Trop J Nat Prod Res, October 2021; 5(10):1828-1834 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effects of Tang Shen 2 Hao Fang, a Traditional Chinese Medicine, on Diabetic Nephropathy in Sprague–Dawley Rats

Anh N. Dang[1], Wang Yao-Guang[1], Zhao Xi[1], Zhang Jing[1], Hoang N. Nguyen[2]*

[1]*Tianjin University Traditional Chinese Medicine, Teaching Hospital 1, Nephropathy Department, Tianjin 300381, China*
[2]*Department of Pharmacology, Vietnam Military Medical University, 160 Phung Hung street, Ha Dong district, Ha Noi, Vietnam*

ARTICLE INFO

Article history:
Received 09 August 2021
Revised 18 September 2021
Accepted 16 October 2021
Published online 02 November 2021

ABSTRACT

Diabetic nephropathy is a chronic disease characterized by a reduced glomerular filtration rate. Kidney disease is a microvascular complication of diabetes mellitus, which can eventually lead to end-stage renal disease and threaten patient health. This study aimed to evaluate the effects of Tang Shen 2 Hao Fang (TS2HF), a traditional Chinese medicine formulation, on rats with diabetic nephropathy induced by streptozotocin injection and a high-fat diet. Rats were given TS2HF at doses of 11.57 and 23.14 g/kg/day. Rat weight, blood glucose levels, and blood lipid indices were measured, and renal function was assessed by blood urea, creatinine, and 24-hour urine albumin. Antioxidant effects were evaluated via serum superoxide dismutase (SOD) activity and glutathione (GSH). The anti-inflammatory effects of TS2HF were evaluated by measuring the proinflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) using quantitative ELISA. The kidney weights and kidney/body weight ratios were also determined. The results demonstrated that TS2HF reduced kidney injury complications. TS2HF decreased triglycerides, total cholesterol, and low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL). TS2HF decreased the cytokines IL-6 and TNF-α and increased SOD and GSH levels in rat serum. It also protected against weight loss, decreased the glomerular size, decreased glomerular and interstitial fibrosis, and reduced the proliferation of endothelial and intercapillary cells in rats with diabetic nephropathy. These results suggest that TS2HF has anti-diabetic and anti-nephropathic activities.

Keywords: Traditional Chinese Medicine, TS2HF, Diabetic nephropathy, Anti-diabetic, Anti-nephropathic.

Introduction

Diabetic kidney disease (DKD) is increasing in prevalence and severity worldwide.[1] According to the ninth edition of the *Global Diabetes Overview* released by the International Diabetes Federation, the global prevalence of diabetes will increase from 463 million in 2019 to 700 million in 2045.[2,3] Approximately 10–40% of patients with type 2 diabetes (T2D) will develop DKD,[4] and 30–40% of these patients will progress to renal failure.[5,6] Treatment for DKD includes controlling blood pressure and blood glucose, particularly by using inhibitors of the renin-angiotensin-aldehyde ketone system. Although this is the primary treatment for DKD, it is not effective in delaying its progression,[7,8] and approximately 20% of patients with diabetes will develop end-stage renal disease.[9] Diabetic nephropathy (DN) is a chronic disease characterized by a reduced glomerular filtration rate. Kidney disease is a microvascular complication of diabetes mellitus (DM), which can eventually lead to end-stage renal disease and threaten patient health. Although many studies have enterprises, the mechanism is still unclear. Clinically, many traditional medicines are applied to treat diabetes and kidney disease patients, including TS2HF.[10] TS2HF has been used in the treatment of diabetic kidney disease patients at the First Teaching Hospital of Tianjin University of Traditional Chinese Medicine for many years.

Although it has been effective in treating DN in clinical practice, the mechanism by which it works is still unclear. Therefore, we adopted the network pharmacology method of Chinese medicine to determine the mechanism of action of TS2HF based on the composition and structure of each component when combined. TS2HF was studied on an experimental model of the streptozotocin (STZ) intraperitoneal injection of rats to evaluate its effect on renal function in a DN drain model.

Materials and Methods

Tang Shen 2 Hao Fang formulation

The TS2HF components used in this study met the standards of the Vietnamese pharmacopeia V 2020 and included the following: *Astragalus mongholicus* Bunge 30 g, *Euonymus alatus* (Thunb.) Sieb 15 g, *Pheretima* 10 g, *Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm 15 g, *Rheum palmatum* L. 6 g, *Salvia miltiorrhiza* Bunge 15 g, cicada slough 6 g, and Herba lycopi 15 g. The ingredients of the TS2HF remedy were collected from January to February 2021. The voucher specimens of *Astragalus mongholicus* Bunge (DAN-01), *Euonymus alatus* (Thunb.) Sieb (DAN-02), *Pheretima* (DAN-03), *Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm (DAN-04), *Rheum palmatum* L. (DAN-05), *Salvia miltiorrhiza* Bunge (DAN-06), *cicada slough* (DAN-07), and Herba lycopi (DAN-08) were deposited at the Laboratory of Pharmacology, Vietnam Military Medical University. The mixture was extracted with water (1:1 v/v) in an automatic extractor (KTP-EP-25, Korea Techno Pack, Bucheon-si, Korea), and the collected extract was concentrated by vacuum evaporation to a concentration suitable for drug administration to rats. The dosage was calculated according to the number of grams of the dried mixture. The recommended human dose is 112 g per day. Using

*Corresponding author. E mail: nganvmu@gmail.com
Tel: +84-38-268-9686

Citation: Dang AN, Yao-Guang W, Xi Z, Jing Z, Nguyen HN. Effects of Tang Shen 2 Hao Fang, a Traditional Chinese Medicine, on Diabetic Nephropathy in Sprague–Dawley Rats. Trop J Nat Prod Res. 2021; 5(10):1828-1834. doi.org/10.26538/tjnpr/v5i10.20

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

an average human body weight of 60 kg, the human dose is 112/60 g/kg/day. Determining the equivalent dose in rats requires multiplying the human dose by a conversion factor of 6.2,[11] resulting in 11.57 g/kg/24 h.

