



Research article

Antidiabetic effect of *Momordica charantia* saponins in rats induced by high-fat diet combined with STZShuang Jiang^a, Lei Xu^b, Yan Xu^b, Yushan Guo^b, Lin Wei^a, Xueting Li^a, Wu Song^{a,*}^a Changchun University of Chinese Medicine, Changchun 130117, PR China^b Beihua University, Jilin 132000, PR China

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ABSTRACT

Background: The harmful effects of type 2 diabetes mellitus and its complications have become a major global public health problem. In this study, the effects of *Momordica charantia* saponins (MCS) on lipid metabolism, oxidative stress, and insulin signaling pathway in type 2 diabetic rats were investigated.**Results:** MCS could attenuate the tendency of weight loss of the model rats. It could also improve glucose tolerance; reduce fasting blood glucose, nonesterified fatty acid, triglyceride, and total cholesterol; and increase the insulin content and insulin sensitivity index of the rats. The activity of superoxide dismutase and catalase increased, and the content of malondialdehyde decreased in the liver and pancreas tissues of rats in MCS-treated groups significantly. In addition, the expression of p-IRS-1 (Y612) and p-Akt (S473) increased, and the expression of p-IRS-1 (S307) decreased in the liver tissues and pancreas tissues of rats in MCS-treated groups significantly.**Conclusion:** MCS has an antidiabetic effect, which may be related to its improving the lipid metabolism disorder, reducing oxidative stress level, and regulating the insulin signaling pathway.**How to cite:** Jiang S, Xu L, Xu X, et al. Anti-diabetic effect of *Momordica charantia* saponins in rats induced by high-fat diet combined with STZ. Electron J Biotechnol 2020;43. <https://doi.org/10.1016/j.ejbt.2019.12.001>.© 2019 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Diabetes is a disease characterized by glucose, lipid, and protein metabolism disorders caused by the absolute or relative insufficient secretion of insulin, with the typical symptoms of polydipsia, polyphagia and polyuria, and weight loss [1]. Diabetes can be divided into type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and gestational diabetes. Among the above three types of diabetes, T2DM has the highest morbidity, accounting for more than 95% of the overall incidence of diabetes, with the largest hazard [2]. T2DM, a lifelong disease, can cause a variety of acute and chronic complications, involving multiple organs and tissues, with a high disability and mortality rate, and cause a serious impact on the patient's physical and mental health, bringing a heavy burden on the

patients, their families, and the society [3]. The harmful effect of T2DM itself and its complications, together with the high cost of treatment, have become a major global public health problem [4]. Therefore, how to prevent and treat T2DM and its complications has become a serious challenge for global clinicians. Drugs used for the treatment of diabetes include insulin and oral hypoglycemic agents, but they can induce some clinical side effects such as hypoglycemia, liver injury, lactic acidosis and diarrhea, which are caused by high doses of insulin and oral hypoglycemic agents [5]; hence, it is necessary to search for natural antidiabetic drugs with low toxicities.

Momordica charantia is the immature fruit of *M. charantia* L., a *Momordica* plant, *Cucurbitaceae*, and is named for its special bitterness. *M. charantia* has a long history of use as a medicine in many Asian countries and regions. Some biological activities of *M. charantia*, including its antitumor, anti-inflammation, anti-oxidation, enhancing immunity, lowering cholesterol, and anti-AIDS, have been confirmed in several studies [6,7,8], and saponins, phenols, alkaloids, polysaccharides, and peptides from *M. charantia* are considered its main active components [9,10,11]. With the continuous advancement

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of the research, the hypoglycemic and antidiabetic effects of *M. charantia* saponins (MCS) have received increasing attention. It was found that the saponin component of *M. charantia* could inhibit the increase in blood glucose and serum neutral fat [12]. The saponin constituents extracted from *M. charantia* in an aqueous two-phase extraction system could induce a significant hypoglycemic activity in hyperglycemic and normal mice [13]. However, the hypoglycemic mechanism of MCS has not been clearly elucidated. In this study, the effects of MCS on the lipid metabolism, oxidative stress and insulin signaling pathway were evaluated, and the underlying mechanisms were explored to lay a foundation for the subsequent clinical application of MCS preparations.

2. Materials and methods

2.1. Materials

Clean grade male Wistar rats (Laboratory Animal Center of Jilin University, China); MCS (Xi'an Wenzhu Biotechnology Co., LTD, China); Streptozocin (STZ) (Sigma Company, USA); Blood glucose and insulin testing kits (Tianjin Jiuding Biotechnology Co., LTD, China); Non-esterified fatty acid (NEFA), triglyceride (TG) and total cholesterol (TC) test kits (Changchun Huili Biotechnology Co., LTD, China); Superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) test kits (Nanjing Jiancheng Biotechnology Co., LTD, China); Insulin receptor (IR), insulin receptor substrate 1 (IRS-1), p-IRS-1 (Y612), p-IRS-1 (S307), protein kinase B (Akt), p-Akt (S473) and glyceraldehyde phosphate dehydrogenase (GAPDH) antibodies (Abcam, UK).

