Marein Regulates Insulin Resistance in Diabetic Nephropathy Mice by Inducing Autophagy

Marein Regula la Resistencia a la Insulina en Ratones con Nefropatía Diabética Induciendo la Autofagia

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SUMMARY: Marein is the main active substance of Coreopsis tinctoria nutt. It not only has anti-oxidation and anti-tumor effects, but also can lower blood lipid, prevent high blood glucose, improve insulin resistance, inhibit gluconeogenesis and promote glycogen synthesis. However, the exact mechanism of its action is still unclear. Here, we explored the effect and mechanism of Marein on insulin resistance. The mice were divided into db/m, db/db, metformin+db/db, and marein+db/db groups. The body weight and kidney weight were recorded. Serum biochemical and renal function tests were measured after 8 weeks of continuous administration. Kidney tissues were subjected to HE staining, PAS staining, and Masson staining. The effect of marein on PI3K/Akt signal and autophagy pathway was detected by Western blot. After 8 weeks of Marein intervention, the body weight and kidney weight of mice did not change significantly, but the fasting blood glucose and blood lipid levels were significantly reduced than db/db group. Marein significantly improved the insulin resistance index, increased serum adiponectin and improved glucose and lipid metabolism disorders of db/db mice. Moreover, marein improved the basement membrane thickness of glomeruli and tubules, improved glomerular sclerosis and tubular fibrosis, as well as renal insufficiency, thereby protecting kidney function and delaying the pathological damage. Furthermore, marein increased the expression of PI3K and the phosphorylation of Akt/Akt (Ser473), and promoted the expression of LC3II/I, Beclin1 and ATG5. Additionally, it promoted the expression of FGFR1 in the kidney of db/db mice, and promoted the increase of serum FGF21 and FGF23. Marein has a protective effect on the kidneys of diabetic mice. It protects diabetic nephropathy by regulating the IRS1/PI3K/Akt signaling pathway to improve insulin resistance. Therefore, marein may be an insulin sensitizer.

KEY WORDS: Marein; Insulin resistance; Diabetic nephropathy; Autophagy.

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by poor insulin secretion or insulin resistance and elevated blood glucose levels. According to the International Diabetes Federation (IDF) statistics (Cho *et al*., 2018), 425 million people worldwide suffered from diabetes, accounting for 8.8 % of the population aged 20-79 in 2017. It is estimated that by 2045, there will be nearly 629 million diabetic patients in the world, accounting for 9.9 % of the population aged 20-79 (Nov 18, 2019). In addition, in 2017, there were 114 million diabetic patients between the ages of 20 and 79 in China . Clinical statistics show that about 30-40 % of diabetic patients will eventually develop diabetic nephropathy (DN) (Alicic *et al*., 2017).

DN is a common and serious complication of diabetes, mainly manifested as edema, proteinuria, and hypertension, which can lead to life-threatening renal failure (Heerspink *et al*., 2019). Insulin resistance is the core cause of diabetes (Umanath & Lewis, 2018). As the target organ of insulin, the kidney expresses large amounts of insulin receptors, and patients with DN usually have systemic or renal insulin resistance (Wei *et al*., 2019). Therefore, improving insulin resistance is of great significance for the treatment and prevention of DN (Thomas & Karalliedde, 2019). At present, the commonly used drugs for DN are glienet, insulin, and biguanide, which can be used to treat DN by combining strategies such as lowering blood glucose,

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lowering blood lipids, and lowering blood pressure (Müller-Wieland, 2018; Wang, 2018). However, these drugs may cause electrolyte disturbances in the long term, leading to increased kidney load, and orthostatic hypotension, etc. Till now, it is still difficult to prevent the occurrence of DN. Moreover, there is no specific medicine for DN (Yashkin *et al*., 2018).

Coreopsis tinctoria nutt is a plant of the Compositae family, native to North America and cultivated as a garden plant worldwide (Hwang *et al*., 2010; Wang *et al*., 2015). As a rare and unique alpine wild plant, Coreopsis tinctoria nutt is mainly planted in high-altitude and cold regions such as Tianshan, Hotan and Kashgar in Xinjiang, China. Studies have shown that Coreopsis tinctoria nutt has the effects of anti-oxidation, lowering blood pressure, lowering blood glucose, andlowering blood lipids (Yao *et al*., 2015; Jiang *et al*., 2018; Mu *et al*., 2020). Our previous research (Yao *et al*.) showed that the ethyl acetate extract of Coreopsis tinctoria nutt can improve blood glucose, blood lipids and kidney function in patients with DN. In addition, the ethyl acetate extract of Coreopsis tinctoria nutt can also inhibit diabetic nephritis and fibrosis (Dias *et al*., 2010a,b; Yao *et al*., 2015).Marein is one main active substance of Coreopsis tinctoria nutt. It not only has anti-oxidation and anti-tumor effects, but also can lower blood lipid, prevent high blood glucose, improve insulin resistance, inhibit gluconeogenesis and promote glycogen synthesis (Guo *et al*., 2020). However, the exact mechanism of its action is still unclear.

Studies have shown that insulin resistance causes the autophagy pathway of various organs and tissues to be significantly inhibited (Shintani & Klionsky, 2004; Mizushima, 2007). Regulating the autophagy pathway improves insulin resistance and plays a role in the prevention and treatment of DN. In vitro experiments (Jiang *et al*., 2016) have shown that the promotive effect of marein on autophagy pathway may be one of the mechanisms for improving insulin resistance of human glomerular vascular endothelial cells. Therefore, this study aims to investigate the protective effect and underlying mechanism of marein on DN in a mouse model. Our findings may provide important evidence for the clinical management and treatment of DN.

