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Trabajo Original

Obesidad y síndrome metabólico

Anti-diabetic effects of *Inonotus obliquus* extract in high fat diet combined streptozotocin-induced type 2 diabetic mice

Efectos antidiabéticos del extracto de Inonotus obliquus *en ratones diabéticos tipo 2 inducidos por estreptozotocina combinada con una dieta rica en grasas*

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Abstract

Introduction: type 2 diabetes (T2DM) is a complex disease affected by lifestyle and genetic factors. Although the drugs currently used to treat T2DM have certain curative effects, they still have some adverse side effects. Therefore, it is urgent to find new effective drugs with few side effects to cure T2DM.

Objective: to study the role of *Inonotus obliquus* (IO) in diabetic model mice.

Methods: we used high-fat diet (HFD) combined with streptozocin (STZ) to establish a diabetic mouse model. Mice were divided into non-high-fat diet group (ND), diabetes model group (HFD + STZ) and IO-treated diabetes model group (IO). The mice in the IO group were orally treated with IO (150 mg/kg) at 10 ml/kg for five weeks. Body weight, glucose level, food intake and water consumption, glucose tolerance and insulin tolerance were evaluated in all mice. The pathological sections of liver, kidney and pancreas were observed by hematoxylin-eosin staining.

Results: after IO administration, the blood glucose level, water consumption, low-density lipoprotein (LDL) and triacylglycerol (TG) levels of mice decreased. Compared with the HFD + STZ group, the number of normal islet β cells increased and focal necrosis of the liver was significantly reduced in the IO administration group.

Conclusions: IO reduced the levels of blood glucose, restored body weight, and enhanced insulin sensitivity along with insulin tolerance and glucose tolerance in diabetic mice. Additionally, IO also reversed HFD and STZ-induced organ injury.

Keywords:

Inonotus obliquus. Type 2 diabetic mice. Anti-dyslipidemia. Insulin sensitivity.

Resumen

Introducción: la diabetes *mellitus* tipo 2 (T2DM) es una enfermedad compleja influenciada por el estilo de vida y los factores genéticos. En la actualidad, aunque los medicamentos para la diabetes tipo 2 tienen cierto efecto curativo, todavía tienen algunos efectos secundarios. Por lo tanto, es urgente encontrar nuevos medicamentos para la diabetes tipo 2 que tengan un buen efecto curativo y menos efectos secundarios.

Objetivo: estudiar el papel del *Inonotus obliquus* (IO) en ratones diabéticos.

Métodos: se estableció un modelo de ratón diabético con dieta de alto contenido en grasas (HFD) y estreptozocina (STZ). Los ratones se dividieron en el grupo de dieta no alta en grasas (ND), el grupo modelo de diabetes *mellitus* (HFD + STZ) y el grupo modelo de diabetes *mellitus* tratado con IO. Los ratones del grupo IO recibieron 10 ml/kg de IO (150 mg/kg) durante cinco semanas. Se observaron el peso corporal, el nivel de azúcar en sangre, la ingesta de alimentos, la ingesta de agua potable, la tolerancia a la glucosa y la tolerancia a la insulina de los ratones de cada grupo, y se estudiaron muestras de biopsias hepáticas, renales y pancreáticas mediante tinción de hematoxilina eosina.

Resultados: los niveles de glucosa en sangre, el consumo de agua, la lipoproteína de baja densidad (LDL) y los triglicéridos (TG) disminuyeron después de la administración de IO. En comparación con el grupo HFD+STZ, el número de células β pancreáticas normales y la necrosis focal hepática disminuyeron significativamente en el grupo IO.

Conclusiones: el IO redujo el nivel de glucosa en sangre, ayudó a recuperar el peso corporal y mejorar la sensibilidad a la insulina, la tolerancia a la insulina y la tolerancia a la glucosa en ratones diabéticos. Además, el IO revirtió el daño orgánico inducido por HFD y STZ.

Palabras clave:

Inonotus obliquus. Ratones diabéticos tipo 2. Antidislipidemia. Sensibilidad a la insulina.

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INTRODUCTION

Diabetes is a chronic disease caused by many factors, and its prevalence has increased significantly in recent years (1). It is estimated that, by 2045, 629 million people will be affected by diabetes, and 90 % to 95 % of them will have type 2 diabetes (T2DM) (2). Due to the increase in childhood obesity, the incidence of T2DM has increased significantly among adolescents, which has brought a great social burden (3). T2DM is mainly caused by decreased insulin sensitivity, and its symptoms are insufficient insulin secretion and insulin resistance (4). At present, drugs for the treatment of T2DM mainly focus on mechanisms such as promoting insulin secretion and inhibiting sodium-glucose cotransporter 2 (5). Although these drugs have certain curative effects, there are also some adverse reactions (6).

Traditional Chinese medicine has been practiced in China for thousands of years, and has accumulated rich experience in the treatment of various diseases (7). Compared with Western medicine, traditional Chinese medicine has a lower risk of inducing adverse reactions (8). Moreover, traditional Chinese medicine has mild properties, and it is considered safe to consume them as a preventive treatment for chronic diseases (9). Traditional Chinese medicine has shown great potential in the treatment of various complex diseases in modern society, such as cardiovascular diseases, infectious diseases, metabolic diseases and neurodegenerative diseases (10). A number of studies have shown that Chinese medicine extracts have the effect of treating T2DM (11,12).

