BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 24(5), 536-542 (2016)

Evaluating Pharmacological Effects of Two Major Components of Shuangdan Oral Liquid: Role of Danshensu and Paeonol in Diabetic Nephropathy Rat

Ying Chen[1,2], Zhuying Liu[3], Fuxing Zhou[4], Hang Zhao[1,2], Qian Yang[1,2], Hua Li[1,2], Jiyuan Sun[1,2], and Siwang Wang[1,2,*]

[1]Department of Natural Medicine, School of Pharmacy, Fourth Military Medical University, Xi'an 710032, [2]Collaborative Innovation Center for Chinese Medicine in Qinba Moutains, 169 West Changle Road, Xi'an 710032, [3]State Key Laboratory of Military Stomatology, Department of Orthodontics, School of Stomatology, Fourth Military Medical University, Xi'an 710032, [4]Department of Gynecology and Obstetrics, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China

Abstract

Shuangdan oral liquid (SDO) containing *radix Salviae miltiorrhizae* (Chinese name Danshen) and cortex moutan (Chinese name Mudanpi) is a traditional Chinese medicine using for treating vascular diseases. Danshensu (DSS) is a main effective monomer composition derived from *radix Salviae miltiorrhizae* and paeonol (Pae) from cortex moutan. Although the two herbs are widely used in traditional Chinese medicine, the pharmacological functions of their active compositions were not reported. Therefore, the research of DSS and Pae in mechanisms and pharmacodynamics interaction can provide scientific evidence to support clinical application. The diabetic nephropathy (DN) rats which were induced by streptozotocin (STZ) were treated with SDO, DSS, Pae, and DSS+Pae for eight weeks. The positive effects on DN animal models were investigated by detection of physiological and biochemical indexes and oxidative stress markers, within five treatments: SDO, DSS, Pae, DSS+Pae and insulin group. Compared with the model group, the DSS+Pae group improved the renal function, blood lipid metabolism and blood viscosity, increased the vitality of T-SOD or T-AOC and decreased the level of MDA or NO after the treatment. The study was successfully showed that the DSS+Pae group could delay the process of DN, especially in the renal injury part of histopathology changes. Our results suggest that the co-administration of DSS and Pae significantly may play a protective role in DN rats through decreasing the oxidative stress and improving the blood lipid metabolism mechanisms.

Key Words: Danshensu, Paeonol, Diabetic nephropathy, Oxidative stress, Blood lipid metabolism, Histopathology

INTRODUCTION

Shuangdan oral liquid (SDO), a famous traditional Chinese prescription made from cortex moutan (Mudanpi named in Chinese) and radix Salviae miltiorrhizae (Danshen named in Chinese) was authorized to sell by State Food and Drug Administration (SFDA, Product with code number: Z20093086, Lot No. 140323) of China for the treatment of vascular diseases. Because of the complexity of chemicals in compound prescriptions, the SDO is still not widely received in the European and American areas. There are lots of reports about the pharmacological effect of the bioactive constituents of SDO in treating diseases such as atherosclerosis, myocardial infarction and coronary heart disease. However, few reports are concerning to the effect of SDO on diabetic nephropathy (DN), which is a severe microvascular complication of diabetes mellitus (DM). Expounding the effective constituents and illuminating the functional mechanisms of the SDO should be a matter of prime importance.

Danshensu (DSS) ([2R]-3-[3,4-di-hydroxyphenyl]-2-hydroxypropanoic acid) is proved to be an active component isolated from *radix Salviae miltiorrhizae* (Danshen named in Chinese), another famous the Chinese herbal medicines (TCHM) used for improving body function (Chan *et al*., 2004; Hu *et al*., 2005; Lam *et al*., 2007), while paeonol (Pae) (4-methoxy-2-hydroxyacetophenone) is a main effective ingredient

Open Access **http://dx.doi.org/10.4062/biomolther.2015.191**

Received Nov 26, 2015 **Revised** Feb 22, 2016 **Accepted** Jun 30, 2016
Published Online Sep 1, 2016

***Corresponding Author**
E-mail: wangsiw@fmmu.edu.cn
Tel: +86-29-84772519, Fax: +86-29-84772519

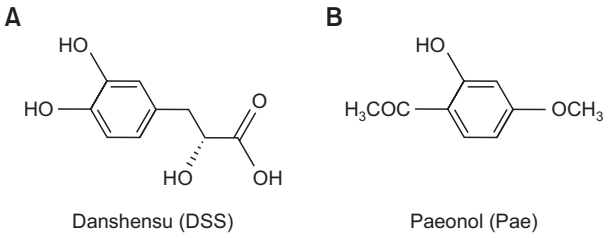

Fig. 1. Molecular structures of the investigated components: Chemical structures of Danshensu (A) and Paeonol (B).

in cortex moutan according to the previous pharmacological research (Mi *et al*., 2005; Lau *et al*., 2007; Wu *et al*., 2008; Li *et al*., 2011). The chemical structures of DSS and Pae are shown in (Fig. 1). We previously reported that the combination of DSS and Rhein could treat the chronic kidney disease by inhibiting the TGF-β/Smad3 pathway (Guan *et al*., 2015), and DSS could also significantly inhibit platelet aggregation and reduce blood viscosity (Ji *et al*., 2008). Meanwhile, the combination of Pae and salvianolic acid B could improve the blood hemorrheology and reduce oxidative injury (Yang *et al*., 2010), and Pae could also significantly inhibit lipid peroxidation and reduce low-density lipoprotein (LDL) (Dai *et al*., 2000). Furthermore, the investigation showed that the concentration of Pae could increase by the combination of DSS in the kidney tissues (Li *et al*., 2012) and increases pharmacological activity in treating vascular diseases (Wang *et al*., 2007). However, the functional mechanism of the characteristic ingredients is not well understood.

