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Dendrobium Officinale Kimura et Migo Ameliorates Insulin Resistance in Rats with Diabetic Nephropathy

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Background: Emerging evidence suggests the potential of *Dendrobium officinale Kimura et Migo* (DO) in treating the complications of diabetes mellitus (DM). We evaluated the therapeutic potential of DO in treating diabetic nephropathy (DN) by preventing insulin resistance.

Material/Methods: A DN model was established. Mean glomerular volume of rats was estimated by the method of Weibel-Gomez. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to determine the expression of mRNAs and we used Western blot assay to determine the expression of proteins. The levels of fasting insulin (FINS) and glucagon (GLU) were measured and we assessed the levels of high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) using the enzyme-linked immunosorbent assay (ELISA).

Results: Compared with the Normal rats, the levels of urinary glucose, albuminuria, Scr, albuminuria/Scr and BUN, and the expression levels of CaN, TLR-2, TLR-4, MyD88, hs-CRP, TNF- α , and IL-6, the level of FINS, GLU, and HOMA-IR were increased in DN, DO 1.0, DO 2.0, and DMBG groups. Compared with the DN rats, in DO 1.0, DO 2.0, and DMBG groups the glomerular volume was smaller, the levels of urinary glucose, albuminuria, Scr, albuminuria/Scr, and BUN, the expression levels of CaN, TLR-2, TLR-4, MyD88, hs-CRP, TNF- α , and IL-6, the level of FINS, GLU, and HOMA-IR were decreased.

Conclusions: We found that DO prevents insulin resistance in rats with DN. This may be associated with reduction of TLRs and inflammatory response, which should be further verified by loss of DO effects on DN after treatment of inhibitors of TLRs.

MeSH Keywords: **Cytokines • Diabetic Nephropathies • Insulin Resistance • Toll-Like Receptors**

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Background

Diabetes mellitus (DM) is a heterogeneous metabolic disorder resulting from variable degrees of insulin resistance and a progressive reduction in insulin secretion, and it is associated with a higher risk of microvascular/macrovacular disease [1,2]. DM is known to cause nephropathic retinopathy and neuropathy. Diabetic nephropathy (DN) is an important long-term microvascular complication of uncontrolled hyperglycemia, which develops in approximately 30% to 40% of all DM patients [3]. The initiation of microalbuminuria is widely considered as the first clinical sign of DN, which results in macroalbuminuria and subsequent reduction in glomerular filtration rate (GFR) [4]. DN is a major cause of end-stage renal disease (ESRD) and its pathophysiology appears to be involved in the interaction between insulin resistance and inflammation [5]. It is likely that a novel therapeutic strategy targeting this interaction would effectively prevent and slow the progression of DN.

Traditional Chinese medicine (TCM) has been widely used for clinical treatment in China for centuries and recently is becoming popular across the globe, especially in the treatment and management of DM and its complications. However, more extensive investigation of their effectiveness and safety and their underlying mechanisms are still urgently needed [6,7]. Dried stems of the plant *Dendrobium officinale* Kimura et Migo (DO), also known as “Tiepi Shihu” in Chinese, is a famous and prized TCM with good tonic efficacy and has been used in China and Southeast Asian countries [8]. For thousands of years, it has been reported that *Dendrobium officinale*, from which about 190 compounds have been isolated, has a wide range of pharmacological actions: anticancer, gastric ulcer protective, hepatoprotective, hypoglycemic, and antifatigue effects [8]. Previously, Zhang et al. reported the cardioprotection of *Dendrobium officinale* against diabetic cardiomyopathy by the reduction of cardiac lipid accumulation, cardiac fibrosis, oxidative stress, and pro-inflammatory cytokines, suggesting the potential of *Dendrobium officinale* in treating the complications of DM [9]. Toll-like receptors (TLRs) are a family of pattern recognition receptors that are responsible for the development of inflammatory and immune responses and their changes have been implicated in the pathogenesis of DN [10]. Interestingly, in a study reported by Zheng et al., the underlying mechanism of TCM and expression of TLRs in acute kidney injury (AKI) were investigated [11]. Here, the purpose of our study was to evaluate the therapeutic potential of 5 and 10 ml/kg/d DO in treating DN by preventing insulin resistance.