Animals

Healthy male Sprague–Dawley rats, weighing 200 ± 20 g, were provided by the Laboratory Animal Supply Department of the Vietnam National Military Medical Academy. They were acclimated to the animal laboratory for at least one week before the experiment. Rats were determined to be healthy if they had smooth hair, clear eyes, and a dry anus, and demonstrated normal activity, movement, eating, and waste production. Two experienced technicians selected the research rats following the protocols of the Vietnam National Military Medical Academy. Experimental rats were fed according to the standards of research animals, and they had free access to boiled and cooled clean water. Rats were monitored daily. The experimental protocol was approved by the Vietnam Military Medical University, Hanoi, Vietnam (Permission number IACUC-0102/21 issued on February 02, 2021).

Equipment and chemical reagents

Chemicals for detecting urea, creatinine, glucose, and blood lipids (total cholesterol, triglycerides, HDL, and LDL) were purchased from Erba Diagnostics (Magstadt, Germany). The following were purchased from Sigma–Aldrich (St. Louis, MO, USA): glutathione assay kit II, catalog number 354103; superoxide dismutase assay kit II, catalog number 574601; rat IL-6 ELISA kit, SKU RAB0311-1KT; rat tumor necrosis factor α ELISA Kit, SKU RAB0479-1KT; BCP albumin assay kit, SKU MAK125-1KT, ALB (24186); streptozotocin (STZ), catalog number S0130. Equipment included a chemical analyzer (3000 EVOLUTION, Biochemical Systems International, Campi Bisenzio, Italy), an ELISA analyzer (Awareness Technology, Palm City, FL, USA), a decoction and extraction machine (KTP-EP-25, Korea Techno Pack, Bucheon-si, Korea), and an electronic scale (BSA124S, Sartorius, Goettingen, Germany).

Animal experimental protocol

Experimental modeling of diabetic nephropathy in Sprague–Dawley rats was carried out using streptozotocin (STZ) in combination with a high-fat diet, according to a method described previously.[12] A total of 70 male rats were selected randomly and divided into five groups: group 1 (G1, n = 10) was the negative control, rats with no diabetic kidney disease; group 2 (G2, n = 15 animals) rats were given STZ and a high-fat diet to induce diabetic nephropathy; group 3 (G3, n = 15 animals) rats had diabetic nephropathy induced and were treated with 11.57 g/kg/24h TS2HF in drinking water; group 4 (G4, n = 15 animals) rats had diabetic nephropathy induced and were treated with 23.14 g/kg/24h TS2HF in drinking water. Group 5 (G5, n = 15 animals) rats had diabetic nephropathy induced and were treated with 200 mg/kg/24h metformin. Rats in G1 were fed a normal diet (4% calories from fat) for four weeks, then injected peritoneally with buffered citric acid (0.1 mol/L, pH 4.3). Diabetic nephropathy was induced in rats in G2–G5 by feeding high-fat food (40% calories from fat) for four weeks. The rats were then injected peritoneally with a single dose of STZ (30 mg/kg) dissolved in 0.1 mol/L citric acid buffer (pH 4.3). A high-fat diet administered to rats induces insulin resistance so that a low dose of STZ (30 mg/kg) causes T2D after a single injection. Blood glucose levels were measured on day three after STZ injection. Rats with a glycemic index greater than 16.7 mmol/L were considered diabetic. Rats with a glycemic index lower than 16.7 mmol/L were considered non-diabetic in G2–G5. The following number of diabetic rats met the glycemic index criterion and were therefore included: G2, n = 11; G3, n = 12; G4, n = 12; G5, n = 12. The groups were then given distilled water only or TS2HF in water until the end of the experiment (12 weeks).

At the end of the experiment, rats were euthanized with an overdose of 10% chloral hydrate via peritoneal injection at a dose of 4 mg/kg to obtain kidney samples for histopathology. Blood was collected by percutaneous cardiac aspiration at the time of euthanasia and placed in tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). After the blood was centrifuged at 3000 rpm for 15 min at 40°C, the supernatant was collected for measurement of glucose, urea, creatinine, triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol.

Rat weight, blood glucose levels, and blood lipid indices (triglycerides, total cholesterol, HDL, and LDL) were measured, and renal function was assessed by blood urea, serum creatinine, and 24-hour urine albumin. The antioxidant effects were evaluated via serum SOD activity. The anti-inflammatory effects of TS2HF were evaluated by measuring the proinflammatory cytokines IL-6 and TNF-α using quantitative ELISA. The kidney weights and kidney/body weight ratios were also determined.

For renal histopathological assessment, rat kidneys were fixed in 10% formalin, dehydrated using an ethanol and acetone series, cast in paraffin, and then stained with hematoxylin-eosin and Masson's trichrome. Microscopic images of the liver were obtained, and these images were assessed by the pathology department (103 Military Hospital, Vietnam).

Statistical analysis

Data were analyzed using Student's t-test and Avant-après test and expressed as mean ± standard deviation. Differences were considered statistically significant when $p < 0.05$.

Results and Discussion

Rat weights

The initial weights of the rats were 190–193 g, and there were no statistically significant initial weight differences ($p > 0.05$) between the groups. However, the weight of rats in the diabetic nephropathy group (G2) and the treatment groups G3 (11.57 g/kg/24 h) and G4 (23.14 g/kg/24 h) increased rapidly due to the high-fat diet. After four weeks on the high-fat diet, the weights in these groups were 394.92 ± 29.25 and 401.50 ± 27.62 g, significantly higher than rats in the control group ($p < 0.01$).

At eight and 12 weeks, the weight of G2 mice was lower than that of the control group ($p < 0.01$), which is unsurprising, as weight loss is one of the symptoms of diabetes. The weight in the G3 and G4 groups also decreased relative to the control group, but the decrease was smaller than for G2. G3 was different from the control group at 12 weeks ($p < 0.05$). At eight weeks, the weight of G4 rats was higher than G2 rats ($p < 0.05$). At 12 weeks, the weight of the rats in G3, G4, and G5 was higher than in G2 ($p < 0.01$). At eight and 12 weeks, there were no statistically significant differences in weight between the G4 and G3 groups ($p > 0.05$) (Table 1).