MATERIAL AND METHOD

Animals. Six-week old db/db (a model for DN) and db/m mice (control for DN model) with average body weight of 28±2 g were from Changzhou Avanse Experimental Animals. The animals were kept in a specific pathogen free environment at 22 ± 2 °C and 45 ± 5 % humidity and fed with normal diet. This study was conducted in strict accordance with the recommendations of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. All animal experiments were conducted according to the ethical guidelines of Animal Experimental Center, Xinjiang Medical University. All efforts were made to minimize animal suffering. All experimental protocols were approved by the Ethics Committee of Xinjiang Medical University.

Animal grouping, treatment and sampling. The mice were further randomly divided into db/m group (control, n=10), db/db group (DN model, n=10), db/db+metformin (280 mg/kg) (Brisol-Myers Squibb, USA) group (n=10), and db/db+marein (50 mg/kg) (Extrasynthese, France) group (n=10). Metformin and Marein were dissolved in 0.5 % (0.5g/100 mL) sodium carboxymethyl cellulose and was administrated by gavage once every day for 8 weeks. The control group was treated with distilled water containing 0.5 % (0.5g/100 mL) sodium carboxymethyl cellulose every day for 8 weeks. At 7 weeks of administration, 24 h urine samples were collected from mice. The body weight of mice was monitored every week. At the end of the experiments, all mice were sacrificed by cervical dislocation. The blood samples were collected from bulbus oculi. The left kidneys were rinsed with cold physiological saline solution, placed immediately in liquid nitrogen, and stored at -80 °C until use. The right kidneys were weighed and fixed in 10 % formaldehyde.

Blood and urine biochemical analysis. The collected blood samples were centrifuged at 3000 rpm for 10 min. Serum samples were separated for detection of total cholesterol (TC), triglycerides, blood urea nitrogen (BUN), and serum creatinine (SCr), fibroblast growth factor 21 (FGF21), and fibroblast growth factor 23 (FGF23), on an automatic biochemical analyzer (BS-120; Mindray, China). Blood glucose concentrations were measured by Accu-Chek Acti-ve Blood Glucose Meter (Roche, Germany). Plasma insulin was measured by Ultra-Sensitive Mouse Insulin ELISA Kit (Morinaga, Tokyo, Japan). Urinary microalbumin excretion was measured by Murine Microalbumin ELISA (Exocell, Philadelphia, PA). HOMA-IR was calculated as [fasting serum insulin (mU/L) ¥ fasting plasma glucose (mmol/L)] / 22.5. Serum adiponectin (APN) was detected using mouse adiponectin ELISA Kit (Cusabio china).

Histopathological staining. Right kidney tissues were fixed in 10 % formaldehyde, embedded in paraffin, and cut into 3μm sections. The tissue sections were stained with hematoxylin and eosin (H&E), Masson's reagent and PAS for histological examination. The images were captured by light microscopy (SZX7-1093; Olympus, Japan) and Image-Pro Plus 6.0 software was used to analyze results.

Trasmission electron microscopy. Renal specimens were fixed in 2 % glutaraldehyde/0.1 M phosphate buffer (pH 7.4) and 1 % osmium tetroxide/0.1 M phosphate buffer (pH 7.4). Samples were then dehydrated, embedded, and cut into ultrathin sections. A transmission electron microscope (H-7500; Hitachi, Tokyo, Japan) was used to visualize ultrathin sections stained with uranyl acetate and lead citrate. Glomerular basement were measured to assess the function of glomeruli by Image-Pro Plus 6.0 software.

Immunohistochemical analysis. After deparaffinization and hydration, the sections were rinsed three times with PBS and treated with endogenous catalase inhibitors. After blocking with goat serum (Bioss Biotechnology, Beijing, China) for 30 min at room temperature, the samples were incubated with anti-p62 (Cell Signaling Technology), at 4 °C overnight. On the next day, the secondary horseradish-peroxidase-conjugated antibody (Bioss Biotechnology) was added and incubated at 37 °C for 30 min. After rinsing three times with PBS, tissue sections were developed with diaminobenzidine tetrahydrochloride. The slices were observed by light microscopy (Olympus BX-43; Olympus Optical, Tokyo, Japan), and Image-Pro Plus 6.0 software was used to analyze results.

Western blot. The left kidneys were subject to lysis with RIPA (Thermo, USA) and protein samples were obtained after sonification and centrifugation. Protein concentration was determined by using BCA Protein Assay Kit (Thermo). The protein samples were separated by SDS-PAGE and then transferred to PVDF membranes (Millpore, USA). The

PVDF membranes were then blocked in a 5 % skimmed milk solution for 2 h at room temperature and subsequently incubated with primary antibodies overnight at 4 °C. The primary antibodies included those against p-AKT, p62, mTOR, AKT, IRS-1, PI3K, Beclin1, ATG5 and FGFR1 (fibrous growth factor receptor) (1:1000; Cell Signaling Technology, Danvers, MA, USA), and GAPDH (1;2000) were incubated at 4°C overnight. The membranes were then rinsed 3 times and incubated in the corresponding secondary antibodies (1:2000, Dingguo, China) at room temperature for 2 h. After rinsing three times, bands were developed with ECL system (Amersham Biosciences, Little Chalfont, UK) and analyzed by an image analyzer (BioRad Laboratories). Image J software was used for densitometry measurements of protein bands on Western blots.

Statistical analysis. Statistical analyses were measured by SPSS 17.0 (Chicago, IL, USA). All data are presented as mean ± standard deviation (SD) of three independent experiments. The comparison of two groups or multiple groups were analyzed using the Unpaired Student's t test and one-way ANOVA (S-N-K method for post hoc analysis), respectively. A p value <0.05 was considered to be statistically significant.

