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ORIGINAL ARTICLE

# *Alisma orientalis* (Sam.) Juzep Polysaccharide-Regulated Glucose-Lipid Metabolism in Experimental Rats and Cell Model of Diabetes Mellitus with Regulation of miR-126

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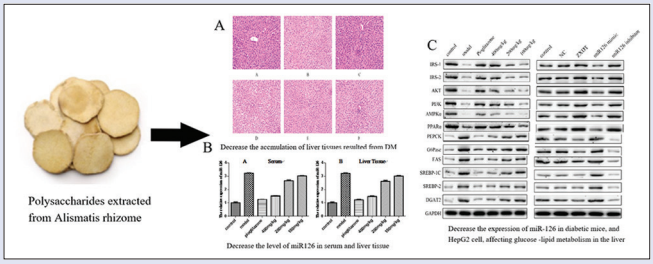
## ABSTRACT

**Background:** Alismatis rhizome (AR) is a popular traditional Chinese medicine, used for hyperlipidemia and diabetes in China for centuries; *Alisma* polysaccharides (AP) are the bioactive components in AR. This study investigates if glucose–lipid metabolism can be regulated by AP via miR-126 *in vivo* and *in vitro*. **Materials and Methods:** The diabetes group (diabetes mellitus) was injected intraperitoneally with streptozotocin (35 mg/kg) for 7 days. Diabetic mice treated with AP at an oral dose of either 400 mg/kg (high), 200 mg/kg (middle), or 100 mg/kg (low); and mice treated with pioglitazone (15 mg/kg) each day. The hypoglycemic and hypolipidemic effects of AP were demonstrated by measuring fasting blood glucose (FBG) levels, fasting insulin level, total cholesterol, triglyceride, low-density lipoprotein and high-density lipoprotein, observing insulin-sensitizing effects, and testing oral glucose tolerance. In addition, the expression of both miR-126 and related glucose–lipid genes in the primary hepatocytes was examined in diabetic mice. **Results:** The FBG, long-term blood glucose, and the ability to resolve blood glucose by insulin in AP groups were significantly better than that in model group. AP could reverse the expression level of miR-126 and genes. The addition of AP could reduce the effect of miR-126, reversing the expression of glucose–lipid genes in HepG2 cell. **Conclusion:** The results demonstrate that the expression of miR-126 can be directly decreased by AP in diabetic mice, especially in HepG2 cell, and AP also can ameliorate the glucose–lipid metabolism.

**Key words:** *Alisma orientalis*, diabetes mellitus, glucose–lipid metabolism, miR-126, polysaccharide

## SUMMARY

- The aim of the study was whether glucose–lipid metabolism can be regulated by AP via miR-126 *in vivo* and *in vitro*. The expression of miR-126 can be directly decreased by AP in diabetic mice, especially in HepG2 cell, and AP also can ameliorate the glucose–lipid metabolism.




**Abbreviations used:** DM: Diabetes mellitus; TCM: Traditional Chinese medicine; AOP: *Alisma orientalis* polysaccharide; ISI: Insulin sensitivity index; FBG: Fasting blood glucose; FINS: Fasting insulin level; GSP: Glycated serum protein; OGTT: Oral glucose tolerance test; HbA1c: Glycosylated hemoglobin; TC: Serum total cholesterol; TG: Triglyceride; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; qPCR: Quantitative polymerase chain reaction.

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## INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disorder characterized by chronic hyperglycemia, which was caused by defecting insulin secretion and resistance.[1] Sustained hyperglycemia might ultimately result in chronic damage and dysfunction of various organs with DM, which causes serious damage to human physical and mental health.[2] Currently, DM and its complications have become one of the major diseases that threaten human health, and the mortality rate is only second to that of cancer and cardiovascular disease.[2] Therefore, it becomes urgent to prevent and treat diabetic patients effectively. At present, western medicine, such as insulin and hypoglycemic agents, are frequently used for clinical treatment for DM with certain therapeutic effect and still exist some problems, such as low curative effect, low tolerability, and big side effects.[3] However, traditional Chinese medicine (TCM) has distinct characteristics and advantages on DM treatment, with the mild lasting effect, small side effects, and multiple components, multiple targets, and multiple effect mechanism.

Alismatis rhizome (AR, named Zexie in China), the dried rhizome of *Alisma orientalis* (Sam.) Juzep, is a popular TCM. The Latin name is *Alisma plantago-aquatica* L. Var. Orientale Samuels. It has been used for diuretics and some symptoms of hyperlipidemia and diabetes in China for centuries,[4,5] which was first recorded in "Shen Nong's Herbal Classic" (Shennong Ben Cao Jing). It is officially listed with the identification code of 0212 in Pharmacopoeia of the People's Republic of China (2015), which states the effects of AR as removing dampness, promoting diuresis, purging heat, and reducing lipid.[6] Many modern pharmacological studies have revealed not only the diuretic activity[7,8]



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but also the activities of antihyperlipidemic,[9] antiatherosclerosis,[10] nephroprotective,[11] immunoenhancing,[12] antidiabetic,[13] anticancer,[14] and anti-inflammatory.[15] Chemical and pharmacological researches have shown that terpenoids and polysaccharides are the principal bioactive components in AR, and especially polysaccharide have been reported to have important bioactivities, including hypoglycemic effects.[16-19] The mechanism of action of AP has not been fully elucidated, although some useful studies about hypoglycemic activity of AP have been performed. Therefore, it is of utmost importance to elucidate the underlying mechanisms of AP.