DN is a common microvascular complication in patients with poorly controlled diabetes (Ritz *et al*., 1999; Moon *et al*., 2014), and one of the most common microvascular complications of diabetes mellitus, greatly affecting the life quality and survival of the patients. DN is now considered to be the major cause of end-stage renal disease (ESRD) (Gregg *et al*., 2014). The prevention of the disease or at least the postponement of its progression has emerged as a key issue. Adverse outcomes of kidney failure can be prevented or delayed through early detection and treatment (Levey *et al*., 2003). Genetic and dietary factors (Azadbakht *et al*., 2008; Azadbakht and Esmaillzadeh, 2009) are determinants of poor glycemic control, insulin resistance (IR), and elevated glycated hemoglobin (HbAlc), which are the primary determinants of microalbuminuria (Haffner and Miettinen, 1997), a prognostic marker of kidney disease. Current therapy for diabetic nephropathy still primarily relies on the anti-proteinuric, anti-hypertensive and nephro-protective effects of renin-angiotensin system (RAS) blockers (Verbeke *et al*., 2014; Zitkus, 2014). However, these standard therapies are insufficient to prevent progression to ESRD in a substantial number of patients with residual proteinuria (albuminuria) (Perkins *et al*., 2003).

Herbal medicines normally contain many constituents. It is hypothesized that only a few constituents with favorable drug-like properties, rather than all the constituents present, are responsible for the pharmacologic effects of an herbal medicine (Lu *et al*., 2008). The main bioactive components are DSS and Pae in SDO produced by Jiulong pharmaceutical factory, Beijing. Previously, we found that DSS+Pae had the ability to increase synergistic protective effects in the type 2 DM animal models (Hu *et al*., 2012). However, further investigations of DSS+Pae in the renal injury in the type 2 DN rats have not been reported. Therefore, the purpose of this research is to explore the therapeutic efficacy of DSS+Pae on DN animal models compared with the SDO group and all other treatment groups.

MATERIALS AND METHODS

Animals

Eighty-two male Sprague-Dawley rats (6 weeks old) weighting 220 ± 20 g were obtained from the Fourth Military Medical University, China. The rats were maintained on the following experimental conditions: 12 h light/dark regime and a constant ambient temperature (20°C ± 3°C). After seven days acclimatization, 10 of the rats were randomly selected as the normal group with normal rodent diet. The rest 72 of rats were used to make diabetes models which were induced by feeding with a high-fat diet (HFD) for 8 weeks and complying with a single intraperitoneal (i.p.) injection of STZ at a dose of 30 mg/kg. STZ was dissolved in a freshly prepared sodium citrate buffer (pH 4.2). The normal rats were injected intraperitoneally with a similar volume of citrate buffer. Blood glucose concentrations were measured after 72 h by a glucometer (Accutrend; Bayer, Mannheim, Germany). Rats with a blood glucose level of >16.7 mmol/ L were selected for the study. After confirmation of diabetes, the rats were randomly divided into six groups: (1) Untreated model SD rats (Model group, n=12), (2) DN rats treated with SDO (10 mL/bottle, Jiulong Pharmaceutical Co., Ltd., Beijing, China, 5 mL/kg, orally, n=12), (3) DN rats treated with DSS (98.5% purity, Xiao Cao Botanical Development Co., Ltd., Xi'an, China, 5 mg/kg, orally, n=12), (4) DN rats treated with Pae (99.0% purity, Fei Da Bio-tech. Co., Ltd., Xi'an, China, 2.5 mg/kg, orally, n=12), (5) DN rats treated with Pae and DSS (1.25 mg/kg Pae coupled with 2.5 mg/kg DSS, orally, n=12), (6) DN rats treated with insulin (Wanbang Biochemical Pharmaceutical Co., Ltd., Jiangsu, China, serves as positive control, 10 u/kg, i.h.; n=12). The above groups were all dosed 2 times a day lasting for 8 weeks. All animal protocols were approved by the Ethics Review Committee for Animal Experimentation of the Fourth Military Medical University.

Measurement of physiological and biochemical indexes

Glucose level in the blood was measured by blood glucose monitor and related test paper (Accu-Chek Performa, ROCHE Co., Berlin, Germany). HbAlc level in the blood was measured by Semi-automatic biochemical analyzer (leidu RT-9600, Leidu Biotechnology Co., New York, USA). 24 h Urine level in the blood was measured by Urine analyzer (youlite-180, Youlite Biotechnology Co., Guilin, China). Kidney weight and body weight of DN rats was measured by Electronic balance (Sartorius Co., Hamburg, Germany). Blood urea nitrogen (BUN), serum creatinine (SCr), total cholesterol (T-CHO), triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) in DN rats were measured by commercial assay kits from huili Biotechnology Co., Ltd., Changchun, China. The whole blood viscosity was measured by automatic blood viscosity analyzer (shidizhongle Science Instrument Co., Ltd., Beijng, China).

