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Berberine attenuates cardiac dysfunction in hyperglycemic and hypercholesterolemic rats

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ABSTRACT

The positive effects of berberine (30 mg/kg/day, i.g. for 6 weeks) on cardiac dysfunction were evaluated in the rat model of hyperglycemia and hypercholesterolemia. Hyperglycemia and hypercholesterolemia were induced by feeding high-sucrose/fat diet (HSFD) consisting of 20% sucrose, 10% lard, 2.5% cholesterol, 1% bile salt for 12 weeks and streptozotocin (30 mg/kg, i.p.). The plasma sugar, total cholesterol, and triglyceride levels were significantly increased (422, 194 and 82%, respectively) in the HSFD/streptozotocin-treated rats, when compared with control animals receiving normal diet and vehicle. Berberine treatment reduced the plasma sugar and lipid levels by 24–69% in the rat model of hyperglycemia and hypercholesterolemia. Cardiac functions signed as values of cardiac output, left ventricular systolic pressure, the maximum rate of myocardial contraction (+d*p*/d*t*max), left ventricular end diastolic pressure and the maximum rate of myocardial diastole (−d*p*/d*t* max) were injured by 16–55% in the hyperglycemic/hypercholesterolemic rats. Berberine increased cardiac output, left ventricular systolic pressure and +d*p*/d*t*max by 64, 16 and 79%, but decreased left ventricular end diastolic pressure and −d*p*/d*t*max by 121 and 61% in the rats receiving HSFD/streptozotocin, respectively, when compared with the drug-untreated rats of hyperglycemia and hypercholesterolemia. Berberine caused significant increase in cardiac fatty acid transport protein-1 (159%), fatty acid transport proteins (56%), fatty acid beta-oxidase (52%), as well as glucose transporter-4 and peroxisome proliferator-activated receptor-γ (PPARγ), but decrease in PPARα mRNA and protein expression in hyperglycemic/hypercholesterolemic rats. These results indicated that berberine exerted protective effects on cardiac dysfunction induced by hyperglycemia/hypercholesterolemia through alleviating cardiac lipid accumulation and promoting glucose transport.

1. Introduction

The term of "diabetic cardiomyopathy" was appeared in the early 1970's (Hamby et al., 1974; Rubler et al., 1972). The diabetic cardiomyopathy is rarely clinically apparent unless associated with hypertension and/or with myocardial ischemia in the asymptomatic diabetic patients (Bell, 2003). Experimental and clinical evidences have indicated that the diabetic cardiomyopathy is mainly characterized by left ventricular diastolic dysfunction, systolic dysfunction and structural remodeling (Airaksinen et al., 1984; Fang et al., 2004). Although diabetic cardiomyopathy is increasing recognized, the underlying mechanisms are still under controversial. Recently, the putative pathological mechanism involves metabolic disturbances, myocardial fibrosis, small vessel disease, cardiac autonomic neuropathy, insulin resistance (Fang et al., 2004). The current important information of diabetic cardiomyopathy is mainly obtained from the animal models of diabetes, obesity or insulin resistance (Bugger and Abel, 2009).

Berberine, an isoquinoline alkaloid, derived from medicinal herbs including *Berberis*, *Hydrastis canadensis*, *Coptis chinensis* Franch. and *Cortex Phellodendri Chinensis*, has antibacterial and anti-inflammatory activities (Jeong et al., 2009). Recent studies have also demonstrated the reduction of blood glucose and lipids (Cui et al., 2009; Gulfraz et al., 2008), increase in insulin sensitivity (Wang et al., 2011) in type 1 or type 2 diabetic animals, antiarrhythmia, as well as inhibition of cardiac hypertrophy with berberine treatment (Lau et al., 2001; Wang et al., 2009; Zhao et al., 2007; Zhou et al., 2009). Clinical researches have indicated that berberine can improve metabolic dysfunction and decrease ventricular premature complexes in the patients with dyslipidemia and congestive heart failure (Gu et al., 2010; Zhang et al., 2010). Up to now, however, little attention has been focus on the role of berberine for treating diabetic cardiomyopathy. Hence our experiments were aimed to explore the effects of berberine on cardiac dysfunction and metabolic disorders in the rat model of hyperglycemia and hypercholesterolemia induced by high-sucrose/fat diet (HSFD) and streptozotocin.

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2. Materials and methods

2.1. Chemical and reagents

Both berberine chloride and streptozotocin were from Sigma-Aldrich in USA. Berberine was suspended in 0.5% sodium carboxymethylcellulose solution and intragastrically administered (i.g.) at 10 ml/kg. Streptozotocin was dissolved in citrate buffer dilution (0.1 M, pH = 4.5) and given with single intraperitoneal injection (i.p.) at 3 ml/kg. TRIzol was purchased from Invitrogen Life Technologies in USA. Antibodies of glucose transporter-4 (GLUT4), peroxisome proliferators-activated receptor-α (PPARα) and PPARγ were from Santa Cruz (USA) and prepared to 1:1000, 1:3000 and 1:1000 dilution. Biochemical kits of plasma and cardiac samples were purchased from Nanjingjiancheng Bioengineering Institute (China), and ELISA kits were obtained from Groudwork Biotechnology Diagnosticate Ltd. (Canada).

2.2. Animal treatment

Male Wistar rats weighing 160–180 g were purchased from Vital River Lab Animal Co. Ltd. (grade II, Certificate No: SCXK-2007-0001). Rats were maintained at 23 ± 1 °C and 60–70% humidity with a 12 h light/dark cycle. The regular diet was a standard chow diet. The high-sucrose/fat diet (HSFD) containing 2920 kcal/kg was composed of 20% sucrose, 10% lard, 2.5% cholesterol, 1% bile salt, and 66.5% regular diet to produce the final HSFD. Water and the various diets were given to all animals *ad libitum*.