2.2. Establishment of animal model, grouping, and administration

All rats were housed individually in cages at $20 \pm 1^\circ\text{C}$ and in 40–70% humidity, subjected to 12-h light/dark cycle with free access to food and water. The animal experiments were approved by the Institutional Animal Care and Use Committee of Changchun University of Chinese Medicine. All the experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of People's Republic of China.

After 1 week of adaptive feeding, 10 rats were selected and randomly allocated to a normal control group (CON group), which received the normal feeding procedure, while the remaining rats were allocated to the experimental group and fed with a high-fat diet. After 4 weeks of feeding, rats in the experimental group were fasted for 12 h prior to the intraperitoneal injection with STZ (30 mg/kg) prepared with the fresh citric acid and sodium citrate buffer. A fasting blood glucose (FBG) level of ≥ 11.1 mmol/L after 1 week was considered to indicate the success of the diabetic model. The high-fat diet was continued in diabetic rats throughout the course of the study. The diabetes model rats were divided into four groups ($n = 10$ per group): one group served as the diabetic control (DM group); rats in the high-, middle- and low-dose MCS groups were intragastrically given 400 mg/kg, 200 mg/kg and 100 mg/kg MCS once daily successively for a period of 4 weeks, respectively. Finally, all rats were anesthetized with chloral hydrate and sacrificed. The blood and the liver and pancreas tissues were removed immediately for further analysis.

2.3. Glucose tolerance test, and fasting blood glucose, fasting insulin and insulin sensitivity index measurement

The intraperitoneal glucose tolerance test (IPGTT) was performed one day before the last drug administration. The rats were given 2 g/kg glucose solution (50%) intraperitoneally after a 12-h fasting period. According to the manual operation steps, blood samples were

collected from the tail vein at 0, 30, 60, 90 and 120 min, respectively, and blood glucose was measured by the glucose oxidase method. At 24 h after the IPGTT, the FBG was measured by glucose oxidase and the fasting insulin (FINS) by radioimmunoassay. The insulin sensitivity index (ISI) was calculated according to the content of FBG and FINS. The calculation formula is shown in [Equation 1]

$$ISI = \text{Ln}(\text{FBG} \times \text{FINS})^{-1} \quad [\text{Equation 1}]$$

2.4. NEFA TG and TC content determination

After the last drug administration, the rats were sacrificed after a 12-h fasting period. The rats were anesthetized with 350 mg/kg chloral hydrate, and their blood samples were collected through their abdominal aorta. According to the instructions of kits, the serum NEFA contents were detected by colorimetry, the serum TG contents by GPO-PAP and the serum TC contents by COD-PAP, respectively.

2.5. Determination of antioxidant capacity

Liver and pancreas tissues were washed in precooled saline to rinse off the blood on the surface of the tissues, and the tissues were dried with a piece of filter paper. The cleaned tissues were put in precooled Tris-HCl (pH 7.4) at the ratio of 1:9 and cut into pieces with a surgical scissors immediately to prepare the tissue homogenates by a tissue homogenizer, then the homogenates were centrifuged at 4°C (3000 rpm, 15 min) to obtain the supernatants, and the supernatants were kept in a -80°C refrigerator for use. The total protein in the supernatants was determined by the Bradford method, and the SOD and CAT activities and MDA contents were detected according to the instructions of the kits.

2.6. Insulin signaling pathway protein detection

Liver and pancreas tissues were washed in precooled saline to rinse off the blood on the surface of the tissues, and then the tissues were dried with a piece of filter paper, respectively. The cleaned tissues were put in the lysis buffer and cracked on ice for 30 min; after centrifugation (3000 rpm, 15 min), the total protein in the tissue supernatants was determined by the Bradford method. A total of 20 μg of denatured protein sample were separated by 12% of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and after the transmembrane and blocking of the protein, IR, IRS-1, p-IRS-1 (Y612), p-IRS-1 (S307), Akt, p-Akt (S473) and GAPDH antibodies, and horseradish peroxidase-labeled secondary antibodies were added onto the membranes. The enhanced chemiluminescence (ECL) kit was used for the color development, and the ratios between the gray values of the target bands and those of IRS-1 or GAPDH bands were used for the semi-quantitative analysis.

2.7. Histopathology analysis

The remaining liver and pancreas tissues were immediately fixed in 10% formalin. Twenty-four hours later, the tissues were placed in 0.01 mol/L PBS and dehydrated by gradient ethanol. The tissues were paraffin-embedded and then sliced (4 μm thick). The slices were stained by haematoxylin-eosin (HE), then dehydrated by gradient ethanol, and the ethanol was replaced with xylene. The slices were mounted by neutral balsam, and examined and photographed under a light microscope.

