**Regular Article**

**Dendrobium officinale** Attenuates Myocardial Fibrosis via Inhibiting EMT Signaling Pathway in HFD/STZ-Induced Diabetic Mice

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Cardiac fibrosis is a major contributor for diabetic cardiomyopathy and *Dendrobium officinale* possessed therapeutic effects on hyperglycemia and diabetic cardiomyopathy. To further investigate the possible mechanisms of the *Dendrobium officinale* on diabetic myocardial fibrosis in mice, water-soluble extracts of *Dendrobium officinale* (DOE) from dry stem was analyzed by HPLC and phenol-sulfuric acid method. Diabetic mice were induced by intraperitoneal injection of streptozotocin (STZ) (30 mg/kg) for 4 consecutive days after intragastric administration of a high-fat diet (HFD) for 2 weeks. The groups were as follows: control group, model group, DOE low, medium, high dose group (75, 150, 300 mg/kg) and Metformin positive group (125 mg/kg). The results showed that DOE dose-dependently lower serum insulin, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and grew the high-density lipoprotein cholesterol (HDL-C) after 12 weeks of daily administration with DOE. Hematoxylin-eosin staining and Sirius red staining showed obvious amelioration of cardiac injury and fibrosis. In addition, the result of immunoblot indicated that DOE increased the expression of peroxisome proliferator activated receptor-α (PPAR-α), phosphorylation of insulin receptor substrate 1 (p-IRS1) and E-cadherin and repressed the expression of transcription factor β1 (TGF-β1), phosphorylation of c-Jun N-terminal kinase (p-JNK), Twist, Snail1 and Vimentin. The present findings suggested that DOE accelerated lipid transport, inhibiting insulin resistant and suppressing fibrosis induced by epithelial mesenchymal transition (EMT).

**Key words** *Dendrobium officinale*; diabetic cardiomyopathy; fibrosis; epithelial mesenchymal transition

**INTRODUCTION**

Diabetes mellitus is a serious threat to human health, and 451 million people were estimated to suffer from diabetes in 2017, more than 90% of whom are type 2 diabetes mellitus (T2DM), with the number increasing to 693 million in 2045. T2DM features chronic hyperglycemia with disturbances of fat metabolism which result from deficiency of insulin secretion and/or insulin resistance. Metabolic dysregulation caused by diabetes increases the risk of developing atherosclerosis, myocardial infarction, cardiomyopathy, and heart failure. Diabetes cardiomyopathy (DCM) is featured by impaired myocardial insulin signal, endoplasmic reticulum stress, mitochondrial dysfunction, sympathetic nervous system activation, excessive oxidative stress, aggravation of inflammation, abnormal coronary microcirculation and maladaptive immune response. These pathophysiological changes give rise to fibrosis and hypertrophy. Myocardial fibrosis is present recognized as the majority of DCM, giving rise to cardiac remodeling, cardiac dilatation and congestive heart failure.

Epithelial–mesenchymal transition (EMT) refers to the phenomenon that epithelial cells transform into interstitial cells, losing their epithelial cell characteristics under physiological and pathological conditions. There are three types of EMT, and type II occurs when specific cells are lost and replaced by fibrotic tissue in the course of a disease. Increasing evidence showed that EMT can lead to myocardial fibrosis due to non-synchronous heart failure through mechanical heterogeneity in the canine model. Fibroblasts in cardiac fibrosis are derived from endothelial cells. In patients with Crohn disease, an expression pattern of EMT was found in areas of fibrosis in the colon. Transforming growth factor β1 (TGF-β1) is an important profibrotic factor to induce EMT in fibrosis under physiological conditions. Novel therapeutic strategy targeting the interaction of TGF-β1 and EMT would effectively prevent cardiac fibrosis and slow the progression of DCM.

*Dendrobium officinale* Kimura et Migo (*Dendrobium catenatum* Lindley), a functional food and medicine herbal, has shown great pharmacological activity on diabetes and hypertension. Polysaccharides, the major constituent in *Dendrobium* species, were recently reported to possess various potent pharmacological effects, including antioxidant, antiapoptotic, antitumor, and immunomodulation activity. The potential mechanism may be that pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and 6 and oxidative stress are inhibited. Our previous study suggested that oral administration of water-soluble extracts of *Dendrobium officinale* (DOE) could remarkably lowered blood glucose and prevent the development of DCM in streptozotocin (STZ) induced diabetic mice. However, the potential effects of *Dendrobium officinale* on cardiac fibrosis in diabetes still unclear. In the present study, we investigated whether DOE prevented insulin resistance and whether DOE reduced cardiac fibrosis through EMT in high-fat diet (HFD)/STZ-induced DCM mice.