Material and Methods

Experimental animals

Fifty 8-week-old female Sprague-Dawley (SD) rats (SPF grade), purchased from Shanghai Silaike Laboratory Animal Company (license No. SCXK2007-0005), were raised in our laboratory and given free access to drinking water and food. After 1-week adaptation, in 50 selected Sprague-Dawley rats, we randomly allocated 10 rats into a normal group and 40 rats were used for establishment of DN models. The normal group received an intraperitoneal injection of sodium citrate buffer (pH 4.5) and the model group was fed a high-fat/sucrose diet (41.4 kJ/kg) including 10% lard, 20% sucrose, 2.5% cholesterol, 1.0% cholate, and 66.5% basal feed. Four weeks later, these rats underwent an overnight 12–16 h fast and received a single intraperitoneal injection of 0.5% streptozotocin (STZ, 30 mg/kg, every 2 weeks, twice, Sigma-Aldrich Biotech., St. Louis, MO). One week after the last injection, blood samples were collected from the tail vein of STZ-treated rats. Fasting blood glucose ≥ 7 mmol/L lasting for 2 weeks indicated successful establishment of T2DM models. Subsequently, these T2DM rats were fed a high-fat/sucrose diet for 8 weeks. T2DM rats had random glycemia ≥ 16 mmol/L with low insulin sensitivity, and trace albuminuria, indicating successful establishment of the diabetic nephropathy model [12]. The study protocols were approved by the Animal Ethics Committee and were in strict accordance with the International Association for the Study of Pain (IASP) ethics guidelines for the use of awake animals in pain research.

Extraction of DO and animal treatment

DO (purchased from Bright Food Group, Yunnan Dendrobium Biotechnology Development Co., Ltd.) was dried and shattered. After passing through a 10-mesh screen, 250 g of DO powder was immersed in 2.5 L water, and 1 h later was boiled for 30 min. The juice obtained was concentrated 3 times to 125 ml (equal to 2 g/ml of crude DO extractum). Successfully established 40 DN models were randomly assigned to DN, DO 1.0, DO 2.0, and DMBG groups. The rats in DO 1.0 were given intragastric administration of 5 ml/kg/day DO, those in the DO 2.0 group were given intragastric administration of 10 ml/kg/day DO, and those in the DMBG group were treated by 0.0042 g/kg/day dimethylbiguanide (DMBG). The DMBG was purchased from Sigma-Aldrich Biotech. (St. Louis, MO). Those rats in the normal and DN rats were given same volume of menstruum. After treatment, no rats received insulin and they were given free access to drinking water and food. Observation lasted for 4 weeks.

Specimen collection

Twenty-four-hour urine samples were collected from rats in each group, centrifuged at 3000 rpm for 15 min, and stored at -80°C .

Rat tail arterial pressure was measured using the RBP-1 rat tail blood pressure measuring instrument (QianSheng Biotech, Shanghai). The rats were subjected to intraperitoneal injection of 3% pentobarbital sodium (0.1 ml/100g) for anesthesia, followed by right common carotid artery ligation. Collected blood samples were centrifuged at 3000 rpm for 15 min to separate plasma. The plasma was stored at -80°C until further use. The left kidney was removed from each rat, weighed, frozen in liquid nitrogen, and stored at -80°C until further use. After the kidney surfaces were completely blanched by continuous irrigation using near-freezing saline (4°C), the right kidney was cut into slices 8 mm in length, fixed in 4% paraformaldehyde, and embedded in paraffin. Additionally, the renal cortex (1×1 mm) was collected and fixed in 2.5% glutaraldehyde.

Laboratory data

The levels of blood glucose, total cholesterol (TC), triglyceride (TG), serum creatinine (Scr), alanine aminotransferase (AST), and aspartate aminotransferase (ALT) were measured in our laboratory. The concentration of albuminuria (g/L) was determined using Coomassie Brilliant Blue (CBB) staining method according to the kit's instruction. The concentration of urinary glucose (mmol/L) was tested by the glucose-oxidase/peroxidase method. The concentration of blood urea nitrogen (mmol/L) (BUN) was measured by FEARON'S color reaction according to the kit's instruction.

Periodic acid Schiff (PAS) staining

The slices were dewaxed in xylene, rehydrated in graded alcohol, and oxidized in 1% periodic acid for 15 min. After rinsing with distilled water, the slices were immersed in Schiff reagent for 30 min, stained with hematoxylin for 1.5 min, and rinsed with 1% hydrochloric acid ethanol. Subsequently, the slices were blued in ammonia, lasting for 5 s. Thereafter, all samples were examined using light microscopy. Mean values for glomerular cross-sectional area were estimated by computer-assisted image analysis. Glomerular volume= $\beta/k\times(\text{area})^{3/2}$ with $\beta=1.38$ and $k=1.1$.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated from the tissues using TRIzol kits (Invitrogen, Carlsbad, CA) according to the manufacturers' instructions. The RNA was dissolved in diethylpyrocarbonate (DEPC)-treated ultrapure water. The quality of the RNA was determined by the ratio of optical density (OD) at 260 nm to that at 280 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) was used for the preparation of cDNA (Applied Biosystems, Foster

Table 1. The primer sequences used quantitative real-time polymerase chain reaction (qRT-PCR).

