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# Hypoglycemic, antioxidant and hepato- and nephroprotective effects of *Teucrium orientale* in streptozotocin diabetic rats

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## Abstract

Hyperglycemia causes protein glycosylation, oxidation and alterations in enzyme activities, which are the underlying causes of diabetic complications. This study was designed to examine the effects of methanol extract from *Teucrium orientale* (TO) on streptozotocin (STZ) induced diabetic rats by measuring glycemia, malondialdehyde (MDA), total sulfhydryl groups (TSH) and activity of some antioxidant enzymes in liver and kidney. This study demonstrated that blood glucose and MDA levels were significantly increased ($p<0.05$), whereas catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and TSH levels were significantly decreased ($p<0.05$) in liver and kidneys of STZ induced diabetic rats. The administration of TO to diabetic rats at a dose of 200 mg $kg^{-1}$ bw resulted in a significant elevation of TSH content and CAT, GPx, SOD activities associated with a reduction in blood glucose and MDA levels in liver and kidneys of TO treated rats in comparison with diabetic group. We conclude that administration of methanol extract of TO may be effective for correcting hyperglycemia and preventing diabetic complications.

Key words: Hypoglycaemia, Teucrium orientale, antioxidant enzymes, streptozotocin

## Introduction

Increased free radical generation and oxidative stress may play a pivotal role in pathogenesis of diabetes mellitus (DM) and its late complications (1). DM is related to a group of metabolic alterations that continues to be a major health problem worldwide. It is characterized by absolute or relative deficiencies in insulin secretion, action or both associated with chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism (2). Indeed, there is widespread acceptance of the possible role of reactive oxygen species (ROS) generated as a result of hyperglycemia, a key clinical manifestation of diabetes mellitus, in causing many of the secondary complications of diabetes such as nephropathy, retinopathy, neuropathy and cardiomyopathy (3-5).

Induced diabetes performed in experimental animals using drugs as streptozotocin (STZ) is belong to the insulin-dependent diabetes because of its ability to destroy the β-cells of pancreas possibly by an excessive generation of reactive oxygen species such as, $H_2O_2$, $O_2^-$ and $OH^-$ (6).

Plants with an antioxidant property still remain a major source for drug discovery in spite of the great development of synthetic molecules. Consequently, the uses of traditional plants in the treatment of various diseases as diabetes have been flourished (7).

*Teucrium orientale* (TO) L. var. *orientale* is a wild aromatic plant belonging to the labiatae family. According to a recent survey in the southeastern region of Turkey (Erzurum), TO possess potent antioxidant and DPPH radical scavenging activities that these activities related to the presence of flavonoids and other phenolic compounds (8).

However, some species of s *Teucrium* genus belonging to genus have been reported to possess hypoglycemic activity in folk medicine and professional studies (9). The present study was undertaken in STZ-diabetic rats in order to investigate [1] mechanism of hypoglycemic effect and [2] to evaluate the antioxidant activity of *T. oriental* methanol extract in liver and kidneys of treated rats.

## Methods

### *Chemicals and reference marker compound*

Streptozotocin (STZ) was purchased from Pharmacia and Upjohn (USA),trichloroacetic acid (TCA) were obtained from Sigma (St. Louis, MO). 5, 5´-dithiobisnitrobenzoic acid (DTNB), hydrogen peroxide ($H_2O_2$), thiobarbituric acid (TBA), solvents and other salts were obtained from Merck (Darmstadt, Germany).

### *Plant material*

Aerial parts (stems, leaves, flowers) of *T. orientale*, growing wild in Iran, were collected in May 2010 from Mishow-Dagh, Marand (North-Weast of Iran). The aerial parts of the plant were gently washed in tap water and completely dried under room temperature (25±2°C) for 2 weeks protected from direct heat or sunlight.

### *Preparation of T. oriental methanol extract (TOME)*

The powdered plant material (160 g) was extracted with methanol (MtOH) (90%), at room temperature (RT) overnight. The extraction was repeated three-times and the solvent was evaporated in vacuum, and dried extracts were stored at 4°C until use (10).

### *Animals*

The study was conducted on thirty matured Wistar male albino rats, were obtained from the experimental animal care centre of Faculty of Pharmacy, Tehran University of Medical Science, 15 weeks old, weighing 200 -250 g which were housed in colony cages (four rats per cage) at an ambient temperature of 25 ± 2°C with 12 h-light and 12 h-dark cycle. The rats were fed normal diets purchased commercially from vendors and also had free access

to water ad libitum. The animals were allowed to acclimatize to the laboratory environment for one week and then randomly divided into various groups.

