Safranal Treatment Improves Hyperglycemia, Hyperlipidemia and Oxidative Stress in Streptozotocin-Induced Diabetic Rats

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ABSTRACT - Purpose. Clinical research has confirmed the efficacy of several plant extracts in the modulation of oxidative stress associated with diabetes mellitus. Findings indicate that safranal has antioxidant properties. The aim of the present study was the evaluation of possible protective effects of safranal against oxidative damage in diabetic rats. **Methods.** In this study, the rats were divided into the following groups of 8 animals each: control, untreated diabetic, three safranal (0.25, 0.50, 0.75 mg/kg/day)-treated diabetic groups. Diabetes was induced by streptozotocin (STZ) in rats. STZ was injected intraperitoneally at a single dose of 60 mg/kg for diabetes induction. Safranal (intraperitoneal injection) was administered 3 days after STZ administration; these injections were continued to the end of the study (4 weeks). At the end of the 4-week period, blood was drawn for biochemical assays. In order to determine the changes of cellular antioxidant defense systems, antioxidant enzymes including glutathione peroxidase (GSHPx), superoxide dismutase (SOD) and catalase (CAT) activities were measured in serum. Moreover we also measured serum nitric oxide (NO) and serum malondialdehyde (MDA) levels, a marker of lipid peroxidation. **Results.** STZ-induced diabetes caused an elevation ($p < 0.001$) of blood glucose, MDA, NO, total lipids, triglycerides and cholesterol, with reduction of GSH level and CAT and SOD activities. The results indicated that the significant elevation in the blood glucose, MDA, NO, total lipids, triglycerides, cholesterol and reduction of glutathione level and CAT and SOD activity were ameliorated in the safranal–treated diabetic groups compared with the untreated groups, in a dose dependent manner ($p < 0.05$, $p<0.01$, $p < 0.001$). **Conclusion.** These results suggest that safranal has antioxidant properties and improves chemically-induced diabetes and its complications by modulation of oxidative stress**.**

INTRODUCTION

Diabetes mellitus is a metabolic disease as old as mankind and its incidence is considered to be high (4-5%) all over the world. It is defined as a group of disorders characterized by hyperglycemia and altered metabolism of lipids, carbohydrates and proteins. Diabetes mellitus is the principal factor responsible for renal failure, blindness and non-traumatic amputations; the connection between diabetes with poor metabolic control and the high prevalence of mortality due to coronary heart disease, retinopathy, nephropathies and neuropathies has been well established. Furthermore, diabetes mellitus in turn leads to hyperlipidemia to cardiovascular morbidity and mortality. Nevertheless, control of plasma glucose and lipid concentrations inhibit micro-vascular complications. For these considerations the main objective of medical treatment in patients with diabetes is metabolic control (1).

Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both (2). Implication of oxidative stress in the pathogenesis of diabetes is suggested, not only by oxygen free-radical generation, but also due to nonenzymatic protein glycosylation, auto-oxidation of glucose,

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impaired glutathione metabolism, alteration in antioxidant enzymes and lipid peroxides formation (3). In addition to glutathione (GSH), there are other defense mechanisms against free radicals, including the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) whose activities contribute to elimination of superoxide, hydrogen peroxide and hydroxyl radicals (4-8).

However, evidence that antioxidants can prevent cells against the cytotoxic effects of free radicals is still relatively scarce. In the last decade, much attention was focused on the biological and medical properties of an ancient spice, saffron and its ingredients (9). Recent scientific findings have been encouraging, uniformly showing that saffron and its component (safranal) can affect carcinogenesis and have currently been studied extensively as a most promising cancer chemopreventive agent (10, 11). Furthermore safranal could be a candidate to suppress the development of age-induced damage by protecting against oxidative stress and by increasing antioxidant defenses (12). Different hypotheses for the modes of anticarcinogenic and antitumor actions of saffron and its components have been proposed (13, 14); one of them is the inhibitory effect on free radical chain reactions. In fact, safranal is lipid-soluble and might act as a membrane-associated high-efficiency free radical scavenger, which is connected with its antioxidant properties (15). This study focuses on properties of safranal that significantly decreased the levels of free radicals and act as an antioxidant. Also, in modern pharmacological studies, saffron, or its active constituents, has demonstrated beneficial effects in reducing or preventing tissue damages in pathological conditions (16). Recently, it was shown that saffron extract, crocin and safranal also had significant radical scavenging activities and consequent antioxidant activity (17). These studies indicated that safranal was a potent antioxidant and was able to protect body organs against toxic materials in diabetes. However, there are no reports about the possible mechanisms and clinical uses of safranal in diabetes mellitus and much more basic pharmacological and toxicological studies are needed to support clinical trials to evaluate the safety, tolerability and efficacy of safranal. Thus, the present study was designed to evaluate the effect of safranal on a model of diabetes mellitus and its effects on serum lipid profiles and oxidative stress parameters in this process.