Detection of oxidative stress markers

Total superoxide dismutase (T-SOD), total antioxidant ca-

Table 1. Effects of DSS+Pae on glucose, HbAlc and 24 h Urine in DN rats (mean ± SEM; number of specimens)

Groups	Numbers	Dose	Glucose (mmol/L)	HbAlc (ABS/10 g Hemoglobin)	24 h Urine/ml
Control group	10	5 mL/kg	6.3 ± 0.9**	29.96 ± 4.41**	13.67 ± 4.37**
Model group	12 (3#)	5 mL/kg	26.7 ± 5.1	55.93 ± 4.03	86.87 ± 11.73
SDO group	12 (1#)	5 mL/kg	24.5 ± 5.6	53.16 ± 3.65	62.30 ± 15.84**
Dss group	12 (1#)	5 mg/kg	23.3 ± 4.8	54.09 ± 4.72	61.33 ± 18.06**
Pae group	12 (1#)	2.5 mg/kg	24.8 ± 4.3	54.86 ± 3.83	59.22 ± 24.86**
Dss+Pae group	12 (2#)	3.75 mg/kg	24.3 ± 5.5	52.03 ± 6.24	58.36 ± 19.65**
Insulin group	12 (1#)	10 u/kg	13.4 ± 3.3**	31.26 ± 5.24**	57.80 ± 16.83**

#Rats died in the experiment; **Significantly different from Model group (p<0.01).

Table 2. Effects of DSS+Pae on body weight and kidney weight/body weight in DN rats (mean ± SEM; number of specimens)

Groups	Numbers	Dose	Body weight (g)	Kidney weight/body weight (10^{-3})
Control group	10	5 mL/kg	459.3 ± 34.3**	2.79 ± 0.13**
Model group	12 (3#)	5 mL/kg	302.5 ± 25.6	4.96 ± 0.21
SDO group	12 (1#)	5 mL/kg	350.3 ± 35.6**	4.45 ± 0.24**
Dss group	12 (1#)	5 mg/kg	364.0 ± 36.4**	4.12 ± 0.26**
Pae group	12 (1#)	2.5 mg/kg	363.4 ± 28.1**	4.06 ± 0.18**
Dss+Pae group	12 (2#)	3.75 mg/kg	368.9 ± 12.7**	3.15 ± 0.29**
Insulin group	12 (1#)	10 u/kg	369.0 ± 18.8**	3.09 ± 0.21**

#Rats died in the experiment; **Significantly different from Model group (p<0.01).

Table 3. Effects of DSS+Pae on BUN and SCr in DN rats (mean ± SEM; number of specimens)

Groups	Numbers	Dose	BUN (mmol/L)	SCr (mmol/L)
Control group	10	5 mL/kg	1.07 ± 0.25**	68.04 ± 7.04**
Model group	12 (3#)	5 mL/kg	2.99 ± 0.48	123.33 ± 8.00
SDO group	12 (1#)	5 mL/kg	2.01 ± 0.34**	107.31 ± 6.48**&&
Dss group	12 (1#)	5 mg/kg	1.98 ± 0.27**&	90.21 ± 4.12**&&
Pae group	12 (1#)	2.5 mg/kg	1.86 ± 0.57**&	87.11 ± 3.67**&&
Dss+Pae group	12 (2#)	3.75 mg/kg	1.70 ± 0.40**	84.54 ± 3.28**&&
Insulin group	12 (1#)	10 u/kg	1.94 ± 0.39**	101.10 ± 17.64**

#Rats died in the experiment; **Significantly different from Model group (p<0.01); &Significantly different from Insulin group (p<0.05); &&Significantly different from Insulin group (p<0.01).

Table 4. Effects of DSS+Pae on T-CHO, TG, LDL-C and HDL-C in DN rats (mean ± SEM; number of specimens)

Groups	Numbers	Dose	T-CHO (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Control group	10	5 mL/kg	2.85 ± 0.28**	3.00 ± 0.54**	2.51 ± 0.30**	0.95 ± 0.13**
Model group	12 (3#)	5 mL/kg	7.57 ± 0.92	6.11 ± 0.40	8.73 ± 1.44	0.28 ± 0.03
SDO group	12 (1#)	5 mL/kg	4.78 ± 0.80**&	4.92 ± 0.56	6.59 ± 0.56**&	0.26 ± 0.02**
Dss group	12 (1#)	5 mg/kg	4.75 ± 0.81**&	4.95 ± 0.65	5.02 ± 0.54**&	0.37 ± 0.04**&
Pae group	12 (1#)	2.5 mg/kg	3.54 ± 0.85**	4.73 ± 0.35	4.68 ± 0.42**&	0.42 ± 0.24**&
Dss+Pae group	12 (2#)	3.75 mg/kg	3.31 ± 0.53**	4.00 ± 0.52*	4.57 ± 0.47**	0.74 ± 0.16**&
Insulin group	12 (1#)	10 u/kg	3.34 ± 0.27**	3.16 ± 0.51**	5.14 ± 0.53**	0.56 ± 0.06**

#Rats died in the experiment; *Significantly different from Model group (p<0.05); **Significantly different from Model group (p<0.01); &Significantly different from Insulin group (p<0.05).

pacity (T-AOC), nitric oxide (NO), methane dicarboxylic aldehyde (MDA) in DN rats were measured by commercial assay kits from Jianchen Bioengineering Company, Nanjing, China.