*Induction of experimental diabetes*

STZ-induced diabetes mellitus was produced in a batch of normoglycemic male Wistar rats (fasting blood glucose level of 75 ± 5 mg/dl). STZ freshly dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, was immediately injected intraperitoneally (60 mg/kg) (11). This single dose of streptozotocin produced type-I diabetes mellitus after 24 h of injection and this diabetic state is maintained throughout the experimental schedule.

*Treatment of animals*

Rats were divided into four groups of eight rats each: Group I (C): normal control rats. Group II (STZ): diabetic rats, received STZ in single dose (60 mg/kg bw, intraperitoneal way). Group III (TOME): TOME treated rats, received only TOME (200 mg/kg bw, oral gavage) for 21 days. Group IV (STZ + TO): TOME-treated diabetic rats received by oral gavage, 3 days after STZ treatment, 200 mg/kg bw of TOME for 21 days.

The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The treatment was started on the third after STZ injection and continued for 21 days.

*Tissue preparation*

At the end of experiment, animals were anesthetized by infusion of ketamine (60 mg/kg) and xylazine (10 mg/kg). After surgery of animals each liver and kidney was quickly removed, cleaned and washed in ice-cold saline solution. It was finely minced and homogenized in 4 ml of 100 mM potassium phosphate buffer (pH 7.4), containing 150 mM KCl and 0.1 mM EDTA and centrifuged at 8000g for 20 min at 4°C. The supernatant was used to assay malondialdehyde (MDA), total thiol (TSH) contents and activity of antioxidant enzymes.

*Estimation of blood glucose*

Blood samples for blood sugar determination were obtained from the tail tip of fasted rats. On days 2, 7, 14 and 21 of the experiment, blood glucose level was determined using a glucosemeter ACCUTREND GC (Boehringer Mannheim, Germany).

*Estemination of lipid peroxidation (LPO) in kidney and liver homogenate*

Lipid peroxidation (LPO) in liver and kidney homogenates was studied by measuring thiobarbituric acid reactive substances (TBARS) according to Ohkawa (12).The results are expressed as mM/mg protein of MDA formed in liver and kidney homogenates.

*Estimation of total sulfhydryl groups in kidney and liver homogenate*

Sulfhydryl groups were measured according to the method of Sedlak and Lindsay (13) using DTNB. Briefly, 50 µl of samples was mixed with 1 ml of Tris-EDTA buffer (0.25 M Tris base, 20 mM EDTA; pH 8.2) ($A_1$). Subsequently, 20 µl of a DTNB solution (4 mg/ml in absolute methanol) was added and the samples were incubated for 15 min ($A_2$). The absorbance of the sample at 412 nm was measured and subtracted from a DTNB blank and a blank containing the sample without DTNB (B). The molar absorption coefficient (ε) of 13,600 $M^{-1}cm^{-1}$ was used for quantification.

$$\text{Reduced thiols (\%)} = (A_2\text{-}A_1\text{-}B) \times 1.57 \text{ mM}$$

*Estimation of antioxidant enzymes*

Catalase (CAT) activity was determined according to the Aebi method (14). The rate of $H_2O_2$ decomposition was followed by monitoring absorption at 240

nm. One unit of CAT activity is defined as the amount of enzymes required to decompose 1µmol of hydrogen peroxide in 1 min. The enzyme activity was expressed as µmol $H_2O_2$ consumed/min/mg protein. Superoxide dismutase (SOD) activity was estimated according to the method of Winterbourn (15).The developed blue color in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as U/mg protein. GPx activity, which is coupled to NADPH utilization and the production of $NADP^+$, was measured spectrophotometrically at 340 nm (16). The specific activity of GPx was expressed as millimoles of NADPH consumed per minute per mg protein (i.e. U/mg protein).

*Statistical Analysis*

Values are reported as mean ± SD. One-way ANOVA and Tukey post hoc multicomparison tests were used to analyze data of experimental and control groups. P values less than 0.05 were considered significant.