MATERIALS AND METHODS

Reagents

All purified enzymes, coenzymes, substrates, standards, buffers, kits and also safranal and other chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, USA).

Animals

Wistar albino rats (2 months; 200 ± 13 g) were bred at the university experimental animal care centre. Animals were maintained under standard environmental conditions and had free access to standard rodent feed and water

Study Design

45 male Wistar albino rats were randomly allotted to five experimental groups (n=8 per group) as follows: group 1, control (C); group 2, diabetic (D); group 3, diabetic and safranal-treated (0.25 mg/kg/day) (D + S1); group 4, diabetic and safranal-treated (0.5 mg/kg/day) (D + S2); group 5, diabetic with safranal-treated (0.75 mg/kg/day) (D + S3). Rats were kept in their own cages at constant room temperature (21 ± 2 °C) under a normal 12-h light/dark cycle with free access to food and water. The animals were housed according to regulations for the Welfare of experimented animals. The study was conducted in Mashhad Medical University Experimental Animal Research Laboratory. Protocols were approved by the Ethical Committee (The Ethical Research Committee of Mashhad University of Medical Sciences). On the first day of the study, the diabetic groups were given streptozocin in a single intraperitoneal (i.p.) injection at a dose of 60 mg/kg for induction of diabetes. Blood was extracted from the tail vein for glucose analysis 72 hours after streptozocin injection. Rats with blood glucose levels higher than 250 mg /dl were accepted as being diabetic. In the control groups (C), physiological saline (i.p.) was injected as vehicle. Safranal (i.p. injection) was administered to the treatment groups from 3 days after STZ administration; these injections were continued to the end of the study (for 4 weeks). Blood glucose level and body weights were recorded at weekly intervals. At the end of the 4-week period, animals were killed by pentobarbital

overdose (150 mg/kg, i.p.), and blood was subsequently collected from the retro orbital sinus. Blood and sera were separated by centrifugation at 3000 rpm for 10 min for glucose, lipid profile, malondialdehyde (MDA), GSH, CAT, SOD and nitric oxide (NO) assays.

Measurement of Blood Glucose

Glucose concentrations were measured with the Ames One Touch glucometer (One-Touch Basic; Lifescan, Johnson and Johnson, New Brunswick, NJ) in rat tail vein blood. Blood glucose was estimated using the diagnostic kits (Pars Azmoon kit, IRI) on an automatic analyzer (Abbott, model Alcyon 300, USA).

Measurement of Serum Lipid Profile

The concentrations of glucose, total lipids, triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol in serum were estimated by using diagnostic kits (Pars Azmoon kit, IRI) on an automatic analyzer (Abbott, model Alcyon 300, USA)

Measurement of Serum Reduced Glutathione (GSH)

GSH was determined by the method of Ellman (1959). To the homogenate was added 10% of trichloracetic acid (TCA) and centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm (18).

Measurement of Serum Thiobarbituric Acid Reactive Species (TBARS)

The formation of lipid peroxides was measured in the serum. The formation of MDA, an end product of fatty acid peroxidation was measured spectrophotometrically at 532 nm by using a thiobarbituric acid reactive substance (TBARS), essentially by the method of Genet et al. (2002). Results are expressed as nmole of MDA formed/mg protein (19).

Measurement of Serum Superoxide Dismutase (SOD) activity

The activity of SOD was determined by a method using inhibition of pyrogallol autoxidation at pH 8 (20). The specific activity of SOD is expressed as units per mg protein per minute.

Measurement of Serum Catalase (CAT) Activity

Catalase activity was assayed by H_2O_2 consumption, following Aebi's(1984) method and modified by Pieper et al. (1995). Briefly, ethanol was added (1:100 v/v) to the supernatants and incubated for 30 min in an ice bath. 1% Triton X-100 (1:10 v/v) (Sigma) was then added to the homogenates. This solution was placed in an ice bath for an additional 15 min. 500 µl of this solution was placed into a glass cuvette and 250 µl of 30 mM H_2O_2 (Sigma) in phosphate buffer (50mM, pH 7.0) was then added to start the reaction. After 15 s the absorbance at 240 nm was read every 15 s for 45 s. Catalase activity was expressed as mmol H_2O_2 /min/ mg protein. An enzyme unit was defined as the amount of enzyme that catalyzes the release of one µmol of H_2O_2 per min. Specific activity was calculated in terms of units per mg of protein. The assay was performed at 25 °C (21, 22).

