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Original Research Article

Antidiabetic retinopathy effect of Fufang Danshen Mingmu in rats

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Abstract

Purpose: *To investigate the effect of Fufang Danshen Mingmu (FDM) on streptozotocin-induced diabetic retinopathy rats.*
Methods: *Diabetic retinopathy model rats were prepared using a single intraperitoneal injection of a freshly prepared solution of streptozotocin (50 mg/kg). The rats were randomly divided into 6 groups of ten rats each: negative control group, control group, reference group (glibenclamide, 1 mg/kg) as well as FDM groups, (50, 100 and 200 mg/kg body weight). Blood glucose and plasma insulin levels were determined. Oxidative stress was evaluated in liver and kidney as lipid peroxidation (LPO), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT). Blood serum levels of creatinine and urea were determined in both diabetic control and treated rats.*
Results: *Compared with diabetic rats, oral administration of FDM at a dose of 200 mg/kg daily for 30 days resulted in a significant decrease in fasting blood glucose (120.21 ± 3.37 mg/dL, $p < 0.05$) and increased insulin level (13.31 ± 0.67 uU/mL, $p < 0.05$). Furthermore, it significantly reduced biochemical parameters (serum creatinine, 0.86 ±0.24 mg/dL, $p < 0.05$) and serum urea (41.86±1.59 mg/dL, $p < 0.05$).*
Conclusion: *The results indicate that FDM normalizes impaired antioxidant status in streptozotocin-induced diabetic retinopathy rats, and also exerts a protective effect against lipid peroxidation by scavenging free radicals.*

Keywords: *Fufang Danshen Mingmu, Diabetic retinopathy, Antihyperglycemic, Antioxidant oxidative stress, Fasting blood glucose*

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INTRODUCTION

Diabetes mellitus, a metabolic disease that manifests due to insulin insufficiency and/or insulin resistance has become a serious health problem worldwide. In 2010, approximately 285 million adults between 20 and 79 years of age in the world were affected by diabetes, and it is expected that 439 million adults will be diabetic by 2030. The primary goals in the management

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of diabetes include tight regulation of glucose levels in the blood and the prevention of diabetic complications [1]. Hyperglycemic control is crucial for the prevention and delay of the progression of diabetic complications.

Although diabetes is a non-communicable disease, it is considered one of the five leading causes of death in the world [2]. The disease is a complex metabolic disorder of the endocrine system. It is characterized by high blood glucose levels (hyperglycemia) due to the inability of the body cells to utilize glucose properly [3]. The increased blood glucose levels in diabetes produces superoxide anions which generate hydroxyl radicals via Haber–Weiss reaction, resulting in peroxidation of membrane lipids and protein glycation. This leads to oxidative damage of cell membranes. These radicals further damage other important biomolecules including carbohydrates, proteins and deoxyribonucleic acid (DNA) [4-7].

Medicinal plants are widely used by people in developing countries as alternative therapy. In China, hundreds of plants are used traditionally for the management of diabetes. Unfortunately, only a few of such Chinese medicinal plants have received scientific scrutiny. Despite the long traditional use of FDM in diabetes [8,9], no systematic pharmacological work has been carried out on this plant.

The present study was therefore designed to study the hypoglycemic effect of FDM on streptozotocin-induced diabetic retinopathy in rats.

EXPERIMENTAL

Plant material and extraction

Fufang Danshen Mingmu (FDM) was composed of *Salvia miltiorrhiza Bge., Angelica sinensis, Astragalus propinquus Schischkin, Ligusticum chuanxiong hort, Tetradium ruticarpum, Anemarrhena asphodeloides Bunge., Ophiopogon japonicus (Linn. f.) Ker-Gawl. and Glycyrrhiza uralensis Fisch.*

The medicinal materials of FDM were collected from Nanning City, Guangxi Province in China in March 2019. Taxonomic identification of the plant was performed by Professor Jun Tang of Wuhan University in China. A voucher specimen no. FDM 20190312 was deposited in the herbarium of College of Pharmacy, Wuhan University, China for future reference. Fufang Danshen Mingmu (FDM) was prepared according to the general method of traditional Chinese medicine decoction.

Animals

Specific Pathogen Free (SPF) male Wistar rats weighing 200 - 220 g, were provided by the Experimental Animal Center of Hubei Province (certificate no. SYXK2014 - 0008). The animals had free access to feed and water, and were allowed to acclimatize for at least one week before use. The rat experiment was approved by the Animal Care and Use Committee of The Third Hospital of Wuhan (approval ref no. 20191005), and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [10]. The rats were randomly divided into 6 groups of ten rats each: negative control group, model group, reference group (glibenclamide) as well as FDM groups, given FDM at dose of, 50, 100 and 200 mg/kg body weight. Treatments (in aqueous solution) were given orally once daily for 30 days.