2.8. Statistical analysis

All data in the experiments were presented as mean \pm SD, and SPSS15.0 software was used for the analysis of data. SNK-*q* test was

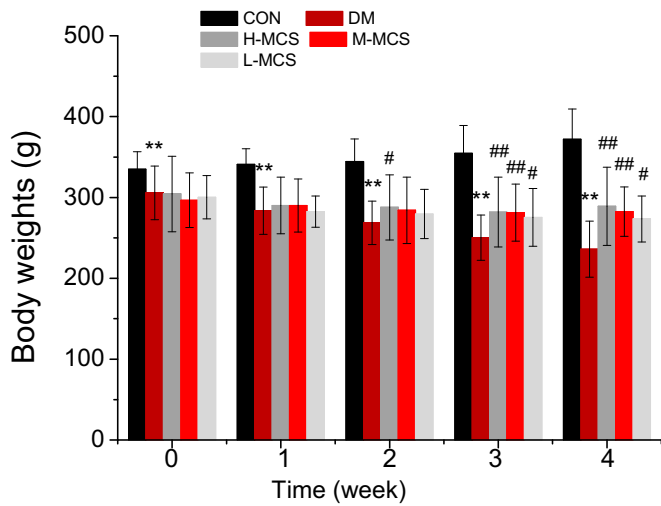


Fig. 1. Changes in body weights of diabetic rats during drug administration (Mean \pm SD, $n = 10$). Compared with the CON group, ** $P < 0.01$; compared with DM group, # $P < 0.05$, ## $P < 0.01$.

adopted for pairwise comparison and one-way ANOVA was used for multiple-group comparison. $P < 0.05$ or $P < 0.01$ was considered as statistically significant difference.

3. Results

3.1. Effect of MCS on the body weight of diabetic rats

During the drug administration, the body weights of rats in the DM group continued to decrease and were significantly different from those in the CON group ($P < 0.01$). Compared with those in the DM group, the rats in MCS groups gained body weight to some extent, in which those in H-MCS group increased significantly at 2 weeks after the administration, and those in M-MCS and L-MCS groups increased significantly at 3 weeks after the administration ($P < 0.05$ or $P < 0.01$) (Fig. 1). The above results indicated that MCS could attenuate the body weight loss of diabetic rats.

3.2. Effects of MCS on the glucose tolerance, FBG, FINS, and ISI of diabetic rats

The glucose tolerance test results showed a blood glucose peak in the CON group, and each MCS-treated group at 30 min after the injection of glucose solution, and that in the DM group at 60 min after the injection. At 30 min after the injection of glucose solution, compared with the DM group, H-, M- and L-MCS could significantly inhibit the increase of blood glucose ($P < 0.05$ or $P < 0.01$), but blood glucose levels in MCS-treated groups at 120 min were still higher than those at 0 min. Compared with that in the CON group, the FBG level in the DM group increased significantly ($P < 0.01$), while the FINS level and ISI decreased significantly ($P < 0.01$).