RESULTS

Changes in body weight and metabolism of db/db mice after receiving marein treatment. The body weights of mice were monitored. As shown in Figure 1A, the body
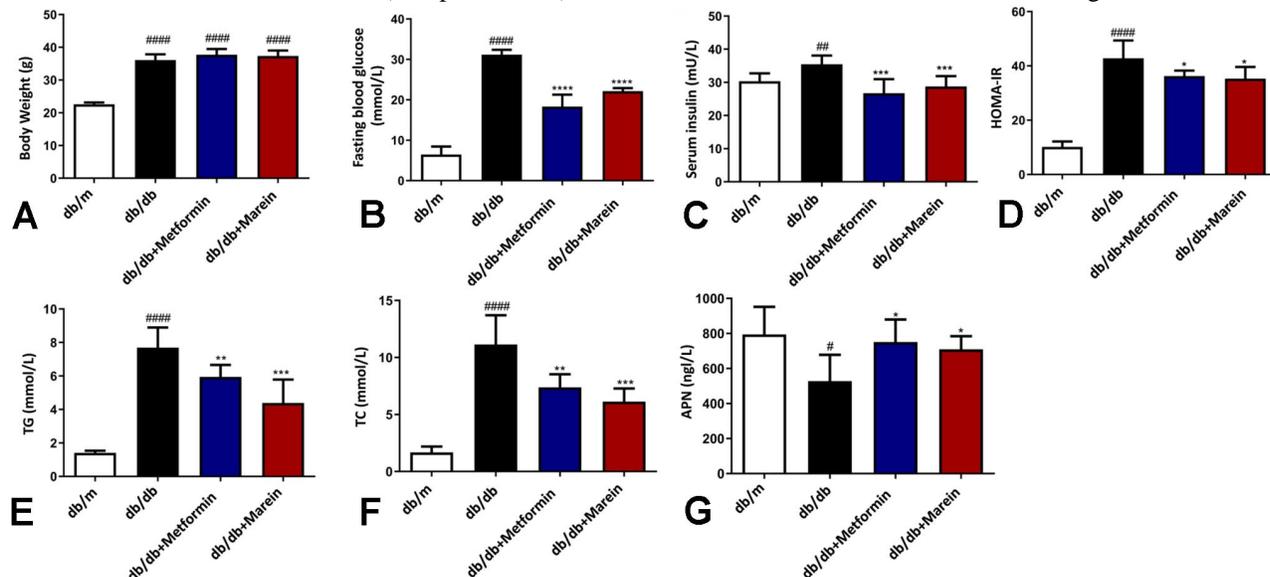
Fig. 1. Changes in body weight and metabolic parameters after treatment with marein. Six-week-old db/db mice received intragastric administration marein per day for 8 weeks. Body weight was monitored. Blood and urine samples were collected for biochemical analysis. (A) Body weight. (B) Fasting blood glucose. (C) Serum insulin. (D) HOMA-IR. (E) TG. (F) TC. (G) APN. The data are presented as the mean ± SD. ## $p < 0.01$, ### $p < 0.001$, versus db/m mice; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, versus db/db mice.

weights of db/db group, db/db+metformin group, db/db+marein group were significantly higher than that of db/m group (P<0.05). No significant difference was observed among db/db group, db/db+metformin group, and db/db+ marein group. Compared with the db/m group, the fasting blood glucose levels (Fig. 1B), the serum insulin (Fig. 1C), HOMA-IR (Fig. 1D), TG (Fig. 1E) and TC (Fig. 1F) of db/db group increased significantly (P<0.05). However, compared with db/db group, the levels of these factors in db/db+metformin and db/db+marein groups were significantly reduced (P<0.05). In addition, the db/db group had significantly lower APN level than db/m group (P<0.05) (Fig. 1G); meanwhile the db/db+metformin and db/db+marein groups had significantly higher APN level than db/db group (P<0.05). These results indicated that marein could improve glucose and lipid metabolism in DN mice.

Marein improves kidney function in db/db mice. In order to examine the effect of marein on improving the kidney function of db/db mice, we measured kidney weight, plasma Scr, BUN and urine microalbumin levels. The results showed that after 8 weeks of treatment, the kidney weights of db/db group, db/db+metformin group, db/db+marein group were significantly higher than that of db/m group (P<0.05) (Fig. 2A). There were no significant differences among db/db, db/db+metformin, and db/db+marein groups. Compared with db/m group, the db/db group had significantly higher Scr (Fig. 2B), BUN (Fig. 2C) and urine microalbumin (Fig. 2D) (P<0.05). Compared with the db/db group, the treatment of metformin and marein significantly decreased the levels of Scr (Fig. 2B), BUN (Fig. 2C) and urine microalbumin (Fig. 2D) (P<0.05). These results indicate that marein may improve kidney filtration function and protect db/db mice from damage.

Marein reduces kidney damage in db/db mice. To evaluate the histological changes, the kidney sections were stained with HE (Fig. 3A). The results showed that the structure of glomerulus and renal tubule in db/m group

was normal, and there was no obvious abnormality. The glomerulus of the model group was irregular in shape, with hyperplasia of fibrous tissue, obviously reduced capillaries, and infiltration of lymphocytes and monocytes. In the metformin and marein groups, the degree of fibrous hyperplasia was reduced, the capillary loop was increased, and inflammatory cells were still infiltrated to a certain extent. HE staining results suggested that marein could alleviate the damage of renal tissue structure in DN mice.

