

Research Article**Ameliorative potential of Chrysoeriol, a bioactive flavonoid on oxidative stress and hepatic marker enzymes in STZ induced diabetic rats****Baskaran Krishnan¹, Kodukkur Viswanathan Pugalendi², Ramalingam Saravanan^{2*}**¹Assistant Professor, Department of Biochemistry, Sree Narayana Guru College, Coimbatore, Tamilnadu, India²Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar, Tamilnadu, India

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Abstract

Objectives: Oxidative stress (OS) has been suggested as a contributory factor in the development and complication of diabetes. The aim of the present study was to evaluate the effects of Chrysoeriol (CS) on altered lipid peroxidation products, enzymatic and nonenzymatic antioxidants and hepatic marker enzymes in streptozotocin (STZ) -induced diabetic rats. **Material and Methods:** Experimental diabetes was induced by a single intraperitoneal (i.p) injection of STZ (40 mg/kg body weight). **Results:** Diabetic rats showed significantly increased levels of plasma glucose, thiobarbituric acid reactive substances (TABRS), lipid hydroperoxides (LOOH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and decreased levels of plasma insulin and body weight. The activities of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and the levels of non-enzymatic antioxidants such as vitamin C, vitamin E and reduced glutathione (GSH) were decreased in diabetic rats. Oral treatment with CS (20 mg/kg/BW) for a period of 45 days showed significant ameliorative effects on all the biochemical parameters studied. **Conclusions:** Biochemical findings were supported by histological studies. These results indicated that CS has potential ameliorative effects in addition to its antidiabetic effect in type 2 diabetic rats.

Keywords: Oxidative stress, Chrysoeriol, intraperitoneal, antioxidants

Introduction

Diabetes mellitus (DM) is a chronic hyperglycemic metabolic disorder and among one of the five leading causes of death in the world. It is predicted that by the year 2025 that world will have increase of 300 million people diabetic patients (King et al., 1998). DM is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids, carbohydrates, proteins metabolism which occurs secondary to an absolute or relative lack of insulin (ADA,2010). The precise cellular and molecular mechanism, which underlies the etiology and progression of diabetes, is still not fully understood. However, increasing evidence suggests that OS plays a crucial role in the pathogenesis of diabetes and its complications (Maritim et al., 2003; Giacco and Brownlee, 2010). Elevated levels of blood glucose can induce

non-enzymatic and auto-oxidative glycosylation, increase polyol and hexosamine pathway, promote protein kinase-C activation and lead to alterations in the levels of inflammatory mediators, as well as in the status of antioxidant defense. These pathways are involved in the generation of reactive oxygen species (ROS) in the diabetic state, which directly contribute to the increase of OS in various organs and tissues (Stevens, 2005; Rains and Jain, 2011).

STZ an antibiotic produced by *Streptomyces achromogenes* has been widely used for inducing diabetes in the experimental animals through its toxic effects on pancreatic β -cells (Rains and Jain, 2011). The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage (Lenz, 2008). LPO is a key marker of the OS. The increased OS, as measured by indices of elevated LPO, depletion of endogenous antioxidant and antioxidant enzyme activities in plasma and tissues, are commonly found in rats with STZ-induced diabetes, and these alterations may cause tissues to be more susceptible to oxidative damage (Nizamutdinova et al., 2009). The significant extent of LPO byproducts that was measured as TBARS has been reported

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in diabetes (Likidilid et al., 2009). Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the cells by oxygen. They act by one or more of the following mechanisms: reducing activity, free radical scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. The formation of ROS is prevented by an antioxidant system that included non-enzymatic antioxidants, enzymes regenerating the reduced forms of antioxidants and ROS-scavenging enzymes (Adewole et al., 2008; Budin et al., 2009). Epidemiological studies have shown that many phytonutrients of fruits and vegetables might protect the human body against damage by ROS. The consumption of natural antioxidant phytochemicals was reported to have potential health benefits (Rangkadilok et al., 2007).

In recent years, considerable focus has been given to an intensive search for a novel type of antioxidants present in plants for treating disease (Srivastava et al., 2003; Fabricant and Farnsworth, 2001) and some of the plants are used by the population as anti-diabetic remedies (Pari and Venkateswaran, 2003). Management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use the natural products with anti-diabetic activity because insulin and oral hypoglycemic drugs possess undesirable side effects (Rao and Rao, 2001). Plants and its active compounds provide useful sources for the development of drugs in the treatment of DM. Clinical research has confirmed the efficacy of several plants and its active compounds in the modulation of the OS associated with diabetes mellitus (Pari and Latha, 2004).

Hypoglycemic sulphonylureas such as glibenclamide can increase pancreatic insulin secretion from the existing β -cells in STZ-induced diabetes by membrane depolarization, and stimulation of Ca^{2+} influx, an initial key step in insulin secretion. Moreover, glibenclamide has shown a protective effect against OS in diabetes (Elmai et al., 2004). Glibenclamide is often used as a reference drug in STZ-induced moderate diabetic model. Though sulphonylureas are valuable in the treatment of diabetes, their use is restricted by their limited action and side effects (hypoglycaemia and liver problems).

