

Original Research Article

Effect of Dachaihu decoction on traditional Chinese medicine-related indices of Type 2 diabetes in rats, and elucidation of its mechanism of action

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Abstract

Purpose: To study the influence of Dachaihu decoction on traditional Chinese medicine (TCM)-related indices of type 2 diabetes mellitus (T2DM) in rats, and the likely mechanism involved.

Methods: Forty-five healthy male SD rats were randomly selected and used to establish T2DM rat model. The rats were randomly assigned to 3 groups: control, model and Dachaihu decoction groups ($n = 15$). Fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), free fatty acids (FFAs), total cholesterol (TC) and oxidative stress were measured. Pathological changes in rats in each group were monitored, and mRNA levels of PDX-1 and MaFA in pancreatic tissue were measured.

Results: The levels of FBG, HbA1c, FFA, TC, MDA and ROS in the Dachaihu decoction group were significantly decreased, while SOD, PDX-1 mRNA and MaFA mRNA were markedly raised, relative to control ($p < 0.05$). In the Dachaihu decoction group, the rats had pink paws; their noses and tongues were glossy and moist, and there was more physical activity, relative to model rats ($p < 0.05$).

Conclusion: These results indicate that Dachaihu decoction is effective against T2DM in rats. The mechanisms of action may be by inhibition of oxidative stress, improvement of the function of islet beta cells, and mitigation of symptoms of fatigue and dehydration. Therefore, Dachaihu decoction may be useful for adjuvant treatment of TCM-T2DM but clinical studies are required to ascertain this.

Keywords: Dachaihu decoction, Type 2 diabetes mellitus, Traditional Chinese medicine (TCM), Pancreas, Islet beta cells

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INTRODUCTION

Diabetes refers to a common chronic ailment that manifests in abnormal glucose metabolism. With improvement in people's life style and changes in habits, the incidence of diabetes is on the increase. China has a high incidence of diabetes. According to statistics, China ranks first in the

number of diabetic patients in the world, with T2DM accounting for 90 % of cases. The pathogenesis of T2DM is unclear, although it has been suggested that it may be related to insulin resistance and islet β cell injury [1]. Oxidative stress is one of the important causes of insulin resistance and islet β cell injury in peripheral tissues [2]. Under continuous stimulation by high

glucose levels in diabetics, oxidative stress response becomes aggravated, and free radical generation is accelerated. Large number of free radicals damage the pancreatic beta cells and further aggravate the condition of the patients. Currently, T2DM is not completely curable. Generally, it requires lifelong medication. With advancements in medical science and technology, various kinds of western medicines have been developed. However, these drugs do not fundamentally restore islet beta cell function, and most of the T2DM patients with insulin resistance are not sensitive to some oral glucose-lowering drugs and insulin, resulting in increased psychological and economic burden on patients [3]. Therefore, it is important to adopt early detection, early diagnosis, early treatment, and blocking of the development of diabetes. The use of TCM in the treatment of diabetes has a long history. In TCM, T2DM is classified as "thirst elimination", and its pathogenesis is considered to be due to *deficiency of Yin* and *fluid, excessive heat* and *dry heat*, abnormal liver function, *stagnation of qi and blood*, *depression and heat loss*, and *hate of symptoms* [4]. Therefore, removal of blood stasis by promoting blood circulation, *dredging of the liver* and relief of depression, *clearing of the liver* and *reducing the liver*, are the major TCM treatment strategies for T2DM. The aim of this study was to investigate the influence of *Dachaihu* decoction on TCM-related indicators in a rat model of T2DM, and the likely mechanism involved.

EXPERIMENTAL

Laboratory animals

Major equipment and reagents

The major instruments and reagents used, and their suppliers (in brackets) were: electronic balance (Shanghai Precision Instruments Co. Ltd, Model: FA2004B); cryogenic high-speed centrifuge (Shanghai Fuze Trading Co. Ltd, Model: TG18.5); -80 °C ultra-low temperature refrigerator (Beijing Nohui Cheng Technology Co. Ltd, Model: DL-86L828); Biomicroscope (Shanghai Yuguang instrument Factory, Model: WMS-1030); H&E dyeing kit (Solibao Technology Co. Ltd); phosphate buffer (Jiangsu Enmo Asai Biotechnology Co. Ltd), and *Dachaihu* decoction (Hanfang Pharmaceutical Co. Ltd).

