Original Article

Teucrium polium L. Improves Blood Glucose and Lipids and Ameliorates Oxidative Stress in Heart and Aorta of Diabetic Rats

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Abstract

Background: Diabetes mellitus (DM) is a prime risk factor for cardiovascular disease. The convincing experimental and clinical evidence indicated that the onset of DM is closely associated with oxidative stress and that the generation of reactive oxygen species increases in both the types of diabetes. The aim of the present study was to evaluate the effect of *Teucrium polium* (TP) hydroalcoholic extract on the blood glucose, cholesterol, triglyceride, and oxidative stress markers of the heart and aorta in streptozotocin (STZ)-induced diabetic rats. **Methods:** The male Wistar rats assigned into six groups (n = 8 in each group): Control, diabetic, and diabetic rats treated with TP extract (100, 200, and 400 mg/kg) or met and metformin (300 mg/kg) formin (300 mg/kg) group, by daily gavage for 6 weeks. Diabetes was induced by injection of STZ (60 mg/kg, i.p). Serum lipids and glucose, malondialdehyde (MDA) level, total thiol level, and also the activities of Cu, Zn-superoxide dismutase (SOD) in the cardiac and aortic tissues were assessed. **Results:** TP extract reduced serum glucose, triglyceride and cholesterol. The MDA levels were reduced significantly in all TP-treated groups and metformin. Total thiol levels were improved in the heart and aorta of TP extract-treated groups and metformin compared to the diabetic rats. The activity of SOD in the cardiac and aortic tissues of TP extract- and metformin-treated groups was higher than diabetic group. **Conclusions:** The results showed that chronic administration of TP in STZ-induced diabetic rats could decrease blood glucose, cholesterol, and triglyceride and also attenuate the oxidative stress in the aortic and cardiac tissues.

Keywords: *Aorta, diabetes mellitus, heart, oxidative stress, Teucrium polium*

Introduction

Diabetes mellitus (DM) is a risk factor for cardiovascular disease. Mounting evidence has established the role of free radicals and oxidative stress in pathogenesis and the development of DM and its associated complications, including retinopathy, nephropathy, neuropathy, accelerated coronary artery disease, and heart failure.[1] Although reactive oxygen species (ROS) are generated under physiological conditions and are known mediators of intracellular signaling cascades, excessive ROS formation results in oxidative stress and could cause oxidative damage to biological macromolecules, especially the plasma membrane.[2] A variety of mechanisms are considered to be responsible for oxidative stress in diabetes including overproduction of oxygen radicals from glucose autoxidation,[3] glycated proteins,[4] and glycation of antioxidative enzymes, which restrict their capacity to detoxify oxygen radicals.[5] There is growing acknowledgment of the potential role for nutraceuticals to diminish health risks and improve well-being quality.

Lamiaceae or Labiatae also known as the mint family comprises about 210 genera and 7000 species. *Teucrium polium* L. (TP) (Lamiaceae) is a popular species of this family which is native to the Mediterranean region and the Middle East. The phytochemistry and medicinal properties of TP which are reviewed by Bahramikia and Yazdanparast[6] TP contain chemical compounds such as flavonoids (rutin, luteolin, apigenin, cirsiliol, salvigenin, and cirsiliol),[7] monoterpenes (α- and β-pinene, sabinene, and myrcene), and sesquiterpenes (germacrene D, β-caryophyllene, and spathulenol).[8,9] TP has been used in traditional medicine mainly for its antidiabetic, antipyretic, and anti-inflammatory.[10] TP has been reported to have antibacterial,[11] anticancer,[12] neuroprotective,[13] and antidiabetic[14,15]

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activity. TP also has cardiovascular effects such as decreasing of blood pressure[16,17] and antihyperlipidemic effects.[15] There is also surmounting evidence demonstrating the antioxidant properties of TP *in vivo*[14,18,19] and *in vitro* studies.[11,20] Thus, this study was designed to investigate the antioxidant properties of TP within the aorta and hearts of diabetic rats.

Methods

Animals and induction of diabetes

The male Wistar rats (250–280 g, 10 weeks old) were housed on a 12 h light/12 h dark cycle, under constant temperature (22°C ± 1°C) and were allowed free access to standard rat chow and drinking water. All experiments were performed under license from the Animal Experimentation Ethics Committee of Mashhad University of Medical Sciences (approval No. 900910).