The microRNAs (miRNAs) are short, noncoding, single-stranded RNAs with a size of 19–25 nucleotides, which are significantly involved in the development of many types of human diseases.[20-22] Since the discovery of miRNAs, an increasing number of them have been found involved in DM pathogenesis.[23,24] Dysregulation of miRNA can lead to profound impairment of glucose and lipid metabolism.[25-27] miRNA expression profiles of various tissues from T2DM patients or hyperglycemia animal models have been established in recent years and make it easier to uncover novel miRNA regulators in diabetes. Recent studies have shown that miR-126 is associated with glucose and lipid metabolism, which can control the insulin signaling pathway.[28] The 12 genes are detected in this study; PEPCK and G6Pase are the key genes in glycosylation. FAS, SREBP-1C, SREBP-2, and DGAT2 are the key genes to regulate the biosynthesis of fatty acids and triglycerides (TGs) involved in lipid metabolism. adenosine 5‘-monophosphate (AMP)-activated protein kinaseα (AMPKα) and peroxisome proliferators-activated receptorα (PPARα) may regulate glucose and lipid metabolism. IRS-1, IRS-2, protein kinase B (AKT), and PI3K are required for glucose metabolism.[28]

This study mainly aims at assessing the hypoglycemic and antihyperlipidemic effect of AP on glucose and lipid profiles in DM rats to confirm and explore their action mechanism in treating DM.

# MATERIALS AND METHODS

## Plant material and extraction

AR (the batch number is ECN000137), purchased from Bozhou, Anhui, in China, was used for isolation of AP. Their botanical origins were identified by Prof. Jinao Duan (Nanjing University of Chinese Medicine, Nanjing, China). The six AR materials (300 g) were extracted twice with water (2:L) for 2.5 h at 100°C. The combined extracts were concentrated to 250 mL using a rotary evaporator (BC-R203, Shanghai Biochemical Equipment Co., Shanghai, China) at 65°C under vacuum. The proteins in the extract were removed by Sevag reagent (The preparation for Sevag reagent: 500 mL trichloromethane and 100 mL n-butanol were measured and then fully mixed and sealed).[29] After removal of the Sevag reagent through rotary evaporation at 30°C and then freeze-drying, 100 mL of anhydrate ethanol was added before the mixture was maintained overnight at 4°C to precipitate polysaccharides. The crude polysaccharides (25 g) were obtained by centrifugation at 3860 g for 15 min.[30,31] The content of AP determined by sulfuric acid–phenol method was 68%.

## Chemicals and reagents

Pioglitazone hydrochloride tablets (Batch No. 9906281) were purchased from Jiangsu Hengrui Medicine Co., Ltd (Jiangsu, China). Streptozotocin (STZ) was obtained from Sigma Aldrich Chemical Co., Ltd. (St. Louis, USA). Glucose assay kit (Batch No. PTT20150909) was purchased from Nanjing Spring and Autumn Biological Engineering Co., Ltd (Jiangsu, China). Glycated serum protein (GSP), glycosylated hemoglobin (HbA1c), total cholesterol (TC), TG, low-density lipoprotein (LDL), test kits were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). Insulin ELISA assay kit and glucagon kit were obtained from Beijing North Biotechnology Research Institute (Beijing, China). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Gibco Invitrogen (Carlsbad, CA, USA). The primary antibodies against triphosphopyridine nucleotide oxidase 1 (NOX1), NOX2, and NOX4, α-smooth muscle actin, E-cadherin, fibronectin, and glyceraldehyde-3-phosphate dehydrogenase (GADPH) were obtained from Abcam (Cambridge, UK. Rabbit-anti-occluding, claudin-1, ZO-1polyclonal antibody were purchased from Gibco Invitrogen (Carlsbad, CA, USA), rabbit β-actin was purchased from Bioss Antibodies Co., Ltd (Beijing, China), Chemiluminescent reagent was purchased from Thermo Fisher Scientific (Waltham, MA,USA), horseradish peroxidase was purchased from Autumn Biological Engineering Co., Ltd (Jiangsu, China) and the blood sugar test paper was from Roche Life Science (Shanghai, China).

## Animal experiments

Sixty Sprague–Dawley rats (with equal ratio of male and female), weighing 220 ± 20 g, were supplied by the Experimental Animal Center of Yangzhou University (Yangzhou, China). All animals were housed in a well-ventilated room (5 rats in one cage) at a constant room temperature by 25°C ± 2°C and controlled humidity (50% ±10%), with a standard condition with 12:12 light/dark cycle and were in free access to chow and water. All procedures for animal care and use were in accordance with the National Institute of Health guidelines for the Care and Use of Laboratory Animals. The rats were allowed to acclimatize for 7 days before the experiments. All rats were randomly divided into six groups of 10 rats each [Table 1].

