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Therapeutic Potential of Some Plant Extracts Used in Turkish Traditional Medicine on Streptozocin-Induced Type 1 Diabetes Mellitus in Rats

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Abstract Diabetes mellitus (DM) is known to impair many physiological functions. Some reports claim that medicinal plants can reduce these alterations caused by DM. The aim of this study was to investigate the therapeutic potential of aqueous-methanol extracts of *Urtica dioica*, *Thymus vulgaris* (TV), *Myrtus communis* (MC), *Scolymus hispanicus* (SH) and *Cinnamomun zeylanicum* (CZ) on streptozotocin (STZ)-induced type 1 DM in rats. Diabetes was induced via a single i.p. injection of STZ (65 mg/kg body weight). After 1 week to allow for development of diabetes, each plant extract was administered to diabetic rats separately at a dose of 100 mg/kg body weight daily for 28 days. The results showed that only SH extract significantly ($P < 0.05$) amended fasting blood glucose level. The lipid profile was ameliorated especially by supplementations of TV, MC and CZ extracts. Almost all plant extract treatments markedly ($P < 0.05$) increased reduced glutathione content and decreased lipid peroxidation levels of erythrocyte, plasma, retina and lens tissues. They also significantly ($P < 0.05$) amended erythrocyte catalase activity, levels of marker serum enzymes (except amylase), urea and blood urea nitrogen when compared to diabetic rats treated with nothing. Furthermore, none of the plant extracts counteracted body weight loss of diabetic rats. Our data revealed that the aforementioned plant extracts have remarkable potential to counteract DM-caused alterations, probably through their antioxidant and free radical-defusing effects.

Keywords Diabetes mellitus · Plant extract · Retina · Lens · Antioxidant · Free radical

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Introduction

Diabetes mellitus (DM) is a worldwide endocrine disorder that impairs many physiological functions of the body. It is primarily characterized by high blood glucose levels (hyperglycemia) induced by insulin insufficiency (Singh et al. 2008). Hyperlipidemia, body weight loss, vascular devastation, nephropathy and retinopathy may also accompany DM (Sepici et al. 2004; Luo et al. 2004; Salahuddin and Jalalpure 2010; Stephens et al. 2009; Coleman 2001). In addition, overproduction of reactive oxygen species (ROS) has been reported in DM (Kataya and Hamza 2008). The main mechanisms that lead to their overproduction in this disorder are nonenzymatic and auto-oxidative glycosylation, metabolic stress which results from changes in energy metabolism, activity of the sorbitol path, localized tissue injury that results from hypoxia and ischemia-reperfusion damage (Akkuş 1995). Escalated ROS levels lead to repression of insulin synthesis (Hartnett et al. 2000) through enhancing apoptosis of pancreatic beta-cells in which insulin secretion is realized (Vijayakumar et al. 2006). Reduction in the biosynthesis of insulin also induces lipolysis in adipose tissue and generates fatty liver (Reaven 1998).

ROS are suppressed or scavenged by cellular antioxidant defense systems consisting of enzymatic (glutathione-*S*-transferase [GST], glutathione peroxidase, glutathione reductase [GR], catalase [CAT] and superoxide dismutase) and nonenzymatic (reduced glutathione [GSH], vitamins A,

C, E) mechanisms. Today, one of the materials being used as an antioxidant source to eliminate excessive ROS are plant extracts (Ozkol et al. 2011, 2012a; Tuluce et al. 2011, 2012).

In many countries, various plants are used traditionally as an alternative strategy for the treatment of diabetes (Bisset 1994). Among these plants, *Urtica diocia* (UD), *Thymus vulgaris* (TV), *Myrtus communis* (MC), *Scolymus hispanicus* (SH) and *Cinnamomun zeylanicum* (CZ) have been traditionally used in Turkey. Although previous studies have reported antiglycemic, antioxidant, antimicrobial, antiulcer and analgesic activities of UD (Ozkol et al. 2012b; Gülçin et al. 2004; Bnouham et al. 2003; Celik and Tuluce 2007); antioxidant, antimicrobial and detoxifying properties of TV (Baranauskiene et al. 2003; Sasaki et al. 2005); anti-inflammatory and hypoglycemic effects of MC (Al-Hindawi et al. 1989; Sepici et al. 2004); antispasmodic and spasmogenic effects of SH (Kırımer et al. 1997); and prohealing and nephroprotective effects of CZ (Kamath et al. 2003; Mishra et al. 2010), the current study is the first comprehensive trial to investigate the therapeutic potential of the foregoing plants on fasting blood glucose (FBG) level, lipid profile, oxidative stress, marker serum enzymes, renal function markers and body weight loss in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Plant Materials and Extractions