Measurement of Serum Nitric Oxide (NO)

NO levels are determined spectrophotometrically by measuring the accumulation of its stable degradation products, nitrite and nitrate. The serum nitrite level was determined by the Griess reagent according to Hortelano et al. (1995). The Griess reagent, a mixture (1:1) of 1% sulfanilamide in 5% phosphoric acid and 0.1% 1-naphthyl ethylenediamine gives a red violent diazo color in the presence of nitrite. The color intensity was measured at 540 nm. Results were expressed as µmol/l using a $NaNO_2$ calibration graph (23).

Measurement of Serum Protein Content

Protein content was determined by the method of Lowry et al. (1951), using bovine serum albumin (BSA) as a standard (24).

STATISTICAL ANALYSIS

All experiments were carried out at least in duplicate. Each group consisted of eight rats. One-way analysis of variance (ANOVA) was performed and Tukey post hoc test was used for multiple comparisons. Statistical analyses were performed using the InStat 3.0 program. The results are expressed as mean ± SEM. The results originated

from analysis of serum. Linear correlation tests were also performed. Differences of $p<0.05$ were considered significant.

RESULTS

During the experimental period (4-week), there was a weight loss in untreated diabetic rats compared with normal healthy rats (control) ($p< 0.001$) (Table 1). However, at the end of the experimental treatment period there was an elevation in body weight of safranal (0.5 and 0.75 mg/kg) - treated diabetic rats compared to untreated diabetic rats ($p<0.05$ and $p<0.01$, respectively), but the elevated body weight in the safranal (0.25 and 0.5 mg/kg)–treated diabetic groups was significantly lower than the control group ($p < 0.01$) (Table 1). At the highest safranal dose (0.75 mg/kg) there were no significant differences in body weight compared to control rats after the 4-week experimental period (Table 1). STZ-diabetic rats exhibited significant ($p < 0.001$) hyperglycemia compared to the control rats (Figure 1). After 4 weeks the safranal dose-dependently decreased blood glucose levels in the diabetic rats compared to the untreated diabetic rats (Figure 1). Safranal (0.25 mg/kg/day) significantly decreased glucose in STZ diabetic rats only at the 4th week of the study ($p<0.05$), while at 0.5 mg/kg/day safranal reduced blood glucose significantly at the 3rd and 4th week from induction of diabetes compared with untreated diabetic rats ($p<0.05$ and $p<0.01$, respectively). At the highest dose of safranal (0.75 mg/kg/day) blood glucose of diabetic rats was significantly reduced beginning from the first week of treatment ($p < 0.05$, $p <0.01$, $p < 0.001$) (Figure 1).

STZ-injected rats showed significant increases in the serum levels of total lipids, triglycerides, total cholesterol and LDL-cholesterol (LDL-C), and significantly decreased serum HDL-cholesterol (HDL-C) level compared to the control group (Figure 2). Safranol dose-dependently reduced the