After 7 days with free access to regular food and water, the mice were randomly divided into two groups. The normal group was fed on regular food. The diabetes group (DM) was injected intraperitoneally with STZ (35 mg/kg) for 7 days. After 7 days, the rats were caudal venous blood with fasting 12 h and the rats with fasting blood glucose (FBG) ≥11.1 mmol/L were confirmed as having diabetes.[32,33] Rats were then randomly divided into five groups: diabetic mice without any drug treatment (DM) and a continued high-fat diet; diabetic mice treated with AP at an oral dose of either 400 mg/kg (high), 200 mg/kg (middle), or 100 mg/kg (low); and mice treated with pioglitazone (15 mg/kg) each

**Table 1:** Effects of AP on fasting blood glucose, fasting insulin level, insulin sensitivity index, glycated serum protein, and glycosylated hemoglobin of diabetic rats ($\bar{x}$±s, $n$=8)

| Groups | FBG (mmol/L) | FINS (mU/L) | ISI | GSP (mmol/L) | HbA1c (OD/10 g) |
|---|---|---|---|---|---|
| Normal | 5.76±0.68** | 21.51±0.82** | −4.84** | 1.48±0.11** | 14.20±0.21** |
| STZ-induced DM | 26.63±3.89 | 31.83±3.91 | −6.74 | 4.42±0.24 | 35.36±0.06 |
| STZ-induced DM + pioglitazone (15 mg/kg) | 8.91±2.96** | 22.65±2.96* | −5.30** | 2.28±0.19** | 19.28±0.12** |
| STZ-induced DM + AP (400 mg/kg) | 12.84±7.04** | 24.92±2.45** | −5.76** | 2.42±0.22 | 21.84±0.23** |
| STZ-induced DM + AP (200 mg/kg) | 15.92±3.28* | 27.18±1.87** | −6.07** | 2.68±0.09 | 21.37±0.15** |
| STZ-induced DM + AP (100 mg/kg) | 18.67±8.23* | 31.36±1.74* | −6.37* | 3.04±0.15 | 34.66±0.19 |

*$P$<0.05 and **$P$<0.01 versus STZ-induced DM group. STZ: Streptozotocin; DM: Diabetes mellitus; AP: *Alisma* polysaccharide; FBG: Fasting blood glucose; FINS: Fasting insulin; ISI: Insulin sensitivity index; GSP: Glycated serum protein; HbA1c: Glycosylated hemoglobin

day. After 30 days of treatment, an oral glucose tolerance test (OGTT) was detected and fasting plasma was collected for further measurement other biochemical assays. At the end of the study, the mice were killed and the livers were isolated and immediately stored at 80°C until further analysis.

## Biochemical assays

After 30 days, OGTT was performed and the blood was collected from mice which were fasted for 12 h from orbital venous plexus and plasma was separated by centrifugation at 3000 g for 10 min. The mice were orally given 25% glucose in water at 5 g/kg body weight after 12 h of fasting. Blood samples were collected at 0, 30, 60 and 120 min to measure the glucose levels through glucose oxidase method. The plasma insulin levels were analyzed by ELISA according to the instructions. Insulin sensitivity index (ISI) was evaluated based on glucose concentrations and fasting insulin. Enzymatic and automatic biochemical analyzer was used to detect FBG, fasting insulin (FINS), GSP, HbA1c, TC, TG, LDL, high-density lipoprotein (HDL) according to the manufacturer's protocols of different commercial kits.

## Histological evaluation

Liver was fixed in 10% neutral-buffered formalin, embedded in paraffin wax, sectioned at 4–5 m by rotary microtome, stained with hematoxylin-eosin, and examined by a laboratory microscope (Olympus, Tokyo, Japan) to assess the histopathological changes.

## Cell culture and transfection

Normal hepatocellular cell G2 was supplied by the Cell Bank of Chinese Academy of Science and cultured in DMEM (Gibco, Grand Island, NY, USA) combined with 10% FBS (Gibco, Grand Island, NY, USA), and 1:100 penicillin and streptomycin (Biosharp, Hefei, China). The cells were cultured in a humidified chamber at 37°C with 5% $CO_2$, and then, HepG2 was put on the cell culture dishes. At the same time, commercial growing medium was used to transfected HepG2 with 100 nmol of miR-126 mimic protein/miR-126 inhibitor/miRNA negative control (GenePharma Co., Ltd.) by which was incubated for 6 h.[33] Phosphate-buffered saline was utilized to wash the cells, and quantitative polymerase chain reaction (qPCR) was used to analyze the expression level of miR-126.

## Quantitative polymerase chain reaction analysis

qPCR was performed using SYBRGreen Master Mix (SYBR Premix Ex Tag TMI; TaKaRa Bio; http://www.takara-bio.com), according to the manufacturer's instructions (TaKaRa Biotechnology). qPCR was performed using a Prism 7500 Sequence Detector (Applied Biosystems). The thermal profile for the qRT-PCR was 2 min at 95°C followed by 40 cycles of 15 s at 95°C, 35 s at 60°C, and 5 min at 72°C. Each sample was tested in triplicate. The primers (Invitrogen, USA) are as follows: GAPDH: Forward, 5'-GCCAAGGCTGTGGGCAAGGT-3', Reverse, 5'-TCTCCAGGCGGCACGTCAGA-3' and the DNA template was 1 μg. Relative gene expression was calculated using the formula $2^{-\Delta\Delta Ct}$. For normalization of gene expression, GAPDH was used as an internal standard of mRNA expression and the normal group was used as a reference sample, which was set to 1.