Gene	Sequence
TLR2	F: 5'-AAACTGTGTTCTGCTTCTGA-3'
	R: 5'-CTTCTTCTCAATGGGTCCAG-3'
TLR4	F: 5'-GAATGAGGACTGGGTGAGAAAC-3'
	R: 5'-ACCAACGGCTCTGGATAAAGT-3'
MyD88	F: 5'-ATACGCAACCCAGCAGAAACAG-3'
	R: 5'-TATCATTGGGGCAGTAGCAGA-3'
β -actin	F: 5'-CAGCAAGCAGGAGTATGACG-3'
	R: 5'-GAAAGGTATAACGCAACTAA-3'

City, CA). The PCR reaction system was prepared according to kit's instructions (59-20063, Fermentas GmbH, St. Leon Rot, Germany). The primer sequences used are listed in Table 1. The PCR reaction conditions were: pre-denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, and extension at 70°C for 10 s. PCR was performed using a Bio Rad iQ5 Real Time PCR instrument (Bio Rad Laboratories, Hercules, CA). β -actin was used as a loading control. The data were analyzed using $2^{-\Delta\Delta\text{Ct}}$ method. $\Delta\text{Ct}=\text{Ct}(\text{target gene})-\text{Ct}(\text{loading control})$, $\Delta\Delta\text{Ct}=\Delta\text{Ct}(\text{experimental group})-\Delta\text{Ct}(\text{control group})$. The experiment was conducted 3 times to obtain the average value.

Western blot

The protein extracted from the tissues was boiled for 5 min in sample buffer, separated in 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a nitrocellulose filter (NC) membrane, blocked for 1 h with Tris-buffered saline plus 0.1% Tween 20 (TBST) containing 5% bovine serum albumin (BSA) at room temperature, and probed overnight at 4°C with the following rabbit anti-mouse antibodies: TLR2 (1: 200); TLR4 (1: 200); MyD88 (1: 500); CaN (1: 1000); β -actin (1: 1500). All primary antibodies were purchased from Santa Cruz Biotechnology, Inc, Santa Cruz, CA. After rinsing 3 times with TBST (for 15 min each time), we added diluted goat anti-rabbit immunoglobulin G (IgG) (Abcam Inc., Cambridge, MA, USA) to the membrane at 4°C . At 4–6 h later, the membrane was rinsed 3 times with TBST thrice (for 15 min each time). Chemiluminescence reagent A solution and B solution were mixed at a ratio of 1: 1 and the mixture was added dropwise on the membrane for visualization.

Estimation of insulin resistance

The levels of fasting insulin (FINS), glucagon (GLU), and homeostasis model of assessment insulin resistance

Table 2. The weight, kidney weight relative to body weight, the levels of fasting blood glucose, fasting insulin, and blood pressure among groups.

Group	Weight (g)	Kidney weight relative to body weight (g/kg)	Fasting blood glucose (mmol/L)	Fasting insulin (mU/L)	Blood pressure (mmHg)
Normal	451.31±22.42	0.30±0.06	3.21±0.89	27.6±3.01	102.5±12.9
DN	222.13±18.32*	0.53±0.05*	6.27±1.08*	33.5±2.12*	145.5±11.3*
DO 1.0	230.46±20.13*	0.50±0.07*	5.34±1.13*	31.7±1.92*	138.7±9.6*
DO 2.0	234.30±22.22*	0.49±0.04*	4.16±0.78#	29.2±1.17#	121.4±10.3**
DMBG	235.30±21.98*	0.49±0.03*	4.21±0.90#	29.4±1.46#	120.7±10.8**

DN – diabetic nephropathy; DO – *Dendrobium officinale Kimura et Migo*; DMBG – dimethylbiguanide; * $P < 0.05$ compared with the normal group; # $P < 0.05$ compared with the DN group.