Inonotus obliquus (IO), a medicinal mushroom, is mainly distributed in the sub-cold and cold regions of the Northern hemisphere, such as in Finland (13). Recent studies have confirmed that IO contains more than 200 different types of biologically active ingredients, including polysaccharides, triterpenes, flavones, sterols, polyphenols, melanin, etc., which implies that IO possesses multiple pharmacological effects identical to TCM (14,15). The oral administration of crude polysaccharides extracted from IO has been found to improve the symptoms of streptozocin (STZ)-induced diabetes in rats (16). Thus, IO has several pharmacological effects as a fungus, used both as medicine and food product, which has important clinical value in drug development.

This study explored the role of IO in diabetic mice induced by the combination of high-fat diet (HFD) and STZ, and analyzed the effects of IO on the body weight, blood sugar, blood lipids, insulin sensitivity and organs of diabetic mice.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

STZ was obtained from Sigma Aldrich (USA). The Jiancheng Bioengineering Institute (Nanjing, China) provided biochemical kits for estimating the levels of low-density lipoprotein (LDL), glycogen, insulin, triacylglycerol (TG), total cholesterol (TCHO) and high-density lipoprotein (HDL). The remaining chemicals were analytical-grade reagents. A glucometer was used to estimate blood glucose levels.

ANIMALS

C57BL6/J male mice (age: six weeks) were procured from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were acclimatized for one week to a relative humidity of 55 ± 15 %, light/dark cycle (08:00/20:00), temperature of 23 ± 3 °C, ventilation frequency of 10-20 times/hour, and luminous intensity of 150-300 Lux in the animal room of Center for Drug Safety Evaluation and Research, Zhengzhou University, whose Institutional Animal Care and Use Committee (IACUC) sanctioned all experimental procedures.

EXPERIMENT DESIGN

In this study, mice were first divided into two parts, labeled ND group and diabetes group. The mice in the ND group were fed only normal diet, the mice in the diabetes group were fed HFD (60 kcal % fat) for five weeks, and then an injection of a fresh preparation of 1 % STZ (40 mg/kg) was administered intraperitoneally, thrice a week, in the dark (to avoid inactivation) to induce diabetes in the mice. Next, on days 3, 6, and 9, a drop of tail blood was used to estimate the blood glucose levels (Yuyell, Jiangsu, China). Mice that had a blood glucose > 11.1 mM were labeled as diabetic. Diabetic mice were divided into groups and labeled as HFD+STZ group and HFD+STZ+IO (IO) group (n = 7 in each group). Next, the mice in the IO group were orally treated with IO (150 mg/kg) at 10 ml/kg for five weeks. The corresponding ND and STZ+HFD group were administered a solution of dimethyl sulfoxide (DMSO) and water. Body weight, fasting blood glucose levels, food consumption, and water consumption were measured on a weekly basis. After the five-week measurement period, we performed the oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). After all experiments, all mice were anesthetized with ether and the mice were sacrificed. Fresh blood was obtained from mice and serum was collected by centrifugation; in addition, liver, kidney, and pancreas tissues of mice were obtained for pathological observation.

GLUCOSE TOLERANCE TEST (GTT)

On the third day before the end of the experiment, GTT was done post-overnight fasting in mice. After the measurement of fasting blood glucose levels, the mice were orally administrated an aqueous glucose solution (2 g/kg). The blood glucose levels in the tail vein blood samples were measured at 30, 60, and 90 minutes. Blood glucose change curve was drawn at each time point and the area under the curve (AUC) was calculated.

INSULIN TOLERANCE TESTS (ITT)

On the day before the end of the experiment, ITT was performed after a four-hour fasting period (9:00 a.m.-13:00 p.m.). After the measurement of fasting blood glucose, the mice were

injected with insulin (0.5 U/kg) intraperitoneally. The blood glucose levels in the tail vein blood samples were measured at 15, 30, 45, and 60 minutes. The blood glucose change curve was drawn at each time point and the area under the curve (AUC) was calculated.

THE MEASUREMENT OF BLOOD LIPID CONTENT IN SERUM

ELISA kit was used to detect the content of LDL, HDL, TG and TCHO in mouse serum. All experimental procedures were conducted following the manufacturer's guidelines.

HISTOPATHOLOGY

The mice tissue specimens were fixed in 4 % paraformaldehyde for a minimum of 24 hours. The paraffin-embedded tissues were divided into sections with 4-5 μm thickness. The H&E stained specimens were examined microscopically.

STATISTICAL ANALYSIS

The data are expressed as mean ± standard deviation (SD). A statistical comparison of the data was performed using one way or two-way analysis of variance followed by t-tests in SPSS 21.0 for Windows to evaluate differences between the groups. A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

EFFECTS OF IO ON BODY WEIGHT AND GLUCOSE LEVEL

By recording the body weight of the mice in each week, we found that the body weight of the mice in the HFD+STZ group was slightly higher than that of the other two groups in the first week of administration. After that, the body weight of the mice in the HFD+STZ group began to show a downward trend, and gradually lower than the ND group. As time went by, the weight difference between the ND group and HFD+STZ group mice became more and more obvious. The IO administration reduced the difference in body weight and increased the body weight of the diabetes model mice, making the body weight closer to the ND group (Fig. 1). During treatment, we observed that blood glucose levels of the ND group mice were normal, while the HFD+STZ group mice were in a hyperglycemic state. IO administration significantly reduced the high blood glucose level of mice induced by HFD combined with STZ ($p < 0.01$) (Fig. 2). These results indicated that IO intake can improve weight loss and increased blood sugar levels in diabetic mice.