**MATERIALS AND METHODS**

**DOE Preparation** The dried material of *Dendrobium officinale* Kimura et Migo (batch No: XZ20140301) were pur-
chased from Xi’an Xiaocao Botanical Development Co., Ltd., Xi’an, China in January 2018 and authenticated by professor Zhubu Li (College of Pharmaceutical Sciences, Southwest University, Chongqing, China) in compliance with the identification standard of Pharmacopoeia of People’s Republic of China. The voucher specimens (No. 20140609) were submitted at the Herbarium of Materia Medica, Department of Traditional Chinese Medicine, College of Pharmaceutical Sciences, Southwest University, Chongqing, China. The dry stems were crushed into suitable powder through 350-mesh. The powders were pre-extracted by petroleum ether and 80% ethanol with 60°C. The residues were extracted with double distilled water for 3 times, and thus the crude extracts were filtered, concentrated, dried by lyophilization. Forty seven gram pow-ered extracts were collected from 200 g powders. The content of polysaccharides in DOE was determined by the phenol-sulfuric acid method. Glucose was used as the standard (D-glucose, Sigma, St. Louis, MO, U.S.A.).

Preparation of 1-Phenyl-3-methyl-5-pyrazolone (PMP) Derivatives of Monosaccharide The monosaccharide composition in DOE polysaccharide was analyzed by HPLC. The amount of mannose and glucose were determined by PMP pre-column derivatization method on the basis of the Pharmacopoeia of People’s Republic of China (2015 Edition). As described by Xiang, PMP (Sigma) derivatives of mannose, glucose, galactose, galacturonic acid and arabinose (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) were prepared before HPLC analysis.

HPLC Analysis The RPHPLC (LC 20A, SHIMADZU, Japan) system equipped with Dikma Diamonsil C18 column (150 × 4.6 mm; 5 mm; Dikma Technologies, China) and SPD-20 A detector was performed to separate the PMP-derivatives of monosaccharide. The flow phase is composed of 80% ammonium acetate (A, 0.02 M) and 20% acetonitrile (B). The wavelength of the detector was 250 nm and the column temperature was 30°C. Internal standard method was performed to the quantitative analysis. The amount of mannose and glucose was expressed as percentage of the extracts of Dendrobium officinale.

Animals and DOE Treatment Kunming male mice, 8–10 weeks of age, weighting 20 ± 2 g, were purchased from Chongqing Tengxin Biotechnology Co., Ltd. [SCXK (Yu) 2017-0002]. The mice were treated with 22°C with a 12 h light/dark cycle and free access to food and tap water. All the animal were administrated according to the National Institutes of Health (NIH) guidelines and approved by the Ethical Committee for Animal of Southwest University.

The diabetic mice model was induced by intraperitoneal injection of streptozotocin (Sigma) at the dose of 30 mg/kg body weight (fresco dissolved in 0.1 M sodium citrate buffer pH 4.5) for 4 consecutive days after intragastric administration of HFD (19.67kJ/g, 45% of energy from fat, 35.2% of energy from carbohydrate, 19.8% of energy from protein, purchased from Botai Hongda Biotechnology Ltd., Beijing) for 2 weeks. Blood glucose levels were measured using glucometer (Sinocare Inc Co., Ltd., Changsha, China) for 2h, the membranes were incubated with primary antibodies against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000, Wanlei, China), peroxisome proliferator activated receptor-α (PPAR-α) (1:1000, Wanlei, China), phosphorylated c-Jun N-terminal kinase (p-JNK) (1:1500, Wanlei, China), IRS1 (1:1000, Wanlei, China), JNK (1:1000, Wanlei, China), p-insulin receptor substrate 1 (IRS1) (1:800, Amyjet Scientific, China), IRS1 (1:500, Wanlei, China), Twist (1:500, Wanlei, China), Snail 1 (1:1000, Wanlei, China), E-cadherin (1:1000, Wanlei, China), Vimentin (1:1000, Wanlei, China) overnight at 4°C. Then the membranes were further reacted with appropriate HRP goat anti-rabbit immunoglobulin G (IgG) (1:5000, Antgene Biotech) for 1h. The chemiluminescence signals were recognized by ECL reagents (Advanta, CA, U.S.A.). Blots were visual-
Statistical Analysis  SPSS 16.0 software (SPSS, Inc., Chicago, IL, U.S.A.) was performed to process all data presented as mean ± standard deviation (S.D.). Statistical analyses of the data were performed by one-way ANOVA using post hoc multiple comparisons; p < 0.05 was considered a significant difference.