## Results

Table 1 shows the levels of serum glucose in normal and STZ-induced diabetic rats. Serum glucose level in diabetic rats elicited a significant rise from 93 to 567 mg/dl as compared to the control rats ($P < 0.01$). On the contrary, after 3 weeks, the blood glucose levels of the TO- treated diabetic rats were significantly lower than diabetic rats (2718± mg/dl and 525± 10 mg/dl, respectively; $P < 0.05$). Serum levels of glucose in TO- treated diabetic group were found non-significant change as compared with the controls.

Table 2 and 3 show the levels of lipid peroxidation markers (MDA), total sulfhydryl groups (TSH), indices of protein oxidative damage in the liver and kidney tissue of normal and experimental animals. MDA concentration, were significantly increased in diabetic group when compared to the control group ($p < 0.01$). Oral administration of TO to the diabetic rats showed a significant decrease in MDA in the liver and kidney tissues by 71% and 79%, respectively. Moreover, TSH levels were significantly decreased in the diabetic group when compared to the control group ($p < 0.01$). On the other hand, administration of TO to the diabetic rats showed a significant increase in TSH levels in the liver and kidney tissues of diabetic rats by 21% and 38%, respectively.

Activities of SOD, CAT and GPx, were significantly decreased in the diabetic group when compared to the control group ($p < 0.05$). Diabetic rats, administered with TO, showed a significant increase in the activities of SOD, CAT and GPx by 16%, 39% and 28%, in liver tissue and by 39%, 33% and 8% in kidney tissue respectively (Figures 1 and 2).

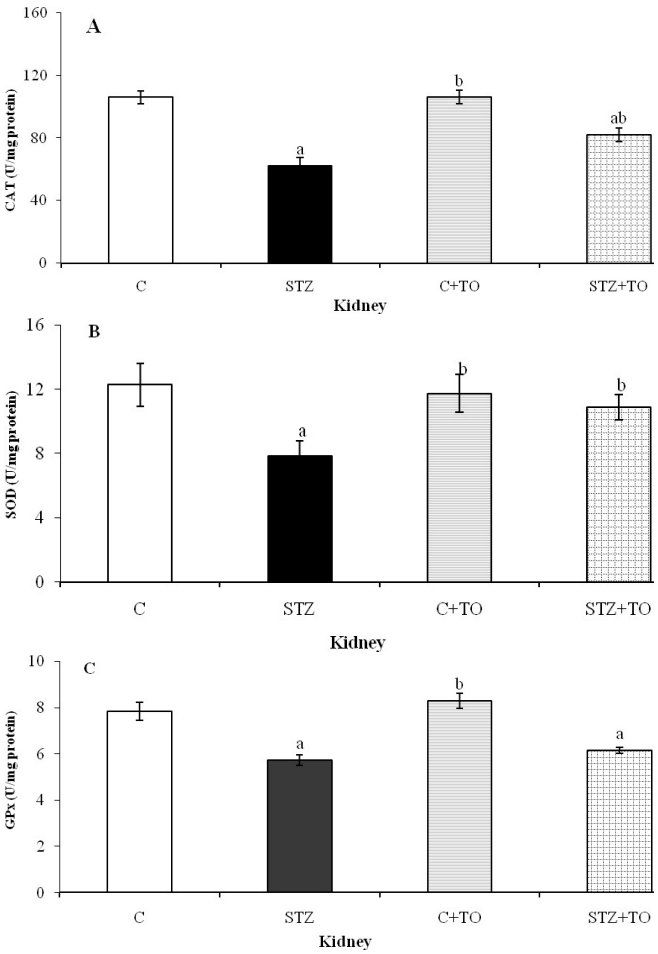


Figure 1. Effect of TOME orally for 21days on (A) CAT, (B) SOD and (C) GPx of kidney in normal and STZ-induced diabetic rats. Values are mean±S.E. of eight animals. Differences of $p<0.05$ were considered significant. Significant differences: a (STZ), (STZ + TO) and (C+TO) vs. C; b (STZ + TO) and (C+TO) vs. (STZ)