(HOMA-IR) together indicated insulin resistance. The renal tissues (150–200 mg) in each group were obtained for preparation of homogenate. The levels of FINS and GLU were determined using a radioimmunoassay according to the manufacturer's instructions (Beijing North Biotechnology Research Institute, China). The homogenate was incubated in the kit at 37°C for 2 h and mixed with 500 µl of separating agent, and the mixture was centrifuged at 3500 rpm for 15 min. $HOMA-IR = FINS (\mu U/mL) \times Fasting\ blood\ glucose (mmol/L) / 22.5$.

Enzyme-linked immunosorbent assay (ELISA)

The renal tissues were homogenized, coated, and incubated overnight at 4°C in an ELISA plate (Shanghai Alpha Biotechnology Co. Ltd., China). The plate was rinsed 3 times with phosphate-buffered saline (PBS) containing Tween20 0.05% (PBST) and blocked for 1 h with 1% BSA at 37°C. After rinsing 3 times with PBST again, to each well in the plate, we added Chemiluminescence reagent A solution and B solution (Shanghai Alpha Biotechnology Co. Ltd., China). Ten minutes later, 2 mo1/L of H₂SO₄ was added to terminate the reaction. Optical density (OD) at 540 nm was assessed. The levels of high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were determined by use of the appropriate kits (Wuhan Boster Biological Technology Ltd., Wuhan, Hubei, China) according to manufacturer's instructions.

Statistical analysis

The statistical software package SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data are expressed as mean ± standard deviation. The *t* test was performed for comparisons between 2 groups. Testing for homogeneity of variance was conducted, followed by two-way analysis of variance (ANOVA) for comparisons among groups, and Fisher's least significant difference (LSD) test was used

for pairwise comparisons. Differences that produced *p*-values less than 0.05 were accepted as significant.

Results

DO reduced fasting blood glucose, fasting insulin, and blood pressure

The rats in the normal group had normal eating and drinking behaviors, as well as normal mental state and daily activity. No rats died. Three days after injection of STZ, the rats developed polydipsia, polyuria, and polyphagia, and these signs became worse as the disease duration increased. At the 4th week, STZ-injected rats had pale hair and showed a slow response. Compared with the rats in the normal group, the rats in DN, DO 1.0, DO 2.0, and DMBG groups had lower body weights ($P < 0.05$), while compared with the rats in the DN group, rats in the DO 1.0, DO 2.0, and DMBG groups were heavier ($P > 0.05$). Compared with the normal group, DN, DO 1.0, DO 2.0, and DMBG group rats had increased kidney weights relative to body weight. Compared with the DN group, levels of fasting blood glucose, fasting insulin, and blood pressure in the DO 2.0 and DMBG groups were decreased ($P < 0.05$), with no significant difference recorded among normal, DO 2.0, and DMBG groups. Blood pressures in the DO 2.0 and DMBG groups were significantly lower than in the DN group. These data suggest that DO reduced fasting blood glucose, fasting insulin, and blood pressure in the rats with DN. The level of blood glucose was lower in the DO 2.0 than in the DO 1.0 group, although the difference was not significant ($P > 0.05$) and there was no significant difference between DO 2.0 and DMBG groups ($P > 0.05$, Table 2).

DO improved renal function in rats with DN

As shown in Table 3, compared with the normal group, DN, DO 1.0, DO 2.0, and DMBG groups had increased levels of urinary

Table 3. The levels of urinary glucose, albuminuria, Scr and BUN among groups.

Group	Urinary glucose (mmol/L)	Albuminuria (mg/L)	Scr (mmol/L)	Albuminuria/Scr	BUN (mmol/L)
Normal	0	27.1±2.06	3.01±0.55	8.15±0.89	255.51±27.88
DN	11.47±0.75*	391.05±37.05*	17.54±1.77*	26.16±1.42*	375.62±29.33*
DO 1.0	6.64±0.46**	299.24±37.12**	12.21±1.26**	23.23±0.54**	310.22±8.45**
DO 2.0	5.53±0.45**	282.34±37.44**	11.47±1.34**	22.43±1.71**	305.23±27.89**
DMBG	5.44±0.44**	275.52±36.23**	11.32±1.17**	21.63±3.90**	303.37±28.82**

DN – diabetic nephropathy; DO – *Dendrobium officinale Kimura et Migo*; Scr – serum creatinine; BUN – blood urea nitrogen; DMBG – dimethylbiguanide; * $P < 0.05$ compared with the normal group; # $P < 0.05$ compared with the DN group

Table 4. The levels of TC, TG, AST and ALT among groups.

