Cissus quadrangularis extract attenuates hyperglycaemia-mediated oxidative stress in streptozotocin-induced diabetic rats

R.K. Lekshmi, B.T. Divya, S. Mini

Department of Biochemistry, University of Kerala, Kariavattom, Trivandrum, Kerala, India

Objective: Hyperglycaemia-mediated oxidative stress plays a major role in the progression of diabetic complications. This study was aimed at evaluating the beneficial effects of *Cissus quadrangularis* stem extract on antioxidant/oxidant status in diabetes mellitus.

Materials and methods: The antioxidant activities of an ethyl acetate fraction of *Cissus quadrangularis* stem (CQSF) at three different doses (50, 100, and 200 mg/kg body weight) were evaluated in rats with experimentally induced diabetes. High performance liquid chromatography analysis was carried out to identify the active components present in the plant fraction.

Results: Induction of diabetes caused deleterious effects including hyperglycaemia, liver dysfunction, significant decline in antioxidants and elevated lipid peroxidation indices. *C. quadrangularis* supplementation significantly improved insulin sensitivity, reduced liver damage, and oxidative changes, and brought back the antioxidants towards normal. Histopathological analysis of the liver also reinforced our findings. Pronounced changes were observed at the doses 100 and 200 mg/kg body weight. In addition, high performance liquid chromatography analysis of *C. quadrangularis* fraction revealed the presence of quercetin.

Discussion: This study suggests an anti-diabetic potential of CQSF, mediated through the modulation of the antioxidant defence system. The ethyl acetate fraction of *Cissus quadrangularis* is rich in quercetin and this indicates that the supplementation of CQSF might be beneficial as a food supplement for the attenuation of diabetic complications.

Keywords: *Cissus quadrangularis*, Diabetes mellitus, Oxidative stress, Antioxidants, Lipid peroxidation, Reactive oxygen species

Introduction

The incidence of diabetes mellitus (DM) is increasing alarmingly throughout the world and is ranked as the fourth leading cause of death by disease globally.¹ Oxidative stress, through the production of reactive oxygen species (ROS), has been reported as the root cause for the development of β -cell dysfunction and impaired glucose tolerance in DM.² The depletion of antioxidants occurs in diabetic patients and leads to diabetes-related complications and onset of other disease conditions like atherosclerosis and coronary heart disease.³ Hyperglycaemia and the characteristic dyslipidemia of DM along with increased oxidative stress have been implicated in the pathogenesis of atherothrombotic macrovascular disease.⁴ Apart from the currently available therapeutic options for diabetes, which have limitations of their own, several medicinal plants have been suggested as a potential source of hypoglycaemic drugs.⁵ So the search for

phytocomponents that normalize hyperglycaemia and ameliorate oxidative stress is an important objective in preventing or minimizing diabetes-associated complications.

Cissus quadrangularis Linn. (CQ) belongs to the family Vitaceae and is a widely cultivated plant in India, Sri Lanka, Malaysia, Thailand, and Africa. In Ayurveda different parts of the plant have been used orally or topically as a medicine for skin infections, constipation, piles, anaemia, asthma, irregular menstruation, burns, and wounds.⁶ The subchronic toxicity study revealed that CQ has no toxic effects at therapeutic doses and can be considered as safe for long-term treatment.⁷ Studies have reported that methanolic extracts from CQ have antimicrobial, anti-ulcer and antioxidant properties.^{8–10} A number of animal studies have claimed the utility of CQ extracts in a wide range of disease conditions like gastric ulcer, bone fracture, and hepatic toxicity.^{11,12} In addition, CQ extract possesses hypoglycaemic and free radical scavenging activities.^{13,14} CQ stem extract effectively reduces the body weight by inhibiting the oxidation

Correspondence to: S. Mini, Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram - 695 581, Kerala, India.
Email: minisaraswathy@gmail.com

of LDL cholesterol and by lowering the blood glucose in obese patients.¹⁵ Previous studies in our laboratory revealed that the ethyl acetate fraction from the methanol extract of *C. quadrangularis* stem possess *in vitro* antioxidant and anti-glycation activities.¹⁶ However, there is a lack of data on the protective effect of CQ on the pathogenesis of DM and its related complications. Therefore, this study endeavours a comprehensive analysis of hypoglycaemic and antioxidant effects of ethyl acetate fraction of *Cissus quadrangularis* stem (CQSF) in streptozotocin-induced diabetes in rats.

