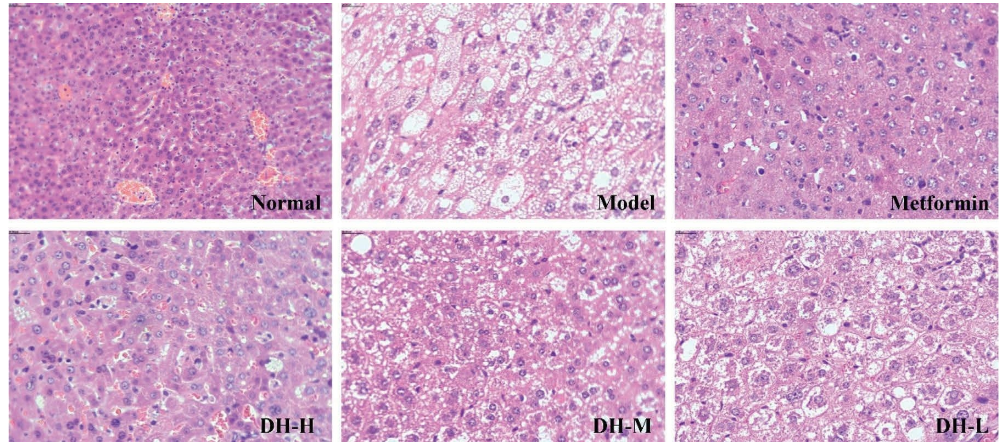


Figure 6: Effects of *D. huoshanense* extract on histological morphology of liver tissues from diabetic mice. Microscopic images with 200x total magnification. Scale bar: 20 μm.