RESULTS

Analysis of DOE  High sensitivity and high consistency calibration curves were performed for glucose. Polysaccharide is the main component of water extract and the concentration of polysaccharide determined by phenol-sulfuric acid method was 44.83%. As shown in the result of RP-HPLC (Fig. 1), the PMP-labeled monosaccharide in DOE were separated by HPLC. The result manifested that DOE contains two major monosaccharides, including mannose and glucose. And the content of mannose and glucose is 28.97 and 4.6%, respectively.

DOE Alleviates Body and Heart Loss in HFD/STZ-Induced DCM Mice  After the model establishment, body weight of all diabetes mice was similar and was significantly lower than that in normal mice (Fig. 2A). After 12 weeks of treatment with DOE, a significant increase in body weight was observed. The body weight in high dose DOE-treatment group increased from 36.12 ± 1.90 to 39.98 ± 3.08 g. In addition, the heart weight and heart-to-body weight ratio (HW/BW) in the diabetic groups were significantly higher than that in the control group. Treatment with DOE reversed their upward trend, and the HW/BW of high dose group was remarkably lower than the model group (Figs. 2B, 2C). The data manifested that DOE may protect heart against cardiac hypertrophy.

DOE Ameliorates Fasting Blood Glucose and Insulin Resistance in HFD/STZ-Induced DCM Mice  After the model establishment, the fasting blood glucose of all diabetes mice were similar and was significantly higher than that in normal mice (Fig. 3A). But the fasting blood glucose levels of all diabetes model kept falling after 12 weeks of gastrointestinal treatment with DOE. The results of the last fasting blood glucose test showed that treatment with DOE reversed their upward trend, and the fasting blood glucose levels of middle and high dose group was remarkably lower than the model group (Fig. 3B). Insulin resistance and abnormal secretion are central to the development of type 2 diabetes. As illustrated in Fig. 3C, the serum fasting insulin of all diabetes model was higher that in normal mouse, and that in high dose group significantly lower than that in model group. The HOMA-IR index was 4-fold higher in the diabetes group than in the control group (Fig. 3D), nevertheless that in the low DOE, middle DOE and high DOE groups was decreased 3-, 2.8- and 2-fold, respectively. These data manifested that DOE distinctly ameliorated fasting blood glucose and relieved insulin resistance.

DOE Accelerates Lipid Transport  To investigate whether DOE treatment could increase fatty acid metabolism in HFD/STZ-induced DCM mice. We measured TC, TG, HDL-C and LDL-C levels in the serum. As showed in Fig. 4, TC, TG, HDL-C and LDL-C levels in the diabetes model group were 4.61 ± 0.30, 3.81 ± 0.07, 1.65 ± 0.21 and 2.79 ± 0.18 (mmol/L), respectively, while they in the control group were 3.43 ± 0.29, 1.85 ± 0.12, 2.97 ± 0.19 (mmol/L) and 1.45 ± 0.07 (mmol/L), respectively. Obviously, TC TG and LDL-C increased and HDL-C decreased in diabetic mice compared to that in control group.
After treatment with DOE for 12 weeks, the TC level significantly decreased to 4.49 ± 0.18, 4.30 ± 0.10 and 4.00 ± 0.33 (mmol/L) in low, middle and high groups, respectively. The TG level decreased to 3.35 ± 0.48, 2.94 ± 0.25 and 2.44 ± 0.15 (mmol/L) in low, middle and high groups, respectively. The HDL-C and LDL-C levels increased to 1.85 ± 0.12, 2.10 ± 0.10, 2.69 ± 0.32 (mmol/L), respectively. Moreover, the LDL-C decreased to 2.51 ± 0.09, 2.00 ± 0.10, 1.78 ± 0.19 (mmol/L), respectively. In DOE groups, TC, TG and LDL-C levels were significantly reduced and HDL-C was significantly increased compared with dyslipidemic-diabetic mice. In addition, Western blotting results revealed that DOE dose-dependently up-regulated the level of PPAR-α and p-IRS1 and down-regulated the level of p-JNK in heart and liver tissue (Fig. 5). The data illustrated that DOE ameliorated fatty acid metabolism via PPAR-α and JNK in HFD/STZ-induced DCM mice.