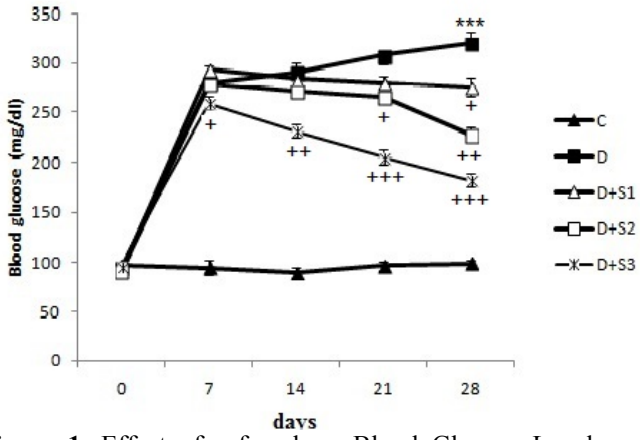
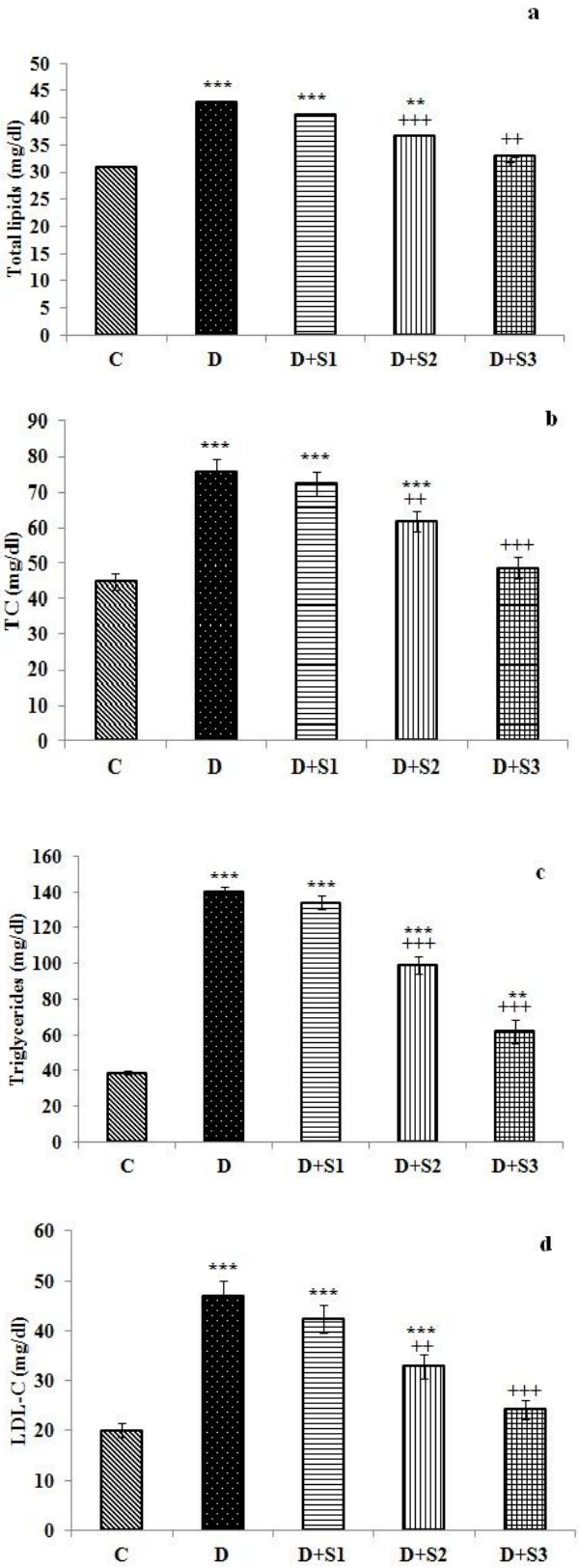


Figure 1. Effect of safranal on Blood Glucose Level (mg/dl). Control (C), untreated diabetic rats (D), safranal (0.25 mg/kg/day) - treated diabetic (D + S1), safranal (0.5 mg/kg/day) - treated diabetic (D + S2) and safranal (0.75 mg/kg/day) - treated diabetic (D + S3) rats during 4 weeks of study (n=8, for each group).Values are the mean ± SEM. Statistical significance for the difference between the data of the control group vs other groups: ***; p <0.001. Statistical significance for the difference between the data of untreated diabetic group vs treated groups: +; p <0.05, ++; p <0.01, +++; p <0.001.

Table 1. Effect of Safranal on Body Weight in STZ-treated Diabetic Rats. Control (C), untreated diabetic rats (D), safranal (0.25 mg/kg/day) - treated diabetic rats (D + S1), safranal (0.5 mg/kg/day) - treated diabetic rats (D + S2) and safranal (0.75 mg/kg/day) - treated diabetic rats(D + S3) during 4 weeks of study

Days 28	0	7	14	21
C Body weight (g) 265 ± 15	180 ± 12	218 ± 10	231 ± 13	245 ± 14
D Body weight (g) 150 ± 15***	175 ± 12	171 ± 12***	164 ± 10***	158 ± 10***
D+S1 Body weight (g) 183 ± 15 **	178 ± 10	174 ± 6**	176 ± 8**	180 ± 13**
D+S2 Body weight (g) 200 ± 15 **,+	179 ± 9	183 ± 9*	190 ± 11*	192 ± 9**, +
D+S3 Body weight (g) 219 ± 15++	182 ± 15	187 ± 11	200 ± 14	211 ± 21+

Each measurement was done at least in triplicate and the values are the means ± SEM for eight rats in each group.
Significantly different from normal control (Group C) rats (*; $P < 0.05$, **; $P < 0.01$, ***; $P < 0.001$).
Significantly different from STZ-treated (Group D) rats (+; $P < 0.05$, ++; $P < 0.01$)