Rats were randomly divided into four groups, each containing 10–12 animals. The first group received regular diet and vehicle (0.5% sodium carboxymethyl cellulose solution). The second group was fed with HSFD for 12 weeks and given a single dose of streptozotocin (i.p.) at 30 mg/kg following a 12 h fast at the seventh week to induce hyperglycemia and hypercholesterolemia. This group was also called HSFD/streptozotocin group (i.e., rat model of hyperglycemia and hypercholesterolemia). In the third and fourth groups, rats were treated as the second group except that they were daily intragastrically administered berberine (15 and 30 mg/kg/day, respectively, for consecutive 6 weeks) 72 h after streptozotocin injection. All experimental protocols were approved by the University Committee of Research Practice of Beijing University of Chinese Medicine.

2.3. Cardiac function, biomarker and histology assessment

At the thirteenth week, rats were anesthetized with pentobarbital sodium (35 mg/kg, i.p.) following a 12 h fast. Stroke volume and cardiac output were detected by means of non-invasive impedance plethysmography. On completion of the cardiac output measurements, a catheter (20 G, Vasocan Braünle, Malaysia) was positioned in the left ventricle via the right carotid artery for measurement of left ventricular systolic pressure, left ventricular end diastolic pressure, the maximum rate of myocardial contraction (+dp/dt max) and the maximum rate of myocardial diastole (−dp/dt max). Data were collected using MP150 systems (BIOPAC Systems, Inc., USA). After that, rat hearts and left ventricles were obtained and weighed to calculate the ratios of heart weight and left ventricular weight to the body weight, respectively.

The homogenate of heart tissues were prepared in the physiological saline (1:9) and centrifuged for 10 min at 2000 g. Myocardial nonesterified free fatty acids were measured by the biochemical method.

Hydroxyproline concentration was determined in heart tissue by alkaline hydrolysis method. Tissue samples were hydrolyzed in 2 mol/L sodium hydroxide at 100 °C for 1 h. Chloramine-T (0.05 mol/L) was used to oxidize for 10 min at room temperature (pH = 6.0–6.8). Then Ehrlich's reagent was added to each sample and the samples were mixed and incubated at 65 °C for 15 min. The absorbance of samples was read at 550 nm using a spectrophotometer to determine the content of hydroxyproline. Left ventricular collagen content was estimated from its hydroxyproline concentration, by multiplying its value by 7.46, since the imino acid represents 13.4% of collagen. Levels of myocardial fatty acid transport protein-1, fatty acid transport proteins and fatty acid beta-oxidase were assessed by ELISA.

Then equator annulus of left ventricles were collected and placed in 10% buffered formalin. Sections (4 μm) were cut and stained with hematoxylin and esosin (H&E). The left ventricular wall thickness and interventricular septum thickness were measured by Image-ProPlus 5.0 image analysis software (USA) on the H&E slices microscopically.

2.4. Cardiac PPARα, PPARγ and GLUT4 mRNA expression assessment

Tissue samples obtained from left ventricles were rapidly frozen in liquid nitrogen and stored at −70 °C prior to quantitative real-time (RT)-PCR analysis. Total RNAs were isolated using TRIzol reagent according to the manufacturer's protocol, and then reverse transcribed to synthesize cDNA. The RT primers were designed by Prime 5.0 software and oligonucleotide sequences were shown as follows. For GLUT4: forward primer 5′-TCCTTTCCTCGCAGCACTT-3′, reverse primer 5′-CCACAGCCTAGCCACAACAC-3′; for PPARα: forward primer 5′-ATTTGCCAAGGCTATCCCA-3′, reverse primer 5′-CAGCATCCCGTCTTTGTTCA-3′; for PPARγ: forward primer 5′-GCGGAAGCCCTTTGGTGA-3′, reverse primer 5′-TGCAGCAGGTTGTCTTGGATG-3′. Total Realtime PCR reaction system was performed in Rotor-Gene 3000 Realtime PCR instrument (Corbett Research, Australia), as previously described (Aslanidi et al., 2007). To allow for comparisons between samples and groups, quantities of all targets in test samples were normalized to the constitutive housekeeping gene glyceraldehyde phosphate dehydrogenase (GAPDH) (Campbell et al., 2002).

2.5. Cardiac PPARα, PPARγ and GLUT4 protein expression assessment

Total GLUT4, PPARα and PPARγ protein expression in the heart homogenate extracts were determined by Western blot as described previously (Lai et al., 2007; Laybutt et al., 1997). GAPDH was probed as an internal loading control. Western blot band density analysis was made using ImageJ. Total GLUT4, PPARα and PPARγ proteins were shown in arbitrary units.

2.6. Plasma profile assessment

Whole blood samples were obtained from the right carotid artery of rats and collected in fresh vials containing anticoagulant, and plasma samples were prepared by centrifuging the whole blood for 10 min at 2000 g. Levels of fasting blood glucose, glycated hemoglobin, fructosamine, glycosylated serum protein, total cholesterol and triglyceride in plasma samples were determined using ultraviolet spectrophtometric method according to the manufacturer's protocol.

2.7. Statistical analysis

All data were presented as mean ± SEM and analyzed by one-way analysis of variance (ANOVA). Multiple group comparisons were made with least significant difference's (LSD) post hoc test by SPSS 17.0. Statistical significant difference was defined as a value of $P<0.05$.

3. Results

3.1. Cardiac function and structure

The HSFD/streptozotocin-treated rats showed the same hemodynamic characteristics of diabetic cardiomyopathy. Stroke volume, cardiac output, left ventricular systolic pressure and +dp/dt max in the hyperglycemic/hypercholesterolemic rats were significantly

Table 1
Effects of berberine (Ber) on cardiac function in hyperglycemic/hypercholesterolemic rats.