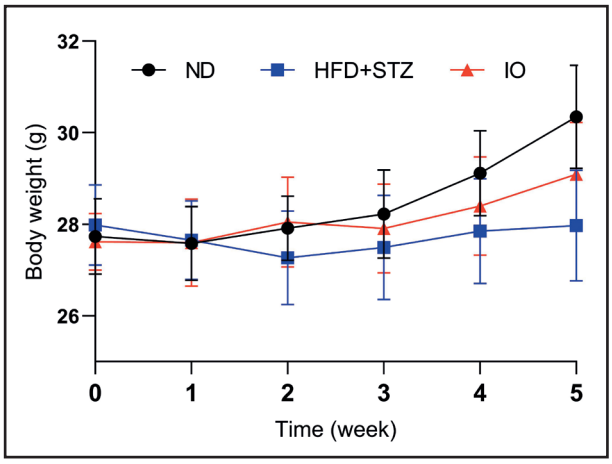

Figure 1.

Mice body weight changes. ND: no high-fat diet; HFD + STZ: high-fat diet + streptozocin; IO: high-fat diet + streptozocin + IO.

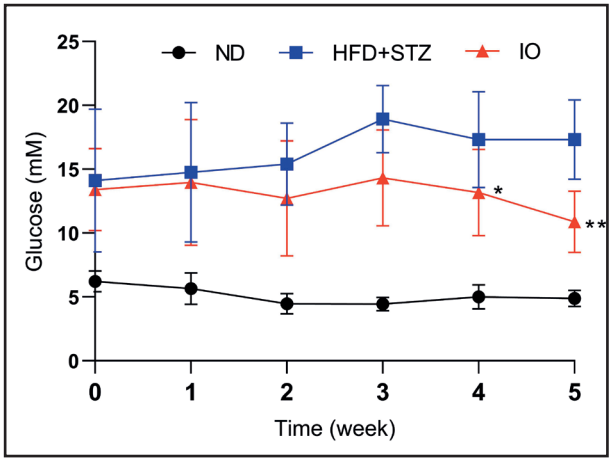

Figure 2.

Mice glucose level. *$p < 0.05$ as compared to the HFD + STZ group. **$p < 0.01$ as compared to the HFD + STZ group. ND: no high-fat diet; HFD + STZ: high-fat diet + streptozocin; IO: high-fat diet + streptozocin + IO.

EFFECT OF IO ON FOOD INTAKE AND WATER CONSUMPTION

By examining the diet of mice, we found that the food intake and water consumption of the diabetic model mice were higher than normal. Compared with the HFD+STZ group, IO administration slightly reduced food intake but did not produce a significant difference (Fig. 3A). After three weeks of IO administration, the water consumption of diabetic mice was significantly reduced ($p < 0.01$) (Fig. 3B). IO administration improved the symptoms of diabetes in mice to a certain extent.

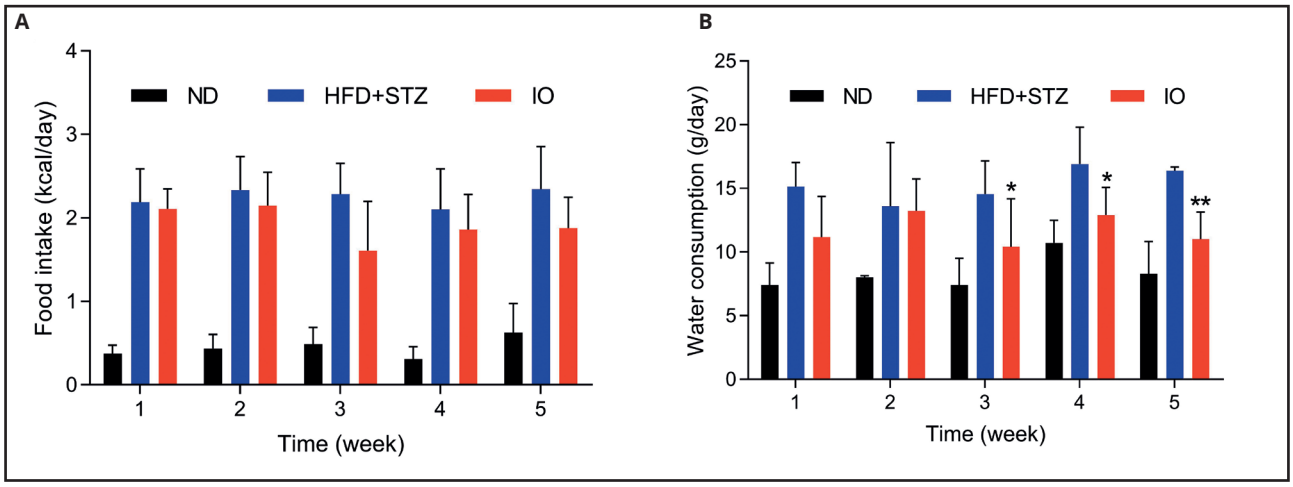

Figure 3.

Consumption of food and water by mice. A. Food intake of mice. B. Mice water consumption changes. *$p < 0.05$ as compared to the HFD + STZ group. **$p < 0.01$ as compared to the HFD + STZ group. ND: no high-fat diet; HFD + STZ: high-fat diet + streptozocin; IO: high-fat diet + streptozocin + IO.