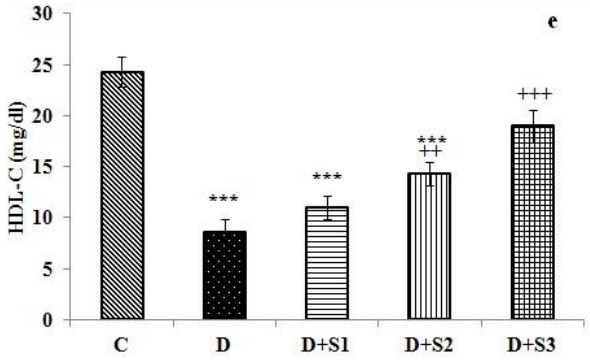


Figure 2. Effect of Safranal on Plasma Lipid Profiles (mg/dl). a: total lipid, b: total cholesterol (TC), c: triglycerides, d: LDL-C, e: HDL-C, in control (C), untreated diabetic rats (D), safranal (0.25 mg/kg/day) - treated diabetic (D + S1), safranal (0.5 mg/kg/day) - treated diabetic (D + S2) and safranal (0.75 mg/kg/day) - treated diabetic (D + S3) rats during 4 weeks of study (n=8, for each group).Values are the mean ± SEM. Statistical significance for the difference between the data of the control group vs other groups: **; p <0.01, ***; p <0.001. Statistical significance for the difference between the data of untreated diabetes group vs treated groups: ++; p <0.01, +++; p <0.001.

serum levels of total lipids, triglycerides, total cholesterol and LDL-C, and increased serum HDL-C level during the experimental period. At the highest safranal dose (0.75mg/kg/day) there was no significant difference in total lipid, cholesterol, LDL-C and HDL-C levels between the STZ-treated rats and the control rats (Figure 2).

STZ injection produced significant changes in oxidative stress parameters in the serum of diabetic rats 4 weeks after diabetes induction, as shown by increased lipid peroxidation product (MDA) and decreased GSH compared to control group (p< 0.001) (Figure 3). Safranal (0.5 and 0.75 mg/kg/day) significantly decreased the serum MDA and increased glutathione (GSH) compared with the untreated diabetic groups (p< 0.01and p< 0.001, respectively).

The increase in GSH was dose-dependent, from 0.2 ± 0.03 at 0.5 mg/kg to 0.37 ± 0.06 at 0.75 mg/kg (p<0.05). In addition, the MDA levels in animals which were administrated with high safranal concentration were significantly lower than the diabetic rats receiving low safranal concentration

(0.24 ± 0.06 vs 0.69 ± 0.08, p<0.001) and MDA levels in diabetic rats treated with the medium concentration (0.5 mg/kg) were significantly lower than the low safranal treated diabetic rats (0.43 ± 0.03 vs 0.69 ± 0.08, p<0.05). Furthermore, there was no significant difference between MDA and GSH levels between control rats and high dose safranal treated diabetic rats (Figure 3).

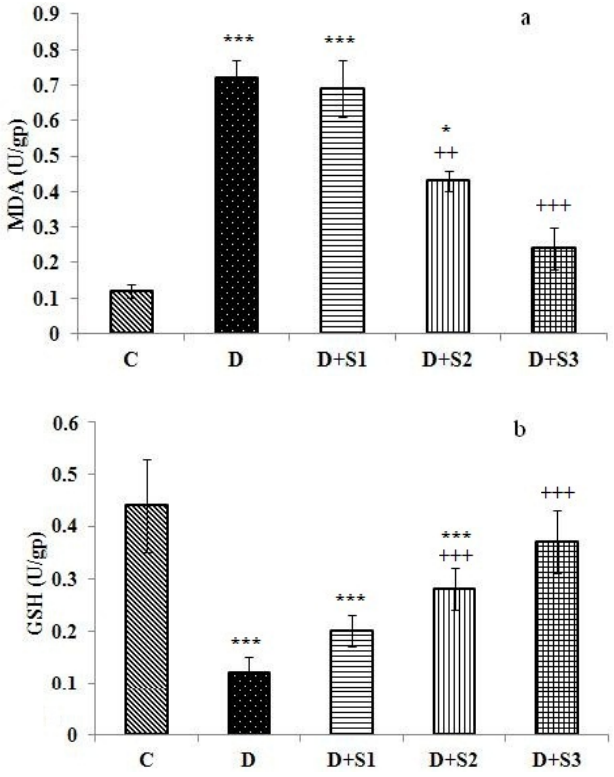


Figure 3. Effect of Safranal on MDA and GSH. Serum MDA (a) and GSH (b) levels (U/gp) in control (C), untreated diabetic rats (D), safranal (0.25 mg/kg/day) - treated diabetic (D + S1), safranal (0.5 mg/kg/day) - treated diabetic (D + S2) and safranal (0.75 mg/kg/day) - treated diabetic (D + S3) rats during 4 weeks of study (n=8, for each group).Values are the mean ± SEM. Statistical significance for the difference between the data of the control group vs other groups: *; p <0.05, ***; p <0.001. Statistical significance for the difference between the data of untreated diabetes group vs treated groups: ++; P<0.01, +++; P<0.001.