DOE Prevents DCM-Induced Myocardial Fibrosis

The development of interstitial fibrosis is also a structural hallmark of diabetic cardiomyopathy. To measure the cardiac fibrosis, Sirius-red staining was used to determine the collagen represented by the red areas in the myocardial tissues (Fig. 7A). Obviously, the red areas of the control groups were the least. As shown in Fig. 7B, the deposition of collagen was 4.5-fold higher in the diabetes group than in the control group, whereas that in the low DOE, middle DOE and high DOE groups was decreased to 3-, 2- and 1.5-fold, respectively. The data demonstrated that DOE obviously decreased the deposition of collagen, downgraded of fibronectin and ameliorated fibrosis in HFD/STZ-induced DCM mice.

DOE Attenuates EMT in HFD/STZ-Induced DCM Mice

Western blotting analysis was performed to determine the progress of EMT. The expression of Vimentin was increased and the expression of E-cadherin decreased in heart tissue of diabetes groups. Of note, the downregulation of epithelial cell junction proteins E-cadherin and the activation of mesenchymal adhesion genes vimentin were characteristic of EMT. DOE treatment increased the expression of E-cadherin and decreased the expression of Vimentin. Meanwhile, the increased expression of twist and snail1, transcription regulators of EMT, also confirmed the enrichment of EMT characteristics in model group (Fig. 8A). In addition, TGF-β1, a potent inducer of EMT was shown obvious dose-dependent...
down-regulation in heart tissue after treatment with DOE (Fig. 8B). Those result demonstrated that DOE attenuated EMT in HFD/STZ-induced DCM mice.

**DISCUSSION**

In the present study, diabetic mice exhibited symptoms of excessive intake, excessive excretion and emaciation. Treatment with DOE for 12 weeks relieved these symptoms and decreased fasting blood glucose in DCM mice. The biochemi-
cal indexes indicated that DOE reduced the level of TC, TG, LDL-C and serum fasting insulin and increased level of HDL-C in DCM mice. The results of HOMA-IR calculation showed that DOE relieved insulin resistance. Hematoxylin-eosin staining and Sirius red staining showed that DOE remarkably decreased cardiac injury and fibrosis. Moreover, Western blotting assay revealed that DOE ameliorated lipid transport and suppressed EMT in HFD/STZ-induced DCM mice.