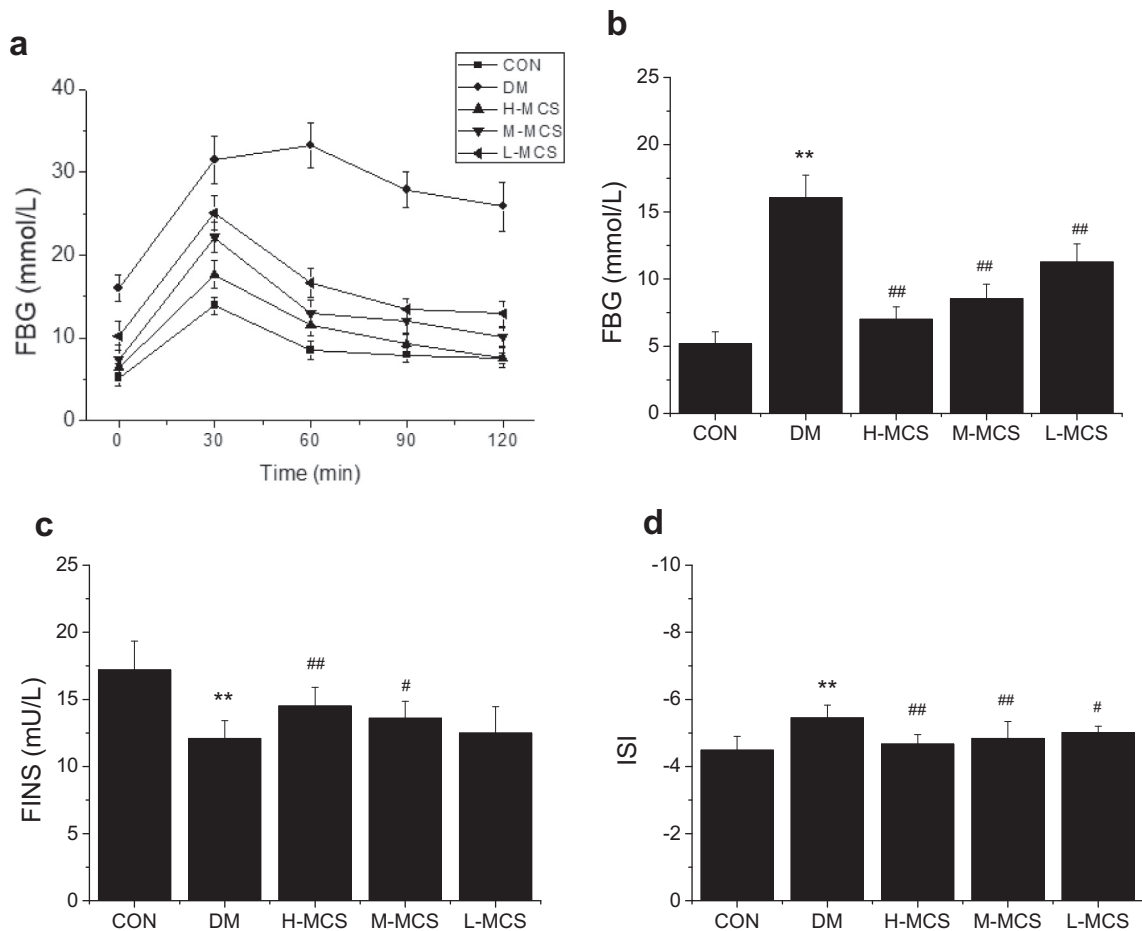


Fig. 2. Effects of MCS on the glucose tolerance, FBG, FINS and ISI of diabetic rats. (Mean \pm SD, $n = 10$). (a): glucose tolerance test; (b): FBG content; (c): fasting insulin content; (d): insulin sensitivity index. Compared with the CON group, ** $P < 0.01$; compared with the DM group, # $P < 0.05$, ## $P < 0.01$.

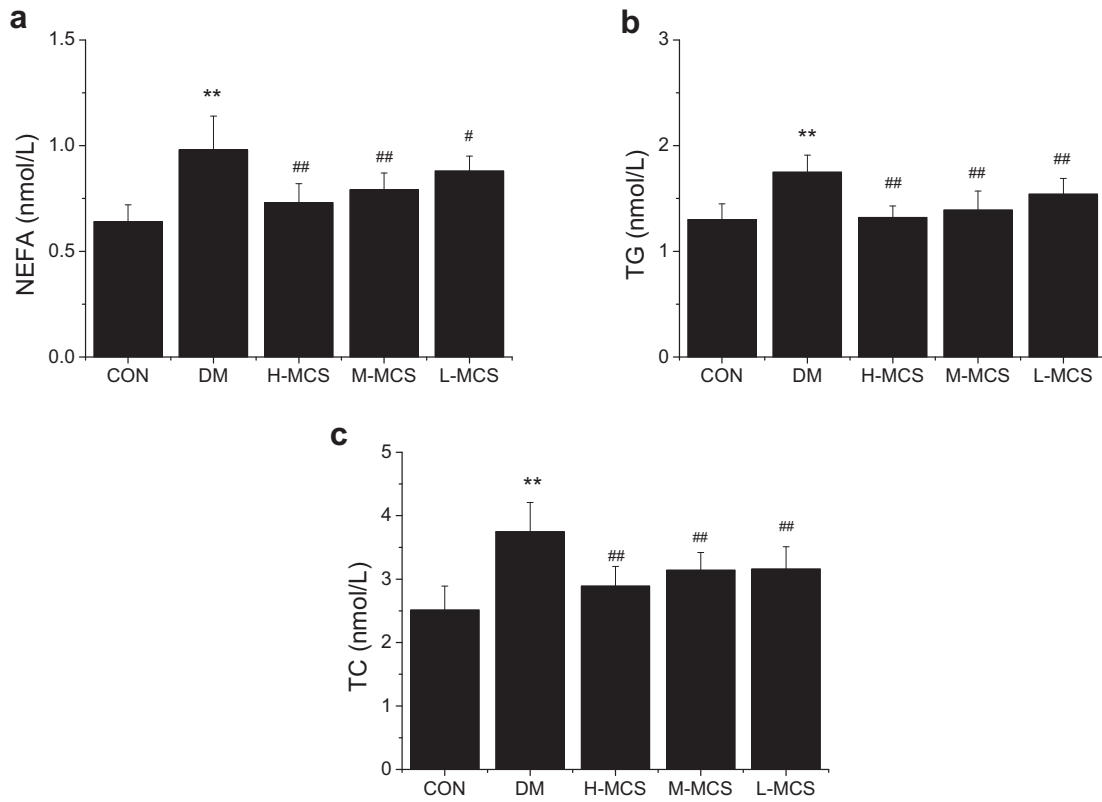


Fig. 3. Effect of MCS on NEFA, TG, and TC contents in the blood of diabetic rats (Mean \pm SD, $n = 10$). (a): NEFA content; (b): TG content; (c): TC content. Compared with the CON group, ** $P < 0.01$; compared with the DM group, # $P < 0.05$, ## $P < 0.01$.