SOD and CAT activities were decreased in the STZ-diabetic group compared with the control group (p< 0.001) (Figure 4). Safranal (0.25, 0.5 and 0. 75 mg/kg/day) treated diabetic rats had significantly increased serum SOD compared with the untreated diabetic rats (p< 0.05, p<0.001 and p< 0.001, respectively). In addition, there was not a

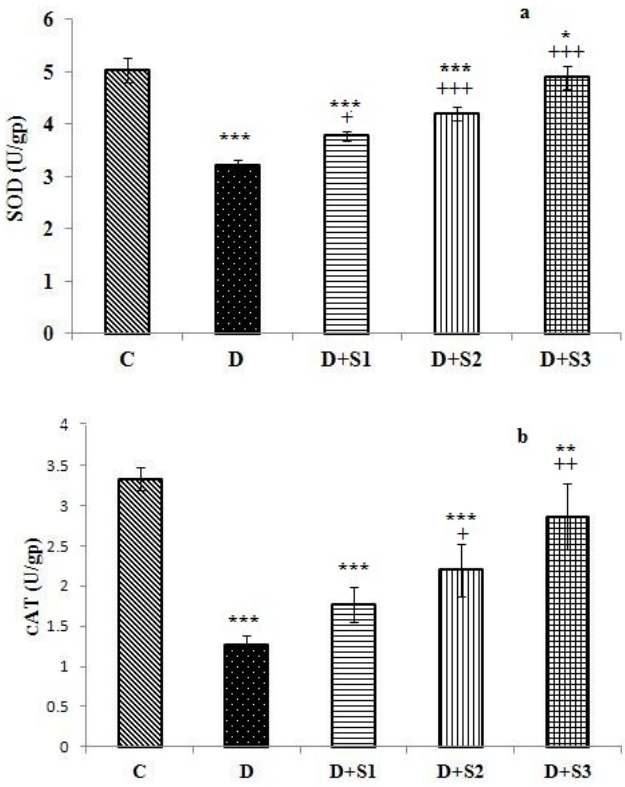


Figure 4. Effect of Safranal on Serum SOD (a) and CAT (b) (U/gp). Control (C), untreated diabetic rats (D), safranal (0.25 mg/kg/day) - treated diabetic (D + S1), safranal (0.5 mg/kg/day) - treated diabetic (D + S2) and safranal (0.75 mg/kg/day) - treated diabetic (D + S3) rats during 4 weeks of study (n=8, for each group).Values are the mean ± SEM. Statistical significance for the difference between the data of control *vs* other groups: *; p <0.05, **; p <0.01, ***; p <0.001. Statistical significance for the difference between the data of diabetes *vs* treated groups: +; p <0.05, ++; p<0.01, +++; p<0.001.

significant difference between diabetic rats treated with high safranal concentration (0. 75 mg/kg/day) and the control group. The CAT activities in safranal (0.5 and 0.75 mg/kg/day)–treated diabetic rats were significantly higher than the untreated diabetic group (p< 0.05 and p< 0. 01, respectively) (Figure 4). The CAT levels in animals which had been administrated high safranal dose were significantly greater than the diabetic rats receiving the low safranal dose (2.86 ± 0.41 vs 1.77 ± 0.22, P<0.05). The effects on SOD and CAT were dose-dependent, the activity of serum SOD in animals administrated with the high safranal dose (0.75 mg/kg) being significantly greater than that in the rats receiving low safranal concentration (0.25

mg/kg) (4.9 ± 0.22 vs 3.78 ± 0.09, p<0.001); CAT activities in diabetic rats treated with medium concentration (0.5 mg/kg) were significantly higher than low safranal treated diabetic rats (4.2 ± 0.13 vs 3.78 ± 0.09, p<0.05) (Figure 4).

STZ injection produced a significant increase of NO compared to the control group (P< 0.001) (Figure 5). Safranal (0.25, 0.5 and 0. 75 mg/kg/day) - treated diabetic rats had significantly decreased serum NO compared with the untreated diabetic rats (p< 0.001). The effects on NO were dose-dependent, serum NO levels of animals having been administrated high safranal dose being significantly lower than those in the diabetic rats receiving the low safranal dose (6.9 ± 0.08 vs 9.9 ± 0.17, p<0.001); and NO levels in diabetic rats treated with a medium concentration (0.5 mg/kg) were significantly lower than the low safranal-treated diabetic rats (8.5 ± 0.21 vs 9.9 ± 0.17, p<0.001).