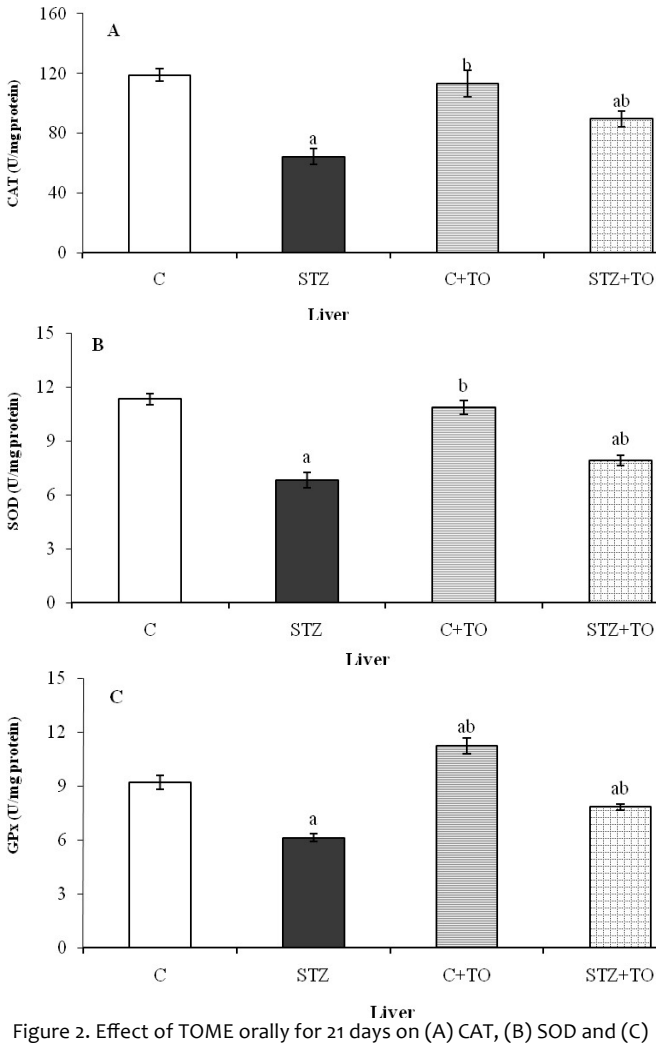


Figure 2. Effect of TOME orally for 21 days on (A) CAT, (B) SOD and (C) GPx of liver in normal and STZ-induced diabetic rats. Values are mean±S.E. of eight animals; CAT= catalase; GPX= glutathione peroxidase; SOD = superoxide dismutase. Differences of $p<0.05$ were considered significant. Significant differences: [a] (STZ), (STZ + TOME) and (C+TO) vs. C; [b] (STZ + TO) and (C+TOME) vs. (STZ)

## Discussion

Diabetes is a complex metabolic disorder, characterized by hyperglycemia together with impaired metabolism of glucose and other energy-yielding fuels, such as lipids and proteins. Persistent and chronic hyperglycemia impairs the prooxidant/antioxidant balance, reducing antioxidant levels and increasing free radicals, which causes many of the secondary complications of diabetes (17). Several studies have demonstrated that STZ has a β-cell cytotoxic and slight carcinogenic effects, which significantly induced diabetes by damaging the cells that causes reduction in insulin release (18). The single high dose STZ-induced diabetic rat is one of theanimal models of human insulin dependent diabetes mellitus (IDDM) or type I diabetes mellitus (19).

It is reported that treatment of diabetic animals with medicinal plant extracts, which are an important source of antioxidant compounds, resulted in activation of β-cell, granulation returned to normal, showing an insulinogenic effect and decreasing in serum glucose level (20). The present study is a preliminary assessment of the antihyperglycemic activity of methanol extract of *T. orientale*. Oral administration of TO to the diabetic rats showed a significant decrease in serum glucose level by 40%. The possible mechanism through which TO its antihyperglycemic effect might have been attributed (i) to a stimulation of langerhans islets, (ii) increased peripheral glucose utilization and (iii) to strong antioxidant properties of *T. orientale* compounds.

Earlier phytochemical investigation of TO led to the characterization of several flavonoids (8) that possess antioxidant properties. Some flavonoids have hypoglycemic properties because they improve altered glucose and oxidative metabolisms of the diabetic states (21, 22). They also exert a stimulatory effect on insulin secretion by changing $Ca^{2+}$ concentration (23).

Lipid peroxidation is a marker of oxidative stress and also one of the prime factors involved in cellular damage caused by free radicals (1). The increase in MDA as a biochemical marker of lipid peroxidation in the diabetic rats might be due to increased levels of oxygen free radicals. In animal studies, TO administration was shown to decrease MDA level due to its potential antioxidant activity. Phenolic compounds of TO can act as scavengers of free radicals oxidative and prevent radical damage.