## Western blotting

The total protein was extracted from cell extracts using RIPA lysis buffer (Beyotime Institute of Biotechnology, China) following the manufacturer's instructions. Protein concentrations were determined with the bicinchoninic acid protein assay kit. Equal amounts of protein were separated by dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (Nanjing Keygen Biotech Co., Ltd.) and transferred to polyvinylidene fluoride membranes. Membranes were incubated overnight at 4°C with primary antibodies: IRS-1, IRS-2, AKT, PI3K, AMPKα, PPARα, PEPCK, G6Pase, Fas, SREBP-1C, SREBP-2, and DGAT2 (1:1000, Epitomics, USA) and then washed and incubated with horseradish peroxidase-conjugated secondary antibodies. The immunoreactive bands were visualized by enhanced chemiluminescence reagents (BioWorld, USA) and were then quantified by densitometric analysis.

## Statistical analysis

All experimental results were expressed as mean ± standard deviation and analyzed by SPSS 19.0 software (SPSS, Inc., Chicago, IL,USA). Statistical significance of all data was determined using *t*-test and one-way ANOVA. $P < 0.05$ was considered statistically significant.

# RESULTS

## Effects of AP on glucose metabolism in diabetic rats

AP and pioglitazone groups could lower FBG levels, FINS, GSP, and HbA1c compared to that in STZ-induced DM group, and the effect was best in the highest dose of AP compared to middle and low dose of AP. Furthermore, AP treatment may increase the level of ISI in diabetic rats [Table 1]. The effect of AP treatment in high- and middle-dose group was significantly improved ($P < 0.01$). The OGTT of diabetic rats is shown in Table 2; the blood glucose levels in DM group were significantly higher than that in normal group.

## Effects of AP on lipid metabolism in diabetic rats

As shown in Table 3, the effect of AP on lipid profiles was analyzed. TG, TC, and LDL were significantly decreased in AP group, compared to that in STZ-induced DM group. However, the HDL level was reversed. As shown in Figure 1, the livers of DM animals exhibited cell swelling, vacuolation, and infiltration of lymphocytes. AP treatment could significantly reduce the phenomenon, which means that AP can decrease the accumulation of liver tissues resulted from DM and can have the effect of protecting the livers.

**Table 2:** Effects of AP on oral glucose tolerance test of diabetic rats ($\bar{x}$±s, $n$=8)

| Group | Glucose (mmol/L) | | | | Area under curve (mmol h/L) |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120 min | |
| Normal | 5.76±0.68** | 9.73±1.65** | 8.84±1.57** | 5.95±0.35** | 15.91±0.78** |
| STZ-induced DM | 26.63±3.89 | 32.08±3.27 | 31.57±4.96 | 29.38±2.67 | 61.07±1.43 |
| STZ-induced DM + pioglitazone (15 mg/kg) | 8.91±2.96** | 29.87±2.69 | 25.82±2.78** | 13.26±2.72** | 43.16±1.98** |
| STZ-induced DM + AP (400 mg/kg) | 12.84±7.04** | 31.56±1.89 | 25.64±2.43* | 15.33±2.12** | 45.39±3.42** |
| STZ-induced DM + AP (200 mg/kg) | 15.92±3.28** | 30.75±3.15 | 30.45±2.01 | 27.45±2.45* | 54.76±3.28 |
| STZ-induced DM + AP (100 mg/kg) | 18.67±8.23** | 31.43±2.83 | 30.06±5.12 | 27.66±2.98 | 56.92±2.19 |

*$P$<0.05 and **$P$<0.01 versus STZ-induced DM group. STZ: Streptozotocin; DM: Diabetes mellitus; AP: *Alisma* polysaccharide

**Table 3:** Effects of *Alisma* polysaccharide on triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein

| Group | TG (mmol/L) | TC (mmol/L) | LDL (mmol/L) | HDL (mmol/L) |
|---|---|---|---|---|
| Normal | 0.85±0.10** | 1.64±0.28** | 0.67±0.23** | 1.11±0.21** |
| STZ-induced DM | 2.01±0.15 | 4.06±1.19 | 6.11±1.42 | 0.64±0.45 |
| STZ-induced DM + pioglitazone (15 mg/kg) | 1.09±0.08** | 2.38±0.58* | 5.53±2.82** | 0.95±0.31** |
| STZ-induced DM + AP (400 mg/kg) | 1.11±0.04** | 2.85±0.51** | 2.96±2.68** | 0.94±0.18** |
| STZ-induced DM + AP (200 mg/kg) | 1.38±0.05** | 3.13±0.25** | 4.08±2.48** | 0.86±0.23* |
| STZ-induced DM + AP (100 mg/kg) | 1.94±0.11 | 3.49±0.63 | 5.67±3.19* | 0.87±0.33* |

*$P<0.05$; **$P<0.01$ versus STZ-induced DM group. STZ: Streptozotocin; DM: Diabetes mellitus; AP: *Alisma* polysaccharide; TG: Triglyceride; TC: Total cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein

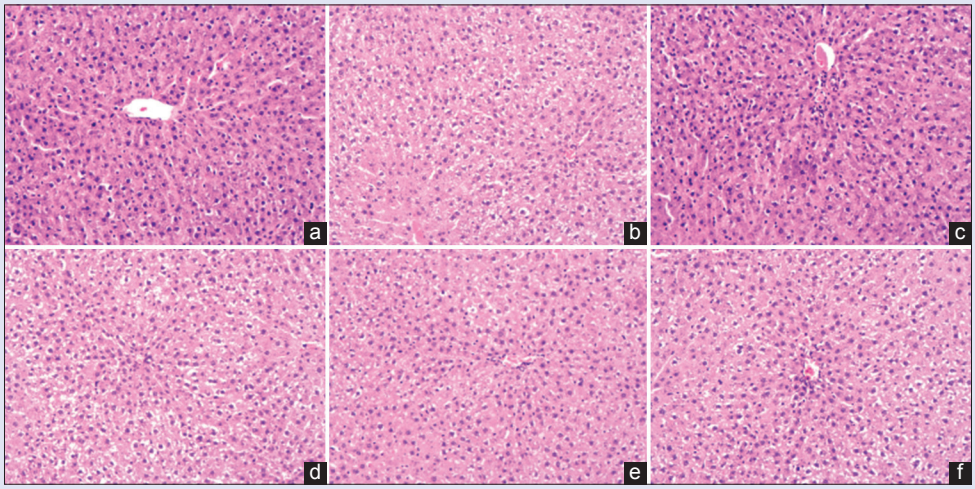


**Figure 1:** Effect of AP on the histological morphology of livers by hematoxylin-eosin staining. (a) control, (b) model, (c) pioglitazone, (d) AP (400 mg/kg), (e) AP (200 mg/kg), (f) AP (100 mg/kg)

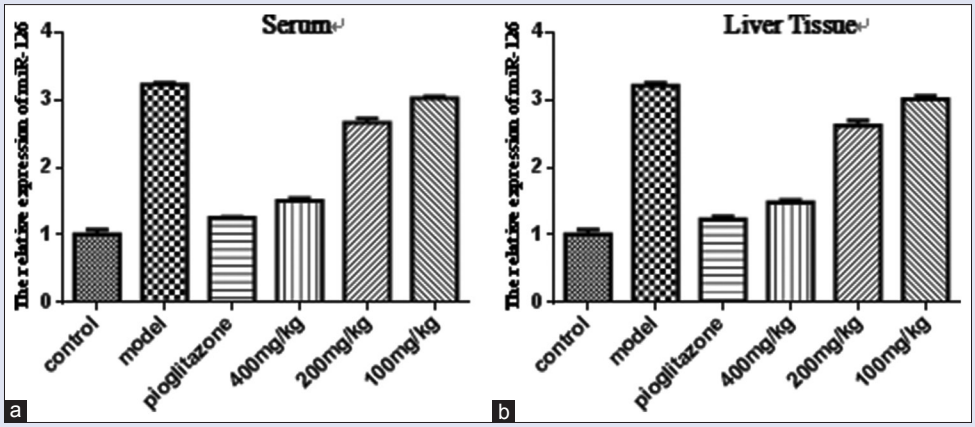


**Figure 2:** The relative expression of miR-126 in six groups in serum (a) and liver tissue (b)

## Effects of AP on miR-126 in diabetic mice

qPCR was used to detect miR-126 levels in serum and liver tissue. The results showed that after treatment with AP, the expression of miR-126 levels was obviously lower than those in the DM group. These findings indicated that AP could ameliorate glucose–lipid metabolism through regulating the expression level of miR-126 in the diabetic mice [Figure 2].

## Effects of AP on downregulation of miR-126 related to glucose–lipid metabolism

As shown in Figure 3, western blot was used to detect the protein expression level of the genes related to glucose and lipid metabolism affected by AP. This result indicated that AP can upregulate the expression of IRS-2, AKT, and AMPKα, reducing the protein expression of PEPCK and G6Pase, PPARα, FAS, and SREBPs.

Western blot was used to analyze the treatment of HepG2 cell with miR-126 in the presence of miR-126 inhibitor or mimic protein. As shown in Figure 4, the miR-126 inhibitor could inhibit the expression of PEPCK, G6Pase, SREBP-1C, FAS, and DGAT2, while enhancing the expression of IRS-2 and AMPK. The expression of PEPCK and G6Pase was significantly increased by the presence of a miR-126 mimic, whereas AP treatment could abolish this activation.