EFFECTS OF IO ON GLUCOSE TOLERANCE AND INSULIN TOLERANCE

Next, we tested the effect of IO extract on glucose tolerance and insulin tolerance in mice before the end of the study. We found that after 30 minutes of glucose administration, the blood glucose level of mice in the IO group decreased faster than that of the HFD+STZ group, and showed a statistical difference at 60 minutes ($p < 0.01$). IO administration significantly reduced the area under the curve (AUC) (Fig. 4A and B). Similarly, after insulin injection, compared with the HFD+STZ group, the blood glucose level of the IO group decreased faster, but the AUC results did not show a difference (Fig. 4C and D). IO administration helped to improve glucose tolerance and insulin resistance of the diabetic mice.

IO CAN IMPROVE SERUM LIPID HOMEOSTASIS AND INSULIN SENSITIVITY IN MICE

By testing the blood lipid levels in the serum of mice, we found that, compared with the ND group, the HFD+STZ group mice had a higher level of LDL, which was significantly reduced after oral administration of IO ($p < 0.05$) (Fig. 5A). In addition, the level of HDL also increased significantly after IO administration (Fig. 5B). Similarly, compared with the ND group, the HFD+STZ group mice showed higher levels of TG and TCHO, as well as IO administration significantly reduced the TG content of diabetic mice ($p < 0.01$) (Fig. 5C). However, IO administration did not change the level of TCHO (Fig. 5D). In addition, a slight decrease in serum insulin levels after IO administration was observed (Fig. 6). Combined with the results of insulin tolerance, we believe that IO administration could improve insulin sensitivity in mice. These results indicated that IO significantly improved the blood lipid levels of diabetic mice.

IO CAN IMPROVE THE STATUS OF ORGANS IN DIABETIC MICE

Next, pathological analysis on the liver, kidney, and pancreas tissues of the mice was performed. Compared with the ND group, the livers of diabetic mice induced by HFD combined with STZ had more white lipid droplets, which were regular in shape and larger in size. The boundary between cell and cell is not clear, and the nucleus is squeezed to one side, accompanied by inflammatory cell infiltration. This may indicate the production of fatty liver and fatty degeneration of liver cells. IO significantly improved this pathological state, reducing the accumulation of lipid droplets and inflammatory cell infiltration in the liver. In the kidney tissue, mice in the HFD+STZ group showed infiltration of renal interstitial inflammatory cells. In pancreatic tissue, mice in the HFD+STZ group showed central venous congestion and a decrease in the number of islet β cells. IO administration reduced the infiltration of inflammatory cells in the kidney and increased the number of islet β cells with normal morphology (Fig. 7). These results indicated that IO can improve the pathological state of various organs in diabetic mice.

DISCUSSION

As a chronic disease, T2DM is a risk factor for hypertension and cardiovascular disease in addition to its health hazards (17). The main features of T2DM are peripheral insulin