Rat blood glucose

All groups had the same blood glucose levels at the start of the experiment ($p > 0.05$) (Table 2). On the third day after STZ injection, the blood glucose levels in the G2, G3, and G4 groups had increased. After excluding rats with a glycemic index lower than 16.7 mmol/L, the blood glucose concentrations were 18.16 ± 1.28 (n = 11) in G2, 18.09 ± 1.11 (n = 12) in G3, and 18.06 ± 1.24 (n = 12) in G4, all of which were significantly higher than the control group ($p < 0.01$). After four and eight weeks of TS2HF administration, the blood glucose concentrations of rats in G3 and G4 were lower than G2 ($p < 0.01$) but higher than in G1 ($p < 0.01$). At eight and 12 weeks, the blood glucose levels in G4 were not statistically significantly different to those in G3 ($p > 0.05$). The blood glucose concentrations in groups G3 and G4 were not statistically significantly different to that in the metformin group, G5 ($p > 0.05$) (Table 2).

Rat blood lipid indexes

The blood lipid indices are shown in Table 3. Compared to rats in the G1 group, those in G2 had elevated triglycerides, total cholesterol, and LDL cholesterol, and reduced HDL cholesterol ($p < 0.01$).

In the G3 group, triglycerides, total cholesterol, and LDL cholesterol concentrations in the blood were lower than in G2 ($p < 0.05$ and $p < 0.01$), but higher than G1 ($p < 0.05$ and $p < 0.01$). The HDL cholesterol concentration in G3 was greater than in G2 ($p < 0.05$), but lower than in the control group G1 ($p < 0.01$).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Rat weight assessment (mean ± SD)

Experiment lots	n	Rat weight (g)			
		1st day	Week 4	Week 8	Week 12
Control, G1	10	193.90 ± 11.57	302.60 ± 20.77	397.70 ± 20.17	435.60 ± 22.11
Diabetic nephropathy,G2	11	190.36 ± 11.35	398.18$^{¥}$ ± 28.20	359.64$^{¥}$ ± 34.53	356.82$^{¥}$ ± 35.89
G-3, 11.57 g/kg/24h	12	191.67 ± 10.11	394.92$^{¥}$ ± 29.25	379.50 ± 27.31	401.58*$^{\Delta}$ ± 32.22
G-4, 23.14 g/kg/24h	12	190.67 ± 12.13	401.50$^{¥}$ ± 27.62	390.92$^{▲}$ ± 26.27	414.33$^{\Delta}$ ± 37.40
G-5, metformin 200 mg/kg/24h	12	192.75 ± 13.22	396.29$^{¥}$ ± 23.69	383.08 ± 21.88	404.50*$^{\Delta}$ ± 36.32

* $p < 0.05$ and $^{¥}p < 0.01$ compared to G1; $^{▲}p < 0.05$ and $^{\Delta}p < 0.01$ compared to G2

Table 2: Assessment of blood glucose concentration in rats (mean ± SD)

Experiment lots	n	Glucose concentration (mmol/L)			
		1st day	Day 3 after Streptozotocin injection	Week 8	Week 12
Control, G1	10	6.05 ± 0.39	6.08 ± 0.42	5.90 ± 0.38	6.10 ± 0.40
Diabetic nephropathy,G2	11	6.07 ± 0.40	18.16$^{¥}$ ± 1.28	20.29$^{¥}$ ± 1.97	23.81$^{¥}$ ± 2.41
G-3, 11.57 g/kg/24h	12	6.04 ± 0.42	18.09$^{¥}$ ± 1.11	18.28$^{¥\Delta}$ ± 1.32	18.63$^{¥\Delta}$ ± 1.48
G-4, 23.14 g/kg/24h	12	6.11 ± 0.43	18.06$^{¥}$ ± 1.24	18.16$^{¥\Delta}$ ± 1.28	18.31$^{¥\Delta}$ ± 1.51
G-5, metformin 200 mg/kg/24h	12	6.09 ± 0.39	18.21$^{¥}$ ± 1.16	18.20$^{¥\Delta}$ ± 0.99	18.40$^{¥\Delta}$ ± 1.29

$^{¥}p < 0.01$ compared to G1; $^{\Delta}p < 0.01$ compared to G2.

Table 3: Assessment of blood lipid levels in rats (mean ± SD)

Experiment lots	n	Blood lipid levels			
		Triglyceride (mmol/L)	Total Cholesterol (mmol/L)	HDL-Cholesterol (mmol/L)	LDL-Cholesterol (mmol/L)
Control, G1	10	0.65 ± 0.06	2.19 ± 0.15	1.27 ± 0.15	0.62 ± 0.06
Diabetic nephropathy,G2	11	3.88$^{¥}$ ± 0.39	8.89$^{¥}$ ± 0.80	0.93$^{¥}$ ± 0.11	6.20$^{¥}$ ± 0.61
G-3, 11.57 g/kg/24h	12	3.55$^{¥▲}$ ± 0.36	8.16$^{¥▲}$ ± 0.62	1.06$^{¥▲}$ ± 0.17	5.48$^{¥\Delta}$ ± 0.59
G-4, 23.14 g/kg/24h	12	3.47$^{¥▲}$ ± 0.35	7.99$^{¥\Delta}$ ± 0.67	1.15$^{\Delta}$ ± 0.19	5.27$^{¥\Delta}$ ± 0.53
G-5, metformin 200 mg/kg/24h	12	3.59$^{¥▲}$ ± 0,22	8.21$^{¥▲}$ ± 0.41	1.05$^{¥▲}$ ± 0.15	5.54$^{¥\Delta}$ ± 0.44

$^{¥}p < 0.01$ compared to G1; $^{▲}p < 0.05$; $^{\Delta}p < 0.01$ compared to G2

In the second TS2HF treatment group (G4), blood triglycerides, total cholesterol, and LDL cholesterol were lower than in G2 ($p < 0.05$ and $p < 0.01$) but higher than in the control group G1 ($p < 0.05$ and $p < 0.01$). The concentration of HDL cholesterol in G4 was greater than in G2 ($p < 0.01$), and was not significantly different to that in G1 ($p > 0.05$). Triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol were not significantly different between the two TS2HF groups ($p > 0.05$). In addition, the concentrations of triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol in G3 and G4 were not statistically significantly different to those in G5 ($p > 0.05$) (Table 3).