Plant samples were obtained from the herbal medicine market in Sanliurfa, Turkey. They were authenticated by Dr. Esat Çetin (Department of Botany, Harran University, Turkey) and stored in air-proof glass boxes in the shade until extraction. The aerial parts of each plant (100 g) were dried in an oven at 50 °C and powdered in an electrical mill. They were extracted with 350 ml methyl alcohol at 25 °C for 5 days after maceration in 225 ml of distilled water. Then, the blends were filtered and stored in a deep freeze for 24 h. Plant extracts were concentrated under reduced pressure and lyophilized (Cryodos; Telstar, Terrassa, Spain) to obtain the dry residues. Finally, extracts were suspended in 0.5 ml of 10 % of gum arabica before administration and given to rats per os (p.o.). Doses of UD, TV, MC, SH and CZ (100 mg/kg body weight) used in this study were adjusted according to earlier studies (Gülçin et al. 2004; Sasaki et al. 2005; Elfellah et al. 1984; Kırımer et al. 1997; Shah et al. 1998).

Chemicals

STZ, 2,4-dinitrophenyhydrazine, nicotinamide adenine dinucleotide phosphate reduced form (NADPH), thiobarbituric acid, 5,5′-dithiobis-(2-nitrobenzoic acid), glutathione oxidized (GSSG) and GSH were purchased from Sigma (St. Louis, MO). All other reagents and chemicals used in this study were of analytical grade.

Experimental Animals

Experiments were performed on 42 Sprague–Dawley male rats (225–250 g) of approximately the same age. Animals were housed in polypropylene cages (six per cage) and kept under standard conditions (12/12 light/dark cycle at 21 ± 2 °C and 50–70 % humidity). They were acclimatized to laboratory conditions for 21 days before treatments. Standard pellet feed and tap water were provided ad libitum. All animals received humane care in compliance with the European Community guidelines on the care and use of laboratory animals.

Induction of Type 1 DM

Type 1 DM was induced by a single i.p. injection of a freshly prepared solution of STZ (65 mg/kg body weight) in 0.1 M citrate buffer (pH 4.3) after a fasting period of 18 h (El-Kashef et al. 1996) on day 0. On the seventh day of STZ injection, diabetes in surviving rats was confirmed by measuring the glucose level of blood obtained from the tail vein. Rats with a plasma glucose level of 200 mg/dl or greater were accepted as diabetic and included in this study (Zhang and Tan 2000).

Experimental Design

Animals were randomly assigned to seven equal groups ($n = 6$ each): control, STZ (alone), STZ + UD, STZ + TV, STZ + MC, STZ + SH and STZ + CZ. All rats except controls were injected with 65 mg/kg STZ in citrate buffer on day 0. Control rats were only exposed to the same amount of citrate buffer. After 1 week to allow for diabetes development, each plant extract was given p.o. daily to diabetic rats in the STZ + UD, STZ + TV, STZ + MC, STZ + SH and STZ + CZ groups at a dose of 100 mg/kg body weight for 28 days. Blood samples were drawn from the tail veins of rats on days of 7, 14, 21, 28 and 35 for monitoring FBG. The mean body weight of each group was also recorded on days 7 (beginning of diabetes) and 35 (last day of the experiment) of the study.

Preparation of Serum Samples, Erythrocyte Lysates and Tissue Homogenates

All rats were anesthetized with ketamine (100 mg/kg i.p.) (Enaida et al. 2002) at the end of the experiment. Blood samples were taken under light anesthesia by injectors from the heart of animals and put separately both in