Diabetes was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ) 60 mg/kg (freshly dissolved in 10 mM sodium citrate, pH 4.5, with 0.9% NaCl). Nondiabetic control animals were received sodium citrate buffer (i.p.). Blood glucose concentrations were determined 3 days after STZ injection. Rats with blood glucose level ≥250 mg/dl following 12 h fasting were regarded as to be diabetic.[21]

Preparation of the extract

TP was purchased from local herbal shop in Mashhad, Khorasan Province, Iran and identified by botanists in the herbarium of Ferdowsi University of Mashhad. Aerial parts of the plant were milled into fine powder. The powder was soaked in hydroalcoholic solution (50% ethanol, 50% water) for 48 h at room temperature. The extract solution was subsequently filtered and subjected to evaporation under vacuum at 40°C until the solvent was evaporated. The extract was preserved at 20°C until use. The dried extract was dissolved in distilled water to obtain the doses of 100, 200, and 400 mg/kg.

Chemicals and drugs

All chemicals were of analytical grade (Merck). STZ and metformin hydrochloride were obtained from Sigma (Germany). Serum cholesterol and triglyceride concentrations were determined by Pars Azmoon kits (Tehran, Iran).

Experimental protocol

Rats were randomly assigned to six groups (n = 8 in each group): Control (C), diabetic (D), diabetic-extract (DE), and diabetic-metformin (DM). Normal saline was administered orally by daily gavage to the C and D groups, and TP extract (100, 200, and 400 mg/kg) and metformin (300 mg/kg) administered daily by gavage to DE and DM groups for 6 weeks.

Preparation and analysis of the samples

After 12 h deprivation of food but free access to water, the animals were anesthetized with ether, and then blood samples were obtained from the orbital sinus. Plasma glucose concentrations were measured in four different periods of the experiment: before STZ injection, 3 days after STZ injection (to confirm STZ had induced diabetes), 21 days, and finally 6 weeks after STZ injection (day 42) by the glucose oxidase method with a glucose analyzer (Glucometer EasyGluco, infopia Co., Korea). Serum cholesterol and triglyceride levels were measured in three different periods of the experiment: before STZ injection, 21 days, and finally 42 days by Pars Azmoon kits.

At the end of experiment, rats were sacrificed under deep anesthesia with ether ventilation. The heart ventricles and thoracic aorta were quickly excised, cleared of adhering fat and rinsed in a cool normal saline. The tissues were homogenized in 10% (w/v) homogenizing buffer (100 mmol $KH_2PO_4 \cdot K_2HPO_4$, pH 7.4, plus 0.1% (w/v) digitonin). The supernatant was obtained by the centrifugation of the homogenates in 5000 g, 4°C for 10 min. The supernatant was used for the measurement of the biochemical markers of oxidative stress.

The animals were weighed at the beginning of experiment (before induction of diabetes), 3 weeks, and 6 weeks later.

Determination of malondialdehyde

Lipid peroxidation was quantified as malondialdehyde (MDA) concentration. MDA was measured using the thiobarbituric acid reactive substances method. In brief, 1 ml of supernatant was added to 2 ml of a complex solution containing thiobarbituric acid/trichloroacetic acid/hydrochloric acid reagent, and the solution was incubated in boiling water bath for 40 min. After reaching room temperature, the solution was centrifuged at 1000 g for 10 min. The absorbance was read at 535 nm.[21] The MDA level was expressed as μmol/g of tissue.

Determination of total thiol

The thiol level was determined in cardiac and aortic tissues using a spectrophotometric method based on the use of Ellman's reagent. 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), which reacts with the thiol group (SH), was used to determine the total thiol groups. The resulting yellow complex has a peak absorbance at 412 nm. In brief, 50 μl of supernatant of homogenized tissue was added to 1 ml Tris-ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.6), and the absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then, 20 μl of

DTNB (10 mM in methanol) was add with the solution and was stored at room temperature for 15 min, and the absorbance was read again (A2). The absorbance of DTNB reagent was also read as blank (B). Total thiol was expressed as mmol/g of tissue.[21]

Assay of Cu, Zn-superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured using the procedure of Madesh and Balasubramanian.[22] This colorimetric assay is based on the production of superoxide pyrogallol autoxidation. The superoxide anion can reduce the tetrazolium dye 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) to its formazan. The Cu, Zn-SOD inhibits the later reaction. Cu, Zn-SOD activity was measured at 570 nm. One unit of Cu, Zn-SOD activity was defined as the amount of enzyme causing 50% inhibition in the MTT reduction rate. The SOD activity was expressed as unit/g tissue.