Compared with those in the DM group, the FBG levels of rats in the different MCS groups were significantly lower ($P < 0.01$), while the FINS levels of rats in H- and M-MCS groups, and the ISI in the different MCS groups increased significantly ($P < 0.05$ or $P < 0.01$) in a dose-dependent manner (Fig. 2).

3.3. Effects of MCS on NEFA, TG and TC contents in the blood of diabetic rats

Effects of MCS on the blood lipid metabolism were evaluated by investigating its effects on the contents of NEFA, TG and TC in the blood of diabetic rats. The results showed that the NEFA, TG and TC

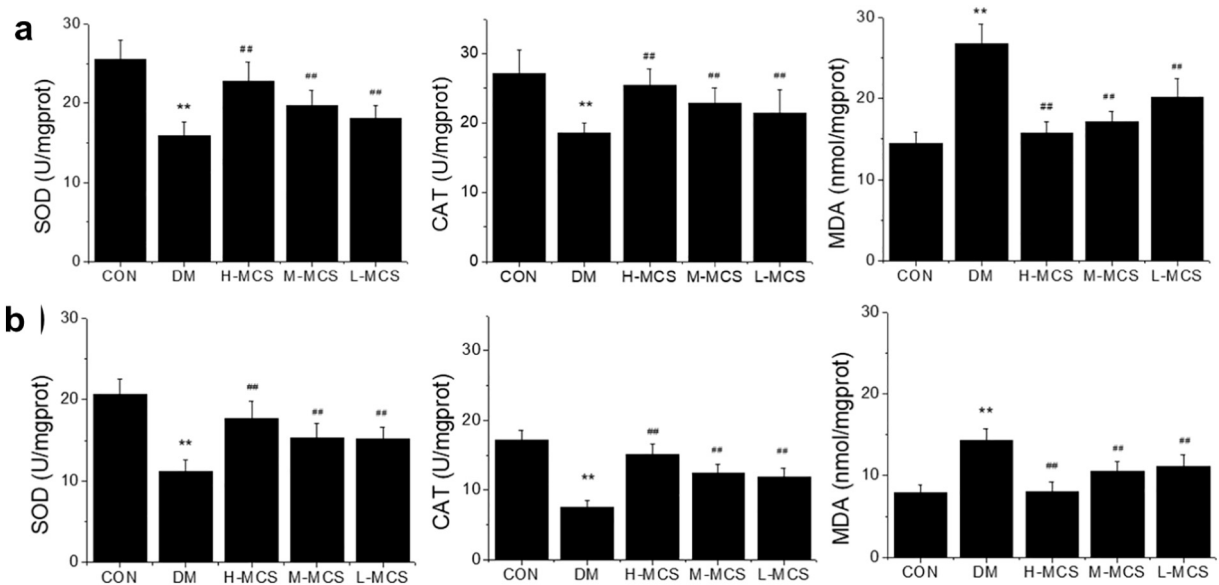


Fig. 4. Effects of MCS on SOD and CAT activities and MDA contents in the liver and pancreas tissues of diabetic rats (Mean \pm SD, $n = 10$). (a): SOD and CAT activities and MDA contents in the liver tissue; (b): SOD and CAT activities and MDA contents in the pancreas tissues. Compared with the CON group, ** $P < 0.01$; compared with the DM group, ## $P < 0.01$.