EDTA-containing tubes and in EDTA-free tubes. Afterward, rats were killed by inhalation of CO_2. While serum samples were obtained via centrifuging blood samples in EDTA-free tubes at 3,000 rpm for 15 min at 4 °C, plasma and erythrocytes were obtained by centrifuging blood samples in EDTA-containing tubes. Erythrocyte lysates were prepared by washing erythrocytes with saline three times. Lens and retina were carefully removed using a posterior approach and rinsed with 6.3 mM EDTA phosphate buffer after enucleation of the eyes. All tissue samples were kept at −80 °C until analysis. Tissues were homogenized in ice-cold (1–4 °C) phosphate buffer solution for 5 min using both mechanic (Heidolf, Schwabach, Germany; Silent Crusher M) and ultrasonic (Bandelin, Berlin, Germany; UW 2070) homogenizers and then centrifuged at 7,000×*g* force for 15 min. The pellet was discarded and the clear supernatant used for the analysis of GSH and malondialdehyde (MDA). All processes were carried out at 4 °C.

Biochemical Analysis

Total cholesterol, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were measured to reflect the lipid profile. Activities of some marker serum enzymes including amylase (AMY), creatine kinase (CK), γ-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured to evaluate the status of some organs such as the liver, heart and pancreas. Creatinine (CRE), urea and blood urea nitrogen (BUN) were assessed to determine renal functions. Among oxidative stress biomarkers, while the lipid peroxidation product MDA was monitored to assess oxidant status, GSH content and GR, GST and CAT activities were determined to reflect antioxidant status in erythrocytes, in retina and lens tissues as well as in plasma.

GR activity was assayed at 37 °C and 340 nm by following the oxidation of NADPH by GSSG (Beutler 1984). GST activity was assayed at 25 °C spectrophotometrically by following the conjugation of GSH with 1-chloro-2,4-dinitrobenzene at 340 nm as described by Mannervik and Guthenberg (1981). CAT activity was defined as the amount of enzyme required to decompose 1 nmol of hydrogen peroxide (H_2O_2) per minute at 25 °C. Decomposition of H_2O_2 in the presence of CAT was measured at 240 nm (Aebi 1974). GSH concentration was assayed by reacting with *o*-phthaldialdehyde (10 mg/10 ml methanol) according to the modified method of Lee and Chung (1999). Pure reduced GSH was used as the standard for calibration. GSH samples were measured using spectrofluorimetry (Jasco 6000; Jasco, Easton, MD), with excitation at 345 nm and emission at 425 nm. Lipid peroxidation product MDA was estimated using the modified thiobarbituric acid-reactive substance method by Hegde et al. (2003). The MDA level was measured spectrofluorimetrically with excitation at 520 nm and emission at 555 nm. Calculations were performed using a linear regression from tetraethoxypropane for the MDA standard curve. Serum biochemical parameters including total cholesterol, TG, HDL, LDL, VLDL, AMY, CK, GGT, LDH, AST, ALT, ALP, CRE, urea and BUN were analyzed by an autoanalyser (Roche, Indianapolis, IN; Cobas Integra 400 plus) using the kits.

Statistical Analysis

Statistical analyses were performed using Minitab 13 for Windows (Minitab, State College, PA). All data were presented as mean ± standard deviation (SD). One-way analysis of variance was used to determine the differences between means of the experimental groups, accepting a significance level of $P < 0.05$.

Results

Effect on FBG

FBG levels of groups are shown in Table 1. In the STZ (alone) group, a significant increase ($P < 0.05$) in FBG level was observed between days 7 and 35 of the experiment. SH supplementation significantly ($P < 0.05$) decreased the elevated FBG levels in the STZ + SH group on days 28 and 35 of the study.

Effect on Lipid Profile

Results for lipid profiles are demonstrated in Table 2. While total cholesterol, TG, LDL and VLDL levels of the STZ (alone) group increased, the HDL level of this group decreased remarkably ($P < 0.05$) compared with control. These parameters were ameliorated especially after TV and MC supplementations.

Effect on Levels of Serum Enzymes

As shown in Table 3, a marked ($P < 0.05$) decrement in the activities of AMY and CK and a remarkable ($P < 0.05$) elevation in GGT, LDH, AST, ALT and ALP were observed in STZ (alone) rats. Treatment with almost all plant extracts significantly increased CK and decreased GGT, LDH, AST, ALT and ALP activities. But none of them amended AMY.