Group	Stroke volume (mL)	Cardiac output (mL/min)	Left ventricular systolic pressure (mmHg)	Left ventricular end diastolic pressure (mmHg)	+d*p*/d*t*max (mmHg/s)	−d*p*/d*t*max (mmHg/s)
Control	0.038 ± 0.008	15.0 ± 3.5	150 ± 17	−11.6 ± 6.8	10,522 ± 3,101	−8,291 ± 2,280
HSFD/streptozotocin	0.026 ± 0.008**	9.4 ± 2.6**	125 ± 14**	−5.2 ± 2.0*	5,906 ± 2,293**	−4,978 ± 2,118**
HSFD/streptozotocin + Ber 15	0.035 ± 0.009	13.3 ± 3.8	145 ± 10***	−8.4 ± 4.6	8,454 ± 2,463	−7,250 ± 3,132
HSFD/streptozotocin + Ber 30	0.040 ± 0.009****	15.4 ± 3.6****	146 ± 24***	−11.5 ± 7.1***	10,595 ± 4,281****	−8,029 ± 3,463***

Rats were fed a high-sucrose/fat diet (HSFD) consisting of 20% sucrose, 10% lard, 2.5% cholesterol, 1% bile salt and 66.5% regular diet for 12 weeks and given a single dose of streptozotocin (30 mg/kg, i.p.) at week 7 to induce hyperglycemia and hypercholesterolemia (i.e., HSFD/ streptozotocin group). HSFD/streptozotocin-treated rats were daily intragastrically administered berberine (Ber) 15 and 30 mg/kg/day for consecutive 6 weeks (from week 7 to week 12), respectively, called HSFD/ streptozotocin + Ber 15 and HSFD/ streptozotocin + Ber 30 groups. Control animals were received regular diet and vehicle (0.5% sodium carboxymethyl cellulose solution). Experiments were carried out at week 13 after the rats feeding HSFD. Values of stroke volume and cardiac output were measured by means of non-invasive impedance plethysmography method. Left ventricular systolic pressure, left ventricular end diastolic pressure, the maximum rate of myocardial contraction (+d*p*/d*t* max) and maximum rate of myocardial diastole (−d*p*/d*t* max) were measure by invasive left ventricular catheterization in anesthetized rats. Values given are mean ± SEM, with n = 12, using one-way ANOVA followed by least significant difference's (LSD) post hoc test.

* $P<0.05$ vs. control rats.
** $P<0.01$ vs. control rats.
*** $P<0.05$, vs. HSFD/streptozotocin-treated rats.
**** $P<0.01$ vs. HSFD/streptozotocin-treated rats.

decreased (32, 37, 16 and 44%, respectively), while left ventricular end diastolic pressure and −d*p*/d*t* max significantly increased (55 and 40%, respectively), when compared with the normal rats. Berberine 30 mg/kg caused a significant increase in stroke volume (54%, $P<0.01$), cardiac output (64%, $P<0.01$), left ventricular systolic pressure (16%, $P<0.05$) and +d*p*/d*t* max (79%, $P<0.01$), but decrease in left ventricular end diastolic pressure (121%, $P<0.05$) and −d*p*/d*t* max (61%, $P<0.05$), when compared with the drug-untreated rats of hyperglycemia and hypercholesterolemia. Berberine 15 mg/kg only increased left ventricular systolic pressure significantly (16%, $P<0.05$), when compared with hyperglycemic/hypercholesterolemic rats (Table 1).

Meanwhile, when rats were treated with the HSFD/streptozotocin, the ratios of heart weight and left ventricular weight to the body weight, left ventricular wall thickness, interventricular septum thickness and myocardial collagen content were significantly increased (8, 12, 38, 80 and 26%, respectively). Berberine 30 mg/kg treatment decreased the ratio of heart weight to body weight (8%, $P<0.05$), the ratio of left ventricular weight to body weight (10%, $P<0.01$), left ventricular wall thickness (46%, $P<0.01$), interventricular septum thickness (20%, $P<0.05$) and collagen content (36%, $P<0.001$) in the HSFD/streptozotocin-treated rats, when compared with the drug-untreated hyperglycemic/hypercholesterolemic rats. Berberine 15 mg/kg just lowered the collagen content significantly in the hyperglycemic and hypercholesterolemic rats($P<0.01$) (Fig. 1).

3.2. Cardiac GLUT4, PPARα and PPARγ mRNA expression

Genes involved in cardiac glucose and lipid metabolism were evaluated by quantitative real-time PCR (RT-PCR). As expected, cardiac mRNA expression of PPARγ and GLUT4 reduced while PPARα increased in the HSFD/streptozotocin- treated rats (all $P<0.05$). Berberine 30 mg/kg treatment increased GLUT4 and PPARγ mRNA expression (both $P<0.05$), and decreased PPARα mRNA levels in the rat model of hyperglycemia and hypercholesterolemia ($P<0.05$) (Fig. 2). This study indicated that berberine 15 mg/kg treatment only showed modest regulating effects on cardiac dysfunction and lipid metabolic disturbances in the hyperglycemic and hypercholesterolemic rats, so tissue samples of which were not chosen for RT-PCR test.

3.3. Cardiac GLUT4, PPARα and PPARγ protein expression

Protein expression of GLUT4 and PPARγ reduced in the hyperglycemic and hypercholesterolemic rats (both $P<0.05$), when compared with the normal rats. Berberine treatment caused an increase in GLUT4 and PPARγ protein expression (both $P<0.05$). However, berberine at the dosage of 30 mg/kg did not produce any detectable