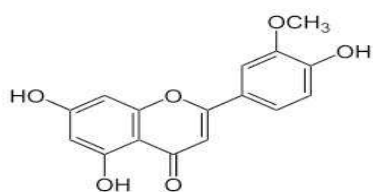


Figure 1. Structure of chrysoeriol

Chrysoeriol (5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromen-4-one) was possessed many biological effects such as antioxidant, anti-inflammatory, and anti-osteoporotic effects (Bui et al., 2009; Hsu and Kuo, 2008). Furthermore, CS were natural pro drugs in cancer prevention (Androutsopoulos et al., 2009; Arroo et al., 2009) and We also viewed the free radical scavenging activity (Baskaran et al., 2015). The structure of CS is depicted as below (Figure1). As yet there is no published study on the role of CS on OS in STZ induced diabetic rats. Hence, the present study aimed to investigate the ameliorative potential of CS on hyperglycemia mediated OS in STZ induced diabetic rats and the effect of CS was compared with glibenclamide, an oral antihyperglycemic drug with antioxidant potential.

Materials and Methods

Isolation of Chrysoeriol from *Cardiospermum halicacabum* leaves

Chrysoeriol was isolated from the leaves *Cardiospermum halicacabum* and tested its antioxidant potency *in vitro* (Baskaran et al., 2015). The isolation of CS was followed by the method (Sundaram et al., 2015).

Animals

Adult Male albino Wistar rats (9 weeks old; 180–200 g) were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and maintained in air-conditioned room ($25 \pm 10^{\circ}\text{C}$) with a 12 h light/dark cycle. Feed and water were provided *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital, Annamalai University (Reg. No. 160/1999/CPCSEA, Proposal No: 539).

Sources of chemicals

All fine chemicals, including STZ, were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of good quality and analytical grade and obtained from Himedia, Mumbai, India.

Induction of experimental diabetes

Experimental diabetes was induced in 12 h fasted rats by single (i.p.) injection of STZ (40 mg/kg B.W.) dissolved in cold citrate buffer (0.1 M, pH 4.5). STZ-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemia. STZ-injected animals exhibited hyperglycemia within a few days. Diabetic rats were confirmed by measuring the elevated plasma glucose (by the glucose oxidase method) 72 h after injection with STZ. The animals with glucose above 255 mg/dl were

selected for the experiment.

Experimental design

The experimental animals were divided into five groups, each group consists of a minimum of six rats detailed as given below. CS and glibenclamide were administered orally for 45 days.

Group I: Normal control (0.5% DMSO)

Group II: Control + Chrysoeriol (20 mg/kg BW)

Group III: Diabetic control (0.5% DMSO)

Group IV: Diabetic + Chrysoeriol (20 mg/kg BW)

Group V: Diabetic + glibenclamide (600 µg/kg BW)

Anesthetized between 8:00 a.m. and 9:00 a.m., using ketamine (24 mg/kg b.w) and sacrificed by cervical decapitation. Blood samples were collected in tubes containing EDTA. The plasma was obtained after centrifugation (2000 × g for 20 min at 4°C) and used for various biochemical measurements. The liver, kidney, and heart were excised immediately, washed with ice-cold isotonic saline and stored at -80°C until analyzed. Liver, kidney and heart were immediately dissected, washed in ice-cold saline to remove the blood.

Analytical procedure

Plasma glucose levels were estimated using a commercial kit (Sigma Diagnostics Private Limited, Baroda, India) by the method (Trinder, 1969). Plasma insulin was assayed by ELISA kit (Boehringer–Mannheim Kit, Mannheim, Germany) respectively. LPO in liver, kidney and heart were estimated UV/VIS spectrophotometer (SL 177, Elico Ltd., Hyderabad, India) by measuring TBARS and LOOH using the methods (Fraga et al., 1988; Jiang et al., 1992). The activity of SOD was assayed by the method (Kakkar et al., 1998). The activity of CAT was estimated by the method (Shinha, 1972). The activity of GPx was measured by the method (Rotruck et al., 1973). GSH was determined by the method (Ellman, 1959). Vitamin C was estimated by the method (Omayya et al., 1979). Vitamin E was determined by the method (Baker et al., 1951). Activities of AST

and ALT were assayed by the method (Reitman and Frankel, 1957) respectively.

Histopathological study

The liver, kidney and heart samples fixed for 48 h in 10% formal saline were dehydrated by passing successfully in the different mixture of ethyl alcohol–water, cleaned with xylene and embedded in paraffin. Sections of liver and kidney (4–5 µm thick) were prepared and then stained with hematoxylin and eosin (H&E) dye, which mounted in a neutral deparaffinized xylene medium for microscopic observations.

Statistical analysis

The statistical significance of the data has been determined using one-way analysis of variance (ANOVA) and significant difference among treatment groups were evaluated by Duncan's multiple range test (DMRT). The results were considered statistically significant at $p < 0.05$. All statistical analyzes were done using SPSS 16.0, SPSS Inc., and Cary, NC.

Results

Figure 2 depicts the values of the initial and final body weight of the normal and experimental rats. BW significantly decreased in diabetic rats compared to normal control rats. Oral administration of CS to diabetic rats protects the loss of BW compared to diabetic control rats.

Figure 3 and 4 show the levels of plasma glucose and insulin in control and experimental animals. There was a significant elevation in plasma glucose level with a significant decrease in insulin levels in STZ induced diabetic rats, compared with control rats. Administration of CS tended to bring plasma glucose and insulin towards near normal levels.