Table 1 Effects of some plant extracts on FBG levels in diabetic rats ($n = 6$)

Groups	FBG concentration (mg/dl)				
	7th day	14th day	21st day	28th day	35th day
Control	101.1 ± 10.1	99.0 ± 9.1	99.6 ± 11.5	101.9 ± 10.9	101.6 ± 9.9
STZ (alone)	363.9 ± 42.5	378.4 ± 49.8	395.3 ± 44.9	406.4 ± 43.1	429.9 ± 45.0*
STZ + UD	358.8 ± 31.4	353.0 ± 40.0	344.7 ± 39.4	344.8 ± 44.5	337.6 ± 44.8
STZ + TV	384.4 ± 47.9	380.4 ± 46.4	376.0 ± 41.7	371.8 ± 47.1	374.2 ± 43.3
STZ + MC	364.5 ± 56.5	355.3 ± 31.1	344.2 ± 49.4	334.8 ± 55.3	311.3 ± 34.1
STZ + SH	372.2 ± 43.5	350.0 ± 34.6	325.6 ± 47.1	301.8 ± 29.4*	308.4 ± 27.9*
STZ + CZ	386.0 ± 47.3	379.2 ± 36.4	373.0 ± 38.8	365.4 ± 44.3	362.4 ± 46.5

Values are given as mean ± SD

* $P < 0.05$ compared with first day

Table 2 Effects of some plant extracts on lipid profiles of diabetic rats ($n = 6$)

Groups	Total cholesterol	TG	HDL	LDL	VLDL
Control	114.7 ± 11.4	97.0 ± 13.5	50.1 ± 7.1	45.2 ± 5.4	19.4 ± 2.7
STZ (alone)	141.7 ± 17.1*	180.0 ± 26.5*	35.2 ± 6.7*	70.4 ± 9.3*	36.0 ± 5.3*
STZ + UD	104.4 ± 12.3**	154.0 ± 21.0*	31.2 ± 9.7*	42.4 ± 7.0**	30.8 ± 4.2*
STZ + TV	131.0 ± 5.9*	119.0 ± 30.5**	49.0 ± 5.7**	58.2 ± 3.7*, **	23.8 ± 6.1**
STZ + MC	98.6 ± 8.4*, **	101.0 ± 9.6**	32.4 ± 2.2*	46.0 ± 7.0**	20.2 ± 1.9**
STZ + SH	112.7 ± 9.4**	181.9 ± 24.5*	37.6 ± 6.8*	38.8 ± 4.4*, **	36.4 ± 4.9*
STZ + CZ	112.9 ± 10.1**	231.0 ± 37.0*, **	36.3 ± 7.9*	30.4 ± 3.8*, **	46.2 ± 7.4*, **

Values are given as mean ± SD

* $P < 0.05$ compared with normal control group, ** $P < 0.05$ compared with diabetic control group

Table 3 Effects of some plant extracts on serum enzyme levels (U/l) of diabetic rats ($n = 6$)

Groups	AMY	CK	GGT	LDH	AST	ALT	ALP
Control	1,407 ± 68	309.0 ± 32.2	2.0 ± 0.1	398.6 ± 27.5	90.8 ± 6.3	59.2 ± 6.1	121.6 ± 7.8
STZ (alone)	1,151 ± 102*	214.3 ± 22.7*	6.1 ± 0.6*	744.0 ± 28.8*	195.0 ± 17.5*	143.4 ± 17.4*	456.6 ± 45.7*
STZ + UD	1,085 ± 156*	275.8 ± 32.1**	3.8 ± 0.4*, **	594.2 ± 33.9*, **	145.6 ± 16.4*, **	101.4 ± 5.9*, **	376.4 ± 18.1*, **
STZ + TV	1,094 ± 98*	277.0 ± 18.2**	3.3 ± 0.4*, **	541.2 ± 82.6*, **	137.6 ± 25.9*, **	97.8 ± 7.2*, **	369.4 ± 25.1*, **
STZ + MC	1,003 ± 162*	312.2 ± 36.6**	5.9 ± 0.5*	545.2 ± 42.4*, **	121.8 ± 10.3*, **	126.2 ± 9.4*	243.4 ± 35.5*, **
STZ + SH	1,140 ± 183*	254.9 ± 27.1*, **	2.4 ± 0.3*, **	401.6 ± 21.2**	100.3 ± 9.7**	98.9 ± 9.2*, **	332.5 ± 43.5*, **
STZ + CZ	1,064 ± 142*	277.4 ± 28.4**	4.3 ± 0.5*, **	421.2 ± 28.9**	110.8 ± 10.2*, **	95.0 ± 8.8*, **	408.0 ± 40.1*