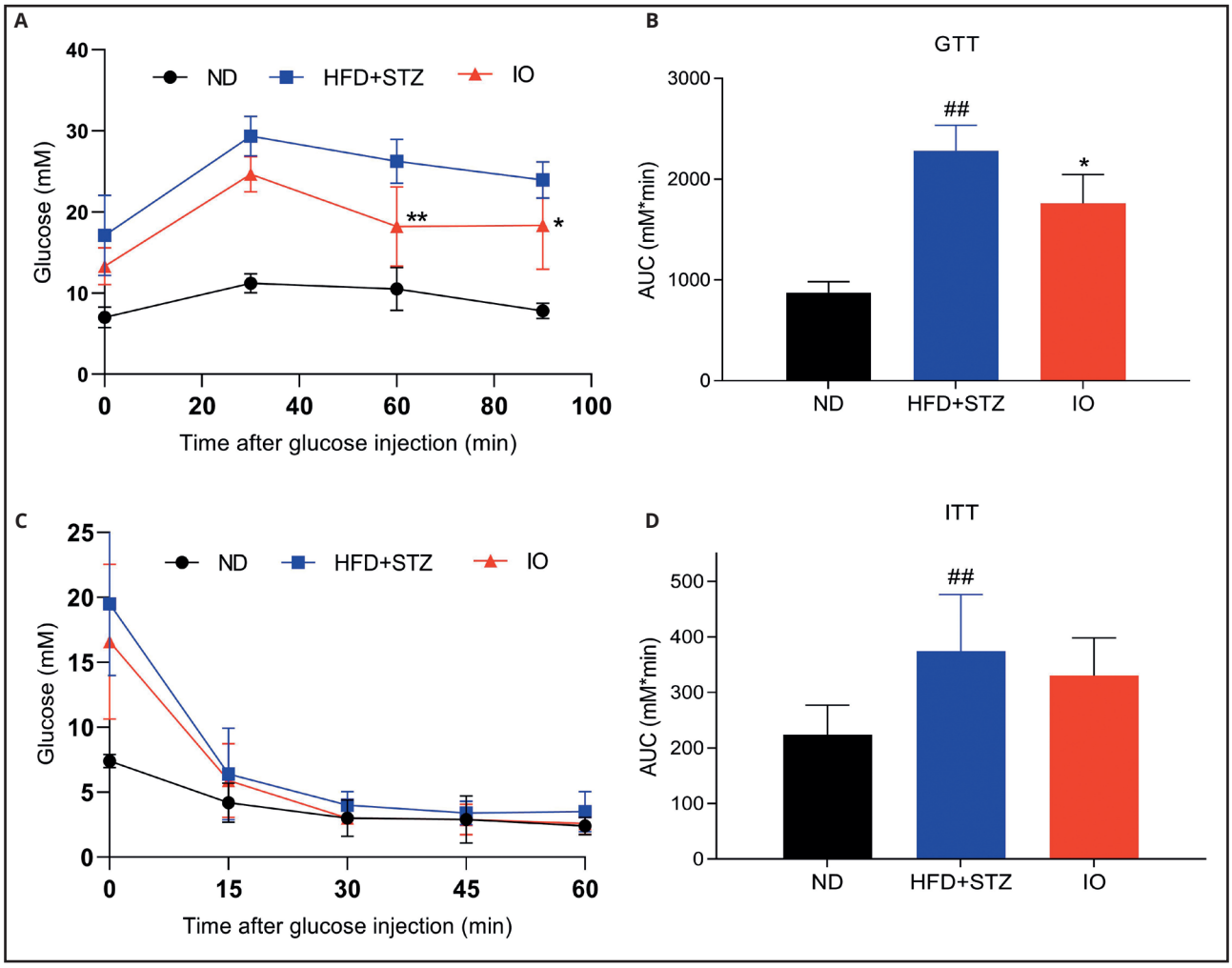

Figure 4.

Changes in glucose tolerance and insulin tolerance. A. Glucose level 0, 30, 60 and 90 minutes after the mice were administrated orally with glucose aqueous solution. B. AUC of glucose tolerance. C. Glucose level 0, 15, 30, 45 and 60 minutes after the mice were injected intraperitoneally with insulin. D. AUC of insulin tolerance. $^{\#\#}p < 0.05$ as compared to the HFD + STZ group. $^{*}p < 0.01$ as compared to the ND group. $^{**}p < 0.01$ as compared to the HFD + STZ group. ND: no high-fat diet; HFD + STZ: high-fat diet + streptozocin; IO: high-fat diet + streptozocin + IO.

resistance, β-cell dysfunction and insufficient compensatory insulin secretion response. The destruction of pancreatic islet function prevents insulin secretion from maintaining glucose homeostasis, resulting in hyperglycemia (18). Improving insulin secretion and controlling blood sugar are currently the main methods for the treatment of T2DM. Many plant ingredients have shown anti-diabetic potential, such as curcumin, rutin, quercetin, etc. (19). In this study, we focused on exploring the role of IO in diabetic mice. The results showed that, compared with the ND group, the HFD+STZ group mice showed lower body weight and high blood sugar levels, while IO treatment improved the mice's weight loss and increased blood sugar levels. The typical symptoms of T2DM are polyphagia, polyuria, and weight loss (20,21). IO treatment also improved the symptoms of polyuria in diabetic mice.

The function of insulin is to bind to the insulin receptor on the plasma membrane of the target cell, regulate the production and output of glucose, and maintain the blood glucose level within a steady-state range (22). This is crucial in the development of T2DM. In general, insulin resistance and hyperinsulinemia are considered to precede the development of hyperglycemia, which only develops when β cells cannot compensate for peripheral insulin resistance (23). Prolonged insulin resistance and compensatory responses will lead to β-cell dysfunction, which will aggravate T2DM (24). In this study, we tested glucose tolerance, insulin tolerance and serum insulin content and found that IO administration helped to improve the glucose tolerance and insulin resistance of diabetic mice, and reduced the serum insulin level to a certain extent. This indicated that IO has the effect of enhancing insulin sensitivity in mice.