Materials and methods

Chemicals

Biochemicals were purchased from Sigma Aldrich (St. Louis, MO, USA), Merck chemical company (Darmstadt, Germany), and Sisco Research Laboratories (Mumbai, India).

Extraction of *Cissus quadrangularis* stem

Stems of *C. quadrangularis* were collected from Thiruvananthapuram, India, during March–April 2012. Authentication was done by Dr Valsala Devi, Department of Botany, University of Kerala, India, and a voucher specimen (Voucher No. KUBH 5805) has been deposited at the herbarium for further reference. *C. quadrangularis* stems (1 kg) were shade dried, powdered, and extracted with methanol (2 l) using Soxhlet extractor. The crude extract was concentrated in vacuum at 40°C in a rotary evaporator (Heidolph, Germany) to give dried methanolic extract (yield: 9.6%). The crude methanolic extract was suspended in water and partitioned with an equal volume of petroleum ether. Then the remaining aqueous phase was again partitioned with ethyl acetate (1:1 v/v) to obtain an ethyl acetate fraction (CQSF-19%) which was dried and supplemented as a drug in the experimental study.

Experimental animals

One to two month old male Sprague Dawley rats (150–200 g body weight (bw), 30 animals in total) bred in our department animal house was used for the study. Animals were housed in polypropylene cages and maintained under standard conditions (12-hour light and 12-hour dark cycle, $25 \pm 10^\circ\text{C}$). All the animal care was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and the experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC-KU-20/2011-12-BC-SM (12)).

Induction of experimental diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (STZ)

at a dose of 40 mg/kg bw in 0.1 M citrate buffer (pH 4.5).¹⁷ The animals were given 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. After 48 hours of STZ induction, blood glucose levels were estimated and rats with a blood glucose ranging between 200 and 400 mg/dl were considered diabetic and used for the experiments.

Experimental design

Animals were randomly divided into five groups of six rats each. The treatment was started on the third day after STZ injection and daily intragastric administration of CQSF was continued for 45 days.

Group I – Normal control rats

Group II – Diabetic control rats

Group III – Diabetic rats received 50 mg/kg bw CQSF

Group IV – Diabetic rats received 100 mg/kg bw CQSF

Group V – Diabetic rats received 200 mg/kg bw CQSF

After the experimental period (45 days), animals were sacrificed; blood and liver tissues were collected for various experimental analyses.

Biochemical parameters

The blood glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured by commercially available assay kits (Agappe Diagnostics, India). Insulin was estimated by the ELISA (rat insulin ELISA kit) method.¹⁸ The activities of antioxidant enzymes were assayed; superoxide dismutase (SOD) by the method of Kakkar *et al.*,¹⁹ catalase (CAT) by the method of Maehly and Chance²⁰ glutathione peroxidase (GPx) by the method of Rotruck *et al.*,²¹ and glutathione reductase (GRd) by the method of David and Richard²² Reduced glutathione (GSH) content in the liver was measured by the method of Patterson and Lazarow²³ Thiobarbituric acid reactive substances (TBARS), hydroperoxides (HP), and conjugated dienes (CD) were assayed by the method of Hiroshi Okhawa *et al.*²⁴

Histopathological analysis of liver

The whole liver from each animal was collected in 10% formalin solution and immediately processed by the paraffin technique. Thin sections (5 μm) were cut and stained with hematoxylin-eosin. The tissue samples were then examined and photographed under a light microscope.

High performance liquid chromatography analysis of CQSF

High performance liquid chromatography (HPLC) analysis was done by the HPLC Waters 2695 system (Canada) and the separation of CQSF was done by isocratic gradient elution using a C18 column (4.6 mm i.d. $\times$ 250 mm, 5 micron particle size). The

mobile phase was methanol (Eluent A), acetonitrile (Eluent B) in 1:1 ratio, the total flow rate was 1.0 ml/minute and the time of analysis was 15 minutes. The detector's wavelength was set at 275 nm. The injection volume was 20 μ l and the temperature of the column was thermostated at 40°C. The chromatographic peaks of the analytes were confirmed and quantified by comparing their retention time and area with corresponding reference standards.

Statistical analysis

The values were expressed as mean $\pm$ SD. The statistical analysis was carried out by one-way analysis of variance using SPSS (version 17) statistical analysis program. Duncan's *post hoc* multiple comparison tests were used to determine significant differences among groups: $P < 0.05$ was considered to be significant.