Proinflammatory cytokines IL-6 and TNF-α in rat blood

The results of the serum proinflammatory cytokine analysis are shown in Table 4. The IL-6 and TNF-α concentrations in G2 were 49.69 ± 5.12 ng/L and 80.18 ± 9.13 ng/L, respectively. These levels were significantly higher than those in G1 ($p < 0.01$). In G3, the IL-6 and TNF-α concentrations were 44.54 ± 4.58 ng/L and 69.77 ± 7.17 ng/L, respectively. They were somewhat lower than in G2 ($p < 0.05$ and $p < 0.01$, respectively) but higher than in the control group G1 ($p < 0.01$). Similarly, the IL-6 and TNF-α concentrations in G4 were lower than in G2 ($p < 0.01$) but still higher than in G1 ($p < 0.01$). The concentrations of IL-6 in G3 and G4 were not significantly different ($p > 0.05$). The concentrations of IL-6 and TNF-α in the G5 group were 43.94 ± 4.77 ng/L and 66.86 ± 6.33 ng/L, respectively, which were not significantly different to the G3 or G4 groups ($p > 0.05$) (Table 4).

Antioxidant effects

The antioxidant effects of TS2HF on diabetic nephropathic mice were determined using measurements of SOD activity and GSH concentration. In the G2 group, the SOD activity and GSH concentration in rat sera were 10.88 ± 2.02 U/L and 9.46 ± 1.41 mg/L, respectively, both significantly greater than in G1 ($p < 0.01$). In the treatment groups, the activity of SOD and concentration of GSH were higher than in G2 ($p < 0.01$) but lower than in G1 ($p < 0.01$). The serum levels of SOD and GSH in G3 and G4 were not different ($p > 0.05$) (Table 5). SOD activity and GSH concentration in G5 were 14.66 ± 2.48 (U/L) and 11.22 ± 1.15 (mg/L), respectively. These results were lower than in G2 ($p < 0.01$) but not significantly different to G3 or G4 ($p > 0.05$) (Table 5).

Blood urea, creatinine, and 24-h albuminuria

In the control group, G1, the urea, serum creatinine, and 24-h urinary albumin were 12.63 ± 1.35 mmol/L, 49.22 ± 5.36 µmol/L, and 0.57 ± 0.16 mg, respectively. These were significantly increased to 25.48 ± 2.65 mmol/L, 93.23 ± 10.62 µmol/L, and 6.02 ± 1.15 mg, respectively, in G2 rats with diabetic nephropathy. In G3, urea, serum creatinine, and 24-h urinary albumin were lower than in G2 ($p < 0.05$), but higher than in G1 ($p < 0.01$).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: IL-6 and TNF-α concentration in rat bloods (mean ± SD)

Experiment lots	n	IL-6 (ng/L)	TNF-α (ng/L)
Control, G1	10	28.48 ± 2.93	43.35 ± 3.47
Diabetic nephropathy,G2	11	$49.69^{¥}$ ± 5.12	$80.18^{¥}$ ± 9.13
G-3, 11.57 g/kg/24h	12	$44.54^{¥▲}$ ± 4.58	$69.77^{¥Δ}$ ± 7.17
G-4, 23.14 g/kg/24h	12	$42.32^{¥Δ}$ ± 4.26	$61.58^{¥Δ†}$ ± 6.44
G-5, metformin 200 mg/kg/24h	12	$43.94^{¥▲}$ ± 4.77	$66.86^{¥Δ}$ ± 6.33

$^{¥}p < 0.01$ compared to G1; $^{▲}p < 0.05$; $^{Δ}p < 0.01$ compared to G2; $^{†}p < 0.01$ compared to G3.

Table 5: SOD and GSH concentration in rat serum (mean ± SD)

Experiment lots	n	SOD (U/L)	GSH (mg/L)
Control, G1	10	39.72 ± 4.61	22.29 ± 3.02
Diabetic nephropathy, G2	11	$10.88^{¥}$ ± 2.02	$9.46^{¥}$ ± 1.41
G-3, 11.57 g/kg/24h	12	$14.32^{¥Δ}$ ± 2.35	$11.08^{¥Δ}$ ± 1.29
G-4, 23.14 g/kg/24h	12	$15.13^{¥Δ}$ ± 3.16	$11.79^{¥Δ}$ ± 1.71
G-5, metformin 200 mg/kg/24h	12	$14.66^{¥Δ}$ ± 2.48	$11.22^{¥Δ}$ ± 1.15

$^{¥}p < 0.01$ compared to G1; $^{Δ}p < 0.01$ compared to G2.

Similarly, the urea, serum creatinine, and 24-h urinary albumin in G4 were lower than in G2 ($p < 0.01$) but higher than G1 ($p < 0.01$). There were no significant differences between the G3 and G4 groups ($p > 0.05$). The concentrations of urea and serum creatinine, and the 24-hour urinary albumin in G3 and G4 were not statistically significantly different to those in G5 ($p > 0.05$) (Table 6).

Rat kidney weights

Rat kidney weight (absolute weight) and the kidney/body weight ratio (relative weight) were significantly higher ($p < 0.01$) (Table 7). The rat kidney weights in G3 and G4 were significantly lower than in G2 ($p < 0.05$) but higher than in G1 ($p < 0.05$). The absolute and relative kidney weights in G3 and G4 were not significantly different ($p > 0.05$). In addition, the absolute and relative kidney weights in G3 and G4 were not statistically significantly different to those in G5 ($p > 0.05$) (Table 7).