Insulin-producing pancreatic endocrine cells are selectively destroyed by STZ. Long periods of hyperglycemia lead to changes in the pathology and function of various organs, such as heart and liver. Elevated blood glucose level, increased drinking water, more food consumption, and blood urea nitrogen production, as well as reduced body weight were shown in diabetes mellitus mice. Type 2 diabetes mellitus is characterized by insulin secretory dysfunction and insulin resistance. The clinical prevention and treatment of type 2 diabetes mellitus mainly begins with the reduction of insulin resistance and insulin secretory dysfunction. HOMA-IR is not only a useful indicator for diagnosis of insulin resistance, but also a follow-up indicator for the treatment of type 2 diabetes. In the present experiment, DOE significantly increased body weights and decreased HOMA-IR in HFD/STZ induced diabetic mice. In addition, peroxisome proliferator-activated receptors is an essential roles in glucose and lipid metabolic processes. Easily binding polyunsaturated fatty acids, PPAR-α accelerates β-oxidation of adipocytes in islet β cells and the clearance of fat in insulin-sensitive organs, resulting in increased insulin secretion under glucose stimulation. It reported that the protection of mice with macrophage-specific JNK deficiency against insulin resistance was associated with reduced tissue infiltration by macrophages. The JNK signaling pathway regulates the PPARα-FGF21 hormone axis. Sustained JNK activity is known to contribute to endoplasmic reticulum stress. The inhibitory serine phosphorylation of IRS-1 by JNK is known to underline inflammatory-as well as free fatty acid-induced insulin resistance. The study indicated that DOE significantly reduces TC, TG, HDL-C and LDL-C in DOE treatment group. Meanwhile, the increased expression of PPAR-α and p-IRS1 and the decreased expression of p-JNK were also found in the present study. In conclusion, the hypoglycemic and hypolipidaemic effects of DOE may prevent the deleterious effects of hyperglycemia and hyperlipidemia on the development of diabetes and diabetic cardiomyopathy. The possible mechanism may be associated with the activation of
The development of fibrosis is one of the structural hallmark and the major causes of diabetes cardiomyopathy.\(^{35}\) Sirius red staining showed that DOE can significantly inhibit fibrosis. The underlying mechanism might be that pro-fibrotic factor TGF-\(\beta\) was inhibited. In the present study we have confirmed that DOE indeed reduced the expression of TGF-\(\beta\) protein. Under long-term hyperglycemia conditions, TGF-\(\beta\) is activated and the activated TGF-\(\beta\) is directly associated with TGF-\(\beta\) receptor II, which raises TGF-\(\beta\) receptor I and lead to phosphorylation of Smad2 and Smad3.\(^{38}\) The activated phosphorylation of Smad2 and Smad3 could promote the deposition of extracellular matrix accumulation proteins, including collagen, elastin, laminin and fibronectin.\(^{39}\) In addition, TGF-\(\beta\) is a potent inducer of EMT, which has been reported by numerous studies.\(^{40}\) Epithelial cells EMT-differentiated transform into mesenchymal phenotypes, producing fibroblasts and myofibroblasts and EMT is widely considered as playing an important role in fibrosis.\(^{35}\) Partial EMT process after epithelial cell injury leads to prolonged cell proliferation, cell cycle stagnation, and secretion of fibrogenic factors, thereby promoting fibrosis and parenchymal injury.\(^{42}\) The Snail family and Twist family were two major groups of EMT-activating transcription factors, which are used to demonstrate the functional significance of EMT.\(^{43}\) During the epithelial mesenchymal process, epithelial polarity and cell connections are absent, while E-cadherin maintains tight junction in cells.\(^{31}\) Similarly, Vimentin is activated and intensifies epithelial mesenchymal fibrosis.\(^{40}\) The current study indicated that the expression of E-cadherin was significantly decreased, while Vimentin, Twist and Snail1 levels were remarkably increased in the model mice. However, DOE up-regulated the expression of E-cadherin and down-regulated the expression of Vimentin, Twist and Snail1 in cardiac tissue. The results demonstrated that DOE rescued EMT and reduce the progression of myocardial fibrosis.

In conclusion, the present study demonstrated that oral administration of DOE effectively ameliorated HFD/STZ induced DCM by accelerating lipid transport, inhibiting insulin resistant. Furthermore, DOE suppressed myocardial fibrosis through inhibiting epithelial mesenchymal transition in DCM mice. Herein, the results indicated that DOE possessed heart-protective effects against DCM and can serve as a potential drug for treating DCM.

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Author Contributions Xiaoyan Zhao and Zhubo Li designed the project; Dongning Li performed the experiments; Dongning Li and Jie Zhang wrote the manuscripts; Jie Zeng analyzed and interpreted data; all authors reviewed the manuscript.

Conflict of Interest The authors declare no conflict of interest.
REFERENCES

1) Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract., 138, 271–281 (2018).

2) DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia, 53, 1270–1287 (2010).

3) Chen PH, Lin YK, Chang CK, Chiang SJ, Tsai SY. Dysregulation of glucose metabolism since young adulthood increases the risk of cardiovascular diseases in patients with bipolar disorder. Kaohsiung J. Med. Sci., 33, 630–636 (2017).

4) Yan B, Singla DK. Transplanted induced pluripotent stem cells mitigate oxidative stress and improve cardiac function through the Akt cell survival pathway in diabetic cardiomyopathy. Mol. Pharm., 10, 3425–3432 (2013).

5) Diao X, Shen E, Wang X, Hu B. Differentially expressed microRNAs and their target genes in the hearts of streptozocin-induced diabetic mice. Mol. Med. Rep., 4, 633–640 (2011).

6) Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ. Res., 107, 1058–1070 (2010).

7) Regan TJ, Ahmed S, Haider B, Moschos C, Weisse A. Diabetic cardiomyopathy: experimental and clinical observations. N. J. Med., 91, 776–778 (1994).

8) Liu M, Liu L, Bai M, Zhang L, Ma F, Yang X, Sun S. Hypoxia-induced activation of Twist/mir-214/e-cadherin axis promotes renal tubular epithelial cell mesenchymal transition and renal fibrosis. Biochem. Biophys. Res. Commun., 495, 3224–3230 (2018).

9) Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. Diabetologia, 57, 660–671 (2014).

10) Zou C, Liu X, Xie R, Bao Y, Jin Q, Jia X, Li L, Liu R. Deferiprone attenuates inflammation and myocardin fibrosis in diabetic cardiomyopathy rats. Biochem. Biophys. Res. Commun., 486, 930–936 (2017).

11) Yan XL, Wang YY, Yu ZF, Tian MM, Li H. Peroxisome proliferator-activated receptor-gamma activation attenuates diabetic cardiomyopathy via regulation of the TGF-β/ERK pathway and epithelial-to-mesenchymal transition. Life Sci., 213, 269–278 (2018).

12) Sun B, Zhang D, Zhao N, Zhao X. Epithelial-to-endothelial transition and cancer stem cells: two cornerstones of vasculogenic mimicry. Cancer Metastasis Rev., 34, 3425–3432 (2013).

13) Nilsson EG, Sayegh MH, Izumo S, Kalluri R. Endothelial-to-mesenchymal transition during pathogenesis of fistulae in Crohn’s disease. Inflamm. Bowel Dis., 24, 1514–1527 (2008).

14) Zhao M, Han J. Dendrobium officinale Kimura et Migo ameliorates insulin resistance in rats with diabetic nephropathy. Med. Sci. Monit. Basic Res., 24, 84–92 (2018).

15) Xu XR, Ma XY, Lei HH, Song HM, Ying QC, Xu MJ, Liu SB, Wang HZ. Proteomic analysis reveals the mechanisms of Mycena dendrobii promoting transplantation survival and growth of tissue culture seedlings of Dendrobium officinale. J. Appl. Microbiol., 118, 1444–1455 (2015).

16) Xiang L, Sze CW, Ng TB, Tong Y, Shaw PC, Tang CW, Zhang YBK. Polysaccharides of Dendrobium officinale inhibit TNF-alpha-induced; apoptosis in A-253 cell line. Inflamm. Res., 62, 313–324 (2013).

17) Xiang L, Shaw PC, Sze CW, Yao T, Zhang Y. Dendrobium officinale polysaccharides ameliorate the abnormality of aquaporin 5, pro-inflammatory cytokines and inhibit apoptosis in the experimental Sjogren’s syndrome mice. Int. Immunopharmacol., 11, 2025–2032 (2011).

18) Zhao X, Dou M, Zhang Z, Zhang D, Huang C. Protective effect of Dendrobium officinale polysaccharides on H2O2-induced injury in H9c2 cardiomyocytes. Biom. Med. Pharmacother., 94, 72–78 (2017).

19) Zang Z, Zhang D, Dou M, Li Z, Zhang J, Zhao X. Dendrobium officinale Kimura et Migo attenuates diabetic cardiomyopathy through inhibiting oxidative stress, inflammation and fibrosis in streptozotocin-induced mice. Biomed. Pharmacother., 84, 1350–1356 (2016).

20) Skovsø S. Modeling type 2 diabetes in rats using high fat diet and streptozocin. J. Diabetes Invest., 5, 349–358 (2014).

21) Wallace TM, Matthews DR. The assessment of insulin resistance in man. Diabet. Med., 19, 527–534 (2002).

22) Li S, Huang Q, Zhang L, Qiao X, Zhang Y, Tang F, Li Z. Effect of CAPE-pN02 against type 2 diabetes mellitus via the AMPK/GLUT4/GSK3β/PPARγ pathway in HFD/SZT2-induced diabetic mice. Eur. J. Pharmacol., 853, 1–10 (2019).