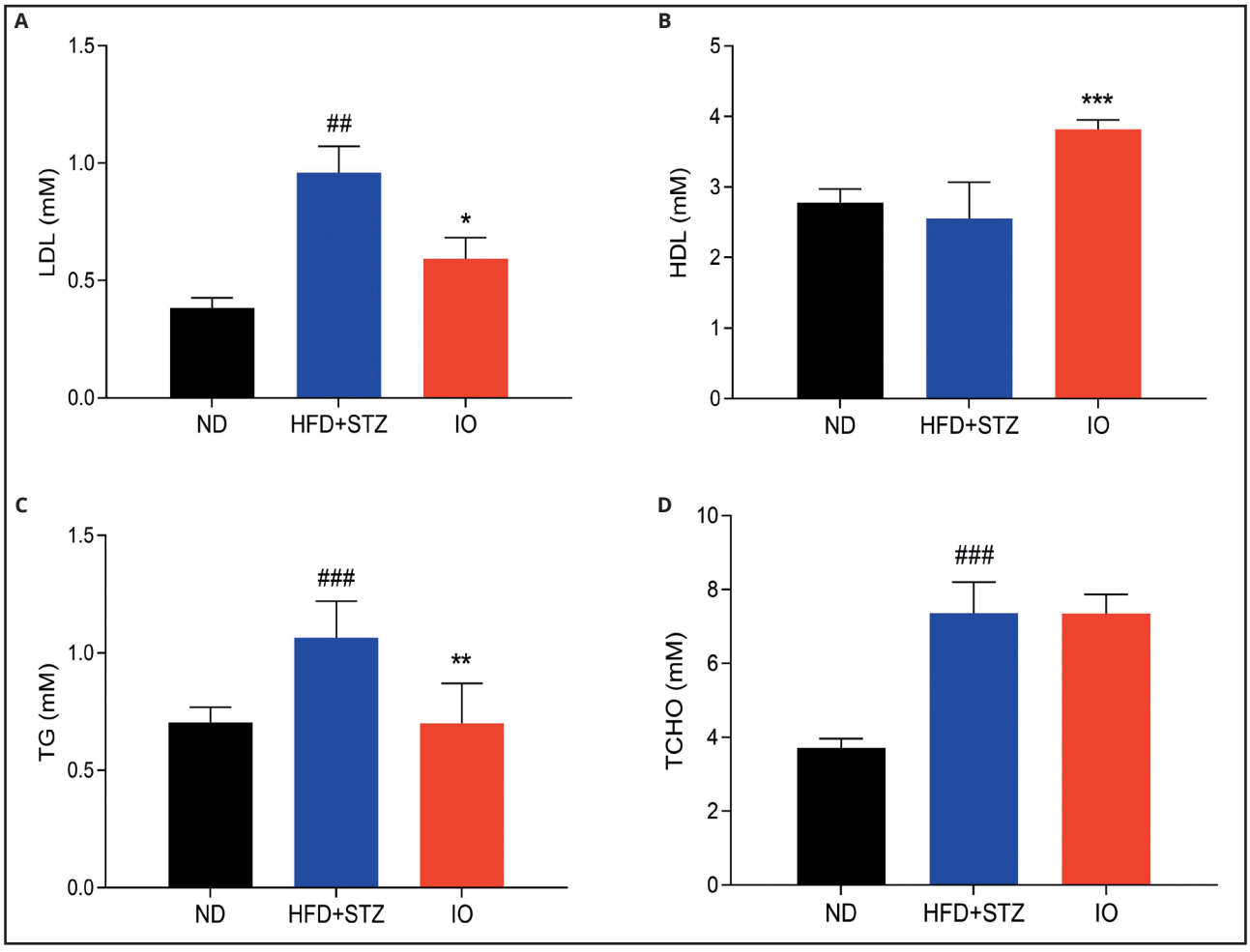

Figure 5.

Effects of IO on serum levels in T2DM mice. A. Low-density lipoprotein (LDL). B. High-density lipoprotein (HDL). C. Triacylglycerol (TG). D. Total cholesterol (TCHO). $^{\#\#}p < 0.05$ as compared to the HFD + STZ group. $^{\#\#\#}p < 0.01$ as compared to the ND group. $^{*}p < 0.001$ as compared to the ND group. $^{**}p < 0.01$ as compared to the HFD + STZ group. $^{***}p < 0.001$ as compared to the HFD + STZ group. ND: no high-fat diet; HFD + STZ: high-fat diet + streptozocin; IO: high-fat diet + streptozocin + IO.

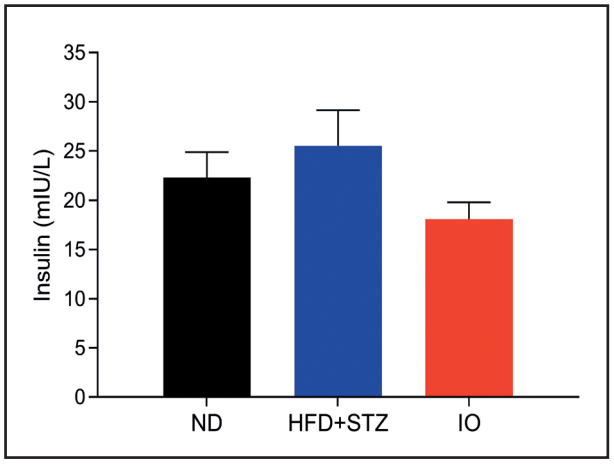

Figure 6.

Effects of IO on insulin sensitivity. ND: no high-fat diet; HFD + STZ: high-fat diet + streptozocin; IO: high-fat diet + streptozocin + IO.

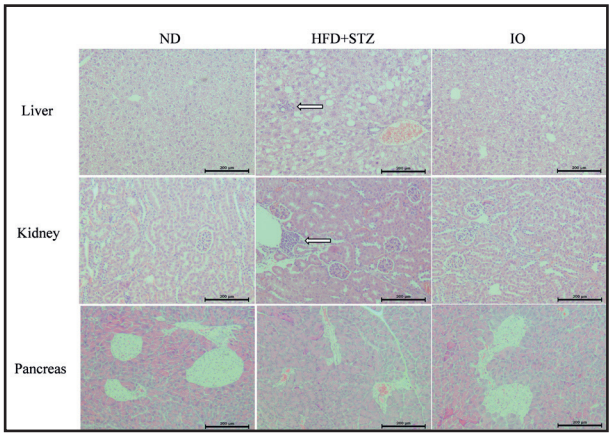

Figure 7.

Effects of IO on the protection of liver, kidney and pancreas against damages in T2DM mice (ND: no high-fat diet; HFD + STZ: high-fat diet + streptozocin; IO: high-fat diet + streptozocin + IO).

Studies have shown that the prevalence of dyslipidemia in T2DM patients is very high. Especially in men, older, obese, and long-term diabetes patients (25). Insulin deficiency and resistance can affect the enzymes and pathways of lipid metabolism, leading to dyslipidemia (26). Dyslipidemia in the context of T2DM increases the risk of cardiovascular disease complications (27). In this study, we found that IO significantly reduced LDL and TG levels in diabetic model mice, and increased HDL levels. IO treatment helped maintain lipid homeostasis, which is essential in T2DM.