The occurrence of protein oxidative stress in the liver and kidney tissues of diabetic rats was also confirmed by evaluation of TSH levels, which the formation of this chemical group likely resulted from the oxidation of essential thiols (24). TSH group levels decreased in the liver and kidney of

diabetic rats. The liver plays a major role in glutathione homeostasis and the main export organ glutathione (25). Treatment with the extract of TO restored TSH levels in the liver and kidney of diabetic rats, which was likely caused by increased glutathione export from muscles into circulation (26).

The present data also show that STZ-induced diabetes disturbs actions of antioxidant enzymes (SOD, CAT and GPx) in liver and kidneys. The decreased activities of SOD, CAT and GPx in tissues during diabetes mellitus may be due to the production of reactive oxygen free radicals that can themselves reduce the activity of these enzymes (27, 28). These enzymes could destroy the peroxides and play a significant role in providing antioxidant defenses to an organism. In the enzymatic antioxidant defense system, SOD and CAT are the two important scavenging enzymes that remove superoxide radicals ($O_2^{\bullet -}$) and hydrogen peroxide, respectively, *in vivo*. Decrease in GPx activity was also observed in tissues during diabetes. GPx is an enzyme with selenium which plays a primary role in minimizing oxidative damage (29) and is known to be involved in the elimination of low $H_2O_2$ concentrations, whereas CAT is sensitive to higher concentrations of $H_2O_2$ (30, 31). Decrease in SOD, CAT and GPx activities may be due to inadequacy of antioxidant defenses in combating ROS production (32).

The positive effect of TO on antioxidant enzymes activity is most probably due to the high contents of flavonoids and polyphenol components of TO, which were probably involved in the healing process of free radical-mediated diseases, including diabetes and its complications.

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| Traetment | Blood glucose mg/dl | | | |
|---|---|---|---|---|
| | 2nd day (initial) | 7th day | 14th day | 21st day (final) |
| Control (C) | 92±5 | 87± 4 [a] | 89±4 | 93±5 |
| Diabetes (STZ) | 525± 10 [a] | 537±9 | 540± 11[a] | 553± 13 [a] |
| Control + TO (C+TO) | 86± 2 [b] | 84±2 [b] | 77±3 [b] | 78±1 [b] |
| Diabetes + TO (STZ+TO) | 271± 8 [ab] | 266± 8 [ab] | 245± 8 [ab] | 222± 9 [ab] |

Table 1.Effect of TOME orally on Blood glucose level at 2, 7, 14 and 21 days in normal and STZ-induced diabetic rats.
Values are mean±'b1S.E. of eight animals. Differences of p<0.05 were considered significant.
Significant differences: a (STZ), (STZ + TOME) and (C+TOME) vs. C b (STZ + TOME) and (C+TOME) vs. (STZ)

| Treatment | MDA ( mM/mg protein) | TSH( µg/mg protein) |
|---|---|---|
| Control (C) | 0.35±0.1 | 5.9±0.1 |
| Diabetes (STZ) | 1.35± 0.31 [a] | 3.7± 0.31[a] |
| Control + TO (C+TO) | 0.32± 0.09[b] | 6.11± 0.09[ab] |
| Diabetes + TO (STZ +TO) | 0.95± 0.15[ab] | 4.45± 0.15[ab] |

Table 2.Effect of TOME orally for 21 days on MDA and TSH of liver in normal and STZ-induced diabetic rats.
Values are mean±'b1S.E. of eight animals. Differences of p<0.05 were considered significant.
Significant differences: a (STZ), (STZ + TO) and (C+TO) vs. C b (STZ + TO) and (C+TO) vs. (STZ)

| Treatment | MDA ( mM/mg protein) | TSH( µg/mg protein) |
|---|---|---|
| Control (C) | 0.5±0.09 | 4.35±0.08 |
| Diabetes (STZ) | 1.35±0.35[a] | 2.87± 0.09 [a] |
| Control + TO (C+TO) | 0.45± 0.08[b] | 5.32± 0.09[ab] |
| Diabetes + TO (STZ +TO) | 0.8± 0.12 [ab] | 3.96± 0.15[ab] |

Table 3.Effect of TO orally for 21 days on MDA and TSH of kidney in normal and STZ-induced diabetic rats.
Values are mean ± S.E. of eight animals; MDA= lipid peroxidation; TSH= total thiol. Differences of p<0.05 were considered significant.
Significant differences: a (STZ), (STZ + TO) and (C+TO) vs. C b (STZ + TO) and (C+TO) vs. (STZ)