The levels of serum ALT, AST, in normal and experimental rats are represented in Figure 5. The activities of ALT, AST were significantly increased in diabetic rats. These values were brought back to near normal levels after treatment with CS.

Table 1. Effect of CS on TBARS in the plasma, erythrocyte and tissues of STZ-diabetic rats.

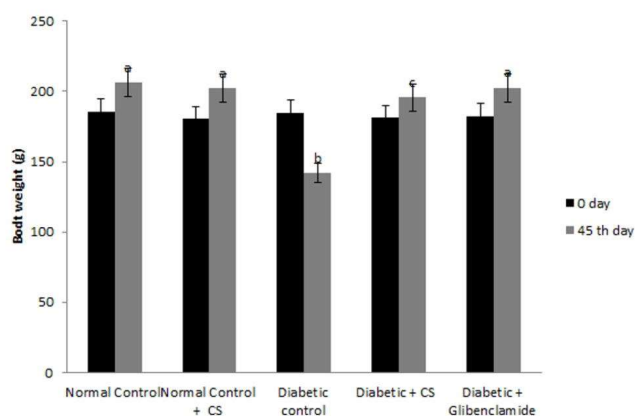
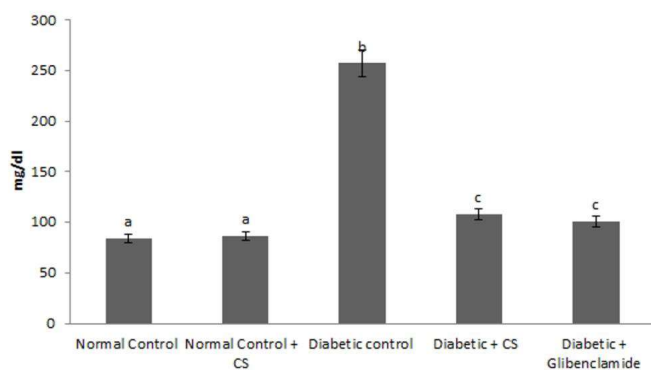
Groups	Plasma (mmol/dL)	Erythrocyte (nmol/mg protein)	Tissue (mmol/100 g wet tissue)		
			Liver	Kidney	Heart
Normal Control	0.27 ± 0.02 ^a	1.44 ± 0.09 ^a	0.86 ± 0.04 ^a	1.47 ± 0.06 ^a	0.64 ± 0.03 ^a
Normal Control + CS (20 mg/kg BW)	0.26 ± 0.01 ^a	1.36 ± 0.07 ^a	0.83 ± 0.04 ^a	1.49 ± 0.09 ^a	0.57 ± 0.05 ^a
Diabetic control	0.38 ± 0.02 ^b	4.22 ± 0.24 ^b	3.88 ± 0.22 ^b	3.75 ± 0.29 ^b	1.84 ± 0.16 ^b
Diabetic + CS (20 mg/kg BW)	0.34 ± 0.03 ^c	1.78 ± 0.04 ^c	1.58 ± 0.03 ^c	1.73 ± 0.04 ^c	0.80 ± 0.05 ^c
Diabetic + Glibenclamide (600 µg/kg BW)	0.20 ± 0.02 ^{ac}	1.56 ± 0.03 ^d	0.92 ± 0.02 ^d	1.59 ± 0.07 ^d	0.69 ± 0.02 ^a

Values are given as means S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 2. Effect of CS on LOOH in the plasma, erythrocyte and tissues of STZ-diabetic rats.

Groups	Plasma (mmol/dL)	Erythrocyte (nmol/mg protein)	Tissue (mmol/100 g wet tissue)		
			Liver	Kidney	Heart
Normal Control	8.39 ± 0.37 ^a	1.02 ± 0.05 ^a	79.79 ± 5.06 ^a	66.79 ± 4.82 ^a	75.95 ± 3.42 ^a
Normal Control + CS (20 mg/kg BW)	8.29 ± 0.22 ^a	0.94 ± 0.04 ^a	75.58 ± 5.13 ^a	66.02 ± 4.57 ^a	77.96 ± 3.68 ^a
Diabetic control	30.64 ± 1.46 ^b	1.44 ± 0.03 ^b	145.11 ± 10.18 ^b	179.21 ± 12.58 ^b	149.97 ± 11.51 ^b
Diabetic + CS (20 mg/kg BW)	13.62 ± 0.97 ^c	1.23 ± 0.07 ^c	113.02 ± 8.06 ^c	98.79 ± 7.35 ^c	86.89 ± 4.35 ^c
Diabetic + Glibenclamide (600 µg/kg BW)	12.83 ± 1.06 ^c	1.18 ± 0.06 ^c	96.74 ± 7.18 ^d	92.88 ± 6.48 ^c	79.15 ± 6.19 ^c

Values are given as means S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

**Figure 2.** Effect of CS on body weight in STZ-induced diabetic rats. Values are given as means S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).**Figure 3.** Effect of CS on plasma glucose in STZ-induced diabetic rats. Values are given as means S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Tables 1 and 2 show the concentration of TBARS and LOOH in plasma, erythrocyte, liver, kidneys and heart of the control and experimental groups of rats. The levels of TBARS and LOOH in diabetic rats were significantly higher than control rats, whereas diabetic rats treated with CS restored the altered values to the near normal.