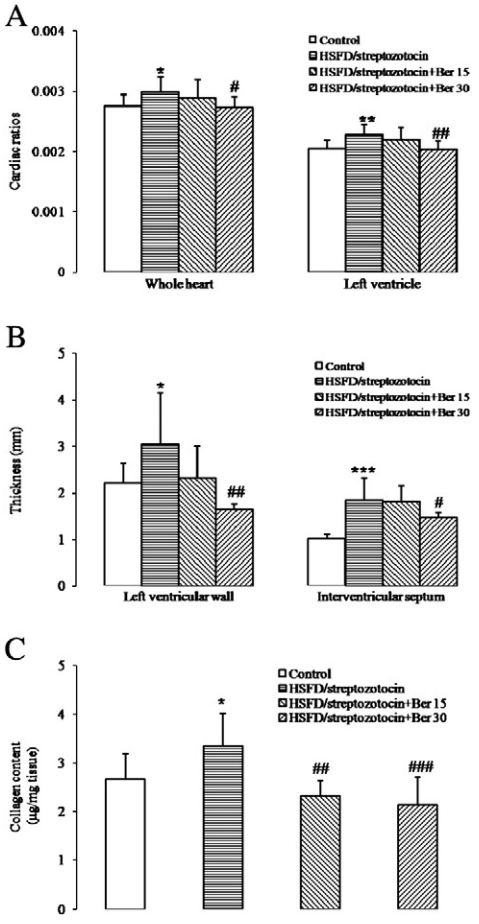

Fig. 1. Effects of berberine (Ber) on cardiac structure in hyperglycemic/hypercholesterolemic rats. Rats were fed a high-sucrose/fat diet (HSFD) consisting of 20% sucrose, 10% lard, 2.5% cholesterol, 1% bile salt and 66.5% regular diet for 12 weeks and given a single dose of streptozotocin (30 mg/kg, i.p.) at the seventh week to induce hyperglycemia and hypercholesterolemia (i.e. HSFD/streptozotocin group). HSFD/streptozotocin-treated rats were daily intragastrically administered berberine (Ber) at the dosage of 15 and 30 mg/kg/d for consecutive 6 weeks (week 7 to week 12), respectively, called HSFD/ streptozotocin + Ber 15 and HSFD/streptozotocin + Ber 30 groups. Control animals received regular diet and vehicle (0.5% sodium carboxymethyl cellulose solution). Experiments were carried out in rats at week 13 after feeding HSFD. The ratios of heart weight and left ventricular weight to the body weight were obtained (A). The left ventricular wall thickness and interventricular septum thickness were measured by Image-ProPlus 5.0 image analysis with H&E staining microscopically (B). Left ventricular collagen content was estimated from its hydroxyproline concentration, by multiplying its value by 7.46 (C). Each bar represents the mean ± SEM, with n = 12. *$P<0.05$, **$P<0.01$ vs. control rats; $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. HSFD/streptozotocin-treated rats, using one-way ANOVA followed by least significant difference's (LSD) post hoc test.

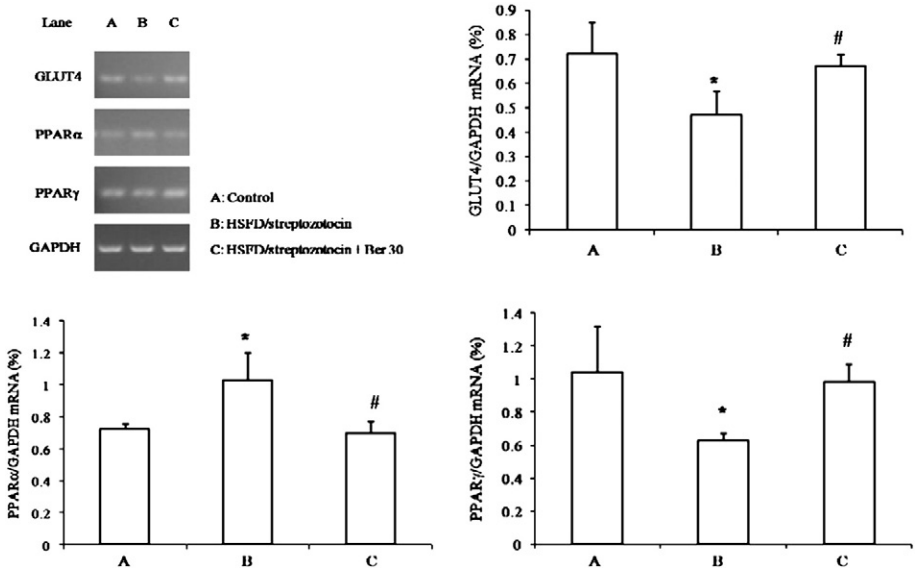

Fig. 2. Effects of berberine on cardiac GLUT4, PPARα and PPARγ mRNA expression in hyperglycemic/hypercholesterolemic rats. Animals were treated as described in Fig. 1. Peroxisome proliferators-activated receptor-α (PPARα), PPARγ and glucose transporter 4 (GLUT4) mRNA gene expression were tested by RT-PCR using GAPDH as internal control. Each bar represents the mean ± SEM, with n = 12. *P<0.05 vs. control rats; #P<0.05 vs. HSFD/streptozotocin- treated rats, using one-way ANOVA followed by LSD post hoc test.

changes in the cardiac PPARα protein expression (Fig. 3). Samples of hyperglycemic and hypercholesterolemic rats that treated with 15 mg/kg berberine were not chosen for western blot test as described before.

3.4. Blood and cardiac biomarkers

In the rat model of hyperglycemia and hypercholesterolemia, plasma fast blood glucose, glycated hemoglobin, fructosamine, glycosylated serum protein, total cholesterol and triglyceride significantly increased (422, 73, 74, 40, 194 and 82%, respectively), when compared with the control group. Berberine 30 mg/kg treatment caused a significant decrease in plasma fast blood glucose (69%, P<0.001), glycated hemoglobin (46%, P<0.001), fructosamine (35%, P<0.05), glycosylated serum protein (40%, P<0.001) and triglyceride (42%, P<0.05), when compared with the drug-untreated rats of hyperglycemia and hypercholesterolemia. Berberine 30 mg/kg tended to decrease plasma total cholesterol in the HSFD/streptozotocin rats, but the change did not attain statistical significance. However, berberine 15 mg/kg just showed significant hypoglycemic effects on hyperglycemic and hypercholesterolemic rats, and deceased plasma fast blood glucose (39%, P<0.05), glycated hemoglobin (24%, P<0.05) and fructosamine (30%, P<0.05), when compared with the drug-untreated rats of hyperglycemia and hypercholesterolemia (Table 2).