Histopathological changes

A small piece of kidney tissue was taken and fixed in 10% formalin, embedded in paraffin wax. Sections were cut at 5 μm

Table 5. Effect of DSS+Pae liquid on the whole blood viscosity in DN rat (mean ± SEM; number of specimens)

Groups	Numbers	whole blood viscosity /mPa·s			
		200 s^{-1}	30 s^{-1}	5 s^{-1}	1 s^{-1}
Control group	10	2.18 ± 0.09**	4.21 ± 0.33**	7.99 ± 0.71**	19.81 ± 1.98**
Model group	12 (3#)	3.74 ± 0.25	6.73 ± 0.31	14.64 ± 0.95	40.18 ± 2.95
SDO group	12 (1#)	3.41 ± 0.12	5.53 ± 0.98&	8.69 ± 1.41*	31.33 ± 4.81
Dss group	12 (1#)	3.12 ± 0.34	4.56 ± 0.31**	8.66 ± 0.65*	30.25 ± 6.54
Pae group	12 (1#)	2.96 ± 0.22	4.31 ± 0.25**	8.56 ± 0.50*	30.06 ± 5.17
Dss+Pae group	12 (2#)	2.23 ± 0.22**	3.18 ± 0.28**	7.67 ± 0.45**	19.08 ± 1.15**
Insulin group	12 (1#)	2.64 ± 0.20**	3.15 ± 0.41**	6.56 ± 0.76**	17.41 ± 2.45**

#Rats died in the experiment; *Significantly different from Model group (p<0.05); **Significantly different from Model group (p<0.01); &Significantly different from Insulin group (p<0.05).

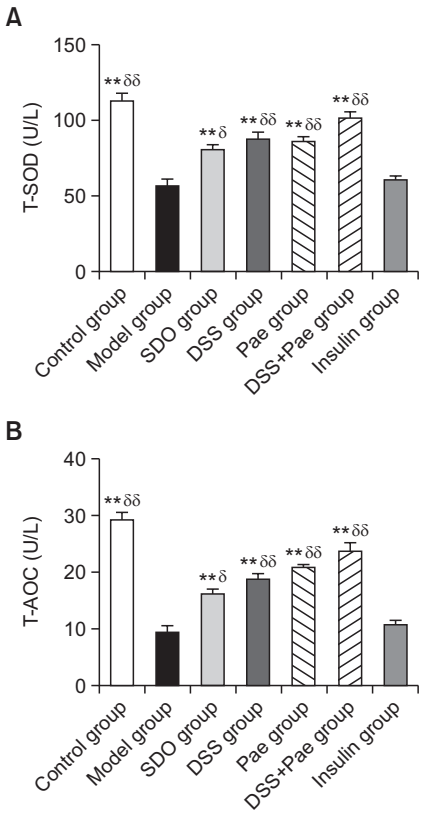

Fig. 2. Effects of DSS+Pae on T-SOD and T-AOC activity in DN rats. The levels of T-SOD (A) activity and T-AOC (B) activity were assessed in the blood (n=10 in the Control group, n=12 in the other groups). **p<0.01 and *p<0.05 compared with the corresponding Model group. $^{\delta\delta}p$<0.01 and $^{\delta}p$<0.05 compared with the insulin group. Results are given as mean ± SEM of three independent experiments.

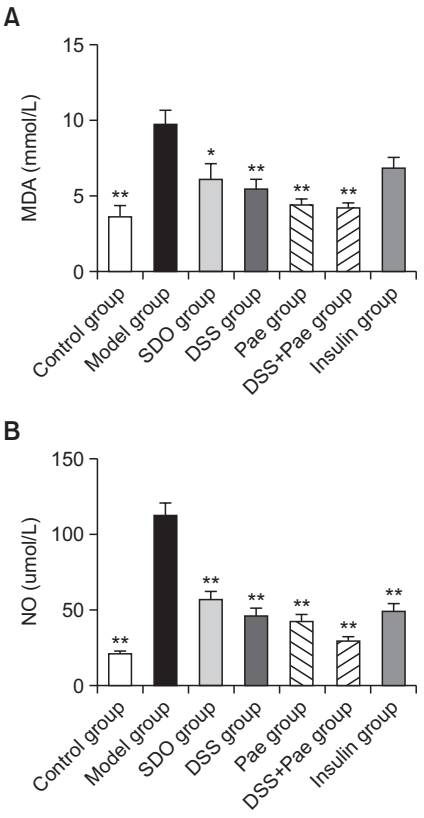

Fig. 3. Effects of DSS+Pae on MDA and NO contents in DN rats. The levels of MDA (A) content and NO (B) content were assessed in the blood (n=10 in the Control group, n=12 in the other groups). **p<0.01 and *p<0.05 compared with the corresponding Model group. Results are given as mean ± SEM of three independent experiments.

thickness and stained with haematoxylin and eosin (H&E). The sections were then viewed under microscope (OLYMPUS-IX3, Olympus Co., Tokyo, Japan) for histopathological changes.

Statistical analysis

All data were presented as mean ± SEM; data were assessed using SPSS 16.0 software. Statistical comparisons were analyzed by *t*-test. Within-group differences were analyzed by one-way analysis of variance (ANOVA). p<0.05 was considered to indicate statistical significance.