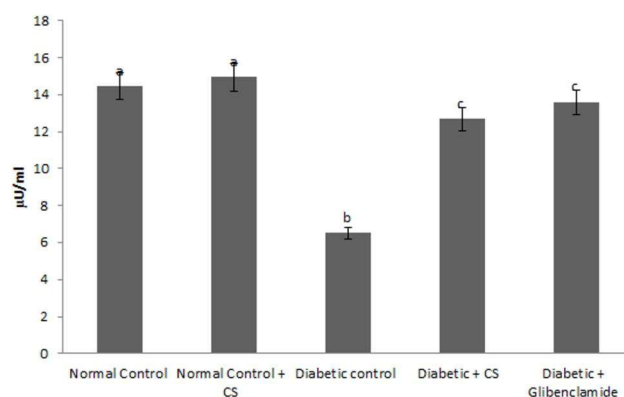
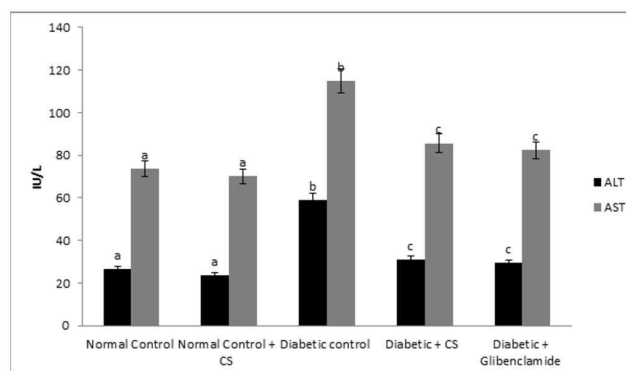
**Figure 4.** Effect of CS on plasma insulin in STZ-induced diabetic rats. Values are given as means S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).**Figure 5.** Effect of CS on ALT and AST in STZ-induced diabetic rats.

Table 3, 4 and 5 represents the activities of antioxidant enzymes (SOD, CAT, GPx) in the erythrocyte, liver, kidney and heart of normal and experimental rats. A fall in the activities of antioxidant enzymes was observed in diabetic rats when compared to normal control. CS administration to diabetic rats significantly improved the activities of the above enzymes.

The levels of plasma, erythrocyte, liver, kidneys and heart non-enzymatic antioxidants such as GSH, vitamin C, and

Table 3. Effect of CS on the activity of SOD in the erythrocyte and tissues of STZ-diabetic rats

Groups	Erythrocyte (U ⁷ /mg Hb)	Tissue (U ⁷ /mg protein)		
		Liver	Kidney	Heart
Normal Control	7.46± 0.56 ^a	9.62 ± 0.64 ^a	15.12 ± 1.36 ^a	4.91 ± 0.31 ^a
Normal Control + CS (20 mg/kg BW)	7.65 ± 0.52 ^a	9.87 ± 0.72 ^a	14.59 ± 1.37 ^a	5.18 ± 0.38 ^a
Diabetic control	3.97 ± 0.27 ^b	5.31 ± 0.35 ^b	7.57 ± 0.53 ^b	3.10 ± 1.96 ^b
Diabetic + CS (20 mg/kg BW)	6.94 ± 0.64 ^c	6.89 ± 0.57 ^c	12.79 ± 1.12 ^c	4.07 ± 0.27 ^c
Diabetic + Glibenclamide (600 µg /kg BW)	7.31 ± 0.68 ^{ac}	7.47 ± 0.69 ^d	13.79 ± 1.38 ^d	4.48 ± 0.34 ^d

Values are given as means ± S.D. from six rats in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT). U^{*} = Enzyme concentration required for 50% inhibition of NBT reduction/minute.

Table 4. Effect of CS on the activity of CAT in the erythrocyte and tissues of STZ- diabetic rats.

Groups	Erythrocyte (U ⁵ /mg Hb)	Tissue (U ⁵ /mg protein)		
		Liver	Kidney	Heart
Normal Control	171.26 ± 10.36 ^a	83.61 ± 6.42 ^a	39.05 ± 3.18 ^a	52.62 ± 3.75 ^a
Normal Control + CS (20 mg/kg BW)	163.29 ± 9.87 ^a	82.28 ± 5.76 ^a	42.39 ± 3.66 ^a	53.66 ± 3.18 ^a
Diabetic control	116.12 ± 6.72 ^b	52.62 ± 4.88 ^b	18.83 ± 1.62 ^b	30.42 ± 2.08 ^b
Diabetic + CS (20 mg/kg BW)	146.37 ± 8.56 ^c	69.87 ± 4.05 ^c	30.11 ± 2.64 ^c	43.56 ± 2.85 ^c
Diabetic + Glibenclamide (600 µg /kg BW)	162.18 ± 11.64 ^c	77.08 ± 5.65 ^d	34.75 ± 2.89 ^d	49.98 ± 3.40 ^a

Values are given as means ± S.D. from six rats in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT). U⁵ = µmol of hydrogen peroxide consumed/minute

Table 5. Effect of CS on the activity of GPx in the erythrocyte and tissues of STZ-diabetic rats.