Preparation of experimental diabetic retinopathy

The animals were fasted overnight and diabetic retinopathy was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (50 mg/kg) in citrate buffer (pH 4.5) [11-12]. After three days of STZ-injection, the rats were fasted for 6 h and blood was withdrawn using retroorbital puncture under ether anesthesia. Hyperglycemia rats (that is, with blood glucose of 250 – 400 mg/dL) were used for the experiment [13].

Biochemical analysis

Rats were fasted overnight and blood was withdrawn using retro orbital puncture under light ether anesthesia on the 1st, 15nd and 30th days post-induction to determine blood glucose and plasma insulin levels. Changes in body weight was determined throughout treatment period in the experimental animals. At the end of 30 days, the animals were sacrificed. Blood was collected from the heart in two different tubes, i.e. one with anticoagulant (for plasma), and another with anticoagulant for (serum separation). Serum was separated by centrifugation 3500 rpm at 25 ^{o}C for 10 min. Fasting blood glucose was estimated using O-toluidine method [14]. Plasma insulin level was assayed using radio-immunoassay method. All biochemical tests were carried out in by using commercial kits obtained from Erba diagnostic Mannheim Gmbh, Germany.

Oral glucose tolerance test

The rats were divided into four groups of 10 animals each. Group I served as control and received distilled water. Group II served as diabetic control and received distilled water. Group III served as positive control, received glibenclamide (1 mg/kg). Group IV received FDM 100 gm/kg orally. The rats were fasted for 18 h and the test was performed using oral administration of glucose (2 g/kg) to diabetic and normal rats 30 min after dosing. Blood samples were collected from the retro-orbital (under light ether anesthesia) immediately (0 h), and 30, 60, 90, and 120 min after the glucose administration and the blood glucose levels were estimated.

Statistical analysis

Data are presented as mean ± standard deviation (SD), and were analyzed using one-way ANOVA followed by Tukey's multiple comparison using SPSS 17.0 software for Windows. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of FDM on blood glucose and plasma insulin levels

Fasting blood glucose levels in the negative group of rats remained unchanged during the course of the experiment. Compared to normal group, level of fasting blood glucose was significantly ($p < 0.01$) higher and the plasma insulin level was significantly decreased in diabetic groups. On the other hand, administration of FDM for 30 days lowered the blood glucose and increased insulin level significantly ($p < 0.01$) in a dose-dependent manner, when compared with control group (Tables 1 and 2).

Effect of FDM on biochemical parameters

Streptozocin (STZ) induced significant ($p < 0.05$) elevations in serum creatinine and urea levels and decrease in total protein, when compared to normal group. However, treatment with different doses of FDM significantly ($p < 0.05$) reduced serum creatinine and serum urea level, and increased total protein, when compared to those of diabetic control groups (Table 3).

Effect of FDM on oral glucose tolerance test (OGTT)

The results indicated that FDM (200 mg/kg) and glibenclamide (1 mg/kg) reduced blood glucose level significantly ($p < 0.05$) after 120 min of oral administration, when compared to diabetic control (Table 4).

DISCUSSION

Diabetes is a metabolic disorder of carbohydrate, fat and protein, which affects a large number of population in the world. Diabetes mellitus is a group of metabolic disorders characterised by chronic hyperglycemia resulting from defects in insulin secretion [1].

Table 1: Effect of FDM on blood glucose level in rats (mean ± SD, n = 10)

Group	Dose (mg/kg)	Blood glucose (mg/dL)		
		Initial	***Day 15***	***Day 30***
Negative	—	58.45 ± 2.51	87.25 ± 2.38**	82.17 ± 2.65**
Model	—	293.15 ± 2.18	343.47 ± 3.29	346.24 ± 3.83
GLI	1	279.36 ± 2.24	176.16 ± 3.08**	126.14 ± 2.26**
FDM-L	50	290.16 ± 3.17	268.36 ± 3.37*	238.24 ± 3.49*
FDM-M	100	284.23 ± 2.38	234.25 ± 4.43**	163.17± 3.14**
FDM-H	200	265.17 ± 2.28	219.34 ± 3.16**	116.24 ± 3.27**

*$P < 0.05$, **$p < 0.01$ vs. control group. GLI: glibenclamide; FDM-L: low dose of FDM; FDM-M: middle dose of FDM; FDM-H: high dose of FDM*

Table 2: Effect of FDM on plasma insulin level in rats (mean ± SD, n=10)