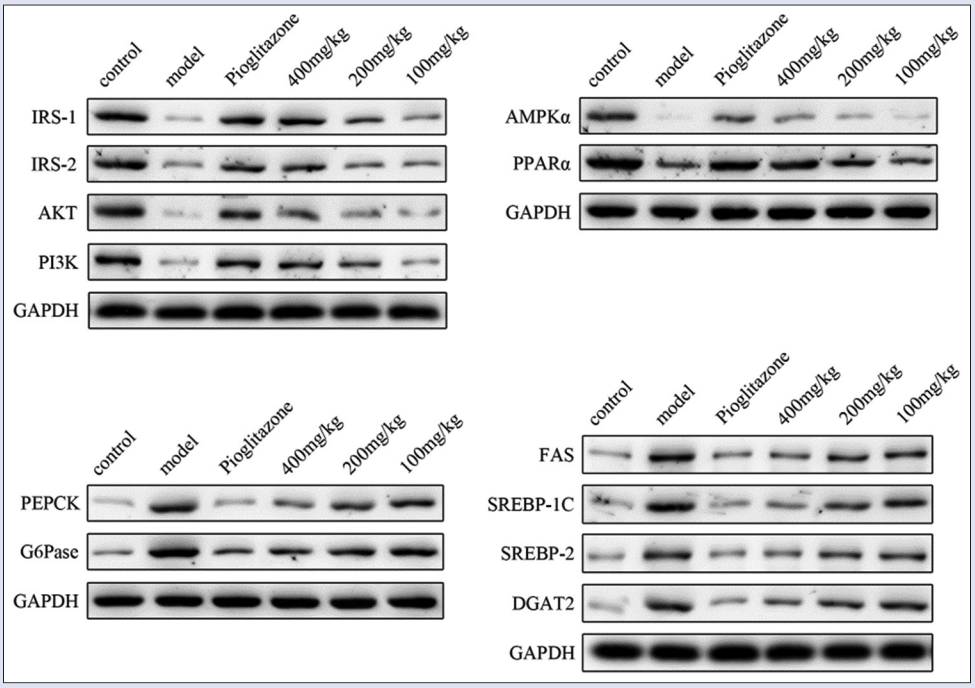


**Figure 3:** The protein expression of the genes related to glucose and lipid metabolism affected by AP in the liver

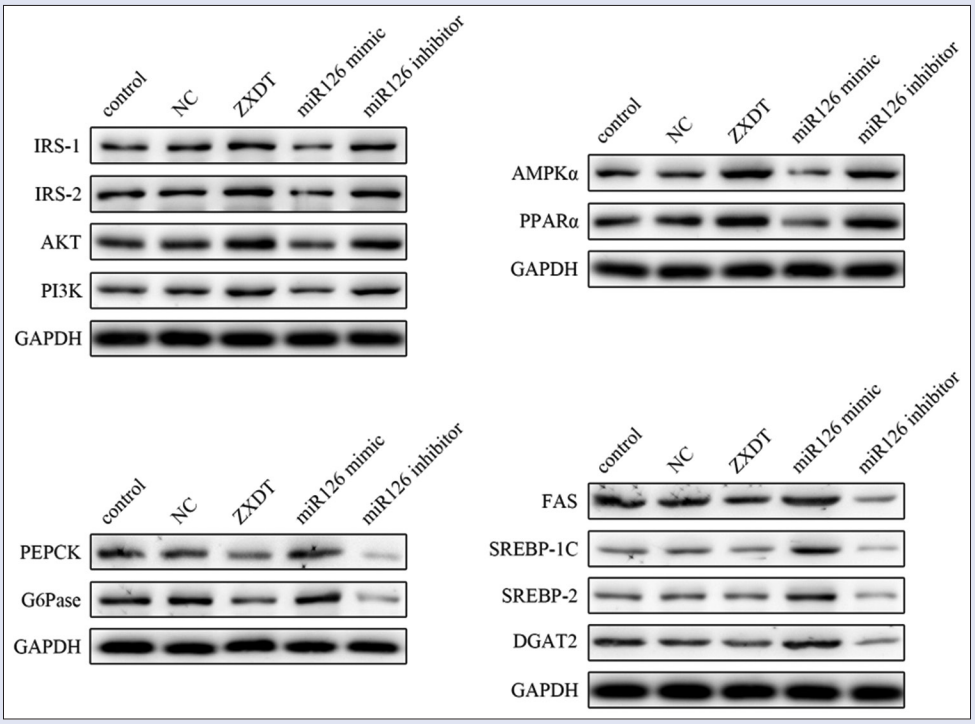


**Figure 4:** Effect of AP on miR-126 in the protein expression of the genes related to glucose and lipid metabolism

## DISCUSSION

Our work is the first study to comprehensively and systematically explore the effect of AP on miR-126 in diabetic mice. The results showed that the contents of FBG, FINS, GSP, and HbAlc in STZ-induced DM group were significantly higher than that in normal and AP group and the contents of these four indexes were lowest in normal group compared to other groups, while AP treatment can significantly increase the level of ISI compared to STZ-induced DM group. The OGTT level in AP treatment was lower than that in STZ-induced DM group, while the OGTT level in group normal was the lowest. AP also can significantly reduce the contents of TC, TG, and LDL compared to STZ-induced DM, and it had an obvious quantitative effect relationship. Furthermore, the contents of TC, TG, and LDL in normal group were significantly lower than that in

AP treatment group, but the content of HDL was reversed. According to the histological findings, the AP treatment could ameliorate livers of DM animals with cell swelling, vacuolation, and infiltration of lymphocytes. Therefore, AP could significantly reduce the blood glucose and regulate blood lipids, improving the disorders of glucose–lipid caused by hyperglycemia.

AP can upregulate the expression of IRS-2[34] and AKT[35] to promote the utilization and intake of glucose, reducing gluconeogenesis to regulate and improve the disorder of glucose metabolism in the liver. AP treatment may reduce the protein expression of PEPCK and G6Pase to restrain gluconeogenesis,[36-38] upregulating the expression of AMPKα to decrease the expression of downstream genes, including PPARα, FAS, and SREBPs, reducing the synthesis of fatty acid glycerin, triglycerin, and cholesterol in the liver.