Groups	Erythrocyte (U [@] /mg Hb)	Tissue (U [@] /mg protein)		
		Liver	Kidney	Heart
Normal Control	15.10 ± 1.01 ^a	8.99 ± 0.67 ^a	7.41 ± 0.53 ^a	6.29 ± 0.39 ^a
Normal Control + CS (20 mg/kg BW)	15.43 ± 0.99 ^a	9.55 ± 0.82 ^a	7.88 ± 0.66 ^a	6.89 ± 0.27 ^a
Diabetic control	6.22 ± 0.40 ^b	4.85 ± 0.35 ^b	3.20 ± 0.22 ^b	3.69 ± 0.20 ^b
Diabetic + CS (20 mg/kg BW)	10.23 ± 0.98 ^c	7.30 ± 0.53 ^c	6.47 ± 0.47 ^c	4.41 ± 0.34 ^c
Diabetic + Glibenclamide (600 µg /kg BW)	11.92 ± 0.82 ^{cd}	8.21 ± 0.58 ^a	7.19 ± 0.62 ^a	5.32 ± 0.29 ^d

Values are given as means ± S.D. from six rats in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT). U[@] = µmol of GSH utilized/minute

vitamin E are represented in Table 6, 7 and 8. Diabetic rats showed a significant decrease in these levels when compared with control rats. Conversely, administration of CS as well as glibenclamide to diabetic rats significantly increased the levels to near control values.

Histopathological observation of experimental rat liver, kidney and heart after 45 days of treatment. Figure 6 represents the photomicrographs of H & E staining of liver tissue of control and experimental rats. (A) Normal control rats liver showing

hepatocytes around the central vein with sinusoidal cords around the central vein and portal tracts. (B) Normal control rats treated with CS shows the normal architecture of the hepatocytes. (C) Diabetic control rats show the congestion of sinusoidal dilatation, inflammation of the central vein and focal necrosis in the hepatocytes. (D) Diabetic rats treated with CS shows near normal hepatocytes, mild sinusoidal dilatation with mild inflammation around central vein. (E) Diabetic rats treated with glibenclamide shows regeneration of hepatocytes and a central vein. Figure 7 represents the

Table 6. Effect of CS on GSH in the plasma, erythrocyte and tissues of STZ-diabetic rats.

Groups	Plasma (mg/dL)	Erythrocyte (mg/g of Hb)	Tissue ($\mu\text{g}/\text{mg}$ protein)		
			Liver	Kidney	Heart
Normal Control	27.73 \pm 2.01 ^a	72.67 \pm 4.58 ^a	13.12 \pm 1.08 ^a	12.01 \pm 0.72 ^a	10.29 \pm 0.64 ^a
Normal Control + CS (20 mg/kg BW)	29.50 \pm 2.35 ^a	70.45 \pm 3.65 ^a	13.77 \pm 1.26 ^a	12.43 \pm 1.08 ^a	10.48 \pm 0.69 ^a
Diabetic control	15.41 \pm 1.09 ^b	41.67 \pm 4.55 ^b	08.88 \pm 0.62 ^b	07.23 \pm 0.57 ^b	05.55 \pm 0.42 ^b
Diabetic + CS (20 mg/kg BW)	19.73 \pm 1.32 ^c	62.56 \pm 3.24 ^c	11.56 \pm 1.01 ^c	10.79 \pm 0.82 ^c	8.81 \pm 0.54 ^c
Diabetic + Glibenclamide (600 μg /kg BW)	23.59 \pm 1.66 ^{ac}	68.13 \pm 4.24 ^{ac}	12.58 \pm 1.15 ^{ac}	11.72 \pm 1.02 ^{ac}	9.48 \pm 0.63 ^{ac}

Values are given as means S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 7. Effect of CS on vitamin C in the plasma, erythrocyte and tissues of STZ- induced diabetic rats.

Groups	Plasma (mg/dL)	Erythrocyte ($\mu\text{g}/\text{mg}$ of Hb)	Tissue ($\mu\text{g}/\text{mg}$ protein)		
			Liver	Kidney	Heart
Normal Control	2.28 \pm 0.19 ^a	1.79 \pm 0.11 ^a	0.90 \pm 0.03 ^a	0.88 \pm 0.05 ^a	0.51 \pm 0.02 ^a
Normal Control + CS (20 mg/kg BW)	2.36 \pm 0.12 ^a	1.76 \pm 0.12 ^a	0.96 \pm 0.04 ^a	0.91 \pm 0.04 ^a	0.54 \pm 0.04 ^a
Diabetic control	0.95 \pm 0.05 ^b	0.65 \pm 0.04 ^b	0.63 \pm 0.05 ^b	0.52 \pm 0.02 ^b	0.25 \pm 0.01 ^b
Diabetic + CS (20 mg/kg BW)	1.83 \pm 0.11 ^c	1.38 \pm 0.11 ^c	0.82 \pm 0.02 ^c	0.77 \pm 0.04 ^c	0.42 \pm 0.03 ^c
Diabetic + Glibenclamide (600 μg /kg BW)	1.98 \pm 0.06 ^d	1.60 \pm 0.12 ^d	0.87 \pm 0.06 ^a	0.81 \pm 0.03 ^a	0.44 \pm 0.03 ^c

Values are given as means S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 8. Effect of CS on vitamin E in the plasma, erythrocyte and tissues of STZ-diabetic rats.