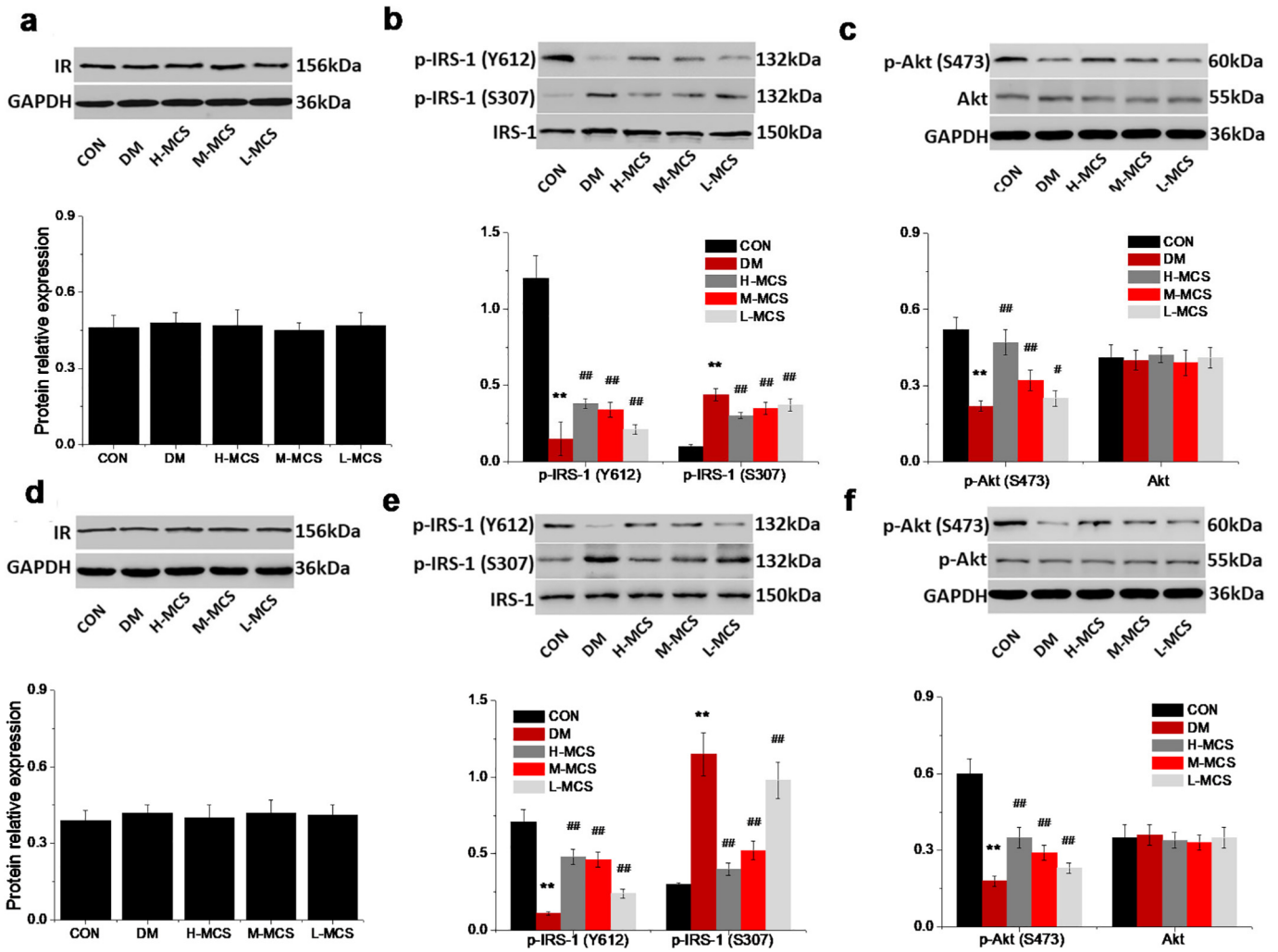


Fig. 5. Effects of MCS on insulin signaling pathway (Mean ± SD, n = 10). (a): relative expression of IR protein in the liver tissue; (b): relative expression of p-IRS-1 (Y612) and p-IRS-1 (S307) in the liver tissue; (c): relative expression of p-Akt (S473) and Akt proteins in the liver tissue; (d): relative expression of IR protein in the pancreas tissue; (e): relative expression of p-IRS-1 (Y612) and p-IRS-1 (S307) in the pancreas tissue; (f): relative expression of p-Akt (S473) and Akt proteins in the pancreas tissue. Compared with the CON group, ** P < 0.01; compared with the DM group, # P < 0.05, ## P < 0.01.

contents in the blood of rats in the DM group were significantly increased compared with those in the CON group (P < 0.01); compared with those in the DM group, NEFA, TG and TC contents in the blood of rats were significantly lower in H-, M- and L-MCS groups (P < 0.05 or P < 0.01) (Fig. 3). The above results indicated that MCS could improve the lipid metabolism disorder in diabetic rats.