Organs involved in the development of T2DM include the pancreas (β cells and α cells), kidney, liver, skeletal muscle, brain, small intestine, and adipose tissue (28). The pancreas is the organ producing pancreatic β-cells (29). Pancreatic β-cells have the ability to sense circulating glucose levels and secrete an appropriate amount of insulin to keep blood sugar within the normal range. The dysregulation of pancreatic or pancreatic islet β-cell function is the main mechanism of T2DM (22). The kidney is responsible for filtering all blood sugar. In the case of hyperglycemia, the increased filtering load has been shown to cause oxidative damage and damage the fragile vasculature and surrounding tubules (30). This will cause diabetic nephropathy. The liver is one of the main organs that control metabolic homeostasis, and it is essential for maintaining normal glucose homeostasis. In T2DM, the liver's ability to regulate blood sugar is imbalanced, leading to hyperglycemia in fasting and postprandial states, which may be due to the occurrence of hepatic insulin resistance (31). In this study, the effects of IO on the liver, kidney, and pancreas were explored. IO improved the pathological state of various organs in diabetic mice. However, the effect of IO in the kidney is not significant, which may be due to the short administration time.

Based on the literature, we found that IO has the effect of reducing blood sugar and anti-diabetes (32). There were differences in the dosage of IO in different studies, including 900 mg/kg, 125 mg/kg and 50 mg/kg (33-35). The difference in the extraction process may lead to differences in the ratio or content of various components in the IO extract, which may affect the therapeutic effect of IO. In this study, we used IO (150 mg/kg) to treat diabetic mice. The dose of IO showed the potential of anti-diabetes. Therefore, the extraction method of IO is feasible, but it also has some limitations. Next, we will continue to separate monomer substances, especially polysaccharides from IO extracts, and verify their ability to resist diabetes.

CONCLUSION

We successfully confirmed the anti-diabetic characteristics of IO in HFD combined with STZ-induced diabetic mice. IO reduced the level of blood glucose and blood lipids in HFD combined with STZ-induced diabetic mice, and enhanced insulin sensitivity. IO treatment improved the pathological changes of organs in diabetic mice. Therefore, IO may be a potential drug for the treatment of T2DM.

REFERENCES

1. Kolb H, Martin S. Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes. BMC Med 2017;15(1):131. DOI: 10.1186/s12916-017-0901-x
2. Captieux M, Prigge R, Wild S, Guthrie B. Defining remission of type 2 diabetes in research studies: a systematic scoping review. PLoS Med 2020;17(10):e1003396. DOI: 10.1371/journal.pmed.1003396
3. Valaiyapathi B, Gower B, Ashraf AP. Pathophysiology of type 2 diabetes in children and adolescents. Curr Diabetes Rev 2020;16(3):220-9. DOI: 10.2174/1573399814666180608074510
4. Melmer A, Laimer M. Treatment goals in diabetes. Endocr Dev 2016;31:1-27. DOI: 10.1159/000439364
5. Artasensi A, Pedretti A, Vistoli G, Fumagalli L. Type 2 diabetes mellitus: a review of multi-target drugs. Molecules 2020;25(8):1987. DOI: 10.3390/molecules25081987
6. Qaseem A, Barry MJ, Humphrey L, Forciea MA. Clinical Guidelines Committee of the American College of Physicians. Oral pharmacologic treatment of type 2 diabetes mellitus: a clinical practice guideline update from the American College of Physicians. Ann Intern Med 2017;166:279-90. DOI: 10.7326/M16-1860
7. Xu L, Zhao W, Wang D, Ma X. Chinese medicine in the battle against obesity and metabolic diseases. Front Physiol 2018;9:850. DOI: 10.3389/fphys.2018.00850
8. Liu R, Li X, Huang N, Fan M, Sun R. Toxicity of traditional Chinese medicine herbal and mineral products. Adv Pharmacol 2020;87:301-46. DOI: 10.1016/bs.apha.2019.08.001
9. Yang M, Chen JL, Xu LW, Ji G. Navigating traditional Chinese medicine network pharmacology and computational tools. Evid Based Complement Alternat Med 2013;2013:731969. DOI: 10.1155/2013/731969
10. Guo R, Luo X, Liu J, Liu L, Wang X, Lu H. Omics strategies decipher therapeutic discoveries of traditional Chinese medicine against different diseases at multiple layers molecular-level. Pharmacol Res 2020;152:104627. DOI: 10.1016/j.phrs.2020.104627
11. Zhao XQ, Guo S, Lu YY, Hua Y, Zhang F, Yan H, et al. Lycium barbarum L. leaves ameliorate type 2 diabetes in rats by modulating metabolic profiles and gut microbiota composition. Biomed Pharmacother 2020;121:109559. DOI: 10.1016/j.biopha.2019.109559
12. Cui X, Qian DW, Jiang S, Shang EX, Zhu ZH, Duan JA. Scutellariae radix and coptidis rhizoma improve glucose and lipid metabolism in T2DM rats via regulation of the metabolic profiling and MAPK/PI3K/Akt signaling pathway. Int J Mol Sci 2018;19(11):3634. DOI: 10.3390/ijms19113634
13. Wang J, Hu W, Li L, Huang X, Liu Y, Wang D, et al. Antidiabetic activities of polysaccharides separated from Inonotus obliquus via the modulation of oxidative stress in mice with streptozotocin-induced diabetes. PLoS ONE 2017;12(6):1-19. DOI: 10.1371/journal.pone.0180476
14. Li Z, Mei J, Jiang L, Geng C, Li Q, Yao X, et al. Chaga medicinal mushroom, Inonotus obliquus (Agaricomycetes) polysaccharides suppress tacrine-induced apoptosis by ROS-scavenging and mitochondrial pathway in HepG2 cells. Int J Med Mushrooms 2019;21(6):583-93. DOI: 10.1615/IntJMedMushrooms.2019030857
15. Wang J, Wang C, Li S, Li W, Yuan G, Pan Y, et al. Anti-diabetic effects of Inonotus obliquus polysaccharides in streptozotocin-induced type 2 diabetic mice and potential mechanism via PI3K-Akt signal pathway. Biomed Pharmacother 2017;95:1669-77. DOI: 10.1016/j.biopha.2017.09.104
16. Diao BZ, Jin WR, Yu XJ. Protective effect of polysaccharides from inonotus obliquus on streptozotocin-induced diabetic symptoms and their potential mechanisms in rats. Evid Based Complement Alternat Med 2014;841496. DOI: 10.1155/2014/841496
17. Petrie JR, Guzik TJ, Touyz RM. Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. Can J Cardiol 2018;34(5):575-84. DOI: 10.1016/j.cjca.2017.12.005
18. Farrugia F, Aquilina A, Vassallo J, Pace NP. Bisphenol A and type 2 diabetes mellitus: a review of epidemiologic, functional, and early life factors. Int J Environ Res Public Health 2021;18(2):716. DOI: 10.3390/ijerph18020716
19. Borse SP, Chhipa AS, Sharma V, Singh DP, Nivsarkar M. Management of type 2 diabetes: current strategies, unfocussed aspects, challenges, and alternatives. Med Princ Pract 2021;30(2):109-21. DOI: 10.1159/000511002
20. Chung IM, Kim H, Yeo MA, Kim SJ, Seo MC, Moon HI. Antidiabetic effects of three Korean sorghum phenolic extracts in normal and streptozotocin-induced diabetic rats. Food Res Int 2011;44(1):127-32. DOI: 10.1016/j.foodres.2010.10.051