There were no abnormal changes in the structure of glomeruli and renal tubules in db/m group, and the collagen fiber staining was normal (Fig. 3B). In the model group, the glomerular basement membrane was thickened; the mesangial membrane showed hyperplasia; and, the collagen fiber staining was obvious. A large amount of blue-stained material was deposited in the renal vesicles and tubules, and the collagen fiber deposition was obvious in the renal interstitium. After administration of metformin and marein, the collagen fibers of the glomerular basement membrane were less stained and the thickening of the basement membrane and mesangium was reduced. Compared with the model group, both marein and metformin significantly improved the deposition of collagen fibers in the glomerular interstitium (P<0.05).

Pathological sections of the kidney of mice in each group were stained with PAS, and morphological changes were observed (Fig. 3C). There was no abnormality in the structure of the glomerulus and renal tubule in the db/m group. The db/db mice in the model group showed hyperplasia of glomerular mesangial cells, obvious glycogen deposition in a mesangial matrix, thickening of basal membrane, and hyaline changes in the capillary loop. Metformin and Marion improved the pathological structure of glomerulus in DN mice. The basement membrane was more intact; and the thickening of basement membrane and the deposition of glycogen in the glomerulus and renal tubules were reduced. Statistically, the glycogen area score was significantly lower (P<0.05).
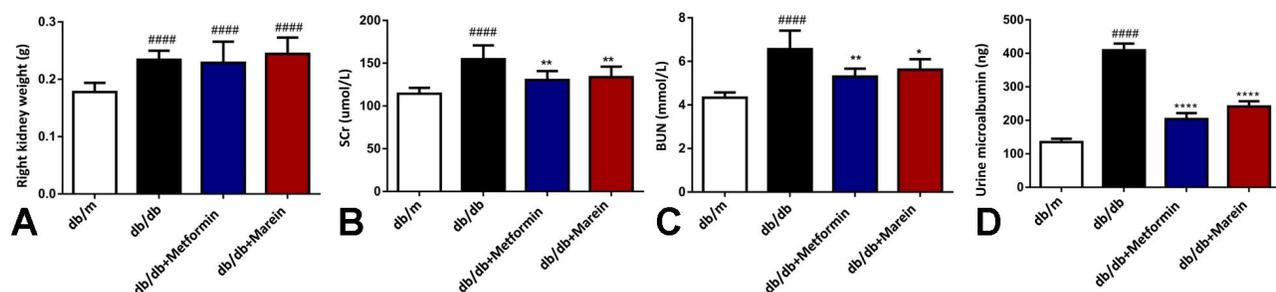
Fig. 2. Marein improved renal function in db/db mice after treatment with marein.(A) Right kidney weight, (B) Scr, (C) BUN, and (D) Urine microalbumin were measured. The data are presented as the mean ± SD. ##p < 0.01, ###p < 0.001, versus db/m mice; *p < 0.05, **p < 0.01, ***p < 0.001, versus db/db mice.

Fig. 3. Pathological changes of kidney tissues. (A) Representative HE staining results. (B) Representative and quantitative Masson staining results. (C) Representative and quantitative PAS staining results. (D) Representative and quantitative electron microscope results. Values are expressed as mean ± SD. ## P<0.01 versus db/m mice; *P<0.05, ** P<0.01, versus db/db mice.
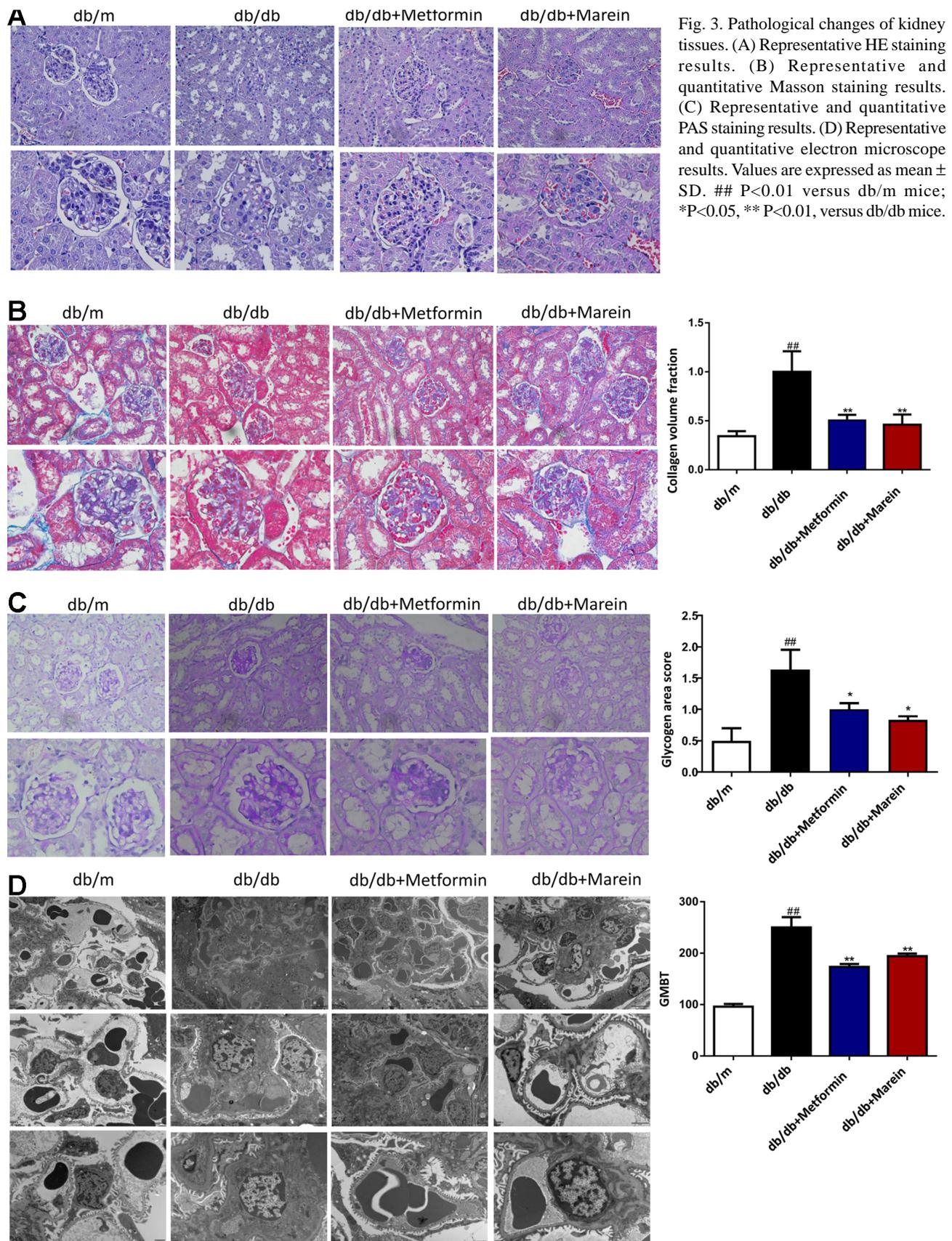
Furthermore, the pathological changes of kidney were observed by electron microscopy (Fig. 3D). In the db/m group, the glomerular structure was normal under electron microscopy, and no obvious abnormalities were observed in endothelial cells, podocytes, and mesangial cells of capillaries. The glomerular basement membrane was normal and uniform in thickness. The morphology of podocytes was complete, and the structure of foot processes was clear, without obvious fusion. The glomerular basement membrane of db/db mice in the model group showed obvious segmental thickening. Electron dense deposition was observed in subendothelial layer and basal membrane. Mesangial matrix was increased. The basal membrane of endothelial cells showed local hyperplasia. Podocyte arrangement was disordered, and podocytes were fused. In the metformin and Marein group, the thickening of glomerular basement membrane was reduced. Only a small amount of basement membrane showed segment thickening. Mesangial cells and matrix proliferation were reduced; the foot process structure was normal; and the disorder of podocyte arrangement was also reduced. Statistically, compared with control group, the glomerular basement membrane thickness in the model group was significantly increased (P<0.01). Compared with the model group, the glomerular basement membrane thickness was significantly decreased in the Marein group and the metformin group (P<0.01). These results indicate that marein may protect DN mice from glomerulopathy and podocyte loss. In addition to glomerular disease, marein treatment reduced renal tubular damage and collagen accumulation in DN mice, indicating that marein could protect the DN kidney from structural and functional damage.