Levels of myocardial nonesterified free fatty acid and enzymes involved in fatty acids transport and oxidation were measured. Myocardial nonesterified free fatty acid in the HSFD/streptozotocin-treated rats was significantly increased (68%), while fatty acid

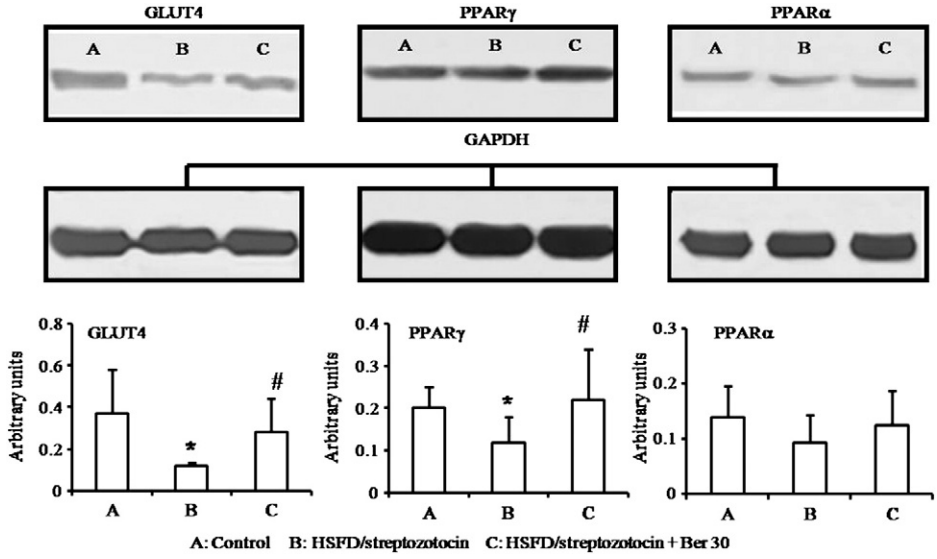

Fig. 3. Effects of berberine on protein expression of cardiac GLUT4, PPARα and PPARγ in hyperglycemic/hypercholesterolemic rats**.** Animals were treated as described in Fig. 1. The protein expression of GLUT4, PPARα and PPARγ in rat heart were analyzed by Western blot and band mean integral optical density. Each bar represents the mean ± SEM, with n = 12. *P<0.05 vs. control rats; #P<0.05 vs. HSFD/streptozotocin-treated rats, using one-way ANOVA followed by LSD post hoc test.

Table 2
Effects of berberine on plasma biomarkers in hyperglycemic/hypercholesterolemic rats.

Group	Fast blood glucose (mmol/L)	Glycated hemoglobin	Fructosamine (mmol/L)	Glycosylated serum protein (mmol/L)	Total cholesterol (mmol/L)	Triglyceride (mmol/L)
Control	3.6 ± 0.3	26 ± 7	2.3 ± 0.8	0.63 ± 0.03	1.7 ± 0.2	0.76 ± 0.27
HSFD/streptozotocin	18.8 ± 5.5***	45 ± 16***	4.0 ± 0.8**	0.88 ± 0.12**	5.0 ± 2.5*	1.39 ± 0.72*
HSFD/streptozotocin + Ber 15	11.4 ± 6.6****	34 ± 10****	2.8 ± 1.2****	0.86 ± 0.13	4.7 ± 2.1	0.94 ± 0.25
HSFD/streptozotocin + Ber 30	5.8 ± 3.4*****	24 ± 7*****	2.6 ± 0.4****	0.53 ± 0.23*****	2.8 ± 0.3	0.80 ± 0.26****

Animals were treated as described in Table 1. Plasma biomarkers were detected using ultraviolet spectrophtometric method according to the manufacturer's protocol after a 12 h fast. Values given are mean ± SEM, with n = 12, using one-way ANOVA followed by LSD post hoc test.
* $P<0.05$vs. control rats.
** $P<0.01$ vs. control rats.
*** $P<0.001$ vs. control rats.
**** $P<0.05$ vs. HSFD/streptozotocin-treated rats.
***** $P<0.001$ vs. HSFD/streptozotocin-treated rats.

transport protein-1, fatty acid transport proteins and fatty acid beta-oxidase were decreased (68, 21 and 52%, respectively), when compared with the normal rats. Berberine at the doses of 15 and 30 mg/kg both caused significant decrease in myocardial nonesterified free fatty acid (26% and 24%, respectively, $P<0.05$), when compared with the rat model of hyperglycemia and hypercholesterolemia. Meanwhile, berberine at the dosage of 30 mg/kg caused an increase in fatty acid transport protein-1 (159%), fatty acid transport proteins (56%) and fatty acid beta-oxidase (52%), and berberine at 15 mg/kg also increased fatty acid beta-oxidase(72%), when compared with the hyperglycemic/hypercholesterolemic rats (Fig. 4).

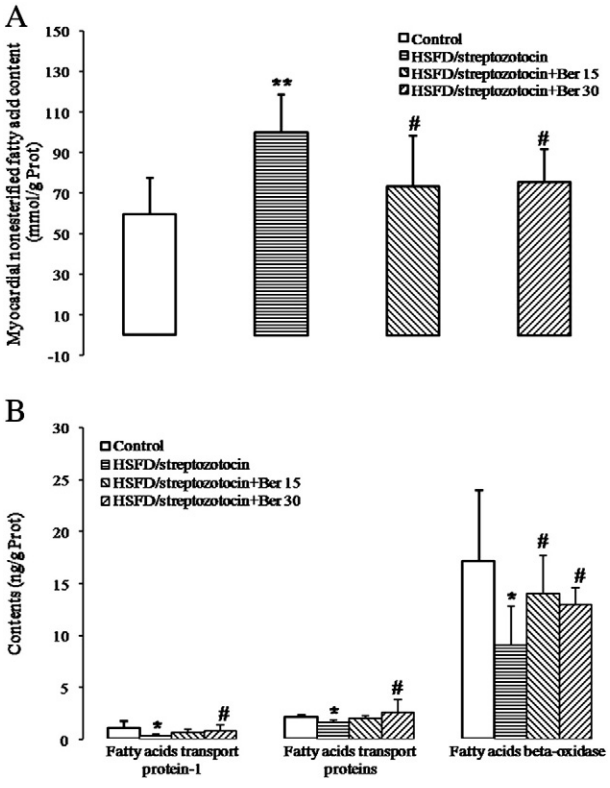

Fig. 4. Effects of berberine on cardiac energy metabolism in hyperglycemic/hypercholesterolemic rats. Animals were treated as described in Fig. 1. Myocardial nonesterified free fatty acids were measured by enzymatic spectrophotometer method (A). Fatty acid transport protein-1, fatty acid transport proteins and fatty acid beta-oxidase in the heart tissue homogenate extracts were measured by ELISA method (B). Each bar represents the mean ± SEM, with n = 12. *$P<0.05$, ***$P<0.001$ vs. control rats; #$P<0.05$ vs. HSFD/streptozotocin-treated rats, using one-way ANOVA followed by LSD post hoc test.