Rat kidney histopathology

Histopathological images of G1 rat kidneys showed that the glomeruli and renal tubules were of normal structure and size without fibrosis. However, histopathological images of the rat kidneys in G2 showed significantly increased glomerular size, an increased number of endothelial and intercapillary cells, glomerular fibrosis, and interstitial fibrosis. In the two treatment groups G3 and G4, the manifestations of kidney damage such as proliferative lesions and fibrotic lesions were significantly reduced compared to G2. There was no difference between the two treatment groups (Figure 2).

The authors of a previous study reported that after feeding rats a high-fat, high-energy diet, blood lipids and blood insulin increased, but blood glucose was normal.[12,13] Peripheral insulin resistance causes the pancreas to increase insulin production to maintain blood glucose levels. STZ is selectively toxic to islet beta cells, and low doses of STZ cause damage to islet β cells in rats. The pancreas maintains normal blood insulin levels, but blood glucose levels will increase due to insulin resistance in the peripheral tissues. A low dose of STZ combined with a high-fat diet is an appropriate T2D animal model, with a mechanism and manifestation similar to T2D in humans. Furthermore, this model has been evaluated in multiple studies.[14-16] In our study, after four weeks on a high-fat, high-energy diet, rats gained substantial weight (Table 1), indicative of obesity. However, blood glucose levels did not increase compared to controls (Table 2). Low-dose STZ (30 mg/kg) was administered four weeks after beginning the high-fat, high-energy diet. After three days of STZ injection, blood glucose increased significantly, to approximately three times that in the control group, and continued to increase at eight and 12 weeks (Table 2). Low-dose STZ causes only mild pancreatic damage. However, blood glucose levels will be greater than normal when combined with peripheral tissue insulin resistance due to obesity. Despite being fed a high-fat diet and experiencing a significant weight gain at week four (before STZ injection), the weight of the diabetic rats decreased at eight and 12 weeks; weight loss is a typical symptom of diabetes.

DN, also known as glomerular sclerosis, is caused by diabetes complications. We investigated a model of T2D nephropathy in white rats fed a high-fat, high-energy diet in combination with a low dose of STZ. The prolonged feeding (four weeks) of a high-fat, high-energy diet caused severe metabolic disorders, demonstrated by high blood lipid indices (Table 3). After low-dose STZ injection, blood glucose increased, and metabolic disorders became more complex.

Increased blood lipid and blood glucose led to inflammation and oxidative stress, with increased expression of IL-6 and TNF-α (Table 4) and decreased antioxidants (SOD and GSH) (Table 5) in rat serum. Together with this increased inflammation and oxidation, metabolic disorders made the glomerular capillaries vulnerable to long-term damage, causing kidney complications.

Reduced kidney function was demonstrated by elevated urea, creatinine, and 24-h urinary albumin levels (Table 6), and renal morphological damage was demonstrated by the increased kidney weight (Table 7). In addition, renal histopathology showed an increased glomerular size, endothelial and intercapillary cell proliferation, glomerular fibrosis, and interstitial fibrosis (Figures 1 and 2). Therefore, the T2D nephropathy model in rats fed a high-fat, high-energy diet combined with low-dose STZ was used completely similar to our modeling results.[12,17,18] In the T2D nephropathy rat model, TS2HF was effective in reversing the pathophysiology of diabetic nephropathy *in vivo.*

Table 6: Assessment of blood urea, creatinine and albuminuria 24h (mean ± SD)

Experiment lots	n	Blood Ure (mmol/L)	Blood Creatinin (µmol/L)	Albuminuria 24h (mg)
Control, G1	10	12.63 ± 1.35	49.22 ± 5.36	0.57 ± 0.16
Diabetic nephropathy,G2	11	$25.48^{¥}$ ± 2.65	$93.23^{¥}$ ± 10.62	$6.02^{¥}$ ± 1.15
G-3, 11.57 g/kg/24h	12	$23.12^{¥▲}$ ± 1.84	$80.94^{¥▲}$ ± 10.69	$4.77^{¥▲}$ ± 1.07
G-4, 23.14 g/kg/24h	12	$22.16^{¥Δ}$ ± 2.23	$76.06^{¥Δ}$ ± 10.92	$4.18^{¥Δ}$ ± 1.18
G-5, metformin 200 mg/kg/24h	12	$23.51^{¥▲}$ ± 1.71	$82.68^{¥▲}$ ± 10.12	$4.94^{¥▲}$ ± 1.10

$^{¥}p < 0.01$ compared to G1; $^{▲}p < 0.05$ and $^{Δ}p < 0.01$ compared to G2.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 7: Weight assessment of rat kidney (mean ± SD)

Experiment lots	n	Rat kidney (g)	Rat kidney/rat (g/kg)
Control, G1	10	2.64 ± 0.28	6.06 ± 0.46
Diabetic nephropathy,G2	11	$3.26^{¥}$ ± 0.35	$9.17^{¥}$ ± 0.65
G-3, 11.57 g/kg/24h	12	$2.95^{*▲}$ ± 0.33	$7.35^{¥Δ}$ ± 0.80
G-4, 23.14 g/kg/24h	12	$2.90^{*▲}$ ± 0.26	$7.02^{¥Δ}$ ± 0.45
G-5, metformin 200 mg/kg/24h	12	$2.97^{*▲}$ ± 0.31	$7.37^{¥Δ}$ ± 0.71

$^{*}p < 0.05$ and $^{¥}p < 0.01$ compared to G1; $^{▲}p < 0.05$ and $^{Δ}p < 0.01$ compared to G2.

The results shown in Tables 2–5 show that TS2HF can regulate metabolic disorders (reducing glucose and blood lipids) and act as an anti-inflammatory (reducing IL-6 and TNF-α) and indirectly as an anti-oxidant (by increasing SOD and GSH). Weight loss (a manifestation of diabetes mellitus) also decreased significantly (Table 1). Furthermore, compared to the nephropathic group that was not administered TS2HF, the TS2HF groups had reduced urea, blood creatinine, 24-h albuminuria (Table 6), and kidney weight index (Table 7). Also, histopathological kidney analyses revealed decreased glomerular size, and less proliferation of endothelial and interstitial cells and less glomerular and interstitial fibrosis (Figures 1 and 2).