Results

Blood glucose levels

STZ-induced diabetic rats showed significant increases in the fasting blood glucose levels when compared to normal rats. A significant hypoglycaemic effect was observed with all the three doses of CQSF studied (Fig. 1). However, CQSF at doses of 100 and 200 mg/kg bw showed maximal effects on blood glucoses levels ($P < 0.05$).

Plasma insulin levels

Plasma insulin level decreased significantly in the diabetic group when compared with other groups and was significantly increased ($P < 0.05$) as a result of treatment with CQSF (Fig. 2).

Toxicity markers

Increased serum activities of liver toxicity markers (AST and ALT) were found in STZ-induced diabetic rats, indicating damage to liver cells. In comparison with the DM group, the activities of ALT and AST were significantly ($P < 0.05$) decreased in diabetic rats treated with CQSF (Fig. 3).

Figure 1 Fasting blood glucose levels. Values are expressed as mean $\pm$ SD of six rats in each group. ^aStatistically significant as compared to normal group. ^{b,c}Statistically significant as compared to diabetic group.

Figure 2 Plasma insulin levels. Values are expressed as mean $\pm$ SD of six rats in each group. ^aStatistically significant as compared to normal group. ^{b,c}Statistically significant as compared to diabetic group.

Effect of CQSF on the activities of antioxidant enzymes in liver

The activities of antioxidant enzymes SOD, CAT, GPx, and GRd were significantly ($P < 0.05$) decreased in STZ-induced diabetic rats compared with controls. But the administration of CQSF significantly ($P < 0.05$) increased the activities of antioxidant enzymes in the DM group (Table 1).

Levels of lipid peroxidation products and GSH content in liver

The lipid peroxidation products TBARS, CD, and HP were significantly increased in STZ diabetic rats compared to normal rats. However, the treatment of STZ diabetic rats with CQSF resulted in marked decrease ($P < 0.05$) in the levels of liver lipid peroxidation products. The concentration of major intracellular antioxidant GSH was significantly ($P < 0.05$) decreased in the livers of the STZ-induced DM group compared to the control group. A significant increase in the GSH content was observed in the liver of the CQSF-treated groups (Table 2).

Histopathological analysis of liver

The histopathological analysis of the liver in control group showed normal cell morphology with hexagonal

Figure 3 Activities of toxicity markers in liver. Values are expressed as mean $\pm$ SD of six rats in each group. ^aStatistically significant as compared to normal group. ^bStatistically significant as compared to diabetic group.

Table 1 Activities of antioxidant enzymes in liver

Groups	SOD (units/mg protein)	CAT ($\times 10^{-3}$ units/mg protein)	GPx (units/mg protein)	GRd (units/mg protein)
I	2.00 $\pm$ 0.19	8.02 $\pm$ 0.73	30.03 $\pm$ 2.73	135.34 $\pm$ 12.98
II	0.45 $\pm$ 0.04*	2.01 $\pm$ 0.19*	9.89 $\pm$ 0.95*	99.44 $\pm$ 9.54*
III	0.73 $\pm$ 0.07 [†]	3.26 $\pm$ 0.30 [†]	14.45 $\pm$ 1.31 [†]	114.81 $\pm$ 9.48 [†]
IV	1.39 $\pm$ 0.13 [†]	4.93 $\pm$ 0.45 [†]	20.27 $\pm$ 1.85 [†]	125.63 $\pm$ 11.88 [†]
V	1.35 $\pm$ 0.13 [†]	3.87 $\pm$ 0.36 [†]	20.31 $\pm$ 1.85 [†]	123.68 $\pm$ 11.86 [†]

Values are expressed as mean $\pm$ SD of six rats in each group; significance accepted at $P < 0.05$.

*Statistically significant as compared to normal group.

[†]Statistically significant as compared to diabetic group.

Table 2 Lipid peroxidation products and GSH content in liver

Groups	TBARS (mM/100 g tissue)	HP (mM/100 g tissue)	CD (mM/100 g tissue)	GSH content (mM/100 g tissue)
I	0.91 $\pm$ 0.07	7.69 $\pm$ 0.73	3.35 $\pm$ 0.30	134.41 $\pm$ 11.22
II	3.28 $\pm$ 0.30*	28.04 $\pm$ 2.69*	14.6 $\pm$ 1.40*	81.31 $\pm$ 9.17*
III	1.64 $\pm$ 0.15 [†]	19.43 $\pm$ 1.87 [†]	9.97 $\pm$ 0.95 [†]	104.77 $\pm$ 10.10 [†]
IV	1.25 $\pm$ 0.11 [†]	12.82 $\pm$ 1.23 [†]	8.13 $\pm$ 0.92 [†]	118.86 $\pm$ 10.30 [†]
V	1.38 $\pm$ 0.13 [†]	14.12 $\pm$ 1.35 [†]	8.08 $\pm$ 0.77 [†]	118.20 $\pm$ 10.30 [†]

Values are expressed as mean $\pm$ SD of six rats in each group; significance accepted at $P < 0.05$.