Data analysis

The results are expressed as mean ± standard error of the mean (SEM). Statistical analyses were made using one-way ANOVA followed by the Tukey's test. Statistical significance was defined as $P < 0.05$.

Results

Effect of *Teucrium polium* on weight

The body weight of diabetic rats reduced significantly during the experiment ($P < 0.001$). The body weight of rats in TP extract- and metformin-treated groups also reduced significantly although the weight loss was lower in TP 100 group [Figure 1].

Effect of *Teucrium polium* on plasma glucose, cholesterol, and triglyceride concentrations

The chronic administration of TP extract reduced blood glucose levels which were significant in TP100 and TP 400 groups ($P < 0.001$ and $P < 0.05$, respectively) compared with the diabetic group [Table 1]. Metformin also reduced glucose levels compared with the diabetic group ($P < 0.05$).

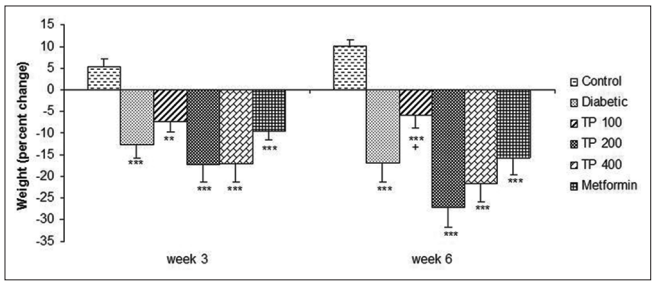

Figure 1: Percent weight changes in control, diabetic, diabetic-metformin, and *Teucrium polium* extract (TP 100, TP 200, TP 400)-treated groups. **$P < 0.01$, ***$P < 0.001$ versus control group and $^{+}P < 0.05$ versus diabetic group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test. TP: *Teucrium polium*

Serum cholesterol level significantly increased at 24 and 42 days in diabetic group compared with the control group ($P < 0.001$) [Table 2]. TP extract and metformin significantly reduced serum cholesterol level ($P < 0.05$ to $P < 0.001$) [Table 2].

The results showed significant elevation of serum triglyceride level of diabetic group compared with the control group at 21 and 42 days ($P < 0.001$) [Table 3]. In TP extract-treated groups, the serum triglyceride level was significantly decreased in TP 200 group at 21 and 42 days ($P < 0.0001$). The triglyceride level was decreased significantly in TP100 and metformin-treated groups at day 42 and in TP 400 at day 21 [Table 3].

Effect of *Teucrium polium* on malondialdehyde

As Figure 2 shows in the aortic and cardiac tissues of diabetic rats, MDA level was increased considerably compared with the control group ($P < 0.001$). The chronic administration of TP extract and metformin significantly reduced the MDA level in the cardiac and aortic tissues ($P < 0.01$ to $P < 0.001$) [Figure 2].

Effect of *Teucrium polium* on total thiol

Total thiol levels were decreased in the aortic and cardiac tissues of diabetic rats compared with the control group ($P < 0.01$) while in TP extract- and metformin-treated groups, total thiol level increased in aortic and cardiac tissues ($P < 0.05$ to $P < 0.01$) [Figure 3].

Effect of *Teucrium polium* on Cu, Zn-superoxide dismutase activity

The activity of Cu, Zn-SOD was decreased in diabetic group compared to the control group ($P < 0.01$) while TP extract- and metformin-treated groups demonstrated a significant increase in SOD activity in the aortic and cardiac tissues ($P < 0.05$ to $P < 0.01$) [Figure 4].

Discussion

The results of this study indicate that chronic administration of TP extract in STZ-induced diabetic rats has hypoglycemic effect. All doses of TP extract reduced blood glucose, but this reduction was significant for 100 and 400 mg/kg doses although no significant differences between 200 and 400 mg/kg doses were seen. Previous studies also reported the hypoglycemic effect of TP.[14,15] Hyperglycemia causes tissue damage through five major mechanisms: increased flux of glucose and other sugars through the polyol pathway, increased intracellular formation of advanced glycation end-products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms and over activity of the hexosamine pathway. Several lines of evidence indicate that all five mechanisms are activated by a single upstream event: mitochondrial overproduction of ROS.[2]

Table 1: The effect of *Teucrium polium* extract on serum glucose level (mg/dl) in streptozotocin-induced diabetic rats