The miR-126 inhibitor can inhibit the expression of miR-126, whereas miR-126 mimic could enhance the expression of miR-126. AP treatment could reduce the expression of PEPCK and G6Pase to restrain gluconeogenesis. The expression of AMPK was decreased with miR-126 mimic while AP treatment could active the expression, which indicated that AP treatment can activate the AMPK phosphorylation of energy metabolism.[39] The expression of IRS-2 was decreased with a miR-126 mimic, whereas AP treatment could increase the expression level of IRS-2 to promote the utilization and intake of glucose in the liver,[40] improving the glucose metabolism. The expressions of SREBP-1C, FAS, and DGAT2[41] were significantly increased with a miR-126 mimic, while AP treatment could reduce this activation to improve the lipid metabolism.

Although the role of miRNA in glucose–lipid metabolism disorders has been researched, it is not clear that if miR-126 is associated with glucose–lipid metabolism in diabetic mice. In this study, we found that the expression level of miR-126 was increased in diabetic mice. In contrast, AP could significantly decrease the expression of miR-126. Furthermore, to demonstrate that AP can regulate genes related to glucose–lipid metabolism directly through miR-126, the results indicated that AP could eliminate the regulation of miR-126 mimic in genes associated with glucose–lipid metabolism in HepG2 cell. This was a new mechanism for AP action in the regulation of glucose–lipid metabolism. Therefore, AP can directly decrease the expression of miR-126 in diabetic mice and in HepG2 cell, affecting glucose–lipid metabolism in the liver.

## CONCLUSION

In conclusion, we found that AP could downregulate the level of miR-126 in serum and liver of diabetic mice, regulating glucose–lipid metabolism in diabetic mice. Furthermore, AP could directly impact the expression level of miR-126 for glucose–lipid metabolism in HepG2 cell. Our study also provides a potential therapeutic application of *Alisma* polysaccharide in DM.

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### Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2004;27 Suppl 1:S5-10.
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
3. Chen R. Analysis of curative effect and drug side effect by glucobay and metformin in the treatment of type 1 diabetes mellitus. China Prac Med 2015;10:28-9.
4. Wu JR, Wang KH, Ji K, Zhang D, Zhang XM, Yang B, *et al.* Medication rules for treating diabetes based on data mining. Chin J Exp Tradit Med Formul 2015;22:214-7.
5. Sun GD, Li CY, Cui WP, Guo QY, Dong CQ, Zou HB, *et al.* Review of herbal traditional Chinese medicine for the treatment of diabetic nephropathy. J Diabetes Res 2016;2016:5749857.
6. National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. Part 1. Beijing: Chemical Industry Press; 2015. p. 229.
7. Zhou X, Xu TH, Liu YH. *Alisma orientale* (Sam.) Juzep., a common traditional Chinese medicine plant as a diuretic agent. Ethnomed Source Mech II 2010;28:461-80.
8. Tian T, Chen H, Zhao YY. Traditional uses, phytochemistry, pharmacology, toxicology and quality control of *Alisma orientale* (Sam.) juzep: A review. J Ethnopharmacol 2014;158 (Pt A):373-87.
9. Dan H, Wu J, Peng M, Hu X, Song C, Zhou Z, *et al.* Hypolipidemic effects of alismatis rhizome on lipid profile in mice fed high-fat diet. Saudi Med J 2011;32:701-7.
10. Qin JG, Wang YH, Liang JP, Zhang Y, Wang SR, Guo WQ. Effect of *Alismaceae* on regulating the expression of liver basal membrane heparan sulfate proteoglycan in ApoE-knock out atherosclerosis mice. Chin Arch Tradit Chin Med 2007;25:696-8.
11. Cao ZG, Liu JH, Yin CP, Zhu YP, Zhou SW, Wu JZ, *et al.* Effect of active constituents of *Alisma orientalis* on bikunin expression in a rat urolithiasis model. China J Modern Med 2006;11:1601-5.
12. Kubo M, Matsuda H, Tomohiro N, Yoshikawa M. Studies on alismatis rhizoma. I. Anti-allergic effects of methanol extract and six terpene components from alismatis rhizoma (dried rhizome of *Alisma orientale*). Biol Pharm Bull 1997;20:511-6.
13. Li Q, Qu H. Study on the hypoglycemic activities and metabolism of alcohol extract of alismatis rhizoma. Fitoterapia 2012;83:1046-53.
14. Fong WF, Wang C, Zhu GY, Leung CH, Yang MS, Cheung HY. Reversal of multidrug resistance in cancer cells by rhizoma alismatis extract. Phytomedicine 2007;14:160-5.
15. Han CW, Kwun MJ, Kim KH, Choi JY, Oh SR, Ahn KS, *et al.* Ethanol extract of alismatis rhizoma reduces acute lung inflammation by suppressing NF-κB and activating nrf2. J Ethnopharmacol 2013;146:402-10.
16. Yang XB, Huang ZM, Chen HY, Wei YP, Wang JH, Cao WB, *et al.* Effects of different solvent extracts from *Alisma orientalis* on blood glucose and biochemical indicators in diabetic mice. Pharm J China PLA 2006;6:419-21.
17. Yang XB, Huang ZM, Cao WB, Chen HY, Tiand WH, Wang JH. Effect of extract from rhizoma alismatis on normal and alloxan midel in mice. Chin J Exp Tradit Med Formul 2002;8:24-6.
18. Yang XB, Huang ZM, Cao WB, Chen HY, Zhen M, Zhang JZ. Effects of Rhizoma alismatis (RA) on blood glucose in normal and diabetes mice. Pharm Clin Chin Mater Med 1998;14:29-30.
19. Yang XB, Huang ZM, Cao WB, Chen HY, Wang JH, Xu L. Therapeutic and protective effects of water-ethanolic extract from Rhizoma Alismatis on streptozotocin-induced diabetic mice. Pharm J Chin PLA 2002;18:336-9.
20. Vimalraj S, Miranda PJ, Ramyakrishna B, Selvamurugan N. Regulation of breast cancer and bone metastasis by microRNAs. Dis Markers 2013;35:369-87.
21. Wang L, Wang J. MicroRNA-mediated breast cancer metastasis: From primary site to distant organs. Oncogene 2012;31:2499-511.
22. Liu X, Gong J, Xu B. MiR-143 down-regulates TLR2 expression in hepatoma cells and inhibits hepatoma cell proliferation and invasion. Int J Clin Exp Pathol 2015;8:12738-47.
23. Simpson K, Wonnacott A, Fraser DJ, Bowen T. MicroRNAs in diabetic nephropathy: From biomarkers to therapy. Curr Diab Rep 2016;16:35.
24. Feng J, Xing W, Xie L. Regulatory roles of microRNAs in diabetes. Int J Mol Sci 2016;17. pii: E1729.
25. Belgardt BF, Ahmed K, Spranger M, Latreille M, Denzler R, Kondratiuk N, *et al.* The microRNA-200 family regulates pancreatic beta cell survival in type 2 diabetes. Nat Med 2015;21:619-27.
26. Wei S, Zhang M, Yu Y, Lan X, Yao F, Yan X, *et al.* Berberine attenuates development of the hepatic gluconeogenesis and lipid metabolism disorder in type 2 diabetic mice and in palmitate-incubated HepG2 cells through suppression of the HNF-4α miR122 pathway. PLoS