Marein regulates insulin resistance in db/db mice. In order to explore the effect of marein on insulin resistance in db/db mice, we detected the expression of insulin resistance-related proteins in the kidney tissue of db/db mice by Western blot.

Figures 4A and 4B showed that there was no significant change in IRS among four groups (all P>0.05). Compared with the db/m group, the level of PI3K (Fig. 4C and 4D) and p-Akt/Akt (Figs. 4E and 4F) in the kidney tissue of the db/db group decreased significantly (P<0.01). However, the db/db+metformin and db/db+marein groups had significantly lower level of PI3K (Figs. 4C and 4D) and p-Akt/Akt (Figs. 4E and 4F) (P<0.05). These results indicated that marein might improve insulin resistance and insulin sensitivity by PI3K/Akt signaling pathway in db/db mice.

Marein promotes the activation of the autophagy in the kidney of db/db mice. In the state of insulin resistance, the autophagy of kidney tissue is defective. Therefore, regulating autophagy can improve insulin resistance and prevent the development of DN. We tested the expression of autophagy-related proteins, including LC3II, LC3I, ATG5, p62 and
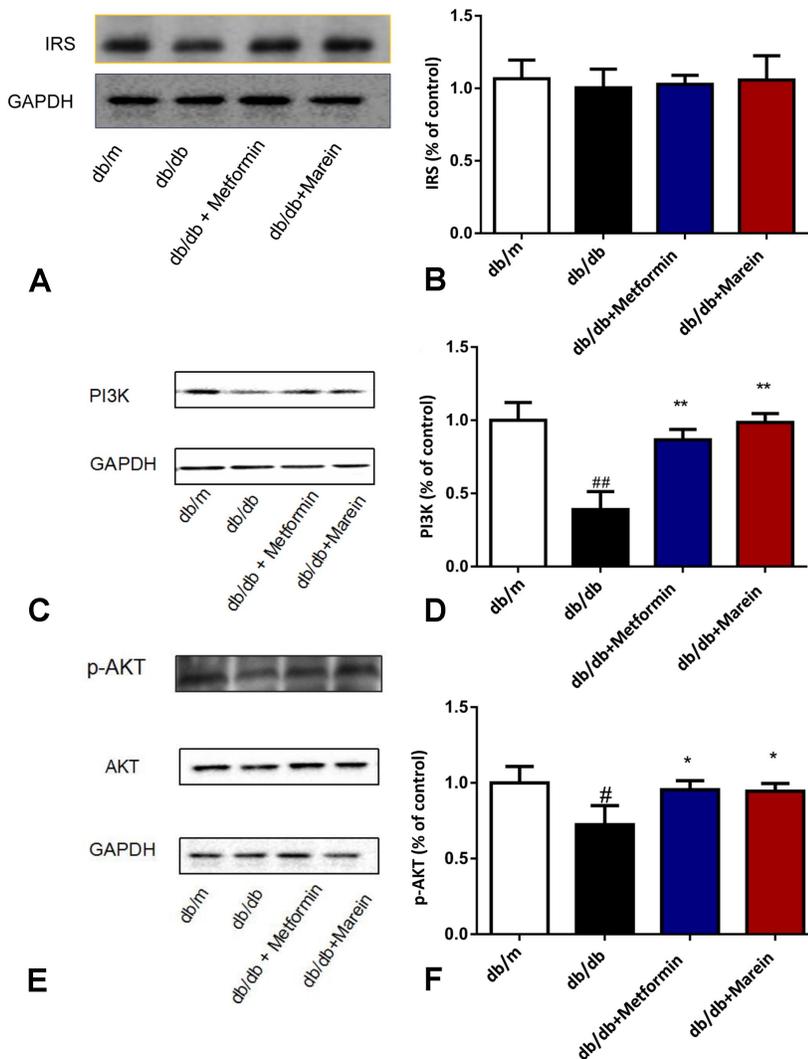
Fig. 4. Marein treatment changed renal insulin resistance. Western blot was conducted to detect protein expression levels of (A and B) IRS, (C and D) PI3K, (E and F) p-Akt and Akt in kidney tissues. The data are presented as the mean ± SD. #p < 0.05, ##p < 0.01, versus db/m mice; *p < 0.05, **p < 0.01, versus db/db mice.