Group	Day 0	Day 3	Day 24	Day 45
Control	92.34±4.15	-	113.59±7.59	108.60±5.22
Diabetic	92.60±5.13	471.4±9.7***	456.3±15.8***	488.58±14.5***
Diabetic + extract 100 mg/kg	98.14±4.5	416.28±6.13***	150.96±6.71^{+++}	134.24±8.4^{+++}
Diabetic + extract 200 mg/kg	109.6±3.76	337.2±8.82***	343.14±12.17***	306.51±8.1**
Diabetic + extract 400 mg/kg	102.40±2.71	318.88±11.2***	292±15.4**	226.8±14.84*$^{,+}$
Diabetic + metformin	98.46±1.67	302.43±10.32***	278.99±26.11**	247.6±21.3*$^{,+}$

Values are expressed as means±SEM (n=8). *P<0.05, **P<0.01, and ***P<0.001 versus to control group, ^{+}P<0.05, ^{+++}P<0.001 versus diabetic group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test. SEM=Standard error of mean

Table 2: The effect of *Teucrium polium* extract on serum cholesterol level (mg/dl) in streptozotocin-induced diabetic rats

Group	Day 0	Day 24	Day 45
Control	74.91±2.3	73.66±1.3	72.63±3.4
Diabetic	71.05±1.4	118.62±3.6***	129.42±4.7***
Diabetic + extract 100 mg/kg	75.7±2.9	90.3±3.1^{+}	93.7±3.2^{+}
Diabetic + extract 200 mg/kg	70.8±1.7	86.4±3.17^{++}	85.9±5.1^{++}
Diabetic + extract 400 mg/kg	69.5±1.5	95±5.7	91.8±3.2^{+}
Diabetic + metformin	72±1.3	84.3±3.2^{+++}	80.7±2.9^{+++}

Values are expressed as means±SEM (n=8). ***P<0.001 versus control group, ^{+}P<0.05, ^{++}P<0.01, ^{+++}P<0.001 versus diabetic group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test. SEM=Standard error of mean

Table 3: The effect of *Teucrium polium* extract on serum triglyceride levels (mg/dl) in streptozotocin-induced diabetic rats

Group	Day 0	Day 24	Day 45
Control	53.42±2.8	57.8±4.1	56.43±3.64
Diabetic	56.81±4.1	282.4±8.2***	251.3±7.6***
Diabetic + extract 100 mg/kg	65.7±3.4	241.3±9.1***	124.7±6.2**$^{,+}$
Diabetic + extract 200 mg/kg	60.5±2.7	76.4±3.17^{+++}	70.9±5.1^{+++}
Diabetic + extract 400 mg/kg	57.9±3.5	85.3±6.7^{+++}	119.4±9.2*$^{,+}$
Diabetic + metformin	62.6±3.4	268.4±15.1***	204.60±18.31***$^{,+}$

Values are expressed as means±SEM (n=8). *P<0.05, **P<0.001, ***P<0.001 versus control group, ^{+}P<0.05, ^{+++}P<0.001 versus diabetic group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test. SEM=Standard error of mean

The data of this study have shown that total thiol significantly decreased in the heart and aorta of diabetic rats while the chronic administration of TP extract increased total thiol level in the aortic and cardiac tissues of diabetic rats. Total thiol groups are very susceptible to oxidation and considered as one of the most important body sacrificial antioxidants. When the cells are exposed to oxidative stress, thiol groups are the first antioxidants that

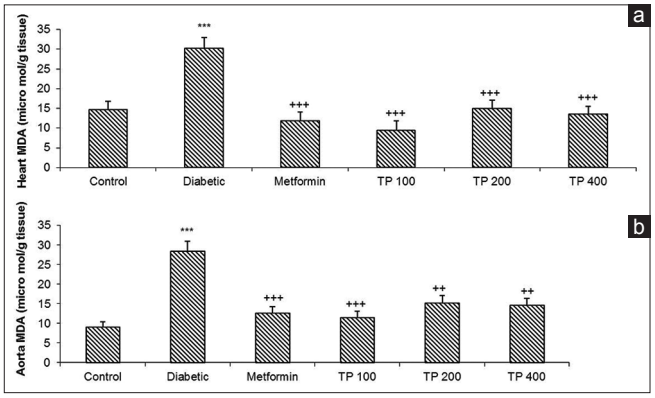

Figure 2: The malondialdehyde level in the cardiac (a) and aortic (b) tissues. Values are expressed as means ± standard error of the mean (n = 8). ***P < 0.001 versus control group and ^{++}P < 0.001, ^{+++}P < 0.001 versus diabetic group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test. TP: Teucrium polium

are consumed.[23] Thus, the increase of total thiol in TP- and metformin-treated groups indicate the improvement of antioxidant status. Previous studies also showed total thiol reduced in type 1 diabetes.[21,24]