Animal grouping and treatments

Establishment of T2DM rat model

The rats were housed at laboratory temperature of 24±1°C and humidity of 52 ± 11%, with free

access to diet and water in an environment with a 12-h light/12-h dark cycle, and were adaptively fed for 1 week. Thereafter, they were assigned in random to 3 groups: control, model g and *Dachaihu* decoction groups of 15 rats each. The control rats were given normal feed, while those in model and *Dachaihu* decoction groups received high-fat and high-sugar diet. After feeding for 4 weeks, streptozotocin was injected intraperitoneally (25 mg/kg).

One week later, the same dose of streptozotocin was injected again. After 3 days, blood sugar was measured randomly, and random 2-day blood glucose levels above 16.7 mmol/L indicated successful establishment of T2DM model. Big *Chaihu* decoction group rats were given the drug at a dose of 10.1 g/kg/day, while rats in the control group and the model group were given equivalent volume of saline in place of decoction.

The big *Bupleurum* decoction comprised 400 g of *Bupleurum*, 100 g of *Rhubarb*, *Radix Scutellariae* and *Paeoniflorin* (150 g), 250 g of ginger, 4 g of *Fructus aurantii*, 12 dates, and *Pinellia ternate*. The mixture was subjected to heat immersion for 30 min, and boiled and filtered twice, each for 1 h. The filtrates were combined and concentrated by heating to achieve a concentration of 1 g/mL.

Biochemical assays

Following overnight fast, 3 mL of venous blood was taken from the tail of each rat and used for measuring fasting blood glucose (FBG). Glycosylated hemoglobin (HBALC), free fatty acids (FFAs) and total cholesterol (TC) levels were measured using automatic biochemical analyzer. The concentration malondialdehyde (MDA) was determined with thiobarbituric acid method, and the reactive oxygen species (ROS) levels were determined using immunofluorescence method. Superoxide dismutase (SOD) activity was also assayed.

Three rats selected from each group at the same time were examined for physical changes, and the ratio of change in each characteristic was calculated as a fraction of total number of rats.

Pathological changes in rat pancreas were determined histologically using H&E staining. The pancreatic tissues were removed from anesthetized rats and routinely processed for light microscopy into paraffin sections which were subsequently dehydrated in gradient ethanol, cleared in xylene, sealed, subjected to routine H & E staining, observed under a microscope, and photographed.

The mRNA expression levels of pancreatic promoter 1 (PDX-1) and homologous A gene of tenosynovial fibrosarcoma gene (MaFA) were measured with real-time quantitative PCR. Total RNA was extracted from 100 g of minced pancreatic tissue with TRIZOL reagent in an RNase-free ddH₂O medium. The RNA was reverse-transcribed to cDNA prior to RT-PCR. The primers used are shown in Table 1.

Table 1: Sequences of primers used

Gene		Sequence (5'~3')
PDX-1	F	AGCGGCACATTCGGAGAG
	R	TTGTACAGGTCCCGCTCCTT
MaFA	F	GGAGGTCATCCGACTGAA
	R	CCGCCAATTCTCGTATTTC
GAPDH	F	AACTTTGGCATTGTGGAAGG
	R	ACACATTGGGGGTAGGAACA

Statistical analysis

The data were analyzed using SPSS20.0 software package. Results for FBG, HbA1c, FFA, TC, MDA, SOD, ROS and other measurement data were compared with single factor diversity mean, and independent sample *t*-test was used for comparison of two groups. Statistical significance was assumed at $p < 0.05$.

RESULTS

Serum levels of FBG, HbA1c, FFA and TC s

Table 2 shows that FBG, HbA1c, FFAs and TC levels in model group were markedly higher than the corresponding values in the control group, but they were markedly reduced in the *Dachaihu* group, relative to model group.

Table 2: Levels of serum FBG, HBALC, FFA and TC

Group	FBG (mmol/L)	HbA1c (%)	FFA (mmol/L)	TC (mmol/L)
Control	4.91±0.53	2.21±0.15	0.44±0.03	1.76±0.13
Model	24.81±1.35	11.88±0.48	0.67±0.03	8.24±0.65
<i>Dachaihu</i> decoction	15.33±0.83	8.81±0.87	0.58±0.13	4.37±0.36
<i>F</i>	1596.69	1088.04	32.33	840.68
<i>P</i> -value	<0.001	<0.001	<0.001	<0.001

Values are presented as mean ± SD

Table 3: Serum levels of MDA, SOD and ROS

Group	MDA (ng/mL)	SOD (ng/mL)	ROS (ng/mL)
Control	0.46±0.33	1.28±0.32	48.33±3.65
Model	1.45±0.18	0.83±0.21	80.06±3.98
<i>Dachaihu</i> decoction	0.52±0.05	1.07±0.14	42.96±5.78
<i>F</i>	96.42	13.74	289.11
<i>P</i> -value	<0.001	<0.001	<0.001

Values are expressed as mean ± SD

Levels of MDA, SOD and ROS in serum of rats

There were significantly higher amounts of MDA and ROS in model group than in control group, while SOD activity was significantly decreased ($p < 0.05$). However, *Dachaihu* decoction significantly decreased the levels of MDA and ROS in the rats, while SOD levels were markedly decreased, relative to the model group ($p < 0.05$; Table 3).