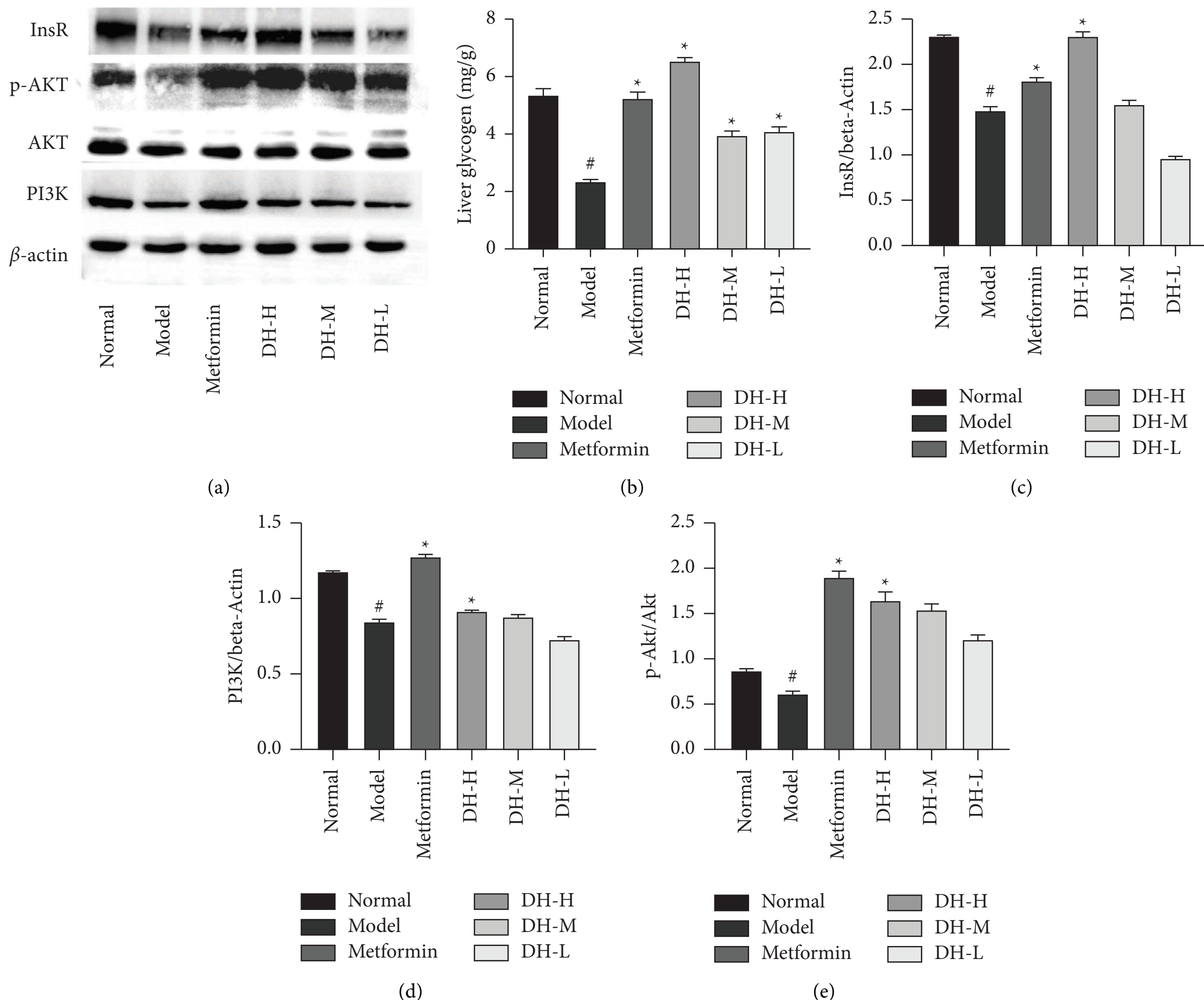


Figure 7: Effect of *D. huoshanense* extract on the expression of Ins R PI3K, p-Akt, and Akt in liver tissues of diabetic mice and the liver glycogen content after five weeks. (a) Protein expression of Ins R, PI3K, p-Akt, and Akt. (c–e) Relative banded protein expression data. (b) Liver glycogen content. Data were expressed as the mean ± SD ($n = 4$ or 8 per group). $^{\#}p < 0.05$, compared with the normal group. $^{*}p < 0.05$, compared with the model group.

levels of fasting glucose and AUC of OGTT, FINS, and HOMA-IR, following 200 mg/kg of *D. huoshanense* extract treatment. *D. huoshanense extract* has been shown to regulate blood glucose and improve glucose tolerance, potentially reducing pancreatic damage and insulin resistance. It can alleviate typical T2DM symptoms, which we considered to be accomplished by protecting pancreatic beta cells and improving insulin resistance [18, 28, 29].

The liver is the primary organ for lipid metabolism which maintains cholesterol homeostasis [30]. Diabetes mellitus is characterized by abnormally elevated blood glucose levels as the primary disease feature, and abnormal glucose metabolism results in disruptions in lipid metabolism, which leads to elevated plasma triglycerides (TG) and cholesterol (TC) and reduced HDL-C and elevated LDL-C [31, 32]. In this study, *D. huoshanense* extract (200 mg/kg) increased HDL-C levels and decreased TC, TG, and LDL-C levels. In addition, the results of liver HE staining showed that the addition of 200 mg/kg of *D. huoshanense* extract reduced lipid accumulation in the liver. As a result, our findings indicated that *D. huoshanense* extract (200 mg/kg) can alleviate T2DM symptoms by protecting hepatocytes, lowering lipid accumulation, and restoring lipid metabolism. *Sargassum fusiforme* extracts have now also been shown to have a protective effect on the liver in type 2 diabetes by Wu et al. [33]. However, the current study did not elucidate the mechanism of antihyperglycemia and antihyperlipidemia, so we explored its potential pathways further.

Insulin resistance caused by T2DM reduced the insulin effect on the liver, increased glycogen decomposition, and decreased synthesis, resulting in an abnormal blood sugar level [34]. The PI3K/Akt signaling pathway is believed to be a major target of insulin for blood glucose level. Insulin can activate the cell surface receptor IRS1, triggering the PI3K/Akt signaling pathway. Insulin secreted by pancreatic β-cells interfered with the PI3K/Akt signaling pathway in the pancreas [35–37]. At the same time, it inhibited the PI3K/Akt signaling pathway in the liver and increased glycogen

synthesis [38]. The results of our research indicated that the protein expression of phosphorylated Akt, Ins R, and PI3K was increased, indicating that the PI3K/Akt pathway was activated, and the hepatic glycogen content of the administration group was higher than that of the model group. As a consequence, we confirmed that *D. huoshanense* extract can modulate the PI3K/Akt pathway in the liver and restore the balance between glycogen synthesis and degradation. Many studies have shown that interfering with PI3K/Akt and other pathways can result in hypoglycemic effects [39, 40]. By activating the PI3K/AKT signaling pathway, the aqueous extract of Flos Aureus is also able to improve hepatic insulin resistance in mice [41]. Extracts from a variety of plants, such as white buttercup and Acanthopanax, have also been shown to affect glycolipid metabolism in mice by this pathway [18, 42, 43].