Group	Dose (mg/kg)	Plasma insulin (uU/mL)		
		Initial	***Day 15***	***Day 30***
Negative	—	26.27 ± 0.81	19.59 ± 0.76**	19.37 ± 0.54**
Model	—	6.35 ± 0.37	5.15 ± 0.61	2.38 ± 0.42
GLI	1	5.41 ± 0.47	14.27 ± 0.64**	11.08 ± 0.52**
FDM-L	50	6.37 ± 0.51	7.39 ± 0.53*	9.36 ± 0.64*
FDM-M	100	5.78 ± 0.46	10.56 ± 0.71**	11.22 ± 0.59**
FDM-H	200	6.37 ± 0.44	9.64 ± 0.62**	13.83 ± 0.62**

*$P < 0.05$, **$p < 0.01$ vs. control group. GLI: glibenclamide; FDM-L: low dose of FDM; FDM-M: middle dose of FDM; FDM-H: high dose of FDM*

Table 3: Effect of FDM on biochemical parameters in rats (mean ± SD, n = 10)

Group	Dose (mg/kg)	Hemoglobin (mg/dL)	Glycosylated Hb (Hb%)	Serum creatinine (mg/dL)	Serum urea (mg/dL)	Total proteins (g/dL)
Negative	—	15.36±2.17*	6.34 ±0.67*	0.92 ±0.14*	29.15±1.64*	9.28 ±1.34*
Model	—	8.34±1.41	19.34 ±1.08	2.74 ±0.22	81.34±2.08	4.21 ±1.18
GLI	1	11.27±2.21	14.27 ±0.74	0.89 ±0.18*	34.49±1.42*	7.65 ±1.32*
FDM-L	50	9.53±1.34*	13.18 ±0.72*	1.59 ±0.14*	78.24±2.14*	6.78 ±1.10*
FDM-M	100	11.22±1.37*	12.17 ±0.65*	1.42 ±0.15*	59.34±1.64*	6.25 ±1.13*
FDM-H	200	10.46±1.54*	8.82 ±0.71*	0.89 ±0.22*	40.67±1.45*	7.34 ±1.21*

*$P < 0.05$, **$p < 0.01$ vs. control group. GLI: glibenclamide; FDM-L: low dose of FDM; FDM-M: middle dose of FDM; FDM-H: high dose of FDM*

Table 4: Effect of FDM on fasting blood glucose level in rats (mean ± SD, n = 10)

Group	Dose (mg/kg)	Fasting blood glucose level (mg/dL)				
		0 h	*0.5 h*	*1 h*	*1.5 h*	*2 h*
Negative	—	91.7 ± 0.5	108.4 ± 1.5	118.4 ± 1.2	132.3 ± 1.2	142.3 ± 1.4
Model	—	238.4 ± 1.5	268.3 ± 0.8	288.2 ± 1.1	293.4 ± 1.3	302.2 ± 1.2
GLI	1	262.4 ± 1.2	262.7 ± 1.2*	263.3 ± 1.5*	275.6 ± 1.3*	263.5 ± 1.3*
FDM-H	200	253.3 ± 1.1	253.8 ± 1.0*	262.4 ± 1.3*	287.3 ± 1.1*	269.4 ± 1.1*

*$P < 0.05$, **$p < 0.01$ vs. control group. GLI: glibenclamide; FDM-H: high dose of FDM*

Increased thirst, increased urinary output, ketonemia and ketonuria are the common symptoms of diabetes mellitus, which occur due to the abnormalities in carbohydrate, fat, and protein metabolism. Chinese medicinal herbs have been shown to exert hypoglycemic activities through stimulation of insulin release [15].

Diabetic hyperglycemia induces elevation of serum level of urea and creatinine, which were considered as significant markers of renal dysfunction [16]. Increase in serum level of urea and creatinine in STZ-diabetic rats may indicate diminished ability of the kidney to filter these waste products from the blood and excrete them in the urine. The results indicate that treatment of diabetic with *Scrophularia ningpoensis* extract significantly reduced serum urea and creatinine level. Based on these findings, FDM may enhance the ability of the kidney to remove these waste products from the blood in diabetic rats.

Catalase has been shown to be responsible for the detoxification of H_2O_2, which protects the tissues from highly reactive hydroxyl radicals [17]. The decrease in CAT activity could have resulted from in activation by glycation of enzyme [18]. In the present study, extract-treated groups showed a significant increase in the hepatic and renal SOD and CAT activities of the diabetic rats. This means that the extract reduced potential glycation of enzymes or reduced reactive oxygen free radicals and improved the activities of antioxidant enzymes. This result clearly shows that FDM contains free radical scavenging activity which could exert a beneficial action against pathological alteration caused by the presence of superoxide radicals and hydrogen peroxide radical.

CONCLUSION

FDM normalizes impaired antioxidant status in streptozotocin-induced diabetic retinopathy rats, and thus has the potential for clinical use in the management of diabetic retinopathy in future, but this requires further investigations.

DECLARATIONS

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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.

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