Group	TG (mg/dl)	TC (mg/dl)	AST (IU/L)	ALT (IU/L)
Normal	103.33±12.34	45.11±2.26	54.22±3.55	60.82±3.87
DN	162.22±11.23*	66.87±2.35*	54.32±3.89	62.44±3.78
DO 1.0	166.66±12.32*	67.12±2.47*	58.33±3.56	66.44±3.92
DO 2.0	165.53±12.24*	67.35±2.44*	58.61±3.33	66.81±3.93
DMBG	165.44±11.98*	67.41±2.53*	58.93±3.11	67.01±3.89

TC – total cholesterol; TG – triglyceride; AST – alanine aminotransferase; ALT – aspartate aminotransferase; DN – diabetic nephropathy; DO – *Dendrobium officinale Kimura et Migo*; DMBG – dimethylbiguanide.

glucose, albuminuria, Scr, and BUN ($P < 0.05$). Compared with the DMBG group, DO 1.0 and DO 2.0 groups had increased levels of urinary glucose, albuminuria, Scr, and BUN ($P < 0.05$). The levels of urinary glucose, albuminuria, Scr, albuminuria/Scr, and BUN were lower in the DO 2.0 group than in the DO 1.0 group, although the difference was not significant ($P > 0.05$). These indicators did not differ between DO 2.0 and DMBG groups ($P > 0.05$).

DO exerted no significant effect on blood lipids or liver function in the rats with DN

As shown in Table 4, DN, DO 1.0, DO 2.0, and DMBG groups showed higher levels of TC and TG compared with the normal group ($P < 0.05$); however, the levels of TC and TG did not change among DN, DO 1.0, DO 2.0, and DMBG groups ($P > 0.05$). The levels of AST and ALT did not differ among the 5 groups ($P > 0.05$), suggesting that DO exerted no significant effect on blood lipids or liver function in the rats with DN.

DO reduced the activity of CaN in rats with DN

We found that the expression level of CaN protein did not differ with the activity of CaN, and the expression level of CaN protein reflected the activity of CaN. Compared with the normal group, the expression levels of CaN protein were elevated in the DN, DO 1.0, DO 2.0, and DMBG groups ($P < 0.05$). Compared with the DN group, DO 1.0, DO 2.0, and DMBG groups exhibited higher expression levels of CaN protein ($P < 0.05$), suggesting that DO reduced the activity of CaN in rats with DN. The expression level of CaN protein was decreased in the DO 2.0 group compared with the DO 1.0 group, but the difference was no significant ($P > 0.05$). No significant difference was found in CaN activity between the DO 2.0 and DMBG groups ($P > 0.05$, Figure 1).

DO decreased the expression of TLRs in rats with DN

Compared with the normal group, the expression levels of TLR2, TLR4 and MyD88 mRNA and protein were higher in the DN, DO 1.0, DO 2.0, and DMBG groups ($P < 0.05$). In comparison with the DN group, DO 1.0, DO 2.0, and DMBG groups had lower expression levels of TLR2, TLR4, MyD88 mRNA, and

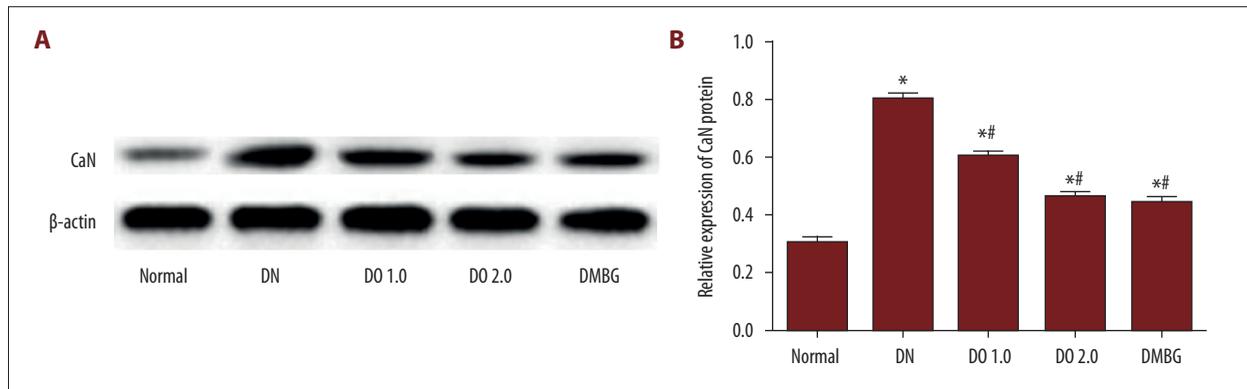


Figure 1. The expression levels of CaN protein among normal, DN, DO 1.0, DO 2.0, and DMBG groups. (A) The gray value of CaN protein band; (B) the expression level of CaN protein; * $P < 0.05$ compared with the normal group; # $P < 0.05$ compared with the DN group.