RESULTS

Physiological and biochemical indexes

As shown in Table 1, the level of blood glucose and glyco-

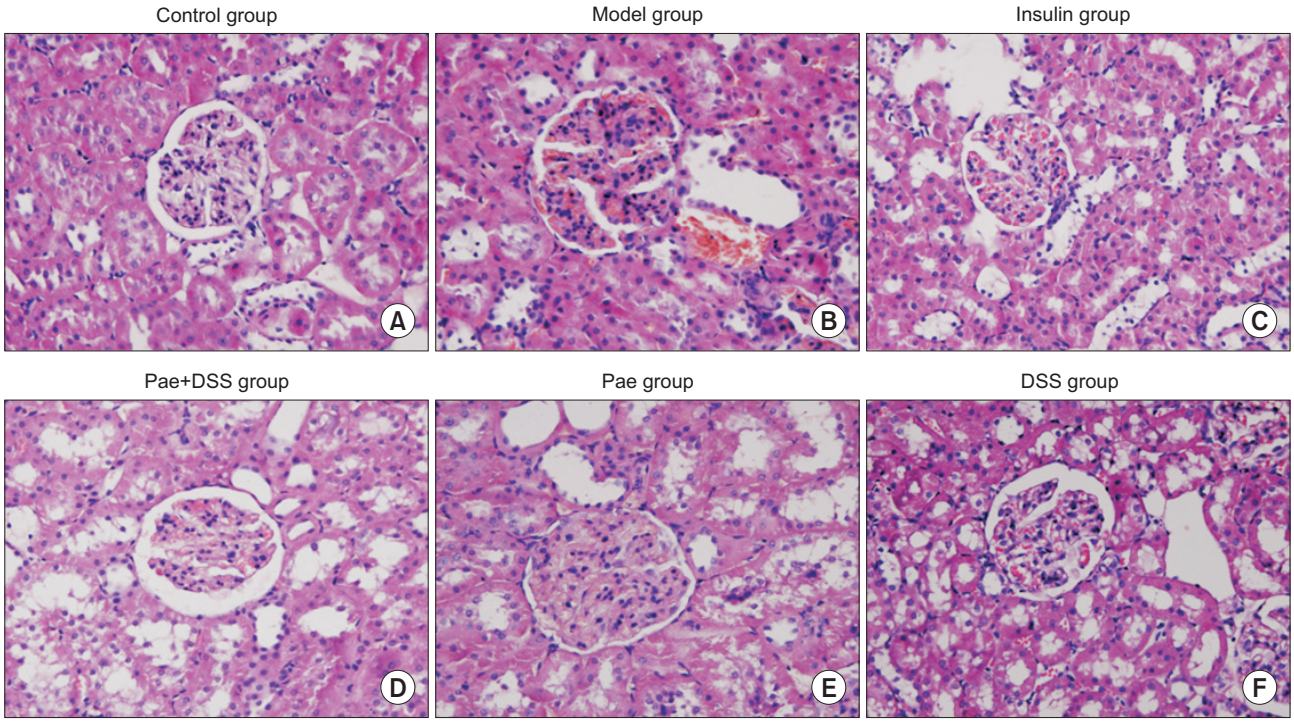

Fig. 4. Histopathology changes of kidneys in DN rats after DSS+Pae treatment: (A) Control group; (B) Model group; (C) Insulin group; (D) Pae+DSS group; (E) Pae group; (F) DSS group. With Pae+DSS treatment, histopathology changes of kidneys was markedly improved in DN rats (original magnification, ×400).

sylated hemoglobin is significant higher in model group than the control (p<0.01). However, there is no difference in glucose and glycosylated hemoglobin among SDO, DSS, Pae, and DSS+Pae treatment groups (p>0.05). Furthermore, 24h urine output is severely decreased in DSS+Pae treatment group and insulin group (p<0.01). Compared with the control, the kidney/body weight, BUN, SCr, T-CHO, TG, LDL-C and whole blood viscosity are increasing, except that the body weight and HDL-C are decreasing in the model group (p<0.05) (Table 2-5). However, compared with the model group, the kidney/body weight, BUN, SCr, T-CHO, TG, LDL-C and whole blood viscosity are decreasing but the body weight and HDL-C are increasing in DSS+Pae treatment group (p<0.05) (Table 2-5). Above all, the DSS+Pae group has shown the most effective function compared with the SDO group and the individual treatments of DSS or Pae.

Oxidative stress markers

T-SOD, T-AOC, MDA and NO are common oxidative stress markers. As shown in Fig. 2, 3, compared with the model group, all treated groups (SDO, DSS, Pae, and DSS+Pae) exhibited increased T-SOD, T-AOC activities and decreased MDA, NO contents. Moreover, the DSS+Pae group showed more reduction in oxidative stress compared with DSS and Pae groups. In addition, compared with the insulin group, the DSS+Pae group also exhibited more reduction in oxidative stress.

Histopathology changes of kidneys

As shown in Fig. 4, the volume of glomerulus is normal and the organization structure is integrated in the control. There are no abnormal histopathology changes in glomerulus, kidney tubules and mesenchyma. However, hypertrophy of glomerulus and kidney tubules, thickening of basement membrane, broadening mesangial area, vacuoles degeneration, expansion and irregular shape of epithelium in partial kidney tubules, compression subsidence in part of the capillary loops are obviously observed in model group. Compared with model group, histopathology changes are clearly catabatic in DN rats after Pae+DSS treatment. Moreover, the DSS+Pae group showed more improved compared with individual treatments of DSS or Pae.