Values are given as mean ± SD

* $P < 0.05$ compared with normal control group, ** $P < 0.05$ compared with diabetic control group

Effect on Serum Renal Function Markers

Results of renal function markers are shown in Table 4. Serum CRE, urea and BUN concentrations elevated dramatically ($P < 0.05$) in the STZ (alone) group compared to control. None of the plant extracts remarkably ($P > 0.05$) diminished CRE levels, whereas treatment with SH and CZ markedly ($P < 0.05$) improved urea and BUN levels. Unlike SH and CZ, treatment with MC led to dramatic escalations of urea and BUN even compared to the STZ (alone) group.

Effect on GSH and MDA Levels

As shown in Tables 5, 6, erythrocyte, plasma, retina and lens GSH contents were markedly lower ($P < 0.05$) and MDA concentrations were notably higher ($P < 0.05$) in the STZ (alone) group compared to control. Treatment with

Table 4 Effects of some plant extracts on serum renal markers (U/l) of diabetic rats ($n = 6$)

Groups	CRE	Urea	BUN
Control	0.42 ± 0.03	41.0 ± 5.4	19.4 ± 2.5
STZ (alone)	0.53 ± 0.05*	94.1 ± 12.9*	43.9 ± 6.3*
STZ + UD	0.48 ± 0.09	88.8 ± 9.9*	41.4 ± 4.7*
STZ + TV	0.49 ± 0.05*	84.6 ± 7.6*	39.4 ± 3.8*
STZ + MC	0.52 ± 0.09	128.8 ± 16.6*, **	59.8 ± 7.8*, **
STZ + SH	0.47 ± 0.07	63.3 ± 9.9*, **	29.6 ± 4.6*, **
STZ + CZ	0.49 ± 0.05*	65.6 ± 10.1*, **	30.4 ± 4.5*, **

Values are given as mean ± SD

* $P < 0.05$ compared with normal control group, ** $P < 0.05$ compared with diabetic control group

each plant extract resulted in a significant ($P < 0.05$) escalation in the GSH content of all studied tissues except plasma. With regard to MDA, plant extracts reduced its concentration in all tissues but not always statistically significantly.

Effect on Activity of Erythrocyte Antioxidant Enzymes

Erythrocyte antioxidant enzyme activities are shown in Table 7. GR, GST and CAT showed a similar trend of significantly ($P < 0.05$) lower activity in the STZ (alone) group compared to controls. None of the plant extracts significantly ($P > 0.005$) increased the GR and GST activities, whereas CAT activity was increased ($P < 0.05$) by each plant extract supplementation.

Effect on Body Weight Loss

Significant ($P < 0.05$) body weight loss was observed between days 7 and 35 in all groups of diabetic rats. On the contrary, body weight gain of 5 % was observed in the control group between days 7 and 35.

Discussion

Today, as DM has become one of the main problems of our age, efforts are needed to provide effective protection against its detrimental influences. Some experimental trials have investigated the medical influence of a number of plant extracts in this regard. The current study is the first comprehensive one to evaluate whether UD, TV, MC, SH and CZ extracts could ameliorate some impairments caused by STZ-induced type 1 DM in rats.

Table 5 Effects of some plant extracts on GSH level in various tissues of diabetic rats ($n = 6$)

Groups	Erythrocyte (nmol/ml)	Plasma (nmol/ml)	Retina (nmol/g)	Lens (nmol/g)
Control	33.46 ± 2.18	2.72 ± 0.19	18.87 ± 0.85	20.44 ± 1.08
STZ (alone)	24.15 ± 1.68*	2.12 ± 0.15*	10.35 ± 0.84*	14.38 ± 1.36*
STZ + UD	29.42 ± 2.26*, **	2.18 ± 0.22*	12.59 ± 1.07*, **	16.05 ± 0.95*, **
STZ + TV	29.39 ± 2.22*, **	2.28 ± 0.23*	12.40 ± 0.87*, **	17.89 ± 1.33*, **
STZ + MC	26.57 ± 1.77*, **	2.34 ± 0.40	14.27 ± 0.91*, **	16.76 ± 1.30*, **
STZ + SH	26.03 ± 1.27*, **	2.52 ± 0.19**	12.78 ± 1.35*, **	18.51 ± 2.06**
STZ + CZ	26.68 ± 1.46*, **	2.17 ± 0.09*	14.81 ± 1.72*, **	18.02 ± 2.82**