3.4. Effects of MCS on SOD and CAT activities and MDA contents in the liver and pancreas tissues of diabetic rats

The antioxidant activity of MCS was evaluated by testing SOD and CAT activities and MDA contents in the liver and pancreas tissues of diabetic rats. The results showed that compared with those in the CON

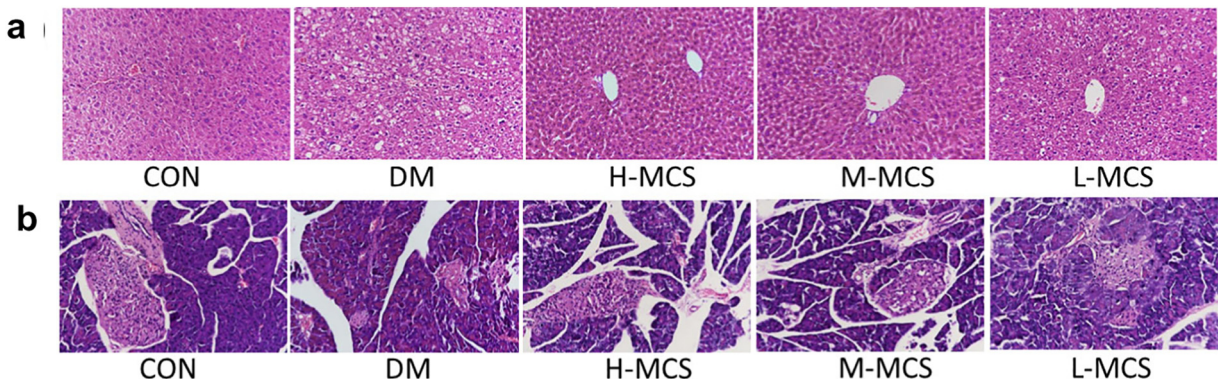


Fig. 6. Effects of MCS on the pathological morphology of liver and pancreas tissues (HE staining). (a): liver tissue; (b): pancreas tissue.

group, the SOD and CAT activities decreased and the MDA contents increased significantly in the liver and pancreas tissues of rats in the DM group ($P < 0.01$); this indicated that the oxidative stress level of the rats was elevated in the DM group. The SOD and CAT activities increased and the MDA contents decreased in the liver and pancreatic tissues of rats in H-, M- and L-MCS groups significantly compared with those in the DM group ($P < 0.01$) (Fig. 4), indicating that MCS should have an antioxidant capacity to decrease the oxidative stress level of diabetic rats.

3.5. Effect of MCS on the insulin signaling pathway

The effect of MCS on the insulin signaling pathway was evaluated by detecting the expression of IR, p-IRS-1 (Y612), p-IRS-1 (S307), Akt and p-Akt (S473) proteins in the liver and pancreas tissues of diabetic rats by western blot. The results showed that compared with those in the CON group, the p-IRS-1 (Y612) and p-Akt (S473) protein expressions decreased, and the p-IRS-1 (S307) expressions increased in the liver tissues and pancreas tissues of rats in the DM group significantly ($P < 0.01$). Compared with those in the DM group, the p-IRS-1 (Y612) and p-Akt (S473) protein expressions increased, and p-IRS-1 (S307) decreased in the liver and pancreas tissues significantly ($P < 0.01$ or $P < 0.01$). There was no significant change in the expression of IR and Akt proteins in the liver and pancreas tissues of diabetic rats ($P > 0.05$) (Fig. 5). The above results suggested that MCS could regulate the insulin signaling pathway.

3.6. Effects of MCS on the pathological morphology of liver and pancreas tissues

The rat liver lobule was regular, the boundaries of hepatocytes were clear, the central vein-centered hepatic cords were arranged in a radial order, and there was no infiltration of inflammatory cells in the liver tissue of rats in the CON group. In the DM group, the liver cells were swollen, and the volume of liver cells was enlarged; moreover, there were more vacuolization in the cells, and the cells were arranged haphazardly. There were vacuoles in the hepatic cells and the infiltration of inflammatory cells in the liver tissue of rats in MCS-treated groups, but less than those in the DM group (Fig. 6a).

The islets of rats showed a round or oval mass, with a clear boundary, and the B cells were evenly distributed in the islet, with dense arrangement and abundant cytoplasm in the CON group. The number of islets was decreased, their volume was smaller, the boundary was blurred, the B cells in the islet were sparse, the cells were significantly reduced, swollen, necrotic, and presented a vacuolar degeneration in the DM group. In MCS-treated group, the pancreatic islets were relatively intact, the boundary of the islets was clear, the number of B cells increased, the cells were relatively normal in morphology, and there was no obvious swelling and necrosis (Fig. 6b).

4. Discussion

The antidiabetic effect of *M. charantia* has been widely reported. Animal experiments have shown that *M. charantia* fruit juice has good anti-diabetic and antioxidant activities, with a great potential for both the prevention and the treatment of diabetes mellitus [14]. Clinical studies have also confirmed that *M. charantia* powder can reduce the glycosylated hemoglobin A1c and postprandial blood sugar level in type 2 diabetes mellitus, increase the secretion of insulin, and have an anti-type 2 diabetic effect [15]. However, there are few reports on the anti-diabetic effect and mechanism of saponins from *M. charantia*. In this study, after the intragastric administration of different doses of MCS in type 2 diabetic rats, it was found that MCS could slow down the trend of weight loss in model rats, increase glucose tolerance, decrease fasting blood sugar, and increase the insulin content and insulin sensitivity index, indicating that MCS should have a good anti-type 2 diabetic effect. The occurrence and development of diabetes are

closely related to lipid metabolism disorder, and furthermore, the lipid metabolism disorder is also the underlying cause of T2DM and a variety of the complications, characterized by elevated NEFA, TG and TC levels [16,17]. In this study, the levels of NEFA, TG and TC decreased significantly after the MCS administration for 4 weeks, indicating that MCS could regulate the blood lipid metabolism disorder in diabetic rats.