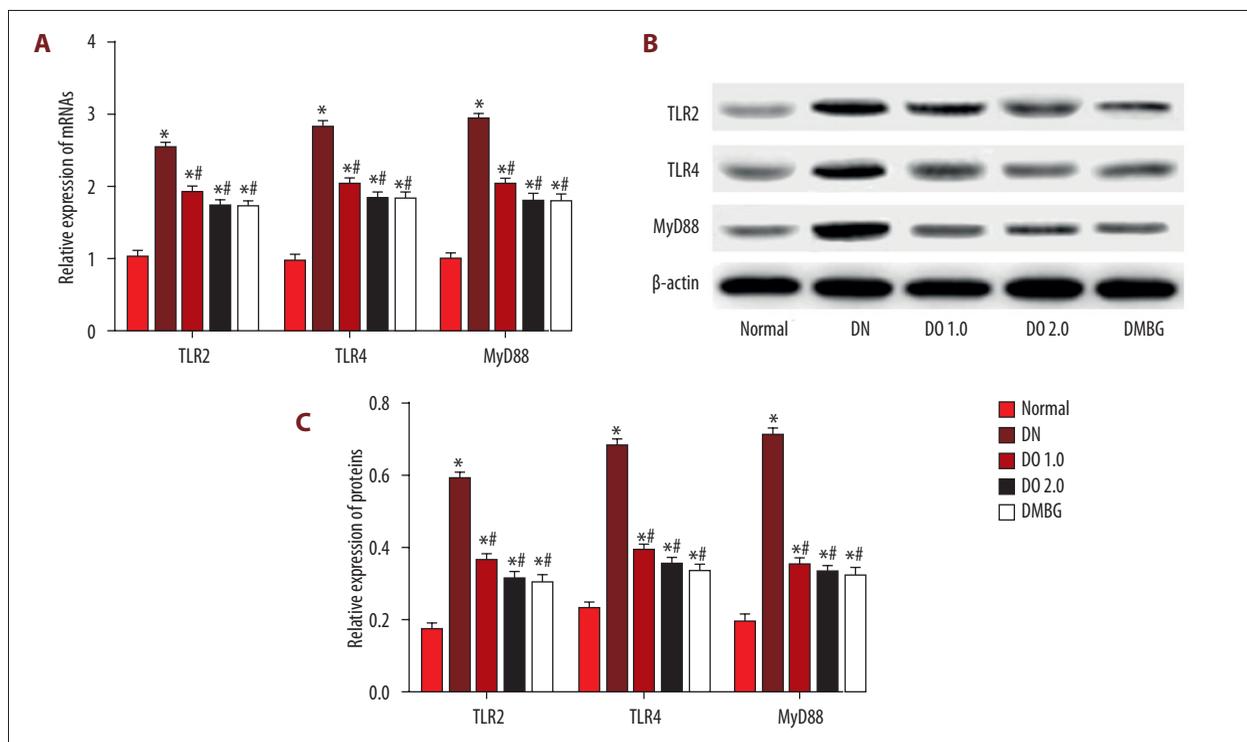


Figure 2. The expression levels of TLR2, TLR4, and MyD88 mRNA and protein among normal, DN, DO 1.0, DO 2.0, and DMBG groups. (A) The expression levels of TLR2, TLR4 and MyD88 mRNA; (B) the gray values of TLR2, TLR4, and MyD88 protein bands; (C) the expression levels of TLR2, TLR4, and MyD88 protein; * $P < 0.05$ compared with the normal group; # $P < 0.05$ compared with the DN group.

protein ($P < 0.05$), suggesting that DO decreased the expression of TLRs in rats with DN. The DO 2.0 group had lower expression levels of TLR2, TLR4, and MyD88 mRNA and protein compared with the DO 1.0, but the difference was not significant ($P > 0.05$). No significant difference was found in the expression levels of TLR2, TLR4, and MyD88 mRNA and protein between DO 2.0 and DMBG groups ($P > 0.05$, Figure 2).