Groups	Plasma (mg/dL)	Erythrocyte ($\mu\text{g}/\text{mg}$ of Hb)	Tissue ($\mu\text{g}/\text{mg}$ protein)		
			Liver	Kidney	Heart
Normal Control	2.11 \pm 0.14 ^a	1.65 \pm 0.12 ^a	5.92 \pm 0.27 ^a	3.81 \pm 0.22 ^a	3.95 \pm 0.29 ^a
Normal Control + CS (20 mg/kg BW)	1.93 \pm 0.11 ^a	1.56 \pm 0.10 ^a	5.76 \pm 0.31 ^a	3.86 \pm 0.19 ^a	3.76 \pm 0.25 ^a
Diabetic control	0.40 \pm 0.20 ^b	0.51 \pm 0.22 ^b	3.66 \pm 0.36 ^b	1.73 \pm 0.06 ^b	2.05 \pm 0.17 ^b
Diabetic + CS (20 mg/kg BW)	2.36 \pm 0.22 ^c	2.21 \pm 0.11 ^c	5.03 \pm 0.24 ^c	3.12 \pm 0.27 ^c	2.80 \pm 0.13 ^c
Diabetic + Glibenclamide (600 μg /kg BW)	2.21 \pm 0.18 ^{ac}	1.84 \pm 0.13 ^d	5.35 \pm 0.19 ^a	3.61 \pm 0.23 ^a	3.42 \pm 0.21 ^d

Values are given as means S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

photomicrographs of H & E staining of kidney tissue of control and experimental rats. (A) Normal control rat kidney showing normal tubules and glomeruli. (B) Normal control rats treated with CS present of normal tubules and glomeruli. (C) Diabetic control rats show degeneration of glomeruli and severe tubular degeneration. (D) Diabetic rats treated with CS shows restoring of glomeruli and renal cells. (E) Diabetic rats treated with glibenclamide shows normal tubules with focal glomerular changes. Figure 8 represents the photomicrographs of H & E staining of heart tissue of control and experimental rats. (A) Normal control group shows normal histopathology of the heart, (B) Normal control rats treated with CS shown heart architecture was observed, (C) Diabetic control shown increased interstitial space and distort the intercalated disc, (C) Diabetic rats treated

with CS shown decreased interstitial space and intercalated disc (F) Diabetic rats treated with glibenclamide shown the normal histopathology of heart.

Discussion

STZ-induced diabetes is characterized by as ever loss in BW. The decrease in BW is due to the increased muscle destruction or degradation of structural proteins (Salahuddin and Jalalpure, 2010). When diabetic rats were treated with CS, they showed an improvement in the BW in comparison to the diabetes control treated group, which signifies its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis. Moreover, the ability to protect BW loss seems to be the result of its ability to reduce hyperglycemia.

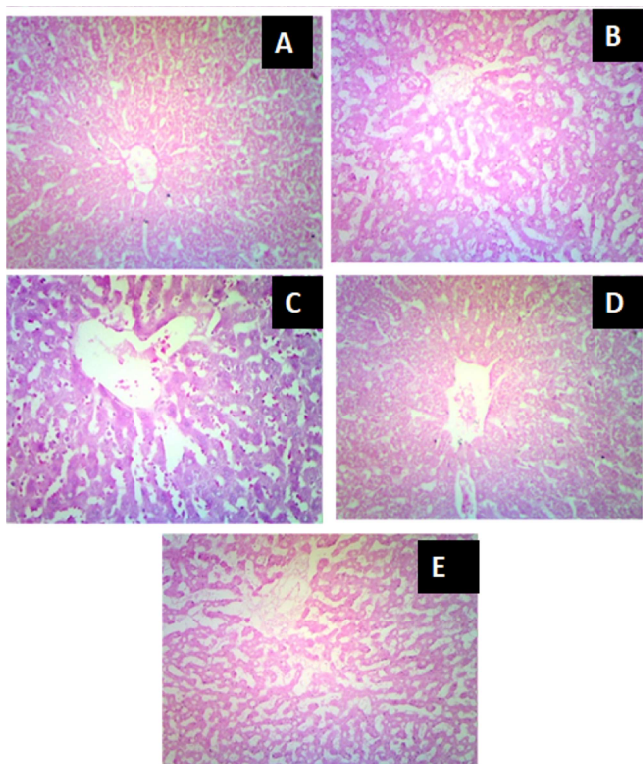


Figure 6. Effect of CS on histopathologic changes in the liver of control and experimental rats. H and E staining, from normal control (A), normal + CS (20 mg/kg) treated rats (B), diabetic control (C), diabetic + CS (20 mg/kg) treated diabetic rats (D) and diabetic + glibenclamide (600 µg/kg) treated diabetic rats (E). Original magnification 40×.