Physical changes in rats

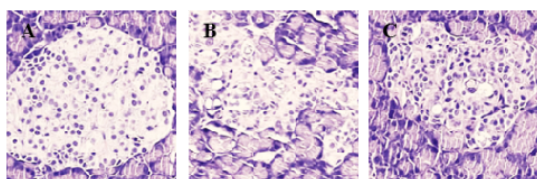
Compared with the control group, rats in the model group had dark red claws, less gloss on noses and lips, less body fluid on tongues, dull eyes and less physical activity ($p < 0.05$). However, compared with the model group, the claws of rats in the *Dachaihu* decoction group were pink, their noses and tongues were shiny and wet, and physical activity was increased ($p < 0.05$). Although the eyes were red and bright, and their lips were shiny and moist, there were no significant differences between rats in the two groups ($p < 0.05$). These data are shown in Table 4.

Pathological changes in pancreatic tissue

The pancreatic tissues of the control group were clear and orderly, with complete pancreatic vesicle lobules. In contrast, pancreatic tissues of the model group rats were disorganized, and there were evidence of inflammatory infiltration, massive reduction in number of pancreatic islets, and some bleeding and necrotic follicular lobules. In contrast, pancreatic tissues from rats in *Dachaihu* decoction group were significantly improved, when compared with the model group. These results are presented in Figure 1.

Table 4: Representative changes in rats (n =15)

Group	Control	Model	Dachaihu decoction	χ^2	P-value
Color of claws					
Pink	45/45	14/45	37/45	56.034	<0.001
Dark red	0/45	31/45	8/45		
Luster of nose					
Shiny and wet	45/45	17/45	38/45	49.140	<0.001
Less gloss	0/45	28/45	7/45		
Luster of tongue					
Shiny and wet, less body fluid	45/45	10/45	38/45	71.129	<0.001
	0/45	35/45	7/45		
Eyes					
Bright eyes, less activity	45/45	20/45	28/45	38.598	<0.001
Dull eyes	0/45	21/45	10/45		
Squint/closed eyes	0/45	4/45	7/45		
Luster of lips					
Shiny and wet	45/45	10/45	28/45	57.491	<0.001
Less gloss	0/45	35/45	17/45		
activity					
No significant changes, with less activity	45/45	10/45	38/45	71.129	<0.001
	0/45	35/45	7/45		

**Figure 1:** Pathological changes in pancreatic tissue of each group. A: Control; B: Model; C: *Dachaihu* decoction

Levels of PDX-1 and MaFA mRNA in pancreatic tissues of rats

There were downregulated mRNA expression levels of PDX and MaFA in the pancreatic tissues of the model group, relative to the control. However, mRNA concentrations of PDX-1 and MaFA in pancreatic tissue of rats in *Dachaihu* decoction group were significantly increased, when compared to the model rats, as presented in Table 5 ($p < 0.05$).

Table 5: mRNA expression levels of PDX and MaFA in pancreatic tissues of rats

Group	PDX-1 mRNA	MaFA mRNA
Control	1.01±0.01	1.01±0.01
Model	0.11±0.03	0.12±0.04
<i>Dachaihu</i> decoction	0.58±0.11	0.54±0.09
F	696.07	910.26
P	<0.001	<0.001

Values are expressed as mean ± SD

DISCUSSION

Type 2 diabetes mellitus (T2DM) is the more common type of diabetes mellitus: more than 90 % of diabetic patients have T2DM. The

pathogenesis of T2DM is still not clear, but it is believed to be mainly related to pre-receptor, post-receptor and receptor defects [6]. At present, metformin, insulin growth factor and immunosuppressant drugs are used in clinical treatment of T2DM. However, the therapeutic efficacies of these drugs are not always very satisfactory.