Beclin 1 with Western blot. The results showed that compared with the db/db group, marein inhibited the expression of p-mTOR in db/db mice (Figs. 5A and 5B). Compared with the db/db group, marein treatment promoted the expression of LC3II/LC3I, ATG5 and Beclin 1 in db/db mice, and reduced the level of p62 (Figs. 5C-5F), indicating that marein promotes the activation of the autophagy pathway in db/db mice.

Immunohistochemical staining was used to locate and quantitatively analyze p62 expression in renal tissues. As shown in Figure 5G, p62 expression in the model group was higher than that in other groups. Statistically, compared with db/m normal mice, p62 expression in glomeruli and renal tubules was significantly increased in the db/db group (P<0.05), and both metformin and marein intervention could decrease p62 expression (P<0.05).
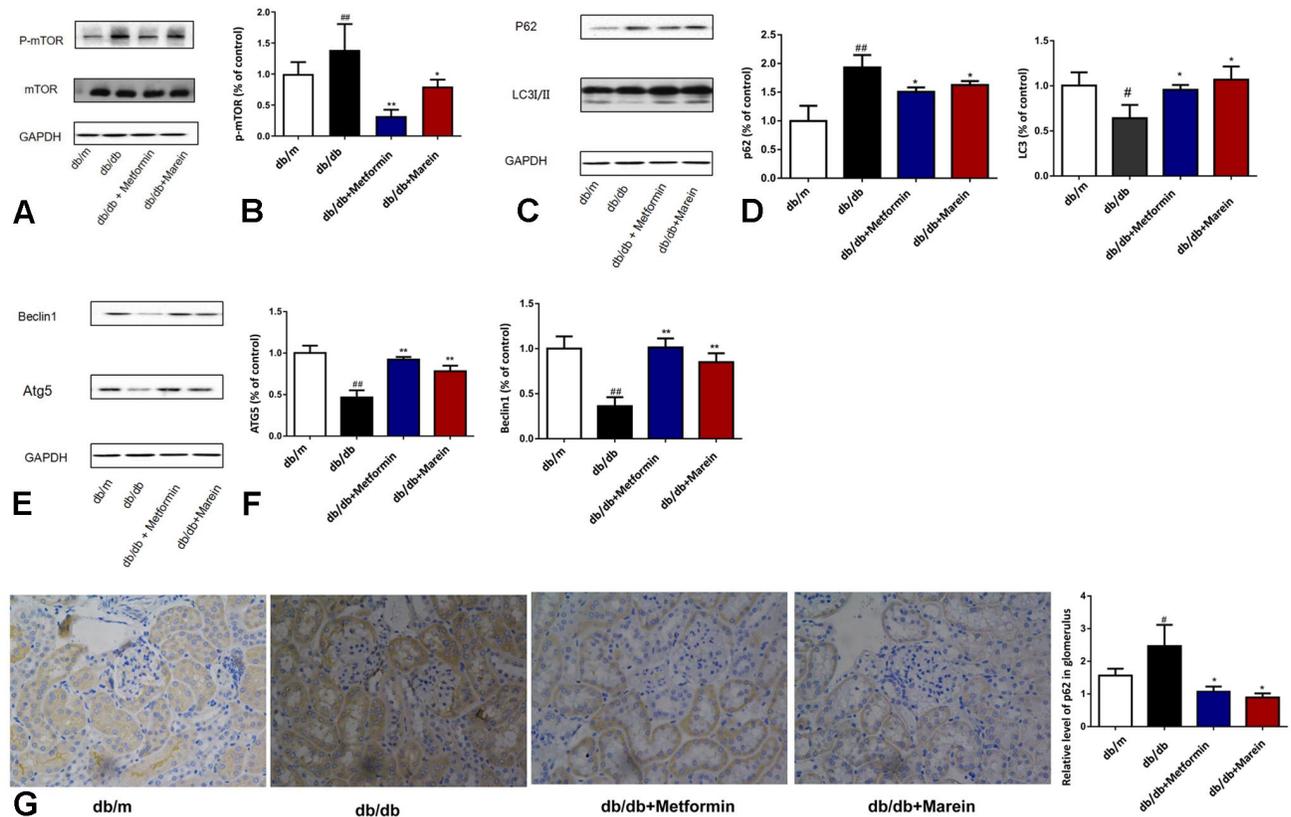
Fig. 5. Marein treatment changed renal autophagy.(A and B) p-mTOR and mTOR, (C and D) LC3I/II and p62, (E and F) ATG5 and Beclin in kidney tissues were detected by Western blot, respectively. (G) Immunohistochemical staining of p62 in kidney tissues. Representative and quantitative results were shown. The data are presented as the mean ± SD. #p < 0.05, ##p < 0.01, ###p < 0.001, versus db/m mice; *p < 0.05, **p < 0.01, versus db/db mice.
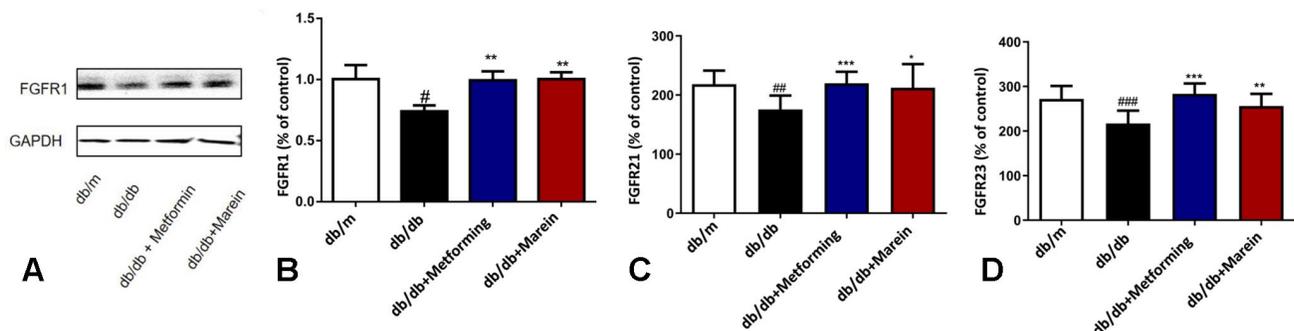
Fig. 6. Effect of marein treatment on FGFR1, FGF21 and FGF23.Western blot was used to analyze protein expression levels of FGFR1 (A and B) in kidney tissues. Serum FGF21 (C) and FGF23 (D) were also detected. The data are presented as the mean ± SD. #p < 0.05, ##p < 0.01, ###p < 0.001, versus db/m mice; *p < 0.05, **p < 0.01, ***p < 0.001, versus db/db mice.