*Statistically significant as compared to normal group.

[†]Statistically significant as compared to diabetic group.

lobular architecture (Fig. 4I). While the STZ-induced DM group (Fig. 4II) exhibited hepatocellular damage in the form of inflammation, sinusoidal dilation, fatty changes, and extensive vacuolization with disappearance of nuclei, the diabetic rats treated with CQSF had less inflammation and the cellular morphology was respoed almost to normal levels (Fig. 4III, IV, and V).

HPLC profiling of CQSF

HPLC profiling of CQSF (Fig. 5a) showed that it contains quercetin (95% w/w) as a major compound when

compared to a corresponding reference standard (Fig. 5b). Retention time for quercetin was 2.28 minutes. The activity of the CQSF may be attributed to the presence of the phenolic compound, quercetin.

Discussion

DM is a clinical syndrome characterized by decreased insulin secretion or insulin resistance. This work is a preliminary study to evaluate the effect of an ethyl acetate fraction of *C. quadrangularis* stem on hyperglycaemia-mediated oxidative stress in rats with STZ-induced diabetes. STZ has been widely used for

Figure 4 Histology of liver in experimental rats after 45 days of treatment. (I) Normal control – normal liver showing normal hepatic cells. (II) Diabetic control – showed extensive hepatocellular damage in the form of mild inflammation, sinusoidal dilation, fatty changes, and extensive vacuolization with disappearance of nuclei. (III), (IV), and (V) Diabetic + CQSF – shows only mild inflammation and there is restoration of normal tissue morphology.

Figure 5 (A) High performance liquid chromatography (HPLC) chromatogram of CQSF at 275 nm using the solvents methanol and acetonitrile 1:1 ratio at a flow rate of 1.00 ml/minute. Quercetin standard chromatogram was given in (B).

inducing diabetes in experimental animals as it is well known for its selective pancreatic β -cell cytotoxicity.²⁵ The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage that culminates in β -cell destruction through the induction of apoptosis and suppression of insulin biosynthesis.^{26,27} The experimentally induced diabetic rats showed severe hyperglycaemia interrelated with a decrease in endogenous insulin secretion and release. Rats treated by the administration of CQSF showed a significant decrease in the level of fasting blood glucose and an increase in the level of plasma insulin. There was no statistically significant difference in the fasting blood glucose and plasma insulin levels between the groups treated with the extract at doses of 100 and 200 mg/kg bw, suggesting that 100 mg/kg is the optimum dose for acquiring good glycaemic control. Here the glucose lowering activity of CQSF may be due to the effect of the polyphenolic compound quercetin in the extract on pancreatic secretion of insulin from regenerated β -cells, which are destroyed by STZ.²⁸ This was supported by the controlled plasma insulin levels, which were decreased in diabetic rats when compared to normal rats.

Diabetes is often associated with the elevated activities of liver marker enzymes like AST and ALT in

serum, which might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream.²⁹ The hepatocellular damage was notably prevented by treatment with even the lowest dose of CQSF and cell integrity was preserved, possibly leading to survival of the functionally active cells. Similar data were obtained from the histopathological analysis of liver where STZ-induced diabetic rats showed hepatocellular damage in the form of inflammation and fatty changes. These changes were ameliorated by treatment with CQSF, as it significantly improved the histological architecture of liver. These results are in agreement with the report of Jainu and Devi¹⁴ who observed the therapeutic action of a methanolic extract of CQ against hepatic damage induced by carbon tetrachloride.