These results demonstrate that this traditional Chinese medicine (TCM) recipe alleviated T2D nephropathy, and that the effects of reducing kidney injury complications were related to the effects on metabolic disorder regulation (decreased glucose and blood lipids), anti-inflammatory activity (reduced IL-6 and TNF-α) and antioxidant activity (increased SOD and GSH). Although TS2HF has been used in the treatment of diabetic kidney disease patients at the First Teaching Hospital of Tianjin University of TCM for many years, this is the first time that the biological effects of this remedy have been evaluated. The results demonstrate that TS2HF was effective in the treatment of diabetic nephropathy *in vivo* when evaluated in rats with T2D nephropathy, similar to results of other TCM remedies.[10] These results are consistent with what is known about the effects of specific ingredients in the TS2HF mixture. According to traditional medicine theories, *Astragalus mongholicus* Bunge lowers blood glucose in type 1 and type 2 diabetes.[19] Astragaloside IV isolated from this plant has been shown to improve renal interstitial fibrosis by inhibiting TLR4/NF-κB-mediated inflammation *in vivo* and *in vitro*,[19] and reducing tissue injury via antioxidants.[20] Calycosin, a major component of *Astragalus mongholicus* Bunge, significantly ameliorated STZ-induced renal injury and dysfunction by modulating the IL-33/ST2 signaling pathway, and reducing inflammatory cytokines, oxidative stress, and fibrosis.[21] *Euonymus alatus* (Thunb.) Sieb has been demonstrated to have good efficacy in the treatment of diabetes mellitus,[22] and diabetic nephropathy,[23] and has anti-inflammatory and antioxidant effects.[24]

The combination of *Astragalus mongholicus* Bunge and *Euonymus alatus* (Thunb.) Sieb has also shown effectiveness in treating diabetes mellitus.[25] A systematic review and meta-analysis showed that rhubarb (*Rheum palmatum* L.) had beneficial effects in animals with diabetic nephropathy.[26] Evaluation of the therapeutic mechanism showed that rhubarb could reduce oxidative stress by improving lipid metabolism, enhancing antioxidant capacity, reducing mitochondrial structural damage, inhibiting β-cell apoptosis, and improving β-cell function.[27]

In TCM, the Tangshen remedy consists of seven components, including the plant *Astragalus mongholicus* Bunge, *Euonymus alatus* (Thunb.) Sieb, and rhubarb. Tangshen extract has been demonstrated to have clear anti-inflammatory and anti-fibrotic effects in rats with diabetic nephropathy.[28,29] Among the ingredients, *Salvia miltiorrhiza* Bunge provides benefits as an antioxidant, anti-fibrotic, anti-inflammatory, anticoagulant, vasodilator, and glucose and blood lipid reducer, demonstrating effectiveness in treating T2D.[30,31] *Pheretima* extract can inhibit alpha-amylase and alpha-glucosidase enzymes, which are important for controlling glucose levels in the body, and are directly related to diabetes.[32] In addition, *Pheretima* has been demonstrated to have anti-inflammatory and antioxidant effects,[33] and has anticoagulant or fibrinolytic activity, facilitating blood circulation.[34] All of these effects are beneficial for the treatment of diabetic nephropathy. *Pseudostellaria heterophylla* (Miq.) and Herba lycopi were shown to improve T2D in diabetic mice with nephropathy.[35,36]

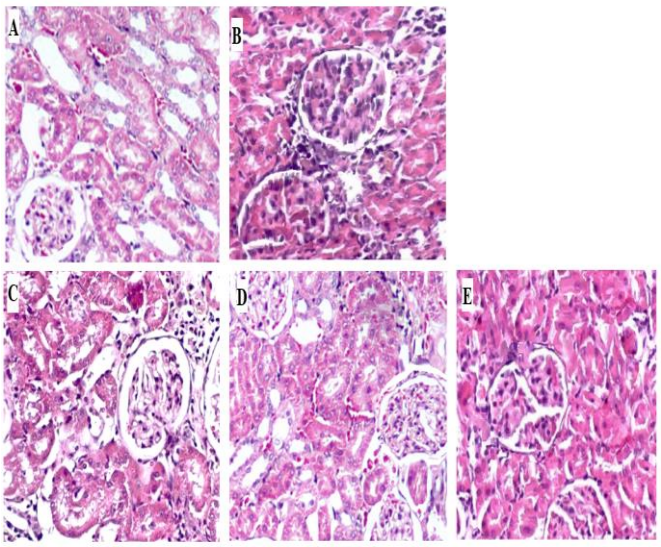

Figure 1: Histopathological image of HE-stained rat kidney (400× magnification).
(A) G1: normal renal tubules, normal glomerulus; (B) G2: glomerular hypertrophy, endothelial and mesangial cell proliferation; (C) G3, 11.57 g/kg/24h; (D) G4, 23.14 g/kg/24h; (E) metformin 200 mg/kg/24h.