DO reduced insulin resistance in the rats with DN

As shown in Figure 3, in comparison with the normal group, the level of FINS, GLU, and HOMA-IR were higher in the DN, DO 1.0, DO 2.0, and DMBG groups ($P < 0.05$). Compared with the DN group, the levels of FINS, GLU, and HOMA-IR were lower in the DO 1.0, DO 2.0, and DMBG groups ($P < 0.05$). The levels of FINS, GLU, and HOMA-IR were lower in the DO 2.0 group than in the DO 1.0 group. No significant difference was found

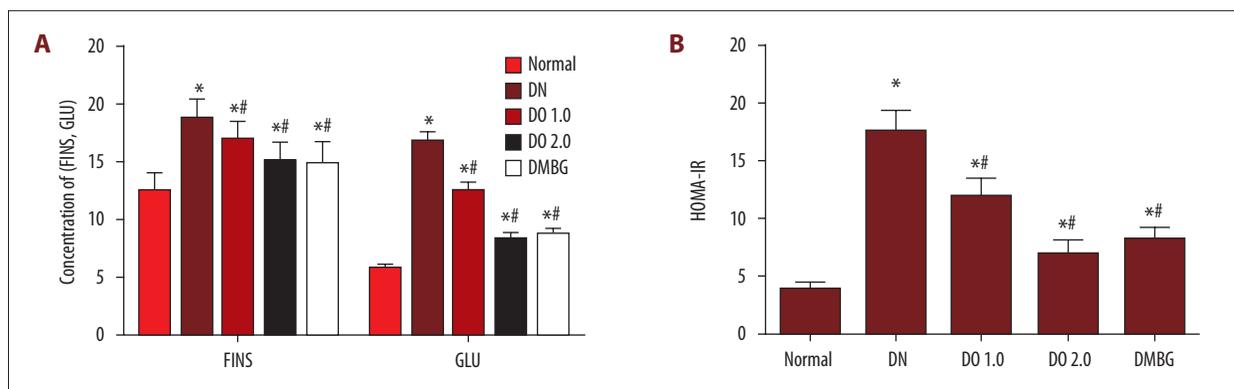


Figure 3. The level of FINS, GLU, and HOMA-IR among normal, DN, DO 1.0, DO 2.0, and DMBG groups. (A) Levels of FINS and GLU; (B) HOMA-IR * $P < 0.05$ compared with the normal group; # $P < 0.05$ compared with the DN group.

Table 5. The levels of hs-CRP, TNF- α and IL-6 among groups.

Group	hs-CRP (mg/L)	TNF- α (ng/L)	IL-6 (ng/L)
Normal	20.23±2.34	10.23±1.26	2.48±0.525
DN	36.66±3.23*	23.22±2.35*	7.67±0.89*
DO 1.0	28.89±3.32**	18.11±2.47**	4.47±0.56**
DO 2.0	27.42±2.24**	14.89±1.74**	3.56±0.33**
DMBG	26.99±2.15**	14.75±1.24**	3.53±0.34**

DN – diabetic nephropathy; DO – *Dendrobium officinale* Kimura et Migo; DMBG – dimethylbiguanide; hs-CRP – high-sensitivity C-reactive protein; TNF- α – tumor necrosis factor- α ; IL-6 – interleukin-6; * $P < 0.05$ compared with the normal group; # $P < 0.05$ compared with the DN group.

between DO 2.0 and DMBG groups ($P > 0.05$). These findings indicate that DO reduced insulin resistance in rats with DN.

DO attenuated inflammatory response in the rats with DN

Compared with the normal group, the levels of hs-CRP, TNF- α , and IL-6 were higher in the DN, DO 1.0, DO 2.0, and DMBG groups ($P < 0.05$). In comparison with the DN group, the levels of hs-CRP, TNF- α , and IL-6 were lower in the DO 1.0, DO 2.0, and DMBG groups ($P < 0.05$), suggesting that DO attenuated inflammatory response in the rats with DN. Compared with the DO 1.0 group, the DO 2.0 group had decreased levels of hs-CRP, TNF- α , and IL-6. No significant difference was found in the levels of hs-CRP, TNF- α , and IL-6 between the DO 2.0 and DMBG groups ($P > 0.05$, Table 5).

Discussion

TCM has been widely used in the treatment of complex chronic diseases due to fewer side-effects, relatively lower cost, and better patient tolerance [13]. Modern pharmacological research has shown the biological properties of TCMs in treating DM, including antioxidant [14], antihyperglycemia [15], anti-lipid

peroxidation, and anti-inflammatory effects [16]. He et al. evaluated antidiabetic potential of selected TCMs in mice with DM and found that 13 out of the selected 34 herbs exhibited a significant effect in decreasing hyperglycemia [17]. However, previous studies that investigated the therapeutic role for treating complications of DM were very limited. The present study evaluated the therapeutic potential of 5 and 10 ml/kg/d DO in treating DN by preventing insulin resistance.