Hyperglycaemia causes the induction of ROS in diabetes, which can lead to increased lipid peroxidation and alterations in antioxidant defence and thereby further impair glucose metabolism in biological systems.³⁰ The liver plays a major role in the regulation of glucose metabolism and various studies have observed the induction of severe oxidative stress in the liver of STZ-induced diabetic rats.^{31,32} The activities of the principal enzymatic and non-enzymatic antioxidant systems are decreased during oxidative

stress.^{33,34} In agreement with the above reports, our results showed that the activities of antioxidant enzymes SOD, CAT, GPx, and GRd and non-enzymatic antioxidant GSH content were significantly decreased in the DM group. Treatment with CQSF reversed the decline in these enzymatic and non enzymatic antioxidants, which might be due to decreased oxidative stress as evidenced by decreased lipid peroxidation products like TBARS, CD, and HP. Similar results were obtained by Chidambaram and Venkatraman with methanolic extract of CQ in rats fed a high-fat, high-fructose diet.³⁵ In our study, the greatest effects were observed at the doses 100 and 200 mg/kg bw and there was no statistically significant difference in most of the antioxidant and lipid peroxidation parameters between the groups treated with the extract at these doses.

The maintenance of prooxidant/antioxidant balance may be due to the presence of quercetin in CQSF, as phenolic compounds are reported to be effective in enhancing antioxidant status and reducing lipid peroxidation. The HPLC analysis of CQSF showed the presence of quercetin (95%) as a major compound. Previous studies have shown that quercetin had strong antioxidant activity due to its ability to scavenge free radicals and to inhibit lipid peroxidation.^{36,37} Quercetin ameliorates hyperglycaemia and dyslipidemia and prevents β -cell damage in type 2 diabetic animals.^{38,39} Natural foods that were rich in quercetin have been reported to improve diabetic status.⁴⁰

Conclusion

Our results showed that supplementation with CQSF enhanced the activity of the antioxidant defense system and thereby conferred protection against DM in rats. Pronounced activities were observed at the doses 100 and 200 mg/kg bw. Thus, CQSF may be effective in preventing diabetic complications such as hyperglycaemia and oxidative damage. This may be mediated by the modulatory action of quercetin in CQSF. Further investigations are necessary to trace out the exact mechanistic pathways.

Disclaimer statements

Contributors

R.K. Lekshmi, B.T. Divya and S. Mini

Funding

The authors are thankful to the University Grants Commission, New Delhi, India, for the financial assistance to carry out the work efficiently.

Conflicts of interest

None.

Ethics approval

The protocol of this study was approved by Institutional Animal Ethics Committee [IAEC-KU-20/2011-12-BC-SM (12)].

References

- Kowluru RA, Chan PS. Oxidative stress and diabetic retinopathy. *Exp Diabetes Res* 2007. Available from: <http://dx.doi.org/10.1155/2007/43603>.
- Wright E, Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. *Int J Clin Pract* 2006;60:308–14.
- Polidori MC, Stahl W, Eichler O, Niestroj I, Sies H. Profiles of antioxidants in human plasma. *Free Radic Biol Med* 2001;30:456–62.
- Nathan DM. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005;353:2643–53.
- Mukherjee P, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. *J Ethnopharmacol* 2006;106:1–28.
- Kirtikar KR, Basu BD. Indian medicinal plants. Allahabad, India: Lalit Mohan Basu Publisher; 2000.
- Attawish A, Chavalitumrong D, Chivapat S, Chuthaputti S, Rattarajarasroj S, Punyamong S. Subchronic toxicity of *Cissus quadrangularis* Linn. *J Sci Tech* 2002;24:39–51.
- Subbu VSV. Mechanism of action of *Vitis* glucoside on myocardial tissue. *Indian J Med Sci* 1970;25:400–3.
- Anoop A, Jagdeesan M. Gastric and duodenal antiulcer and cytoprotective effect of *Cissus quadrangularis* Linn. variant II in rats. *Nigerian J Nat Prod Med* 2002;6:1–7.
- Chidambaram Murthy KN, Vanitha A, Mahadeva Swamy M, Ravishanker GA. Antioxidant and antimicrobial activity of *Cissus quadrangularis* Linn. *J Med Foods* 2003;6:99–105.
- Prasad GC, Udupa KN. Effect of *Cissus quadrangularis* on the healing of cortisone treated fractures. *Indian J Med Res* 1963;51:667–76.
- Chopra SS, Patel MR, Gupta LP, Datta IC. Studies on *Cissus quadrangularis* in experimental fracture repair: effect on chemical parameters in blood. *Indian J Med Res* 1975;63:824–8.
- Chaudhari RL, Patil PS, Chaudhari RY, Bhangale JO. Antihyperglycaemic activity of ethanolic extract of *Cissus quadrangularis* (L.) leaves in alloxan induced diabetic rats. *J Appl Pharm Sci* 2013;3:73–7.
- Jainu M, Shyamala Devi CS. *In vitro* and *in vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*. *Pharm Biol* 2005;43:773–9.
- Oben J, Kuate D, Agbor G, Momo C, Talla X. The use of a *Cissus quadrangularis* formulation in the management of weight loss and metabolic syndrome. *Lipids Health Disease* 2006;5:24.
- Lekshmi RK, Mini S. Evaluation of antioxidant and antiglycation activities of various solvent fractions of *Cissus quadrangularis* stem. *Int J Pharm Biosci* 2013;4:1259–68.
- Ramesh B, Pugalendi KV. Antihyperglycemic effect of umbelliferone in streptozotocin-diabetic rats. *J Med Food* 2006;9:562–6.
- Grassi J, Pradelles Ph. Compounds labelled by the acetylcholinesterase of *Electrophorus Electricus*. Its preparation process and its use as a tracer or marker in enzyme-immunological determinations. United States patent, No. 1,047,330; 1991.
- Kakkar P, Dos B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 1984;21:130–2.
- Maehly AC, Chance B. The assay of catalases and peroxidases. In: Glick D(ed.) *Methods of biochemical analysis*. Vol. 1. New York: Interscience; 1954. p. 357–424.
- Rotruck JT, Pope AL, Ganther HE. Selenium: biochemical roles as a component of glutathione peroxidase. *Science* 1973;179:588–90.
- David M, Richard JS. Glutathione reductase. In: Bermeyer HU(ed.) *Methods of enzymatic analysis*. New York, USA: Academic Press; 1983. p. 258–65.
- Patterson JW, Lazarow A. Determination of glutathione. In: Glick D, (ed.). *Methods of biochemical analysis*. New York: Interscience; 1955. p. 259.
- Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric reaction. *Anal Biochem* 1979;95:351–8.