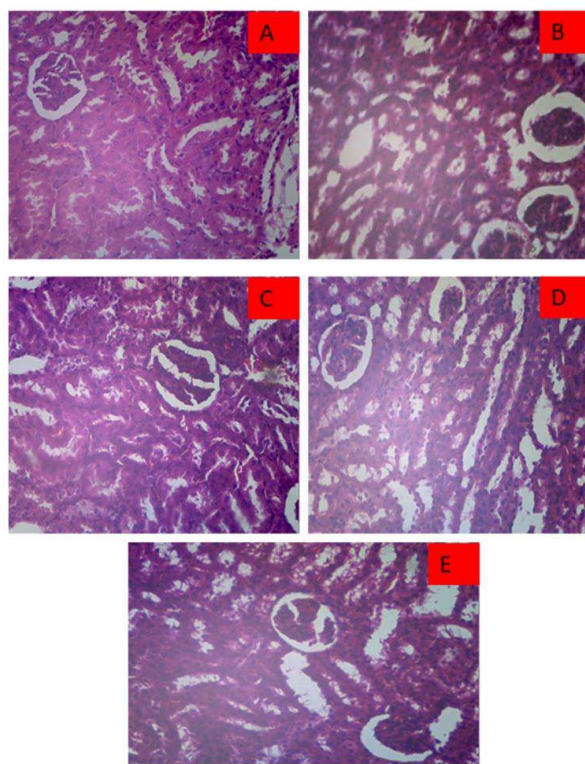


Figure 7. Effect of CS on histopathologic changes in the kidney of control and experimental rats. H and E staining, from normal control (A), normal + CS (20 mg/kg) treated rats (B), diabetic control (C), diabetic + CS (20 mg/kg) treated diabetic rats (D) and diabetic + glibenclamide (600 µg/kg) treated diabetic rats (E). Original magnification 40×.

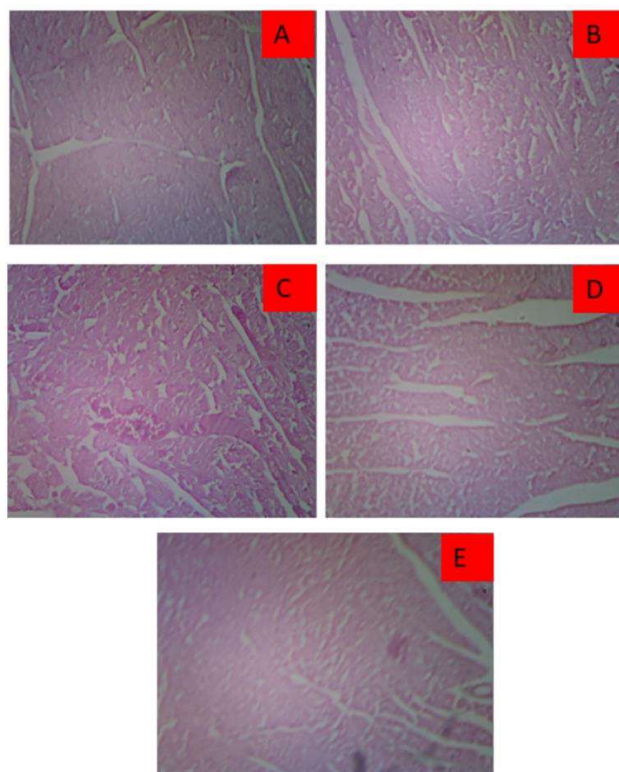


Figure 8. Effect of CS on histopathologic changes in the heart of control and experimental rats. H and E staining, from normal control (A), normal + CS (20 mg/kg) treated rats (B), diabetic control (C), diabetic + CS (20 mg/kg) treated diabetic rats (D) and diabetic + glibenclamide (600 µg/kg) treated diabetic rats (E). Original magnification 40×.

STZ-induced hyperglycemia has been described as a useful experimental model to study the effect of hypoglycemic agents. The mechanism by which STZ brings about its diabetic state includes selective destruction of pancreatic β -cells which make cells less active, leading to a poor sensitivity of insulin for glucose uptake by tissues and hyperglycemia (Burns and Gold, 2007). A low dose of STZ (40 mg/kg b.w) destroys some population of pancreatic β -cells in rats, leading to insufficient insulin secretion causing type 2 diabetes model. We observed an increase in the level of plasma glucose and decrease in the level of plasma insulin. The ability of CS significantly reduces fasting plasma glucose level in diabetic rats, which could be due to its potential to stimulate insulin secretion from the remnant β -cells.

STZ induced DM results in abnormal values for liver enzymes. This phenomenon is attributed to free radical production that causes membrane damage, especially in the liver tissues. In DM, raised activities of transaminase enzymes (ALT and AST) are employed as the evidence of liver injury (Prangthip et al., 2013). The reversal of ALT, AST activities in CS treated diabetic rats towards near normalcy indicate the liver protective nature. These results are in agreement with (Srinivasan and Pari, 2012) who reported that diosmin, a flavanoid improved hepatic enzymes in hepatotoxicity.

Increased free radical observed in diabetic rats is attributed to chronic hyperglycemia that damage, antioxidant defense system (Kumar et al., 2008). Free radicals may also be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radical produced may react with polyunsaturated fatty acids in cell membrane leading to lipid peroxidation. LPO will in turn result in the elevated production of free radicals (Lery et al., 1999). Increased LPO impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Baynes, 1991). Its products are harmful to most of the cells in the body and are associated with a variety of diseases (Xing and Tan, 2000). Our present study showed a significant increase of tissue TBARS and LOOH level in diabetic rats. The increased TBARS content of diabetic rats suggests that peroxidative injury may be involved in the development of diabetic complications. TBARS and LOOH levels in plasma, erythrocyte, liver, kidneys and heart were significantly lower in the CS treated group compared to the diabetic control rats. The above result suggests that the CS may exert antioxidant activities and protect the tissues from the LPO.