1641

Effect of marein on kidney FGFR1, serum FGF21 and FGF23 in db/db mice. We performed Western blot to test the effect of marein on the expression of FGFR1 in the kidney tissue of db/db mice. As shown in the Figures 6A and 6B, compared with the db/m group, the expression level of FGFR1 in the db/db group was significantly reduced. After 8 weeks of metformin and marein treatment, the expression level of FGFR1 in the kidney tissue increased significantly (P<0.01) (Figs. 6A and 6B). Moreover, the FGF21 (P<0.01) (Fig. 6C) and FGF23 (P <0.001) (Fig. 6D) of the db/db mice were significantly reduced compared with the db/m group. Metformin and marein significantly increased FGF21 and FGF23 (P<0.01).

DISCUSSION

In this study, we demonstrated the renal protective effect of marein on db/db mice. After 8 weeks of treatment with marein, the blood glucose and lipid metabolism disorders of diabetic mice were improved. In addition, marein treatment significantly improved the renal function, including BUN, SCr and urine microalbumin. Histopathological analysis also showed that marein reduced glomerular and tubular damage and collagen accumulation in db/db mice.

Abnormal blood glucose and blood lipids are risk factors for DN (Li *et al*., 2020). Clinical studies (Calle & Hotter, 2020; Donate-Correa *et al*., 2020; Nunes *et al*., 2020) have shown that persistent high blood glucose and large amounts of hyperlipidemia will increase the production of reactive oxygen species in the body and in-duce kidney inflammation and oxidative stress. The main pathological changes of DN are the thickening of the glomerular basement membrane and the increase of the mesangial matrix, which leads to a decrease in glomerular filtration rate, accompanied by proteinuria, urea nitrogen and creatinine increase (Porrini *et al*., 2015; Alicic *et al*.). Here, we found that marein improved glucose and lipid metabolism disorders in DN mice, reduced glomerular and tubular damage caused by DN, and improved kidney function.

As the target organ of insulin, the kidney has a large number of insulin receptors. DN has systemic or renal insulin resistance (Pina *et al*., 2020). Therefore, improving the insulin sensitivity and insulin resistance of kidney tissue is a prevention and treatment strategy for DN (Xu *et al*., 2020). We showed that marein improved glucose and lipid metabolism in DN mice, inhibited the decline of renal function, glomerular and tubular damage, and up-regulated PI3K and pAkt/Akt expression, suggesting that marein can improve insulin resistance and insulin sensitivity in db/db mice. Therefore, marein may be an insulin sensitizer.

PI3K/AKT is closely related to the occurrence and development of diabetes (Huang *et al*., 2018), and its regulatory signals and downstream molecules are considered to be the most promising targets for the treatment of DN (Maffei *et al*., 2018). In the state of insulin resistance, a large number of dysfunctions and components in cells are damaged, leading to intracellular stress response and inflammation (Moreno *et al*., 2018; Sagoo & Gnudi, 2018). Therefore, the regulation of autophagymay improve insulin resistance in type 2 diabetes (Yang *et al*., 2020). We believe that autophagy may be a new prevention and treatment strategy to improve insulin resistance. In our study, db/db mice treated with marein showed a significant increase in the activation of the autophagy pathway, including related proteins (ATG5, Beclin1 and LC3B-II). All these indicate that enhanced autophagy activity can help delay the development of diabetes.