Values are given as mean ± SD

* $P < 0.05$ compared with normal control group, ** $P < 0.05$ compared with diabetic control group

Table 6 Effects of some plant extracts on MDA level in various tissues of diabetic rats ($n = 6$)

Groups	Erythrocyte (nmol/ml)	Plasma (nmol/ml)	Retina (nmol/g)	Lens (nmol/g)
Control	15.72 ± 1.80	2.40 ± 0.26	5.31 ± 0.57	3.69 ± 0.41
STZ (alone)	19.59 ± 2.34*	3.39 ± 0.35*	9.03 ± 0.98*	4.86 ± 0.50*
STZ + UD	19.10 ± 2.19*	2.60 ± 0.36**	7.63 ± 0.63*, **	4.40 ± 0.46*
STZ + TV	15.50 ± 1.91**	2.96 ± 0.39*	6.27 ± 0.66*, **	4.57 ± 0.49*
STZ + MC	15.77 ± 1.68**	2.24 ± 0.26**	9.19 ± 0.67*	4.09 ± 0.41**
STZ + SH	15.80 ± 1.65**	2.77 ± 0.34**	8.64 ± 0.72*	3.95 ± 0.32**
STZ + CZ	18.24 ± 2.20	2.45 ± 0.23**	6.13 ± 0.68**	4.68 ± 0.47*

Values are given as mean ± SD

* $P < 0.05$ compared with normal control group, ** $P < 0.05$ compared with diabetic control group

Table 7 Effect of some plant extracts on antioxidant enzymes activity (U/ml) in erythrocytes of diabetic rats ($n = 6$)

Groups	GR	GST	CAT
Control	0.30 ± 0.03	12.43 ± 1.45	114.60 ± 10.77
STZ (alone)	0.26 ± 0.02*	9.03 ± 1.09*	67.30 ± 6.79*
STZ + UD	0.29 ± 0.03	9.41 ± 0.88*	81.71 ± 8.28*, **
STZ + TV	0.26 ± 0.02*	9.56 ± 1.26*	98.44 ± 10.80*, **
STZ + MC	0.26 ± 0.04	9.09 ± 0.91*	102.50 ± 11.22**
STZ + SH	0.29 ± 0.03	9.08 ± 0.91*	84.34 ± 8.57*, **
STZ + CZ	0.30 ± 0.04	9.40 ± 1.18*	86.17 ± 9.72*, **

Values are given as mean ± SD

* $P < 0.05$ compared with normal control group, ** $P < 0.05$ compared with diabetic control group

The FBG test is ordinarily used in the detection of DM. In our study, a marked increment in FBG level occurred between days 7 and 35 of the experiment in the STZ (alone) group (Table 1). A similar result was observed in previous studies in which STZ was used to produce diabetes (Murugan and Pari 2006; Kim et al. 2006). Among all of the plant extracts used in this study, only SH treatment significantly decreased the elevated FBG levels in the STZ + SH group on days 28 and 35. However, both of these values were still higher than the normal reference range of FBG. To the best of our knowledge, a hypoglycemic effect of SH was shown for the first time in this study. The SH extract might have such a role through elevating insulin secretion of intact pancreatic beta-cells (Bhandari et al. 2007).

High blood glucose content is accompanied by elevation of serum lipid levels in STZ-induced diabetes (Kim et al. 2006). Alterations of lipid profile are also reported as prevalent in diabetic patients (Howard et al. 1978). In the STZ (alone) group of the present study, increased total cholesterol, TG, LDL and VLDL levels and decreased HDL level are in accordance with the results of Cho et al. (2002). Remarkable hyperlipidemia in this group might be associated with uninhibited activities of lipolytic hormones on the fat depots (Al-Shamaony et al. 1994). All serum lipid constituents were ameliorated, especially after TV and MC supplementations (Table 2). The hypolipidemic effect of these extracts may be related to their reducing impact on cholesterologenesis and fatty acid synthesis (Bopanna et al. 1997). These effects of TV and MC extracts have been demonstrated for the first time in the current study. Interestingly, TG and VLDL levels of the STZ + CZ group increased even when compared with the STZ (alone) group. These elevations might be attributed to an enhancing effect of this extract on fatty acid synthesis (Yoshino et al. 1996).