However, we had to concede that the key active ingredients of *D. huoshanense* extract are unidentified still and the relevance between the PI3K/Akt signaling pathway and the effects of *D. huoshanense* extract on diabetic mouse is not absolute owing to the insufficiency of the experimental evidence on inhibitor treatment or gene knockout mouse. Therefore, the key active ingredients and mechanism of *D. huoshanense* extract need further investigation.

5. Conclusion

In the research, we investigated the hypoglycemic and hypolipidemic effects of *D. huoshanense* extract on T2DM mouse and the substance basis of it. Overall, the UPLC Triple-TOF MS/MS showed active ingredients of *D. huoshanense* extract, including polysaccharides, flavones, and polyphenol. Also, we explored that *D. huoshanense* extract at 200 mg/kg showed a significant and similar effect on the diabetes mellitus as that of metformin. However, the lower doses of 50 mg/kg and 100 mg/kg of *D. huoshanense* extract had no significant effect on diabetes mellitus. It was also suggested that *D. huoshanense* extract could alleviate the symptoms of diabetes mellitus by protecting pancreatic β-cells and the liver and could activate the PI3K/Akt signaling pathway in the liver, maintaining the equilibrium of hepatic glycogen synthesis and decomposition.

Abbreviations

Akt: Protein kinase B
AUC: Area under the curve
DM: Diabetes mellitus
ECL: Enhanced chemiluminescence
FBG: Fasting blood glucose
FINS: Fasting insulin
HDL-C: High-density lipoprotein cholesterol
HE: Hematoxylin and eosin
HFD: High-fat diet
HOMA-IR: Homeostasis model of assessment of insulin resistance
InsR: Insulin receptor
LDL-C: Low-density lipoprotein cholesterol
OGTT: Oral 60 glucose tolerance test
PI3K: Phosphatidylinositol-3 kinase
STZ: Streptozotocin
TC: Total cholesterol
TG: Triglyceride
T2DM: Type 2 diabetes mellitus.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Additional Points

Practical Applications. Type 2 diabetes mellitus (T2DM) has become a major global public health threat due to its high prevalence and complications. Effective glycaemic control, improved insulin secretion, and reduced insulin resistance are essential in the treatment of T2DM. The findings of the present research revealed that *D. huoshanense* extract could alleviate diabetic symptoms, improve insulin resistance in peripheral tissues, activate the Akt/PI3K signalling pathway in the liver, and restore the balance of hepatic glycogen synthesis and catabolism by protecting pancreatic β-cells and the liver. Therefore, this study suggests that *D. huoshanense*, as a medicinal herb, has potential and industrial value as a hypoglycaemic and hypolipidemic drug.

Ethical Approval

All animal handling procedures were performed strictly in accordance with the P.R. China Legislation on the Use and Care of Laboratory Animals and were approved by the Animal Care Review Committee, Anhui University of Traditional Chinese Medicine (license number: AHUCM-mouse-2022048).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xiaoqian Zhang, Jing Fang, Ruipeng Ge, and Jing Wu prepared and revised the manuscript. Xiaoqian Zhang, Jing Fang, Ruipeng Ge, Mengjuan Ye, and Xiao Cai participated in the animal experiments. Guanghui Deng, Mengzhen Ma, Xiaoqian Zhang, and Jiahui Lv performed the UPLC Triple-TOF MS/MS experiments and data analysis. Nianjun Yu, Liang Yao, and Daiyin Peng were responsible for the study design and approved the article. All the authors have read and approved the final manuscript. Xiaoqian Zhang and Jing Fang contributed equally to this work.

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Supplementary Materials

Supplementary material includes graphic summaries and animal group and administration. Supplementary materials are available at Journal of Food Biochemistry online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author. (*Supplementary Materials*)

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