Although the molecular mechanisms of diabetes and its complications are not entirely clear, many evidences show that oxidative stress plays an important role in the process of the progression of diabetes and its complications [18]. The antioxidant effect of MCS extracts had been reported; hence, it was speculated that the antidiabetic effect of MCS might also be related to the antioxidant effect [19,20]. In order to verify this hypothesis, the effect of MCS on the antioxidant capacity of the liver and pancreas tissues of the diabetic model rats was investigated in this study, in which SOD, CAT and MDA were used as the evaluation indexes. SOD is the most important antioxidant enzyme to scavenge free radicals in the body; it is widely distributed in all kinds of organisms and can scavenge O_2^- to protect cells from oxidative damage [21]. Studies have shown that there are more than 60 kinds of diseases caused by O_2^- , while SOD can block various damages caused by O_2^- to cells and repair the function of damaged cells [22]. CAT is widely distributed in various tissues, among which its content is higher in the liver and pancreatic tissues, and can quickly eliminate the toxic metabolites of cells [23]. MDA, a lipid peroxidation product, can reflect the level of lipid peroxidation in the body directly and the degree of damage to the cells indirectly [24]. After the administration of MCS for 4 weeks, the activities of SOD and CAT increased and the content of MDA decreased in the liver and pancreas tissues of diabetic rats, suggesting that MCS can counteract the oxidative stress to prevent the oxidative damage by increasing the activity of antioxidant enzymes and reducing the content of lipid peroxides in the model rats, thereby playing an anti-diabetic role.

Different mechanisms contribute to the antidiabetic activity of *M. charantia*, including increasing the secretion of pancreatic insulin, decreasing insulin resistance and increasing the glucose utilization of peripheral and skeletal muscular cells, and inhibiting glucose absorption through the gastrointestinal tract and suppressing the key enzymes in the gluconeogenic pathways [25]. The results of this study showed that the expression of p-IRS-1 (Y612) in the liver and pancreas of rats in the DM group was decreased, and the expression of p-IRS-1 (S307) was increased, consistent with the research results reported by Tian et al. [26], while MCS could increase the expression of p-IRS-1 (Y612) in the liver and pancreas, and reduce the expression of p-IRS-1 (S307). In addition, this study further explored the effect of MCS on the phosphorylation of the IRS-1 downstream cascade protein Akt, and the results showed that MCS could promote the expression of p-Akt (S473) in the liver and pancreas tissues of model rats. The insulin signaling pathway is the target of various anti-diabetic drugs, with a high importance for the development of antidiabetic drugs [27]. IRS protein family is a key mediator in the insulin signaling pathway, of which IRS-1 is mainly distributed in the liver, pancreas, skeletal muscle, and fat, and plays a major role in the process of insulin signaling transduction [28]. The phosphorylated tyrosine 612 of IRS-1 (p-IRS-1 (Y612)) can bind to PI3Kp85 protein to promote the phosphorylation of Akt, thereby accelerating the translocation of Glut4 from the inside of the cells to the membrane to regulate the uptake of glucose by the target cells [29]. Bonala et al. [30] found that IRS-1 (Y612) could decrease the phosphorylation level and participate in the process of insulin resistance in diabetic and obese patients. Serine 307 phosphorylation of IRS-1 (p-IRS-1(S307)) plays an important role in mediating the regulation of insulin negative feedback, not only interfering with the interaction of IRS-1 with insulin receptors, but also hindering the phosphorylation of IRS-1 (Y612), thus affecting the downstream transduction of insulin signals, which is considered the main index at the molecular level for the insulin resistance in

diabetes and obesity at present [31]. The above results indicate that MCS can play an anti-diabetic role by regulating the insulin signaling pathway.

5. Conclusion

This study confirmed that MCS could attenuate the body weight loss of diabetic rats induced by high-fat diet combined with STZ, improve glucose tolerance, reduce FBG, and increase the FINS and ISI, with an anti-type 2 diabetes mellitus effect. Further studies found that MCS could improve the lipid metabolism disorder, reduce stress level, and regulate the insulin signaling pathway in diabetic rats, indicating that MCS may exert its anti-diabetic effect by improving the lipid metabolism disorder, reducing the oxidative stress level, and regulating the insulin signaling pathway in diabetic rats.

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Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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