Serum enzyme activities can be used as useful biomarkers for monitoring the cytotoxicity of xenobiotics including STZ. In the current study significant increases of GGT, LDH, AST, ALT and ALP activities and remarkable reductions of AMY and CK were determined in the STZ (alone) group compared with the control group. Treatment with almost all plant extracts significantly increased CK and decreased GGT, LDH, AST, ALT and ALP activities. But none of them amended AMY (Table 3). Decrease in AMY activity probably resulted from alteration of the parenchyma in pancreatic tissues (Hegyi et al. 2000). Diminished CK activity might be attributed to the diabetic state, as previously reported (Christopher et al. 2003). Similar to our results, some earlier studies noted increases in GGT activity in the liver and plasma of STZ-induced diabetic rats (Hemmings and Pekush 1994; Lu et al. 1997). It was reported that STZ-induced diabetes changes the expression of GGT, which catalyzes the conversion of the glutathione conjugate into the cysteine conjugate (Watkins and Sherman 1992). LDH activity increases after liver, muscle and heart damage (Aldrich 2003). It was reported that escalated LDH levels in diabetic rats indicate cardiac muscular damage (Bhandari et al. 2007). Serum AST and ALT are elevated in cardiac or hepatic damage (Murray et al. 1993). Escalated ALP activity indicates bone and liver diseases or bile tract blockage (Mayne 1996). When compared to the STZ (alone) group, significant reductions in serum activities of GGT, LDH, AST, ALT and ALP were observed in plant extract-treated groups, consistent with the study of Rai et al. (2010). It is most likely that leakage of enzymes from tissues to serum was reduced after supplementation with these extracts. They might have provided muscle integrity and ameliorated injuries of liver and heart tissues in diabetic rats (Rai et al. 2010). In addition, the current study demonstrates for the first time that SH extract has a remedial effect on activities of serum enzymes in diabetic rats.

The most common way to evaluate kidney function is to measure serum CRE, urea and BUN concentrations. These parameters increased dramatically in the STZ group (Table 4). None of the plant extracts remarkably diminished the CRE level, whereas treatment with SH and CZ noticeably improved urea and BUN levels. Unlike SH and CZ, treatment with MC led to significant escalations of urea and BUN even when compared to the STZ (alone) group. BUN is a scale of the amount of nitrogen as urea in the blood and provides data concerning renal function. An increase in the BUN level indicates renal dysfunction (Danda et al. 2005). In the diabetic state BUN and CRE are noticeable indexes for assessment of glomerular filtration rate (He et al. 2006). Our results indicate that SH and CZ extracts might have a curative effect on renal injury in STZ-induced diabetic rats (Liu et al. 2008). SH extract was

more effective compared with the other extracts in this regard.

There is increasing evidence that complications associated with diabetes may be related to oxidative stress induced by the production of free radicals (Armstrong and Al-Awadi 1991). Several studies have shown that antioxidant treatment reduces diabetic complications (Wohaieb and Godin 1987; Siman and Eriksson 1997). One of the materials being used as an antioxidant source to eliminate free radicals generated in diabetes are plant extracts (Liu et al. 2008; Rodrigues et al. 2012). MDA is a noxious end product of lipid peroxidation and a considerable biomarker of oxidative membrane damage. GSH is a nonprotein thiol and the leading antioxidant defense mechanism in xenobiotic metabolism, and it protects tissue from damage. GSH reacts with superoxide anion ($O_2^{-\bullet}$), singlet oxygen and peroxy radicals directly. The drug-metabolizing enzyme GST is a GSH-dependent antioxidant enzyme. It has a crucial role in the metabolism of xenobiotics and the detoxification of peroxidized lipids (Valavanidis et al. 2006). Ancillary enzyme GR reduces GSSG to GSH in the presence of NADPH. CAT is an enzyme present in almost all living organisms which are exposed to oxygen (O_2). It converts H_2O_2 to H_2O and $½O_2$.