P62 is an autophagy receptor, which can bind to autophagosomes that degrade LC3B after fusion of ubiquitinated proteins with lysosomes (Wen *et al*., 2020). Therefore, it can be used as an indicator of autophagy degradation, acting as an autophagy receptor and signaling hub (Liu *et al*., 2020). In this study, immunohistochemical staining showed that the expression of p62 in the kidneys of diabetic mice was significantly higher than that in the control group, while treatment with marein significantly reduced the expression of p62. These results indicate that autophagy is inhibited in diabetic mice, and marein could promote autophagy.

We also found that after 8 weeks of marein intervention in db/db mice, the expression level of mTOR protein in kidney tissues decreased. mTOR is a downstream signal protein of PI3K/AKT pathway (Zhang *et al*., 2019) and plays a central role in autophagy (Li *et al*., 2017). When the human body is over-nourished, mTOR activation can inhibit autophagy activity. When the human body is in a state of starvation, nutritional deficiency and cellular stress, mTOR is inhibited and autophagy is initiated (Lantier *et al*., 2020). The activation of mTOR signaling pathway can aggravate the development of DN (Wen *et al*.). Here, we found that marein could inhibit mTOR, thereby activating the autophagy pathway and playing a role in kidney protection.

Our results showed that treatment of db/db mice with marein for 8 weeks significantly up-regulated the

expression of FGFR1 in kidney tissues and increased the levels of FGF21 and FGF23 in serum. A large number of studies (Yuan *et al*., 2017; Wang *et al*., 2018) have shown that FGFR1 can promote autophagy.FGF21 and FGF23 are important members of the fibroblast growth factor family (Yamamoto *et al*., 2020). FGF21 is a new metabolic regulator that can increase the body's metabolic level and promote glucose uptake (Aljohani *et al*., 2019). In addition, FGF21 participates in the metabolism of glycolipid substances in the body, thereby increasing the body's energy consumption, regulating blood lipids, enhancing the absorption of glucose by fat cells, and inhibiting the secretion of glucagon. Studies (Talukdar *et al*., 2016; Mottillo *et al*., 2017) have shown that increased expression of FGF21 can significantly reduce the body weight of type 2 diabetic mice, lower blood glucose levels, promote blood glucose reabsorption, and maintain the function and number of islet cells. FGF23 is a hormone-like fibroblast growth factor newly identified in recent years (Bär *et al*., 2019). It is mainly produced by bone cells and secreted into the blood. It participates in the regulation of calcium and phosphorus levels and vitamin D (Trummer *et al*., 2019). It is also related to pathological processes such as abnormal glucose, lipid metabolism and insulin resistance (Fayed *et al*., 2018). Abnormal serum FGF-23 levels in patients with T2 diabetes are closely related to the occurrence of DN and other microangiopathy (Zhang *et al*., 2018).

CONCLUSIONS

In conclusion, we demonstrated that marein reduced kidney damage, glomerular and tubular damage, and collagen accumulation in db/db mice. We also found that marein induced autophagy and enhanced the insulin sensitivity of the kidney by activating the PI3K/AKT signaling pathway, thereby improving DN. Our findings may have a positive role in the mechanism research and clinical drug treatment of DN.

FUNDING

LI, T.; ABULA, Z.; KADIER, K.; GUO, Y.; WANG, L.; ZHANG, Y.; RAN, Z.; SONG, Z.; ZHANG, F. & MAO, X. Marein regula la resistencia a la insulina en ratones con nefropatía diabética induciendo la autofagia. *Int. J. Morphol., 39(6)*:1635-1645, 2021.

RESUMEN: Marein es la principal sustancia activa de Coreopsis tinctoria nutt. No solo tiene efectos antioxidantes y antitumorales, sino que también puede reducir los lípidos en sangre, prevenir la glucemia alta, mejorar la resistencia a la insulina, inhibir la gluconeogénesis y promover la síntesis de glucógeno. Sin embargo, el mecanismo exacto de su acción aún no está claro. Se analizó el efecto y el mecanismo de Marein sobre la resistencia a la insulina. Los ratones se dividieron en grupos db / m, db / db, metformina + db / db y mareína + db / db. Se registró el peso corporal y el peso de los riñones. Se midieron las pruebas de función renal y bioquímica sérica después de 8 semanas de administración continua. Los tejidos renales se sometieron a tinción HE, tinción PAS y tinción Masson. El efecto de la mareína sobre la señal de PI3K / Akt y la vía de autofagia se detectó mediante Western blot. Al término de 8 semanas de tratamiento con mareína, el peso corporal y el peso de los riñones de los ratones no cambiaron significativamente, pero los niveles de glucosa en sangre y lípidos en sangre en ayunas se redujeron significativamente en relación a los del grupo db / db. Marein mejoró significativamente el índice de resistencia a la insulina, aumentó la adiponectina sérica y mejoró los trastornos del metabolismo de la glucosa y los lípidos de los ratones db / db. Además, la mareína mejoró el grosor de la membrana basal de los glomérulos y túbulos, mejoró la esclerosis glomerular y la fibrosis tubular, así como la insuficiencia renal, protegiendo la función renal y retrasando el daño patológico. Además, la mareína aumentó la expresión de PI3K y la fosforilación de Akt / Akt (Ser473), y promovió la expresión de LC3II / I, Beclin1 y ATG5. Además, promovió la expresión de FGFR1 en el riñón de ratones db / db y el aumento de FGF21 y FGF23 en suero. Marein tiene un efecto protector sobre los riñones de ratones diabéticos. Protege la nefropatía diabética regulando la vía de señalización IRS1 / PI3K / Akt para mejorar la resistencia a la insulina. Por tanto, la mareína puede ser un sensibilizador a la insulina.

PALABRAS CLAVE: Marein; Resistencia a la insulina; Nefropatía diabética; Autofagia.

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