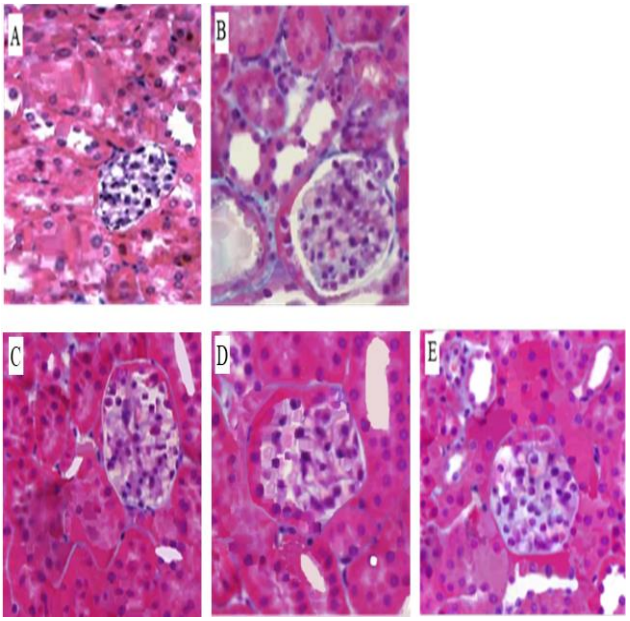

Figure 2: Histopathological image of Masson's trichrome-stained rat kidney (400× magnification).
(A) G1: normal renal tubules, normal glomerulus; (B) G2: the fibrous tissue is stained green, demonstrating glomerular hypertrophy, endothelial and mesangial cell proliferation, and fibrosis of the glomeruli and interstitial tissue; (C) G3: 11.57 g/kg/24h; (D) G4: 23.14 g/kg/24h; (E) metformin 200 mg/kg/24h.

Cicada slough reduces IgA nephropathy,[37] an immune-mediated inflammatory condition of the glomerulus.[38] With its anti-inflammatory and antioxidant effects and the capability to lower blood glucose and blood lipids by different mechanisms, the combination of components in this natural traditional Chinese remedy partially remedied disease-causing diabetic kidney injury in rats.

Conclusion

The advantages of topical microparticles for skin diseases such as a burn or wound therapy have been widely explored. Topical materials commonly used for pharmacological therapy in second-degree burns are synthetic antimicrobial agents and natural oils. Microparticles as one of the drug delivery systems could be an alternative to antimicrobial in second-degree burn wound infection.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors are thankful to the management, the principal of the Department of Pharmacology – Vietnam Military Medical University and Department of Pathology and Forensic Medicine, Military Medical Hospital 103, Vietnam for providing the necessary infrastructure and conducting the experiments.

References

1. Xu Y, Wang L, He J, Bi Y, Li M, Wang T, Wang L, Jiang Y, Dai M, Lu J, Xu M, Li Y, Hu N, Li J, Mi S, Chen CS, Li G, Mu Y, Zhao J, Kong L, Chen J, Lai S, Wang W, Zhao W, Ning G. 2010 China Noncommunicable Disease Surveillance Group. Prevalence and control of diabetes in Chinese adults. J Am Med Assoc. 2013; 310(9):948-959.
2. Zhang L, Long J, Jiang W, Shi Y, He X, Zhou Z, Li Y, Yeung RO, Wang J, Matsushita K, Coresh J, Zhao MH, Wang H. Trends in Chronic Kidney Disease in China. N Engl J Med. 2016; 375(9):905-906.
3. Li G, Qiao Y, Lu Y, Liu S, Ding Y, Chen X, Ke C. Role of handgrip strength in predicting new-onset diabetes: findings from the survey of health, ageing and retirement in Europe. BMC Geriatr. 2021; 21(1):445.
4. Lu CC, Hu ZB, Wang R, Hong ZH, Lu J, Chen PP, Zhang JX, Li XQ, Yuan BY, Huang SJ, Ruan XZ, Liu BC, Ma KL. Gut microbiota dysbiosis-induced activation of the intrarenal renin-angiotensin system is involved in kidney injuries in rat diabetic nephropathy. Acta Pharmacol Sin. 2020; 41(8):1111-1118.
5. Satirapoj B and Adler SG. Prevalence and Management of Diabetic Nephropathy in Western Countries. Kidney Dis. 2015; 1(1):61-70.
6. Tomino Y and Gohda T. The Prevalence and Management of Diabetic Nephropathy in Asia. Kidney Dis (Basel). 2015; 1(1):52-60.
7. Wolf G and Ziyadeh FN. Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. Nephron Physiol. 2007; 106(2):26-31.
8. Al-Waili N, Al-Waili H, Al-Waili T, Salom K. Natural antioxidants in the treatment and prevention of diabetic nephropathy; a potential approach that warrants clinical trials. Redox Rep. 2017; 22(3):99-118
9. Pofi R, Di Mario F, Gigante A, Rosato E, Isidori AM, Amoroso A, Cianci R, Barbano B. Diabetic Nephropathy: Focus on Current and Future Therapeutic Strategies. Curr Drug Metab. 2016; 17(5):497-502.
10. Tang G, Li S, Zhang C, Chen H, Wang N, Feng Y. Clinical efficacies, underlying mechanisms and molecular targets of Chinese medicines for diabetic nephropathy treatment and management. Acta Pharm Sin B. 2021; 11(9):2749-2767.
11. Nair AB and Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016; 7(2):27-31.
12. Peng L, Li J, Xu Y, Wang Y, Du H, Shao J, Liu Z. The Protective Effect of Beraprost Sodium on Diabetic Nephropathy by Inhibiting Inflammation and p38 MAPK Signaling Pathway in High-Fat Diet/Streptozotocin-Induced Diabetic Rats. Int J Endocrinol. 2016; 2016:1690474.
13. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, Reaven GM. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metab. 2000; 49(11):1390-1394.
14. Mansor LS, Gonzalez ER, Cole MA, Tyler DJ, Beeson JH, Clarke K, Carr CA, Heather LC. Cardiac metabolism in a new rat model of type 2 diabetes using high-fat diet with low dose streptozotocin. Cardiovasc Diabetol. 2013; 12:136.
15. Guo XX, Wang Y, Wang K, Ji BP, Zhou F. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. J Zhejiang Univ Sci B. 2018; 19(7):559-569.
16. Wen W, Lin Y, Ti Z. Antidiabetic, Antihyperlipidemic, Antioxidant, Anti-inflammatory Activities of Ethanolic Seed Extract of *Annona reticulata* L. in Streptozotocin Induced Diabetic Rats. Front Endocrinol (Lausanne). 2019; 10:716.
17. Ni Z, Guo L, Liu F, Olatunji OJ, Yin M. *Allium tuberosum* alleviates diabetic nephropathy by supressing hyperglycemia-induced oxidative stress and inflammation in high fat diet/streptozotocin treated rats. Biomed Pharmacother. 2019; 112:108678.
18. Matboli M, Eissa S, Ibrahim D, Hegazy MGA, Imam SS, Habib EK. Caffeic Acid Attenuates Diabetic Kidney Disease via Modulation of Autophagy in a High-Fat Diet/Streptozotocin-Induced Diabetic Rat. Sci Rep. 2017;7(1):2263.
19. Zhou X, Sun X, Gong X, Yang Y, Chen C, Shan G, Yao Q. Astragaloside IV from *Astragalus membranaceus* ameliorates renal interstitial fibrosis by inhibiting inflammation via TLR4/NF-κB *in vivo* and *in vitro*. Int Immunopharmacol. 2017; 42:18-24.
20. Shahzad M, Shabbir A, Wojcikowski K, Wohlmuth H, Gobe GC. The Antioxidant Effects of Radix Astragali (*Astragalus membranaceus* and Related Species) in Protecting Tissues from Injury and Disease. Curr Drug Targets. 2016; 17(12):1331-1340.
21. Elsherbiny NM, Said E, Atef H, Zaitone SA. Renoprotective effect of calycosin in high fat diet-fed/STZ injected rats: Effect on IL-33/ST2 signaling, oxidative stress and fibrosis suppression. Chem Biol Interact. 2020; 315:108897.
22. Zhai X, Lenon GB, Xue CC, Li CG. *Euonymus alatus*: A Review on Its Phytochemistry and Antidiabetic Activity. Evid Based Complement Alternat Med. 2016; 2016:9425714.
23. Chang B, Jin C, Zhang W, Kong L, Yang JH, Lian FM, Li QF, Yu B, Liu WK, Yang LL, Zhao P, Zhen Z. *Euonymus alatus* in the treatment of diabetic nephropathy in rats. Am J Chin Med. 2012; 40(6):1177-1187.
24. Oh BK, Mun J, Seo HW, Ryu SY, Kim YS, Lee BH, Oh KS. *Euonymus alatus* extract attenuates LPS-induced NF-κB activation via IKKβ inhibition in RAW 264.7 cells. J Ethnopharmacol. 2011;134(2):288-293.
25. Chen K, Deng D, Huang Y, Ma X, Li X, Zhang W, Li Z, Liu Y, Wang W, Chen J, Zhao HH. Combination of *Astragalus membranaceus* and *Euonymus alatus* (THUNB.) SIEB. for treatment of diabetes: A network-based pharmacology research. J Tradit Chinese Med Sci. 2016; 3(3):184-191.
26. Zeng JY, Wang Y, Miao M, Bao XR. The Effects of Rhubarb for the Treatment of Diabetic Nephropathy in Animals: A