DISCUSSION

Our study showed that the physiological and biochemical indexes in the blood of DN rats could be partly restored by SDO, DSS, Pae, and DSS+Pae. Furthermore, DSS+Pae had better therapeutical effects compared with the SDO group and the individual treatments of DSS or Pae. The indexes of oxidative stress were reduced by the combination treatment of DSS+Pae in the blood of DN rats. In the kidney, histopathology changes are clearly improved and catabatic in DN rats after the chronic supplementation of DSS+Pae. These results suggest that DSS+Pae may be the new therapeutic to improve the renal injury inpatient with diabetes. However, we did not find any alterations in the blood glucose, the treatments of DSS, Pae, and DSS+Pae did not affect the glycosylated hemoglobin of DN rats as well.

DN which is one of the main chronic complications of DM is a hackneyed capillary vessel syndrome and can lead to end-

stage renal disease. The frequent pathological characteristics of DN are the hypertrophy of glomerulus and kidney tubules, the broadening of mesangial area, the degeneration of vacuoles and the appearing of irregular shape epithelium in partial kidney tubules (Ayo *et al*., 1990; Yano *et al*., 2009), which may ultimately result in glomerular sclerosis and renal fibrosis. In this study, we observed that hypertrophy of glomerular and renal tubular was alleviated, basement membrane was not thickened, mesangial area was not obviously broadened, vacuoles degeneration was reduced in renal tubular epithelia in SDO, DSS, Pae, and DSS+Pae treatment groups. Particularly, the degree of renal recovery in DSS+Pae group was higher than the SDO group and all other treatment groups. It indicated that pathological renal lesion will be effectively improved in DN rats after DSS+Pae treatments.

An increased reactive oxygen species and insufficient antioxidant activity is associated with diabetes mellitus, which is mainly responsible for diabetic pathogenesis (Liu *et al*., 2008). The previous experimental and clinical evidence indicates that oxidative stress may play an important role in the initiation and development of diabetes complications. Further studies showed that oxidative stress contributes to the pathogenesis of diabetic nephropathy (DN) (Giacco and Brownlee, 2010; Rajavel *et al*., 2012). Free radicals play an important role in the metabolic environment balance. And Free radical damage can cause vascular permeability increase and basement membrane thickening in pathological changes, and associated with hemodynamics change of glomerular in early phase of DN (Dewanjee *et al*., 2009; Subash-Babu *et al*., 2014). According to the previous studies, DSS derived from radix Salviae miltiorrhizae inhibited oxidative stress in animal and cell model systems (Zhao *et al*., 1996; Liu *et al*., 1999; Wu *et al*., 2007; Han *et al*., 2009). In this study, compared with model group, the vitality of T-SOD or T-AOC increased and the level of MDA or NO decreased were concluded after treatments (SDO, DSS, Pae, and DSS+Pae). Meanwhile, in the DSS+Pae group, the rate of the increase of T-SOD or T-AOC and the decrease of MDA or NO significantly accelerated compared with those in the Pae, DSS and SDO treated DN groups. According to the present results, the DSS+Pae can be used as a good free radical scavenger of exogenous oxygen.

In the present study, we found that the blood lipid metabolism and the whole blood viscosity played an important role in the onset of DN. And it was reported that LDL-C was one of the characteristic atherogenic risks of DM (Zhang *et al*., 2013; Pang *et al*., 2015; Sakamoto *et al*., 2015). In this study, we draw the conclusion that the DSS+Pae improve the blood lipid metabolism by decreasing T-CHO, TG, LDL-C and increasing HDL-C ($p<0.01$). In addition, DN rats treated with DSS+Pae have significantly improved the whole blood viscosity ($p< 0.01$) than all the other treatment groups. Therefore, the results mean that it is advantaged for the DSS+Pae to improve the renal vascular injury in DN rats.

The above outcomes indicated that the co-administration of DSS+Pae compared with the SDO group and all other treatment groups has better therapeutic effect in protecting the structure and function of the kidney and in delaying the onset and development of DN. These aforementioned results clarify the functional mechanisms of the obvious protective effects after the Pae+DSS treatment in DN rats: decreased the oxidative stress and improved the blood lipid metabolism. This article indicates that the beneficial effects of the DSS+Pae may be developed as a novel therapeutic approach in DN rats, suggesting the DSS+Pae may be a candidate for therapeutic applications in DN.

ACKNOWLEDGMENTS

This work is supported by Shaanxi Province Science and Technology Research and Development Program (NO. 2013K12-07-05).

REFERENCES

Ayo, S. H., Radnik, R. A., Garoni, J. A., Glass, W. F., 2nd and Kreisberg, J. I. (1990) High glucose causes an increase in extracellular matrix proteins in cultured mesangial cells. *Am. J. Pathol*. **136**, 1339-1348.

Azadbakht, L. and Esmaillzadeh, A. (2009) Soy-protein consumption and kidney-related biomarkers among type 2 diabetics: a crossover, randomized clinical trial. *J. Ren. Nutr.* **19**, 479-486.

Azadbakht, L., Atabak, S. and Esmaillzadeh, A. (2008) Soy protein intake, cardiorenal indices, and C-reactive protein in type 2 diabetes with nephropathy: a longitudinal randomized clinical trial. *Diabetes Care* **31**, 648-654.

Chan, K., Chui, S. H., Wong, D. Y., Ha, W. Y., Chan, C. L. and Wong, R. N. (2004) Protective effects of Danshensu from the aqueous extract of Salvia miltiorrhiza (Danshen) against homocysteine-induced endothelial dysfunction. *Life Sci.* **75**, 3157-3171.