Our results showed MDA level, as a lipid peroxidation marker, increased in diabetic rats and chronic administration of TP extract reduced the MDA level in the heart and aorta of diabetic rats. MDA has been documented as a primary biomarker of free radical-mediated lipid damage and oxidative stress. Increased the level of MDA in diabetes suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of decline in defense mechanisms of enzymatic and nonenzymatic antioxidants.[25] In the present study, the SOD activity reduced in the heart and aorta of diabetic rats. TP extract increased SOD activity in the heart and aorta of diabetic rats. SOD plays important protective roles against cellular and histological damages that are produced by ROS. Decline in the level of SOD in diabetic tissue and blood has been reported in many studies.[5,26] Ardestani *et al*. reported chronic administration of TP extract in STZ-induced diabetic rats increased GSH level and SOD activity in the pancreatic tissue while MDA level decreased.[18] Mousavi *et al*. also showed TP extract reduced MDA level in brain of diabetic rats,[27] so regarding

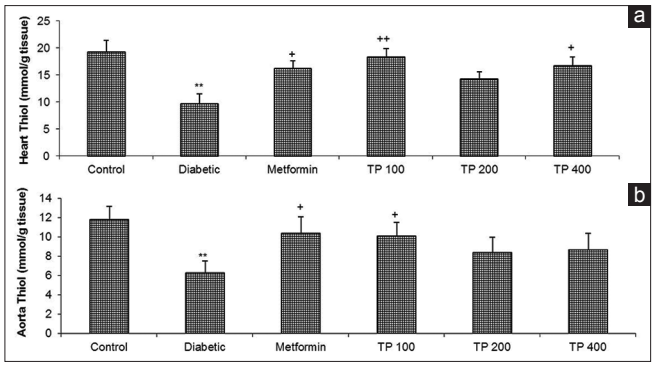

Figure 3: The total thiol level in the cardiac (a) and aortic (b) tissues in control, diabetic, diabetic-metformin, and *Teucrium polium* extract (TP 100, TP 200, TP 400)-treated groups. Values are expressed as means ± standard error of the mean (n = 8). $^{+}P < 0.05$, $^{++}P < 0.01$ versus diabetic group and $^{**}P < 0.01$ versus control group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test. TP: *Teucrium polium*

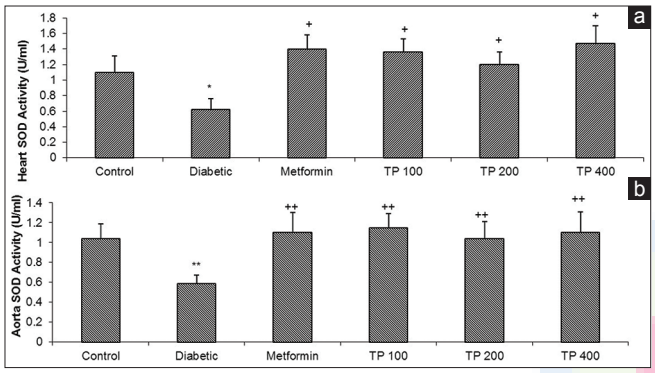

Figure 4: The superoxide dismutase activity (U/100 mg tissue) of the cardiac (a) and aortic (b) tissues in control, diabetic, diabetic-metformin, and *Teucrium polium* extract (TP 100, TP 200, TP 400)-treated groups. Values are expressed as means ± standard error of the mean (n = 8). $^{+}P < 0.05$, $^{++}P < 0.01$ versus diabetic group and $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group. Statistical analyses were made using the one-way NOVA followed by the Tukey's test. TP: *Teucrium polium*

above-mentioned former studies, it could be concluded that our results are another confirmation for the preventive antioxidant role of TP extract in this model of DM.

In general, our results showed that TP hydroalcoholic extract significantly reduced blood glucose and lipids and also ameliorated the oxidative stress in the cardiac and aortic tissues of diabetic rats.

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Conflicts of interest

There are no conflicts of interest.

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