Systematic Review and Meta-analysis. Front Pharmacol. 2021; 12:602816.
27. Cheng FR, Cui HX, Fang JL, Yuan K, Guo Y. Ameliorative Effect and Mechanism of the Purified Anthraquinone-Glycoside Preparation from *Rheum palmatum* L. on Type 2 Diabetes Mellitus. Molecules. 2019; 24(8):1454.
28. Jin D, Huang WJ, Meng X, Yang F, Bao Q, Zhang MZ, Yang YN, Ni Q, Lian FM, Tong XL. Chinese herbal medicine Tangshen Formula treatment for type 2 diabetic kidney disease in the early stage: study protocol for a randomized controlled trial. Trials. 2019; 20:756.
29. Fan XM, Huang CL, Wang YM, Li N, Liang QL, Luo GA. Therapeutic Effects of Tangshen Formula on Diabetic Nephropathy in db/db Mice Using Cytokine Antibody Array. J Diabetes Res. 2018; 2018:8237590.
30. Huang M, Xie Y, Chen L, Chu K, Wu S, Lu J, Chen X, Wang Y, Lai X. Antidiabetic effect of the total polyphenolic acids fraction from *Salvia miltiorrhiza* Bunge in diabetic rats. Phytother Res. 2012; 26(6):944-948.
31. Su CY, Ming QL, Rahman K, Han T, Qin LP. Salvia miltiorrhiza: Traditional medicinal uses, chemistry, and pharmacology. Chin J Nat Med. 2015; 13(3):163-182.
32. Mir MA, Upadhyay S, Mir BA. Inhibition of alpha amylase and alpha glycosidase enzymes by various earth worm extracts. Biomed Pharmacol J. 2018; 11(3):1261-1268.
33. Gupta M, Mazumder UK, Kumar RS, Gomathi P, Rajeshwar Y, Kakoti BB, Selven VT. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. J Ethnopharmacol. 2005; 98(3):267-273.
34. Balamurugan M, Parthasarathi K, Cooper EL, Ranganathan LS. Anti-inflammatory and anti-pyretic activities of earthworm extract-*Lampito mauritii* (Kinberg). J Ethnopharmacol. 2009; 121(2):330-332.
35. Chen Z, Yuan Y, Zou X, Hong M, Zhao M, Zhao Y, Liu Y, Li G, Zhu Y, Luo L, Bao B, Bu S. Radix Puerariae and Fructus Crataegi mixture inhibits renal injury in type 2 diabetes via decreasing of AKT/PI3K. BMC Compl Altern Med. 2017; 17(1):454.
36. Fang ZH, Duan XC, Zhao JD, Wu YJ, Liu MM. Novel Polysaccharide H-1-2 from *Pseudostellaria heterophylla* Alleviates Type 2 Diabetes Mellitus. Cell Physiol Biochem. 2018; 49(3):996-1006.
37. Ebefors K, Granqvist A, Ingelsten M, Mölne J, Haraldsson B, Nyström J. Role of glomerular proteoglycans in IgA nephropathy. PLoS One. 2011; 6(4):e18575.
38. Xia M, Liu D, Peng L, Li Y, Liu H, Wu L, Chen G, Liu Y, Liu H. Coagulation parameters are associated with the prognosis of immunoglobulin a nephropathy: a retrospective study. BMC Nephrol. 2020; 21(1):447.