- 25 Kumar GPS, Arulselvan P, Kumar DS, Subramanian SP. Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. *J Health Sci* 2006;52:283–91.
- 26 Szkudelski T. The mechanism of alloxan and streptozotocin action of β -cells of the rat pancreas. *Physiol Res* 2001;50:537–46.
- 27 Zhang W, Zhao J, Wang J, Pang X, Zhuang X, Zhu X. Hypoglycemic effect of aqueous extract of sea buckthorn (*Hippophae rhamnoides* L.) seed residues in streptozotocin induced diabetic rats. *Phytotherapy Res* 2010;24:228–32.
- 28 Eidi A, Eidi M, Esmaili E. Antidiabetic effect of garlic (*Allium sativum*) in normal and streptozotocin-induced diabetic rats. *Phytomed* 2006;13:624–9.
- 29 Eliza J, Daisy P, Ignacimuthu S, Duraipandiyan V. Antidiabetic and antilipidemic effect of eremanthin from *Costus speciosus* (Koen.) Sm., in STZ-induced diabetic rats. *Chem Biol Interact* 2009;182:67–72.
- 30 Balasubashini MS, Rukkumani R, Viswanathan P. Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytotherapy Res* 2004;18:310–4.
- 31 Ohaeri OC. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. *Biosci Rep* 2001;21:19–24.
- 32 Cotrozzi G, Raggi VC, Relli P. Role of the liver in the regulation of glucose metabolism in diabetes and chronic liver disease. *Annali Italiani di Medicina Interna* 1997;12:84–91.
- 33 Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003;17:24–38.
- 34 Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother* 2005;59:365–73.
- 35 Jaya C, Anuradha CV. *Cissus quadrangularis* stem alleviates insulin resistance, oxidative injury and fatty liver disease in rats fed high fat plus fructose diet. *Food Chem Toxicol* 2010;48:2021–9.
- 36 Sakanashi Y. Possible use of quercetin, an antioxidant, for protection of cells suffering from overload of intracellular Ca^{2+} : A model experiment. *Life Sci* 2008;83:164–9.
- 37 Hollman PC, Katan MB. Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed Pharmacother* 1997;51:305–10.
- 38 Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β -cell damage in rat pancreas. *Pharmacol Res* 2005;51:117–23.
- 39 Jeong SM, Kang MJ, Choi HN, Kim JH, Kim JI. Quercetin ameliorates hyperglycaemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. *Nutr Res Pract* 2012;6:201–7.
- 40 Gregory S, Kelly ND. Quercetin. *Altern Med* 2011;16:172–94.