21. Gomes MB, Rathmann W, Charbonnel B, Khunti K, Kosiborod M, Nicolucci A, et al. Treatment of type 2 diabetes mellitus worldwide: baseline patient characteristics in the global DISCOVER study. Diabetes Res Clin Pract 2019;151:20-32. DOI: 10.1016/j.diabres.2019.03.024
22. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. Physiol Rev 2018;98(4):2133-223. DOI: 10.1152/physrev.00063.2017
23. Rachdaoui N. Insulin: the friend and the foe in the development of type 2 diabetes mellitus. Int J Mol Sci 2020;21(5):1770. DOI: 10.3390/ijms21051770
24. Hudish LI, Reusch JE, Sussel L. β cell dysfunction during progression of metabolic syndrome to type 2 diabetes. J Clin Invest 2019;129(10):4001-8. DOI: 10.1172/JCI129188
25. Mehta RK, Koirala P, Mallick RL, Parajuli S, Jha R. Dyslipidemia in patients with type 2 diabetes mellitus in a tertiary care centre: a descriptive cross-sectional study. JNMA J Nepal Med Assoc 2021;59(236):305-9. DOI: 10.31729/jnma.6278
26. Geberemeskel GA, Debebe YG, Nguse NA. Antidiabetic effect of fenugreek seed powder solution (Trigonella foenum-graecum L.) on hyperlipidemia in diabetic patients. J Diabetes Res 2019;2019:8507453. DOI: 10.1155/2019/8507453
27. Aguilar-Ballester M, Hurtado-Genovés G, Taberner-Cortés A, Herrero-Cervera A, Martínez-Hervás S, González-Navarro H. Therapies for the treatment of cardiovascular disease associated with type 2 diabetes and dyslipidemia. Int J Mol Sci 2021;22(2):660. DOI: 10.3390/ijms22020660
28. Galicia-García U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci 2020;21(17):6275. DOI: 10.3390/ijms21176275
29. Moin ASM, Butler AE. Alterations in beta cell identity in type 1 and type 2 diabetes. Curr Diab Rep 2019;19(9):83. DOI: 10.1007/s11892-019-1194-6
30. Shepard BD. Sex differences in diabetes and kidney disease: mechanisms and consequences. Am J Physiol Renal Physiol 2019;317(2):F456-62. DOI: 10.1152/ajprenal.00249.2019
31. Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. Nat Rev Endocrinol 2017;13(10):572-87. DOI: 10.1038/nrendo.2017.80
32. Szychowski KA, Skóra B, Pomianek T, Gmiński J. Inonotus obliquus: from folk medicine to clinical use. J Tradit Complement Med 2020;11(4):293-302. DOI: 10.1016/j.jtcme.2020.08.003
33. Wang J, Wang C, Li S, Li W, Yuan J, Pan Y, et al. Anti-diabetic effects of Inonotus obliquus polysaccharides in streptozotocin-induced type 2 diabetic mice and potential mechanism via PI3K-Akt signal pathway. Biomed Pharmacother 2017;95:1669-77. DOI: 10.1016/j.biopha.2017.09.104
34. Liu P, Xue J, Tong S, Dong W, Wu P. Structure characterization and hypoglycaemic activities of two polysaccharides from Inonotus obliquus. Molecules 2018;23(8):1948. DOI: 10.3390/molecules23081948
35. Wang J, Hu W, Li L, Huang X, Liu Y, Wang D, et al. Antidiabetic activities of polysaccharides separated from Inonotus obliquus via the modulation of oxidative stress in mice with streptozotocin-induced diabetes. PLoS ONE 2017;12(6):e0180476. DOI: 10.1371/journal.pone.0180476