The use of TCM in the treatment of T2DM has a long history. In TCM, diabetes is placed in the category of "thirst elimination", and it is believed that diabetes is caused mostly by *lack of diet, deficiency of natural resources and imbalance in mood*. The pathogenesis of the disease is thought to be due to loss of body fluids and *excessive dry heat* [7]. However, due to changes in people's lifestyles, the understanding of T2DM by TCM doctors has undergone significant changes. Some scholars believe that the early and middle stages of T2DM are similar to *hate of the spleen*, which is an important stage in the development of *thirst dissipation*, while the early syndrome type of this disease is due to *depression of the liver and deficiency of the spleen and hot nodes* [8]. Therefore, the TCM treatment strategy for T2DM adopts mostly the method of *clearing heat and opening depression*.

Dachaihu decoction is composed of *Bupleurum, Rhubarb, Radix Scutellariae, Paeoniflorin, Ginger, Fructus aurantii, Jujube* and *Pinellia ternate*. *Bupleurum* and *Huang Ling* soothe the liver and gallbladder, *clear heat* and relieve depression. *Rhubarb* and *Fructus aurantii* can be combined to relieve *qi* and *Yang Ming heat knot*. *Rhubarb* and *Paeoniflorin* relieve heat and treat abdominal pain. *Paeoniflorin* and *Fructus aurantii* regulate *qi* and blood, except heart pain. *Ginger*

and *Pinellia ternata* or *Jujube* protect the spleen and stomach [9]. Thus, the combination of these drugs results in *opening liver depression, clearing stomach heat, regulating qi and clearing blood collaterals*.

In most cases, T2DM is caused by excessive eating of fat and sweet-tasting foods, excessive mental tension, sluggish splenic transport, inability of food to *decompress* in time, *obstruction of qi* and blood, *stagnation of dampness and turbidity in the body, change in heat with time, and blood turbidity* [10]. Insulin resistance is a major characteristic of T2DM, and an independent risk factor for the disease. Insulin resistance may aggravate diabetes mellitus due to impairment of glucose and lipid metabolism and abnormal proliferation of vascular smooth muscle cells [11]. The results of this study showed that T2DM rats had abnormalities in glucose and lipid metabolism. However, *Dachaihu* decoction reduced blood glucose, regulated blood lipids and improved insulin resistance in the T2DM rats.

Oxidative stress is an important cause of a variety of diseases. A large number of studies have shown that oxidative stress is closely related to the pathogenesis of diabetes [12]. Under normal circumstances, the free radical generation and scavenging system of an organism are in a dynamic equilibrium, thereby maintaining redox homeostasis. However, under stress, the production of ROS far exceeds the capacity of the body to clear them, leading to tissue and cell damage which lead to a variety of diseases [13]. Malondialdehyde (MDA) is one of the end-products of lipid peroxidation, and is the most reliable index used to determine the level of oxidative stress. The *in vivo* antioxidant system comprises antioxidant enzymes and non-enzymatic antioxidants. Superoxide dismutase (SOD), an important antioxidant enzyme, plays an important role in maintaining balance between oxidation and antioxidants, thereby protecting the functional integrity of various cells [14].

It has been reported that oxidative stress enhances diabetes by inducing impairment of islet beta cell function and inducing peripheral tissue insulin resistance: oxidative stress promotes the production and secretion of insulin in islet beta cells [15]. The pancreatic specific expression transcription factor, PDX-1 promotes the differentiation of β cells and secretion of insulin, and maintains normal function of pancreatic beta cells. Studies have shown that oxidative stress may affect the nucleoplasmic translocation of PDX-1 by inhibiting the binding of PDX-1 to insulin gene promoter, thereby

inhibiting insulin production and secretion. Moreover, PDX-1 is regulated by MaFA [16], an important transcription factor which regulates insulin gene expression and islet β cell maturation. The expression of MaFA occurs specifically in islet β cells, and it regulates insulin secretion as well as the proliferation and differentiation of islet β cell mass.

It has been reported that MaFA acts in synergy with PDX-1, thereby promoting the transformation of non-islet β cells into insulin-secreting cells [17-18]. The present study has demonstrated that the oxidative stress response of T2DM rats was enhanced, the antioxidant capacity was significantly decreased, and the islet β cells were damaged. However, *Dachaihu* decoction enhanced antioxidant capacity and protected islet β cells in T2DM rats.

CONCLUSION

Dachaihu decoction is effective in the treatment of T2DM in rats via inhibition of oxidative stress response, improvement of islet β -cell function, and mitigation of symptoms such as listlessness, fatigue and dehydration. Thus, *Dachaihu* decoction may be useful for adjuvant treatment of TCM-T2DM.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ying Zhang designed the study, supervised the data collection, and analyzed the data. Qiuju Zhang interpreted the data and prepared the manuscript for publication. Zhufeng Wang, Hongliang Xu, Li Qin and Ran An supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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