In our study, erythrocyte, plasma, retina and lens GSH contents were lower and MDA concentrations were higher in the STZ (alone) group (Tables 5, 6). Treatment with each plant extract resulted in a significant escalation in the GSH content of all studied tissues except plasma. With regard to MDA, plant extracts reduced its concentration in all tissues but not always statistically significantly. Several studies have shown elevated MDA levels in the diabetic state (Hamdy and Taha 2009; Parveen et al. 2010). This escalation indicates a rise in ROS. Hyperglycemic conditions of diabetes not only produce excessive ROS but also impair the antioxidant defense system (Liu et al. 2008).

In the present study, GR, GST and CAT showed a similar trend of significantly lower activities in the STZ (alone) group compared to controls. None of the plant extracts significantly increased GR and GST activities, whereas CAT was increased by each plant extract supplementation (Table 7). In some earlier studies it was reported that STZ-induced diabetes could cause a reduction in GSH level as well as activities of GR, GST and CAT (Parveen et al. 2010; Jin et al. 2008; Hamdy and Taha 2009; Bhandari et al. 2007; Muruganandan et al. 2002). CAT has been reported to be involved in the detoxification of H_2O_2 (De duve and Baudhhuin 1996) and to protect tissues from hydroxyl radicals. In our study, decreased CAT activity of diabetic rats is compatible with the findings of Kakkar et al. (1998) but incompatible with those of Cho et al. (2002). These changes may have resulted from the use of different tissues and implementation periods. Our results indicate that plant extract supplementations improved GSH level and CAT activity, thereby overcoming the increased oxidative stress in diabetic rats, resulting in a lower MDA level. Such an effect may have occurred through the free radical-scavenging action of plant extracts (Bhandari et al. 2007). Among extracts used in this trial, the SH extract was determined to have the most antioxidative effect on the investigated tissues of diabetic rats.

There were notably body weight reductions in all groups except control. Maximal reduction was determined as 25 % in the STZ (alone) group, whereas minimal reduction of 17 % was observed in the STZ + SH group (Fig. 1). Elevated muscle wasting and decrement of tissue proteins may have given rise to body weight loss in all STZ-treated groups (Chatterjee and Shinde 2002). Control rats, on the other hand, slightly gained body weight, 5 %. Results obtained in this study show that plant extract applications could not considerably prevent body weight loss.

As a result, the present findings suggest that almost all of the plant extracts used in this study had remarkable

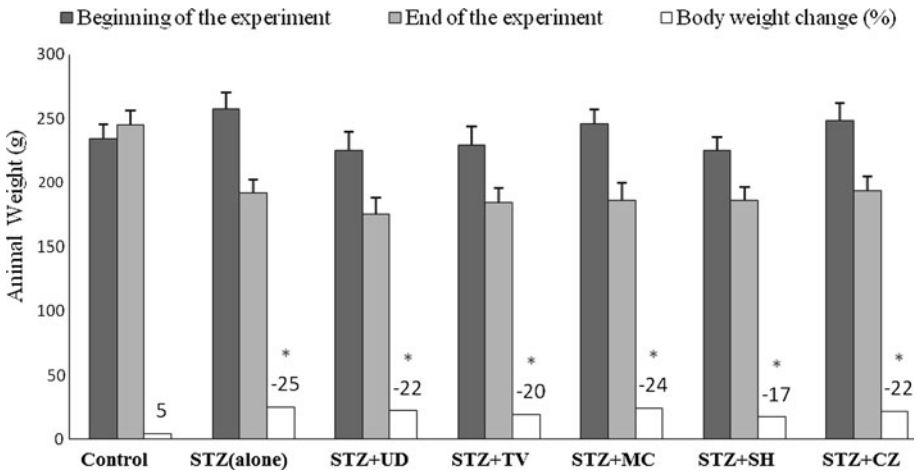

Fig. 1 Changes of body weight in groups ($n = 6$). Values are given as mean ± SD. *Significant ($P < 0.05$) difference in control and other groups

potential to heal the detrimental effects of STZ-induced type 1 DM through their beneficial effects on erythrocytes and some organs such as the liver, heart, eye and kidney, as evidenced from their reducing influences on levels of serum enzymes, renal function markers and lipid peroxidation as well as enhancing effects on levels of some antioxidant defense systems including GSH, GR, GST and CAT. Moreover TV and MC extracts ameliorated the defect in lipid metabolism. In terms of FBG concentration, it can be concluded that the SH extract had a relatively hypoglycemic effect.

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