One 2016;11:e0152097.

27. Dey N, Das F, Mariappan MM, Mandal CC, Ghosh-Choudhury N, Kasinath BS, *et al.* MicroRNA-21 orchestrates high glucose-induced signals to TOR complex 1, resulting in renal cell pathology in diabetes. J Biol Chem 2011;286:25586-603.
28. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, *et al.* Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 2010;107:810-7.
29. Xia Q, Liu G, Ge CL, Xu DJ. Removal of proteins from crude astragalus polysaccharides by sevag method. Anhui Med Pharm J 2007;11:1069-71.
30. Jang MK, Han YR, Nam JS, Han CW, Kim BJ, Jeong HS, *et al.* Protective effects of *Alisma orientale* extract against hepatic steatosis via inhibition of endoplasmic reticulum stress. Int J Mol Sci 2015;16:26151-65.
31. Yang XB, Huang ZM, Chen HY, Wei YP, Wang JH, Cao WB, *et al.* Effects of different solvent extracts from *Alisma orientalis* on blood glucose and biochemical indicators in diabetic mice. Pharm J Chin PLA 2006;22:419-21.
32. Li W, Zhang M, Gu J, Meng ZJ, Zhao LC, Zheng YN, *et al.* Hypoglycemic effect of protopanaxadiol-type ginsenosides and compound K on type 2 diabetes mice induced by high-fat diet combining with streptozotocin via suppression of hepatic gluconeogenesis. Fitoterapia 2012;83:192-8.
33. Zhang XR, Fu XJ, Zhu DS, Zhang CZ, Hou S, Li M, *et al.* Salidroside-regulated lipid metabolism with down-regulation of miR-370 in type 2 diabetic mice. Eur J Pharmacol 2016;779:46-52.
34. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, *et al.* Disruption of IRS-2 causes type 2 diabetes in mice. Nature 1998;391:900-4.
35. Kang C, Jin YB, Lee H, Cha M, Sohn ET, Moon J, *et al.* Brown alga *Ecklonia cava* attenuates type 1 diabetes by activating AMPK and AKT signaling pathways. Food Chem Toxicol 2010;48:509-16.
36. Zhang LN, Xu L, Zhou HY. Diabetes, metabolic syndrome, AMPK. PLoS One 2013;8:1-10.
37. Sutherland C, O'Brien RM, Granner DK. New connections in the regulation of PEPCK gene expression by insulin. Philos Trans R Soc Lond B Biol Sci 1996;351:191-9.
38. Gomis RR, Favre C, García-Rocha M, Fernández-Novell JM, Ferrer JC, Guinovart JJ. Glucose 6-phosphate produced by gluconeogenesis and by glucokinase is equally effective in activating hepatic glycogen synthase. J Biol Chem 2003;278:9740-6.
39. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci U S A 1999;96:13656-61.
40. Brindley DN. Role of glucocorticoids and fatty acids in the impairment of lipid metabolism observed in the metabolic syndrome. Int J Obes Relat Metab Disord 1995;19 Suppl 1:S69-75.
41. Stone SJ, Myers HM, Watkins SM, Brown BE, Feingold KR, Elias PM, *et al.* Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. J Biol Chem 2004;279:11767-76.