Dai, M., Liu, Q. Y., Gu, C. G. and Zhang, H. Y. (2000) Inhibitory effect of paeonol on lipid peroxidational reaction and oxidational decorate of low density lipoprotein. *Zhongguo Zhong Yao Za Zhi* **25**, 625-627.

Dewanjee, S., Das, A. K., Sahu, R. and Gangopadhyay, M. (2009) Antidiabetic activity of Diospyros peregrina fruit: effect on hyperglycemia, hyperlipidemia and augmented oxidative stress in experimental type 2 diabetes. *Food Chem. Toxicol.* **47**, 2679-2685.

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

Gregg, E. W., Li, Y., Wang, J., Burrows, N. R., Ali, M. K., Rolka, D., Williams, D. E. and Geiss, L. (2014) Changes in diabetes-related complications in the United States, 1990-2010. *N. Engl. J. Med.* **370**, 1514-1523.

Guan, Y., Wu, X. X., Duan, J. L., Yin, Y., Guo, C., Wei, G., Wang, Y. H., Zhu, Y. R., Weng, Y., Xi, M. M. and Wen, A. D. (2015) Effects and mechanism of combination of rhein and danshensu in the treatment of chronic kidney disease. *Am. J. Chin. Med.* **43**, 1381-1400.

Haffner, S. M. and Miettinen, H. (1997) Insulin resistance implications for type II diabetes mellitus and coronary heart disease. *Am. J. Med*. **103**, 152-162.

Han, J. Y., Horie, Y., Fan, J. Y., Sun, K., Guo, J., Miura, S. and Hibi, T. (2009) Potential of 3,4-dihydroxy-phenyl lactic acid for ameliorating ischemia-reperfusion-induced microvascular disturbance in rat mesentery. *Am. J. Physiol. Gastrointest. Liver Physiol.* **296**, G36-G44.

Hu, J., Li, Y. L., Li, Z. L., Li, H., Zhou, X. X., Qiu, P. C., Yang, Q. and Wang, S. W. (2012) Chronic supplementation of Paeonol combined with danshensu for the improvement of vascular reactivity in the cerebral basilar artery of diabetic rats. *Int. J. Mol. Sci*. **13**, 14565-14578.

Hu, P., Luo, G. A., Zhao, Z. and Jiang, Z. H. (2005) Quality assessment of radix Salviae miltiorrhizae. *Chem. Pharm. Bull*. **53**, 481-486.

Ji, H. S., Yu, F. and Yang, J. (2008) Comparative research on pharmacodynamics of Danshen co-microemulsion on hemorheology in rats with hyperlipidemia. *Zhong Yao Cai* **31**, 566-569.

Lam, F. F., Yeung, J. H., Chan, K. M. and Or, P. M. (2007) Relaxant effects of danshen aqueous extract and its constituent danshensu on rat coronary artery are mediated by inhibition of calcium channels. *Vascul. Pharmacol*. **46**, 271-277.

Lau, C. H., Chan, C. M., Chan, Y. W., Lau, K. M., Lau, T. W., Lam, F. C., Law, W. T., Che, C. T., Leung, P. C., Fung, K. P., Ho, Y. Y.

and Lau, C. B. (2007) Pharmacological investigations of the anti-diabetic effect of cortex moutan and its active component Paeonol. *Phytomedicine* **14**, 778-784.

Levey, A. S., Coresh, J., Balk, E., Kausz, A. T., Levin, A., Steffes, M. W., Hogg, R. J., Perrone, R. D., Lau, J. and Eknoyan, G.; National Kidney Foundation (2003) National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann. Intern. Med*. **139**, 137-147.

Li, H., Wang, S. W., Zhang, B. L., Xie, Y. H., Yang, Q., Cao, W. and Wang, J. B. (2011) Simultaneous quantitative determination of 9 active components in traditional Chinese medicinal preparation ShuangDan oral liquid by RP-HPLC coupled with photodiode array detection. *J. Pharm. Biomed. Anal.* **56**, 820-824.

Li, H., Wang, S., Zmhang, B., Xie, Y., Wang, J., Yang, Q., Cao, W., Hu, J. and Duan, L. (2012) Influence of co-administered danshensu on pharmacokinetic fate and tissue distribution of Paeonol in rats. *Planta Med.* **78**, 135-140.

Liu, J., Yang, C. F., Lee, B. L., Shen, H. M., Ang, S. G. and Ong, C. N. (1999) Effect of Salvia miltiorrhiza on aflatoxin B1-induced oxidative stress in cultured rat hepatocytes. *Free Radic. Res.* **31**, 559-568.

Liu, Z., Li, J., Zeng, Z., Liu, M. and Wang, M. (2008) The antidiabetic effects of cysteinyl metformin, a newly synthesized agent, in alloxan- and streptozocin-induced diabetic rats. *Chem. Biol. Interact.* **173**, 68-75.

Lu, T., Yang, J., Gao, X.,Chen, P., Du, F., Sun, Y., Wang, F., Xu, F., Shang, H., Huang, Y., Wang, Y., Wan, R., Liu, C., Zhang, B. and Li, C. (2008) Plasma and urinary tanshinol from Salvia miltiorrhiza (Danshen) can be used as pharmacokinetic markers for cardiotonic pills, a cardiovascular herbal medicine. *Drug Metab. Dispos.* **36**, 1578-1586.