4. Discussion

Appropriate animal models play an important role in illuminating the underlying mechanisms of diabetic cardiomyopathy. Recently, the streptozotocin model, transgenic animals (OVE26 mouse, *ob*/*ob* mouse, *db*/*db* mouse, Zucker fatty rat, Zucker diabetic fatty rat), diet-induced obesity and diabetic animals have been used in diabetic cardiomyopathy research commonly (Bugger and Abel, 2009). Rodent models have many characteristics similar to human diabetic cardiomyopathy, such as diastolic dysfunction, left ventricular hypertrophy, increase in cardiac fatty acid uptake and oxidation, decrease in cardiac glucose utilization and cardiac efficiency and increase in myocardial lipid storage (Boudina and Abel, 2007; Bugger and Abel, 2009). In this study, the results illustrated cardiac dysfunction, left ventricular hypertrophy, hyperglycemia and hyperlipidemia in the HSFD/streptozotocin-treated rats, which were also in common to diabetic cardiomyopathy. Berberine treatment remarkably improved cardiac dysfunction, inhibited left ventricular hypertrophy, lowered blood sugar and lipids in the rat model of hyperglycemia and hypercholesterolemia.

The energy demands for the cardiomyocyte predominantly are met by ATP production from beta-oxidation of long-chain fatty acids. Fatty acids enter the cell via transport mechanisms such as fatty acid transport protein-1 and translocase/CD36, or through direct passive membrane diffusion. After then, fatty acids are conjugated with acyl-CoA and transported to the mitochondria to undergo beta-oxidation and generate ATP for cellular energy demands. Fatty-acyl CoA not used for energy production can be esterified to tri-acyl glycerol (triglyceride) for fatty acids storage (Glatz et al., 2010; Ruberg, 2007). Commonly, the process keeps a balance between uptake and portioning in the heart. However, lipid accumulation occurs when increased cardiac myocellular fatty acids contents overwhelms the capacity of cell to expediently metabolize substrate, which is referred as lipotoxicity and is the predominant contributor to the pathophysiology of insulin resistance, apoptosis of cardiomyocytes, morphological and functional changes of mitochondria, and cardiac dysfunction (Di Paola and Lorusso, 2006; Holland et al., 2007; Ouwens et al., 2007; Sharma et al., 2004; Wang et al., 2006).

However, the alteration in myocardial substrate metabolism that occurs in diabetic heart, and the cause and consequences of these abnormalities, are poorly understood. Some research indicated that excess of fatty acid oxidation was a cause of early cardiac dysfunction in parallel with the increase of proteins which participate in fatty acid uptake and oxidation (e.g., FATP1, CD36, FABP-1) in obesity and diabetes (Christoffersen et al., 2003). Whereas, previous researches also showed that in the heart of high fat diet fed rats, db/db mice, obese Zucker rats, and ZDF rats, fatty acid oxidation was either reduced slightly (Bandyopadhyay et al., 2006; Han et al., 2007), unaltered (Smith et al., 2007), or increased (Carley et al., 2007; Coort et al., 2004; Turcotte et al., 2001). The evidence of our present

research indicated an increase of cardiac fatty acids content in parallel with decreased fatty acid transport proteins and fatty acid beta-oxidase in the rat model of hyperglycemia and hypercholesterolemia (4B).

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily, and three subtypes including PPARα, PPARγ and PPARβ/δ have been identified recently. PPARα regulates fatty acid oxidation via fatty acid transport, esterification, biding and beta-oxidation (Finck, 2004; Huss and Kelly, 2004). Increase of PPARα and its coactivator PGC-1α has been discovered in rodent models of insulin-deficient and insulin-resistant diabetes (Belke et al., 2000). Recent researches also indicated that PPARα played a critical role in the development of ventricular hypertrophy and dysfunction in the diabetic heart. It was reported that the development of severe cardiomyopathy in transgenic mice with cardiac-specific overexpression of PPARα (myosin heavy chain-PPARα mice) was associated with marked myocardial lipid accumulation (Finck et al., 2003). Increase of PPARα is also referred as a leading cause to inhibition of glucose uptake and utilization in diabetic heart by compromising glucose transporter (GLUT) 4 and GLUT1 expression (Panagia et al., 2005), which was proved in type 1 and type 2 diabetic animal models (Camps et al., 1992; Carroll et al., 2005; Young et al., 2002). PPARγ mainly expresses in the adipose tissue and regulates expression of genes that participate in lipogenesis and fatty acids storage. Interestingly, the activation of PPARγ acts as an inhibitor of cardiac hypertrophy and improves left ventricular diastolic function in diabetic rats. Evidences showed that PPARγ agonist decreased blood and cardiac and cardiac lipids in inhibited triglyceride uptake and accumulation in the heart (Golfman et al., 2005; Vikramadithyan et al., 2005). The present evidence from our research showed an increase in gene expression of PPARα and decrease in gene and protein expression of PPARγ and GLUT4 in the heart of rat model of hyperglycemia and hypercholesterolemia, which were reversed by berberine treatment.

5. Conclusions

Phenotypes such as hyperglycemia, hypercholesterolemia, cardiac dysfunction and left ventricular hypertrophy, cardiac lipid accumulation, which mimics to the diabetic cardiomyopathy, were found in the rat model induced by HSFD/streptozotocin treatment. Berberine treatment could effectively recover the diastolic and systolic dysfunction, inhibit the cardiac left ventricular hypertrophy, lower plasma sugar and lipids levels in the hyperglycemic and hypercholesterolemic rats. It could also alleviate cardiac lipid accumulation, increase intracellular fatty acid transport proteins and fatty acid beta-oxidase, and impressively increase mRNA and protein expression of PPARγ and GLUT4 and repress PPARα gene expression, indicating a protective effect of berberine on diabetic cardiomyopathy.