23) Tesch GH, Allen TJ. Rodent models of streptozotocin-induced diabetic nephropathy. Nephrol., 12, 261–266 (2007).

24) Delevé AE, Sharma K. New pharmacological treatments for improving renal outcomes in diabetes. Nat. Rev. Nephrol., 6, 371–380 (2010).

25) Prattley RE, Weyer C. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. Diabetologia, 44, 929–945 (2001).

26) Katsuki T, Sumida Y, Gabazza EC, Murashima S, Furuta M, Ariki-Sasaki R, Hori Y, Yano Y, Adachi Y. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. Diabetes Care, 24, 362–365 (2001).

27) Ye G, Gao H, Lin Y, Ding D, Liao X, Zhang H, Chi Y, Dong S. Peroxisome proliferator-activated receptor A/G reprogrammes metabolism associated with lipid accumulation in macrophages. Metabolomics, 15, 36 (2019).

28) Bragt MC, Poepeus HE. Peroxisome proliferator-activated receptors and the metabolic syndrome. Physiol. Behav., 94, 187–197 (2008).

29) Ip E, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPARα-dependent hepatic lipid turnover in dietary steatohepatitis in mice. Hepatology, 38, 123–132 (2003).

30) Han MS, Jung DY, Morel C, Lakhani SA, Kim JK, Flavell RA, Davis RJ. JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. Science, 339, 218–222 (2013).

31) Vernia S, Cavanagh-Kyros J, Garcia-Haro L, Sabio G, Barrett T. The PPARα-TGFβ2 hormone axis contributes to metabolic regulation by the hepatic JNK signaling pathway. Cell Metab., 20, 512–525 (2014).

32) Win S, Tian T, Fernandez-Check J, Kaplowitz N. JNK interaction with Sab mediates ER stress induced inhibition of mitochondrial respiration and cell death. Cell Death Dis., 5, e989 (2014).

33) Altamimi TR, Gao S, Karwi QG, Fukushima A, Rawat S, Wagg CS, Zhang LY, Lopachuk GD. Adiponectin regulates cardiac energy metabolism and improves cardiac function and efficiency. Metabolism, 98, 37–48 (2019).

34) Toedebusch R, Belenchia A, Pulakat L. Diabetic cardiomyopathy: impact of biological sex on disease development and molecular signatures. Front. Physiol., 9, 453 (2018).

35) Balah A, Ezzat O, Akool ES. Vitamin E inhibits cyclosporin A-induced CTGF and TIMP-1 expression by repressing ROS-mediated activation of TGF-β/Smad signaling pathway in rat liver. Int. Immunopharmacol., 65, 493–502 (2018).

36) Miyazono K. TGF-beta signaling by Smad proteins. Cytokine...
39) Yang L, Hu J, Hao HZ, Yin Z, Liu G, Zou XJ. Sodium tanshinone II A sulfonate attenuates the transforming growth factor-β1-induced differentiation of atrial fibroblasts into myofibroblasts in vitro. *Int. J. Mol. Med.*, **35**, 4 (2015).

40) Wu J, Chen X, Liu X, Huang S, He C, Chen B, Liu Y. Autophagy regulates TGF-β2-induced epithelial-mesenchymal transition in human retinal pigment epithelium cells. *Mol. Med. Rep.*, **17**, 3607–3614 (2018).

41) Willis B, Borok Z. TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **293**, L525–L534 (2007).

42) Han Q, Lin L, Zhao B, Wang N, Liu X. Inhibition of mTOR ameliorated bleomycin-induced pulmonary fibrosis by regulating epithelial-mesenchymal transition. *Biochem. Biophys. Res. Commun.*, **500**, 839–845 (2018).

43) Iderzorig T, Kellen J, Osude C, Singh S, Woodman JA, Garcia C, Puri N. Comparison of EMT mediated tyrosine kinase inhibitor resistance in NSCLC. *Biochem. Biophys. Res. Commun.*, **496**, 770–777 (2018).

44) Wang Z, Divanyan A, Jourd'Heuil F, Goldman RD, Ridge KM, Jourd'Heuil D, Lopez-Soler RI. Vimentin expression is required for the development of EMT-related renal fibrosis following unilateral ureteral obstruction in mice. *Am. J. Physiol. Renal Physiol.*, **315**, F769–F780 (2018).