Mi, X. J., Chen, S. W., Wang, W. J., Wang, R., Zhang, Y. J., Li, W. J. and Li, Y. L. (2005) Anxiolytic-like effect of Paeonol in mice. *Pharmacol. Biochem. Behav.* **81**, 683-687.

Moon, E., Lee, SO., Kang, T. H., Kim, H. J., Choi, S. Z., Son, M. W. and Kim, S. Y. (2014) Dioscorea Extract (DA-9801) Modulates Markers of Peripheral Neuropathy in Type 2 Diabetic db/db Mice. *Biomol. Ther. (Seoul)* **22**, 445-452.

Pang, B., Zhao, L. H., Zhou, Q., Zhao, T. Y., Wang, H., Gu, C. J. and Tong, X. L. (2015) Application of berberine on treating type 2 diabetes mellitus. *Int. J. Endocrinol*. **2015**, 905749.

Perkins, B. A., Ficociello, L. H., Silva, K. H., Finkelstein, D. M., Warram, J. H. and Krolewski, A. S. (2003) Regression of microalbuminuria in type 1 diabetes. *N. Engl. J. Med.* **348**, 2285-2293.

Rajavel, V., Abdul Sattar, M. Z., Abdulla, M. A., Kassim, N. M. and Abdullah, N. A. (2012) Chronic administration of oil palm (elaeis guineensis) leaves extract attenuates hyperglycaemic-induced oxidative stress and improves renal histopathology and function in experimental diabetes. *Evid. Based Complement. Alternat. Med.* **2012**, 195367.

Ritz, E., Rychlik, I., Locatelli, F. and Halimi, S. (1999) End-stage renal failure in type 2 diabetes: a medical catastrophe of worldwide dimensions. *Am. J. Kidney Dis.* **34**, 795-808.

Sakamoto, K., Kawamura, M., Kohro, T., Omura, M., Watanabe, T., Ashidate, K., Horiuchi, T., Hara, H., Sekine, N., Chin, R., Tsujino, M., Hiyoshi, T., Tagami, M., Tanaka, A., Mori, Y., Inazawa, T., Hirano, T., Yamazaki, T. and Shiba, T.; RESEARCH Study Group (2015) Effect of ezetimibe on LDL-C lowering and atherogenic lipoprotein profiles in type 2 diabetic patients poorly controlled by statins. *PLoS ONE* **10**, e0138332.

Subash-Babu, P., Alshatwi, A. A. and Iqnacimuthu, S. (2014) Beneficial antioxidative and antiperoxidative effect of cinnamaldehyde protect streptozotocin-induced pancreatic β-cells damage in wistar rats. *Biomol. Ther. (Seoul)* **22**, 47-54.

Verbeke, F., Lindley, E., Van Bortel, L., Vanholder, R., London, G., Cochat, P., Wiecek, A., Fouque, D. and Van Biesen, W. (2014) A european renal best practice (ERBP) position statement on the kidney disease: improving global outcomes (KDIGO) clinical practice guideline for the management of blood pressure in non-dialysis-dependent chronic kidney disease: an endorsement with some caveats for real-life application. *Nephrol. Dial. Transplant.* **29**, 490-496.

Wang, Y., Liu, L., Hu, C. and Cheng, Y. (2007) Effects of salviae mitiorrhizae and cortex moutan extract on the rat heart after myocardial infarction: a proteomic study. *Biochem. Pharmacol.* **74**, 415-424.

Wu, J. B., Song, N. N., Wei, X. B., Guan, H. S. and Zhang, X. M. (2008) Protective effects of paeonol on cultured rat hippocampal neurons against oxygen-glucose deprivation-induced injury. *J. Neurol. Sci.* **264**, 50-55.

Wu, L., Qiao, H., Li, Y. and Li, L. (2007) Protective roles of puerarin and Danshensu on acute ischemic myocardial injury in rats. *Phytomedicine* **14**, 652-658.

Yang, Q., Wang, S., Xie, Y., Wang, J., Li, H., Zhou, X. and Liu, W. (2010) Effect of salvianolic acid B and paeonol on blood lipid metabolism and hemorrheology in myocardial ischemia rabbits induced by pituitruin. *Int. J. Mol. Sci.* **11**, 3696-3704.

Yano, N., Suzuki, D., Endoh, M., Cao, T. N., Dahdah, J. R., Tseng, A., Stabila, J. P., McGonnigal, B. G., Padbury, J. F. and Tseng, Y. T. (2009) High ambient glucose induces angiotensin-independent AT-1 receptor activation, leading to increases in proliferation and extracellular matrix accumulation in MES-13 mesangial cells. *Biochem. J*. **423**, 129-143.

Zhang, Q., Xiao, X., Li, M., Li, W., Yu, M., Zhang, H., Sun, X., Mao, L. and Xiang, H. (2013) Attenuating effect of fufang xueshuantong capsule on kidney function in diabetic nephropathy model. *J. Nat. Med.* **67**, 86-97.

Zhao, B. L., Jiang, W., Zhao, Y., Hou, J. W. and Xin, W. J. (1996) Scavenging effects of salvia miltiorrhiza on free radicals and its protection for myocardial mitochondrial membranes from ischemia-reperfusion injury. *Biochem. Mol. Biol. Int.* **38**, 1171-1182.

Zitkus, B. S. (2014) Update on the american diabetes association standards of medical care. *Nurse Pract*. **39**, 22-32; quiz 32-23.