One of the most important findings of the present study is that *Dendrobium officinale* reduced insulin resistance in the rats with DN. Chinese medicine considers that renal injury occurs due to qi and blood deficiency [18]. *Dendrobium officinale* is a tonic herb in folk medicine and has been used for the treatment and management of yin-deficiency diseases [8]. This may show the possibility of using *Dendrobium officinale* in treating DN. It was found that STZ destroys pancreatic islet beta cells, thereby leading to the reduction of insulin synthesis and secretion [19]. Lin et al. found a possible mechanism by which *Dendrobium officinale* affects islet cells, and considered that *Dendrobium officinale* can protect islet beta cells and promote against proliferation by increasing the expression of GLP-1R receptor [20]. The underlying mechanism of *Dendrobium officinale* on insulin resistance may be explained by the fact that

Dendrobium officinale can promote insulin secretion of pancreatic islet beta cells to improve the level of serum insulin, and inhibit glucagon secretion from pancreatic islet alpha cells to decrease the level of serum glucagon in DN rats [21]. This shows that *Dendrobium officinale* can regulate hormone secretion of pancreatic islet alpha and beta cells to reduce blood glucose levels, suggesting its hypoglycemic action [22]. As reflected in our study, compared with the untreated DN rats, the level of FINS was higher and the level of GLU was lower in the DN rats treated with *Dendrobium officinale*. We also demonstrated that the inhibitory effect of *Dendrobium officinale* might be achieved by inhibition of TLRs. The insulin-resistant state that occurs in DM can increase TLR expression and promote TLR-mediated signal transduction pathways [23]. Activation of TLR2/6 and TLR4 ligands reduces insulin secretion from pancreatic beta cells by a IL-1 β - and IL-6-mediated decrease in insulin gene expression [24]. We found that in DN rats, *Dendrobium officinale* treatment decreased the expression levels of TLR2, TLR4, and MyD88, which indicated *Dendrobium officinale* inactivated TLRs to prevent insulin resistance. Our study is not the only pharmacological study that supported the underlying mechanism of Chinese herbal medicine regulating TLRs in kidney diseases. Zheng et al. reported that TCM can attenuate renal functional damage caused by ischemia-reperfusion injury (IRI)-induced AKI in rats [11].

Another important finding in our study is that *Dendrobium officinale* can alleviate inflammatory response in rats with DN by inhibiting TLRs. *Dendrobium officinale* has been shown to inhibit oxidative stress and pro-inflammatory cytokines [25]. Toll-like receptors (TLRs) are a group of innate immune receptors. TLR2 and TLR4 can combine various pathogen-related molecular patterns and induce signal transduction, thus promoting the release of inflammatory cytokines [26]. Specifically, TLR signaling pathways are the basis of IRI, which is followed by the

production of pro-inflammatory mediators [27]. Aslam et al. found that TLRs appear to be involved in TNF- α production [28] and Souza et al. demonstrated that TLR2 plays an important role in the stimulation of IL-6 [29]. In addition, Fousteris et al. reported hs-CRP levels were positively associated with soluble ST2, which is a member of the Toll/IL-1 superfamily in DM [30]. In our study, we found that DN rats treated by *Dendrobium officinale* showed decreased levels of hs-CRP, TNF- α , and IL-6, suggesting that *Dendrobium officinale* inhibited the expression of TLRs to alleviate inflammatory response in rats with DN.

Conclusions

In conclusion, we demonstrated the therapeutic potential of 5 and 10 ml/kg/d DO in treating diabetic nephropathy (DN) by preventing insulin resistance. The present study provides a better understanding of relative significance of *Dendrobium officinale* as natural anti-DN drugs. We speculate that the anti-DN effect of *Dendrobium officinale* may be associated with reduction of TLRs and inflammatory response, which should be further verified by loss of *Dendrobium officinale* effects on DN after treatment of inhibitors of TLRs. Interestingly, the TLR family acts as a bridge between innate and adaptive immunity and hypotheses of the effect of *Dendrobium officinale* on immune reaction by blocking the TLR signaling pathway should be further confirmed. Whether the mechanism of insulin resistance is regulated by muscle or regulated by protein is still unknown in *Dendrobium officinale*. We will further explore this topic in subsequent research.

Conflicts of interest

None.

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