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References

Airaksinen, J., Ikaheimo, M., Kaila, J., Linnaluoto, M., Takkunen, J., 1984. Impaired left ventricular filling in young female diabetics. An echocardiographic study. Acta Med. Scand. 216, 509–516.

Aslanidi, G., Kroutov, V., Philipsberg, G., Lamb, K., Campbell-Thompson, M., Walter, G.A., Kurenov, S., Ignacio Aguirre, J., Keller, P., Hankenson, K., Macdougald, O.A., Zolotukhin, S., 2007. Ectopic expression of Wnt10b decreases adiposity and improves glucose homeostasis in obese rats. Am. J. Physiol. Endocrinol. Metab. 293, E726–E736.

Bandyopadhyay, G.K., Yu, J.G., Ofrecio, J., Olefsky, J.M., 2006. Increased malonyl-CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; thiazolidinedione treatment reverses these defects. Diabetes 55, 2277–2285.

Belke, D.D., Larsen, T.S., Gibbs, E.M., Severson, D.L., 2000. Altered metabolism causes cardiac dysfunction in perfused hearts from diabetic (db/db) mice. Am. J. Physiol. Endocrinol. Metab. 279, E1104–E1113.

Bell, D.S., 2003. Diabetic cardiomyopathy. Diabetes Care 26, 2949–2951.

Boudina, S., Abel, E.D., 2007. Diabetic cardiomyopathy revisited. Circulation 115, 3213–3223.

Bugger, H., Abel, E.D., 2009. Rodent models of diabetic cardiomyopathy. Dis. Model. Mech. 2, 454–466.

Campbell, F.M., Kozak, R., Wagner, A., Altarejos, J.Y., Dyck, J.R., Belke, D.D., Severson, D.L., Kelly, D.P., Lopaschuk, G.D., 2002. A role for peroxisome proliferator-activated receptor alpha (PPARalpha) in the control of cardiac malonyl-CoA levels: reduced fatty acid oxidation rates and increased glucose oxidation rates in the hearts of mice lacking PPARalpha are associated with higher concentrations of malonyl-CoA and reduced expression of malonyl-CoA decarboxylase. J. Biol. Chem. 277, 4098–4103.

Camps, M., Castello, A., Munoz, P., Monfar, M., Testar, X., Palacin, M., Zorzano, A., 1992. Effect of diabetes and fasting on GLUT-4 (muscle/fat) glucose-transporter expression in insulin-sensitive tissues. Heterogeneous response in heart, red and white muscle. Biochem. J. 282 (Pt 3), 765–772.

Carley, A.N., Atkinson, L.L., Bonen, A., Harper, M.E., Kunnathu, S., Lopaschuk, G.D., Severson, D.L., 2007. Mechanisms responsible for enhanced fatty acid utilization by perfused hearts from type 2 diabetic db/db mice. Arch. Physiol. Biochem. 113, 65–75.

Carroll, R., Carley, A.N., Dyck, J.R., Severson, D.L., 2005. Metabolic effects of insulin on cardiomyocytes from control and diabetic db/db mouse hearts. Am. J. Physiol. Endocrinol. Metab. 288, E900–E906.

Christoffersen, C., Bollano, E., Lindegaard, M.L., Bartels, E.D., Goetze, J.P., Andersen, C.B., Nielsen, L.B., 2003. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. Endocrinology 144, 3483–3490.

Coort, S.L., Hasselbaink, D.M., Koonen, D.P., Willems, J., Coumans, W.A., Chabowski, A., van der Vusse, G.J., Bonen, A., Glatz, J.F., Luiken, J.J., 2004. Enhanced sarcolemmal FAT/CD36 content and triacylglycerol storage in cardiac myocytes from obese zucker rats. Diabetes 53, 1655–1663.

Cui, G., Qin, X., Zhang, Y., Gong, Z., Ge, B., Zang, Y.Q., 2009. Berberine differentially modulates the activities of ERK, p38 MAPK, and JNK to suppress Th17 and Th1 T cell differentiation in type 1 diabetic mice. J. Biol. Chem. 284, 28420–28429.

Di Paola, M., Lorusso, M., 2006. Interaction of free fatty acids with mitochondria: coupling, uncoupling and permeability transition. Biochim. Biophys. Acta 1757, 1330–1337.

Fang, Z.Y., Prins, J.B., Marwick, T.H., 2004. Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. Endocr. Rev. 25, 543–567.

Finck, B.N., 2004. The role of the peroxisome proliferator-activated receptor alpha pathway in pathological remodeling of the diabetic heart. Curr. Opin. Clin. Nutr. Metab. Care 7, 391–396.

Finck, B.N., Han, X., Courtois, M., Aimond, F., Nerbonne, J.M., Kovacs, A., Gross, R.W., Kelly, D.P., 2003. A critical role for PPARalpha-mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. Proc. Natl. Acad. Sci. USA 100, 1226–1231.

Glatz, J.F., Luiken, J.J., Bonen, A., 2010. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. Physiol. Rev. 90, 367–417.

Golfman, L.S., Wilson, C.R., Sharma, S., Burgmaier, M., Young, M.E., Guthrie, P.H., Van Arsdall, M., Adrogue, J.V., Brown, K.K., Taegtmeyer, H., 2005. Activation of PPARgamma enhances myocardial glucose oxidation and improves contractile function in isolated working hearts of ZDF rats. Am. J. Physiol. Endocrinol. Metab. 289, E328–E336.

Gu, Y., Zhang, Y., Shi, X., Li, X., Hong, J., Chen, J., Gu, W., Lu, X., Xu, G., Ning, G., 2010. Effect of traditional Chinese medicine berberine on type 2 diabetes based on comprehensive metabonomics. Talanta 81, 766–772.

Gulfraz, M., Mehmood, S., Ahmad, A., Fatima, N., Praveen, Z., Williamson, E.M., 2008. Comparison of the antidiabetic activity of Berberis lyceum root extract and berberine in alloxan-induced diabetic rats. Phytother. Res. 22, 1208–1212.