The lowered level of cellular oxidative damage is associated with enzymatic and non-enzymatic antioxidant defense systems present in cells. Under chronic hyperglycemia, ROS production is accelerated, which may cause a decreased level of cellular antioxidant enzymes (SOD, CAT, and GPx), thus, promoting the development of antioxidant system (Saravanan and Ponmurugan, 2011). During diabetes, the level of these enzymes decreased due

to the production of ROS (Kaleem et al., 2006).

SOD is an important defense enzyme since it is involved in the direct elimination of ROS and protecting the cells from oxidative damage by converting superoxide radicals into hydrogen peroxide which is further metabolized by catalase to molecular oxygen and water which may have led to diminished toxic effects caused by radical (Ramachandran et al., 2004; McCord et al., 1971). A noticeable decrease of SOD activity in the present study proposes a counterbalance reaction to oxidative stress owing to a rise in endogenous H_2O_2 production. Oral administration CS increased the activity of SOD in diabetic animals. The resulting increased activity of this enzyme clearly shows that CS have free radical scavenging property, which exerts a beneficial action against pathological alterations caused by ROS.

CAT is a heme protein, which catalyses the reduction of H_2O_2 and protects the tissues from highly reactive hydroxyl radicals (Giugliano et al., 1996). The decrease in CAT activity could result from inactivation by glycation of enzyme (Karthikesan et al., 2010). CAT reduces H_2O_2 produced by dismutation reaction. It also prevents the generation of hydroxyl radicals, thereby protecting the cellular constituents from oxidative damage in peroxisomes. The reduced activity of CAT in STZ-treated rats results in the accumulation of H_2O_2 , which produces deleterious effects. In the present study, it was observed that the CS caused a significant increase in the activity of CAT in diabetic rats. This action is predominantly due to the antioxidant nature of CS and could involve a mechanism related to scavenging activity.

GPx is an important antioxidant enzyme whose activities was significantly decreased in diabetic erythrocyte, liver, kidney and heart tissues, indicating impaired scavenging of H_2O_2 and LPO (Friesen et al., 2004). GPx catalyzes the reaction of hydroperoxides with GSH to form glutathione disulfide. GPx uses GSH as a proton donor, converts H_2O_2 to H_2O and molecular oxygen (Winter bourm, 1995). Reduced activity of GPx may result from radical-induced inactivation and glycation of the enzyme (Ewis and Abdel-Rahman, 1995). Decreased GPx activities could be due to excess ROS, which are increased in diabetic rats (Haung and Philbert, 1996). The decreased activities these enzymes may also be due to the decreased availability of their substrate GSH, which has been shown to be depleted during diabetes (Ugochukwn et al., 2004). However, administration of CS reversed the progress of disease and increased the activities of GPx in diabetic rats (Manuel et al., 1999).

GSH is one of the essential compounds for maintaining cell integrity against ROS, as it can scavenge free radicals and

reduce H₂O₂ (Masella et al., 2004). The liver plays a major role in glutathione homeostasis and is the main export organ for glutathione (Townsend et al., 2003). The depletion of GSH below its basal level promotes the generation of ROS and OS with a cascade of effects on the functional and structural integrity of cells and organelle membranes (Deleve et al., 1996). GSH is required for the recycling of vitamin C and acts as a co substrate for GPx which are involved in preventing the deleterious effect of oxygen radicals. Studies have shown that the tissue GSH concentrations of STZ-induced diabetic rats are significantly lower when compared with the control rats (Friesen et al., 2004). Decreased levels of GSH in the liver of diabetic rats may increase susceptibility to oxidative damage. In the present study, the elevation of GSH levels in plasma, erythrocyte, liver, kidneys and heart were observed in the CS treated diabetic rats (Rizk and Sabri, 2009). This indicates that the CS can increase the biosynthesis of GSH and reduce the OS.

Earlier research has shown that diabetes rats have low levels of vitamin C and vitamin E. Supplementation of vitamin E helps to prevent the development of glucose intolerance and diabetes (Ozkan et al., 2005). Vitamin E is a well-known physiological antioxidant and membrane stabilizer. It interrupts the chain reactions of LPO by reacting with lipid peroxy radicals, thus protecting the cell structures against damage. It is suggested to be a beneficial antioxidant for the treatment of diabetic complication in humans as well as in STZ-treated rats (Tiwari et al., 2009). Vitamin C is a major antioxidant that is essential for the scavenging of toxic free radicals in plasma and tissues. The disturbances in vitamin C metabolism in diabetes are might be important in the pathogenesis of diabetic complications (Opoka-Winiarska, 2001). Hyperglycemia has also been shown to inhibit the uptake of dehydro ascorbic acid; the oxidized species of vitamin C. Vitamin C scavenges ferociously destructive hydroxyl radicals. In our study, both the vitamin C and vitamin E significantly decreased in the plasma and tissues of diabetic rats. This could be due to the increased OS. Administration of CS increases the levels of vitamin C and vitamin E by directly scavenging the free radicals which in turn reduced oxidative stress.

In conclusion, the present investigation showed that CS possesses an antioxidant activity and also LPO and enhances its effect on cellular antioxidant defense. This activity contributes to the protection against oxidative damage in STZ induced diabetes.

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