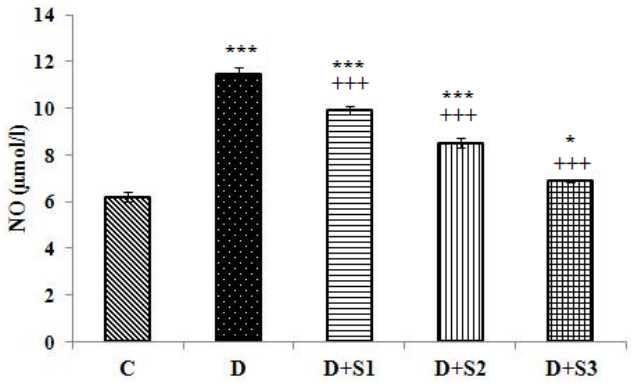


Figure 5. Effect of Safranal on Serum NO (µM/l). Control (C), untreated diabetic (D), diabetic and (0.25 mg/kg/day) safranal (0.25 mg/kg/day) - treated diabetic (D + S1), safranal (0.5 mg/kg/day) - treated diabetic (D + S2) and safranal (0.75 mg/kg/day) - treated diabetic (D + S3) rats during 4 weeks of study (n=8, for each group).Values are the mean ± SEM. Statistical significance for the difference between the data of control *vs* other groups: *; p<0.05, ***; p<0.001. Statistical significance for the difference between the data of untreated diabetes *vs* treated groups: +++; p<0.001.

DISCUSSION

The results of the present study indicate that intraperitoneal injection of safranal significantly ameliorated the adverse metabolic effects in rats treated with STZ.

Safranal injection after STZ treatment resulted in lower serum glucose levels, and improved lipid profile as well as body weight as compared with rats treated with STZ alone. In addition, safranal treatment of diabetic rats were observed to significantly recover the decreased levels of GSH, CAT and SOD, as well as the decline in the lipid profiles and the oxidative stress parameters MDA and NO compared with untreated diabetic group. These results are compatible with the findings reported by other investigators using saffron and its active constituent, crocin, which improved oxidative damage due to STZ and alloxan induced diabetes in rats (25-27). The effects observed in the present study are consistent with amelioration of oxidative stress in the STZ diabetic rats. The results are similar to the findings reported by other investigations using STZ to induce diabetes in rats, accompanied by an increase in the susceptibility to lipid peroxidation (28, 29).

Oxidative stress plays an important role in the pathogenesis and the complications of diabetes. Hyperglycemia results in overproduction of oxygen free radicals, which contributes to the progression of diabetes and its complications (28).

Several studies have shown that STZ produces imbalance between plasma oxidant and antioxidant content, resulting in the development of diabetes mellitus and its complications. STZ enters the β cell via the low affinity glucose protein-2 transporter, inducing the selective destruction of the insulin producing islet β cells and, in turn, a drastic reduction in insulin production. The cytotoxic effect of STZ could result from the combined action of DNA alkylation (30) and the cytotoxic effects of ROS (31) or the intracellular liberation of NO (32, 33) directly or indirectly through the formation of peroxynitrite. Thus, in the STZ-treated model of diabetes in rats, the diabetes that develops after the destruction of the pancreatic islets is associated strictly with the induction of oxidative and nitrosative stress, both systemically and locally. Furthermore, insulin resistance leads to adipocyte dysfunction and decrease in inhibition of the release of free acid into the plasma (34). The consequent increase in circulating free fatty acids (FFA) contributes to an increase in hepatic triglycerides. This increased hepatic triglyceride content contributes to an increase in the production of more

atherogenic small dense LDL. These findings may constitute the predominant mechanism in STZ-induced complications of hyperglycemia (35).

In our experimental model of diabetes mellitus, it was seen that STZ administration led to a significant decrease in plasma GSH content and CAT and SOD activities, accompanied by a significant increase in MDA, NO and overall lipid profile, indicating an increased plasma oxidative stress which may also occur in other tissues in STZ-treated rats. MDA is an aldehydic product of lipid peroxidation that reacts quickly with biomolecules, such as proteins, lipids and nucleic acids and leads to cellular dysfunction, including liver and β - pancreatic cells, hence disturbing glucose regulation (36). The enhanced interaction of superoxide with NO in the oxidant environment of diabetes increases the formation of peroxynitrite, which in turn, oxidizes tetrahydrobiopterin (BH4, a cofactor for eNOS), leading to eNOS uncoupling (37). The diabetic condition favors the uncoupling of eNOS (38) and the increase in nitric oxide synthase (iNOS) expression (39), with a diminished NO bioavailability. Increasing evidence suggests that oxidative stress and changes in nitric oxide formation or action play major roles in the onset of diabetic complications such as atherosclerosis.