Hamby, R.I., Zoneraich, S., Sherman, L., 1974. Diabetic cardiomyopathy. JAMA 229, 1749–1754.

Han, X.X., Chabowski, A., Tandon, N.N., Calles-Escandon, J., Glatz, J.F., Luiken, J.J., Bonen, A., 2007. Metabolic challenges reveal impaired fatty acid metabolism and translocation of FAT/CD36 but not FABPpm in obese Zucker rat muscle. Am. J. Physiol. Endocrinol. Metab. 293, E566–E575.

Holland, W.L., Knotts, T.A., Chavez, J.A., Wang, L.P., Hoehn, K.L., Summers, S.A., 2007. Lipid mediators of insulin resistance. Nutr. Rev. 65, S39–S46.

Huss, J.M., Kelly, D.P., 2004. Nuclear receptor signaling and cardiac energetics. Circ. Res. 95, 568–578.

Jeong, H.W., Hsu, K.C., Lee, J.W., Ham, M., Huh, J.Y., Shin, H.J., Kim, W.S., Kim, J.B., 2009. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. Am. J. Physiol. Endocrinol. Metab. 296, E955–E964.

Lai, Y.M., Mohammed, K.A., Nasreen, N., Baumuratov, A., Bellew, B.F., Antony, V.B., 2007. Induction of cell cycle arrest and apoptosis by BCG infection in cultured human bronchial airway epithelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 293, L393–L401.

Lau, C.W., Yao, X.Q., Chen, Z.Y., Ko, W.H., Huang, Y., 2001. Cardiovascular actions of berberine. Cardiovasc. Drug Rev. 19, 234–244.

Laybutt, D.R., Thompson, A.L., Cooney, G.J., Kraegen, E.W., 1997. Selective chronic regulation of GLUT1 and GLUT4 content by insulin, glucose, and lipid in rat cardiac muscle in vivo. Am. J. Physiol. 273, H1309–H1316.

Ouwens, D.M., Diamant, M., Fodor, M., Habets, D.D., Pelsers, M.M., El Hasnaoui, M., Dang, Z.C., van den Brom, C.E., Vlasblom, R., Rietdijk, A., Boer, C., Coort, S.L., Glatz, J.F., Luiken, J.J., 2007. Cardiac contractile dysfunction in insulin-resistant rats fed a high-fat diet is

associated with elevated CD36-mediated fatty acid uptake and esterification. Diabetologia 50, 1938–1948.
Panagia, M., Gibbons, G.F., Radda, G.K., Clarke, K., 2005. PPAR-alpha activation required for decreased glucose uptake and increased susceptibility to injury during ischemia. Am. J. Physiol. Heart Circ. Physiol. 288, H2677–H2683.
Ruberg, F.L., 2007. Myocardial lipid accumulation in the diabetic heart. Circulation 116, 1110–1112.
Rubler, S., Dlugash, J., Yuceoglu, Y.Z., Kumral, T., Branwood, A.W., Grishman, A., 1972. New type of cardiomyopathy associated with diabetic glomerulosclerosis. Am. J. Cardiol. 30, 595–602.
Sharma, S., Adrogue, J.V., Golfman, L., Uray, I., Lemm, J., Youker, K., Noon, G.P., Frazier, O.H., Taegtmeyer, H., 2004. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. FASEB J. 18, 1692–1700.
Smith, A.C., Mullen, K.L., Junkin, K.A., Nickerson, J., Chabowski, A., Bonen, A., Dyck, D.J., 2007. Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet-induced hyperglycemia. Am. J. Physiol. Endocrinol. Metab. 293, E172–E181.
Turcotte, L.P., Swenberger, J.R., Zavitz Tucker, M., Yee, A.J., 2001. Increased fatty acid uptake and altered fatty acid metabolism in insulin-resistant muscle of obese Zucker rats. Diabetes 50, 1389–1396.
Vikramadithyan, R.K., Hirata, K., Yagyu, H., Hu, Y., Augustus, A., Homma, S., Goldberg, I.J., 2005. Peroxisome proliferator-activated receptor agonists modulate heart function in transgenic mice with lipotoxic cardiomyopathy. J. Pharmacol. Exp. Ther. 313, 586–593.
Wang, J., Song, Y., Wang, Q., Kralik, P.M., Epstein, P.N., 2006. Causes and characteristics of diabetic cardiomyopathy. Rev. Diabet. Stud. 3, 108–117.
Wang, C., Li, J., Lv, X., Zhang, M., Song, Y., Chen, L., Liu, Y., 2009. Ameliorative effect of berberine on endothelial dysfunction in diabetic rats induced by high-fat diet and streptozotocin. Eur. J. Pharmacol. 620, 131–137.
Wang, Y., Campbell, T., Perry, B., Beaurepaire, C., Qin, L., 2011. Hypoglycemic and insulin-sensitizing effects of berberine in high-fat diet- and streptozotocin-induced diabetic rats. Metabolism 2, 298–305.
Young, M.E., Guthrie, P.H., Razeghi, P., Leighton, B., Abbasi, S., Patil, S., Youker, K.A., Taegtmeyer, H., 2002. Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese Zucker rat heart. Diabetes 51, 2587–2595.
Zhang, H., Wei, J., Xue, R., Wu, J.D., Zhao, W., Wang, Z.Z., Wang, S.K., Zhou, Z.X., Song, D.Q., Wang, Y.M., Pan, H.N., Kong, W.J., Jiang, J.D., 2010. Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. Metabolism 59, 285–292.
Zhao, H.P., Hong, Y., Xie, J.D., Xie, X.R., Wang, J., Fan, J.B., 2007. Effect of berberine on left ventricular remodeling in renovascular hypertensive rats. Yao Xue Xue Bao 42, 336–341.
Zhou, J., Zhou, S., Tang, J., Zhang, K., Guang, L., Huang, Y., Xu, Y., Ying, Y., Zhang, L., Li, D., 2009. Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. Eur. J. Pharmacol. 606, 262–268.