The improvement of variable measurements in STZ-diabetic rats after safranal treatment might suggest a protective influence of safranal against STZ action that could be mediated through suppression of oxygen free radicals induced by STZ. A stimulating effect of the formation of GSH by safranal was observed in the present study. The GSH reacts with free radicals and is a crucial substrate for glutathione peroxidase and glutathione-S-transferase, which take part in the cellular defense mechanisms against intermediate oxygen products (40, 41). It may be relevant that the ratio of GSH/GSSG plays a critical role in glucose homeostasis of diabetes because thiol groups are important in intracellular and membrane redox state (41) Safranal induced an increase in plasma GSH content, which might enhance the GSH/GSSG ratio and decrease lipid peroxidation, hence aldehydic concentration, and therefore improve serum glucose regulation. Parallel to these events, plasma CAT and SOD activity were increased in safranal treated diabetic rats as compared with untreated diabetic rats. SOD is responsible for removal of superoxide radicals and catalase decomposes hydrogen peroxide to water and oxygen; thus, these enzymes may contribute to the modulation of redox state of plasma (42). This observation perfectly agrees with those of Rahbani et al (2011) who demonstrated hypoglycemic and antioxidant activity of ethanolic saffron in STZ-induced diabetic rats. Similarly, Kianbakht et al (2011) observed that saffron, crocin and safranal may effectively control glycemia in the alloxan-induced diabetes rat model (16, 26).

The results of the present study indicate that safranal is also effective to prevent hyperlipidemia due to diabetes. Safranal inhibits elevation of the serum lipid profile by controlling oxidative and nitrosative systems. Saffron has been reported to help lower cholesterol and keep cholesterol at healthy levels (43). In agreement with previous studies, one mechanism for the hypolipidemic effect of saffron extract and its constituents has proposed inhibitory effects on the levels of MDA, oxygen free radical and activating superoxide dismutase (44).

Reactive oxygen species are increased by hyperglycemia. Hyperglycemia, which occurs during diabetes (both type 1 and type 2) and, to a lesser extent, during insulin resistance, causes oxidative stress. Elevated glucose in diabetes may also react with lipids, resulting in the generation of ROS (45). Diabetes mellitus often includes lipid abnormalities such as elevated LDL-C and cholesterol, and such effects were shown in this *in vivo* study. These abnormalities may be further exacerbated by the increased oxidizing environment which enhances the formation of oxidized LDLs (oxLDLs), glycated LDL and oxysterols (formed from the oxidation of cholesterol). It has been suggested that these oxidized lipid products can bind to specific receptor proteins or activate inflammatory proteins which generate ROS (45). The import of oxLDLs in the vascular wall is an important mechanism by which ROS and oxidative stress induce atherosclerosis (46). In our study, pro-oxidant-antioxidant balance was evaluated by measuring MDA and NO levels and enzymatic antioxidants in the diabetic rats. Increased MDA, NO and decreased SOD, GST and CAT activities indicated that the balance changed towards pro-oxidation in STZ-induced diabetic rats. Safranal treatment of diabetic rats, which restored SOD, GST and CAT activities, may be due to a decrease in free radical generation by safranal and increased

antioxidant defenses (12). Safranal, a monoterpene aldehyde major constituent of the essential oil of saffron (47), showed good antioxidant activity *in vivo* (48, 49). Safranal administration regulates the expression of antioxidant related genes, and consequently oxidant levels in diabetic animals. In biological systems safranal shows its antioxidant impact via stabilizing membranes (50), inhibiting ROS and reducing peroxidation of unsaturated membrane fatty acids (50). Safranal has been known to function as a radical scavenger inhibiting lipid peroxidation *in vivo* and *in vitro*. It also has been reported that safranal supplementation could decrease lipid peroxidation (47, 51). Safranal modulates antioxidant gene expression and also up-regulates mitochondrial antioxidant genes, leading to a lower mitochondrial oxygen radical generation, which may be responsible at least in part for the improved hyperglycemia, hyperlipidemia and oxidative stress seen in the present study in STZ-induced diabetic rats. Additionally, increased SOD, GST and CAT levels after safranal treatment may play an additional role in decreasing oxidative stress. The present study elucidated the anti-hyperglycemia, hypolipidemic and protective potential of safranal treatment on activities of antioxidant enzymes (SOD, GST, CAT), lipid peroxidation levels and serum NO content in the STZ diabetic rat model.

In summary, the findings in this study show that safranal treatment demonstrates a protective effect in the STZ model of diabetes and its complications by modulation of oxidative stress. Although detailed studies are required for the evaluation of the exact protective mechanism of safranal against diabetic complications in animal models and humans, these *in vivo* experimental findings demonstrate that safranal exhibits antidiabetic effects in the rat diabetic model by potentiating the antioxidant defense system. The findings support the potential efficacy of safranal for diabetes management.

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