Zostera noltii extract lowers blood glucose and restores vascular function in diabetic rats

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Abstract
The antidiabetic effect of seagrass Zostera noltii extract was investigated through a crosstalk between its antioxidant and vasoprotective properties. The extract was orally administered to alloxan-diabetic rats (50, 150, 250 mg/kg body weight). Serum glucose was determined; liver and kidney functions, body weight, total leukocyte counts were measured; liver oxidative markers were assayed. Acetylcholine, phenylephrine and 5-HT responses were tested. eNOS levels and generation of ROS in aortic tissue were quantified. The extract of Z. noltii lowered blood glucose in all tested dose levels. Extract at a concentration of 50 mg/kg failed to preserve the levels of antioxidants and did not alter lipid peroxidation whereas higher doses improved liver oxidative status. Impaired acetylcholine relaxations, augmented phenylephrine and 5-HT contractions in alloxan-diabetic aortic rings were restored by Z. noltii treatment. This recovery was accompanied by increased eNOS synthesis and a reduction in ROS generation. The extract lowers blood glucose and prevents hyperglycemia-induced endothelial dysfunction in alloxan-diabetic rats.

Introduction
Diabetes mellitus is a life-threatening metabolic disorder of multiple etiology. Oxidative stress has been recognized as a critical player in the pathogenesis of diabetes (Murugan and Pari, 2006). Furthermore, chronic hyperglycemia itself, enhances the production of reactive oxygen species (ROS) by glucose auto-oxidation and/or non-enzymatic protein glycosylation (Giugliano et al., 1996; Signorini et al., 2002). Elevated oxidants and markers for oxidative tissue damage have been well documented in patients with diabetes (Rehman et al., 1999; Chowienczyk et al., 2000; Fava et al., 2002; Zitouni et al., 2005). Therefore, antioxidants gained considerable attention in prevention of diabetes and its micro- and macrovascular complications which are regarded as the most common reasons of morbidity and mortality (Guo et al., 2005; Gokce and Haznedaroglu, 2008). While a damaged endothelium is the starting point to diabetic macroangiopathy, impairments in endothelium- and nitric oxide (NO)-dependent microvascular function, may contribute to several other corollaries of diabetes, such as hypertension, dyslipidemia and insulin resistance (Laight et al., 1999).

Antioxidant therapy may conceivably confer both cardiovascular and metabolic benefits in diabetes. This notion is well grounded in the theory surrounding the role of oxidative stress in disease and supported by evidence of reduced antioxidant defences in diabetes and also by experimental findings that antioxidants improve endothelium-dependent vasodilation and insulin sensitivity (Laight et al., 1999). Moreover, there is an increasing demand by patients to use natural anti-diabetic products due to undesirable side effects of insulin and oral hypoglycemic drugs (Rao and Rao, 2001; Gong et al., 2012; Mohan et al., 2014).

Zostera noltii Hornemann (Zosteraceae) is a perennial phanerogam with small leaves growing permanently submerged. It is distributed in shallow sheltered sea...
bays from the southern coasts of Norway to the Mediterranean Sea, the Black Sea, besides northwest Africa coasts (Mireia et al., 2011). As with other seagrasses, Z. noltii plays important roles in marine ecosystems for biodiversity, ecological, sedimentary and economic reasons (Rende et al., 2012). Seagrasses are thought to be chemically defended against herbivores and pathogenic infections such as wasting disease by phenolic compounds (Grignion-Dubois et al., 2012). Z. noltii is rich in phenolic compounds and flavonoids such as, chlorogenic acid, caffeic acid, rosmarinic acid, zosteric acid, apigenin, diosmetin, luteolin and luteolin-7-O-glucoside (Lamaison et al., 1990; Zeljan and Misko, 2000; Achamla et al., 2009; Newberry et al., 2011; Sato et al., 2011). Additionally, crude extracts of seagrasses are shown to exert antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antidiabetic and anti-cancer activities (Papenbrock, 2012). Moreover, we have shown that total extract of another seagrass, Posidonia oceanica has antioxidant, antidiabetic and vasoprotective effects (Gokce and Haznedaroglu, 2008). Present study aims to investigate the glucose lowering activity of Z. noltii in alloxan-induced diabetes, through a crossstalk between its antioxidant and vasoprotective properties.

Materials and Methods

**Plant material:** Z. noltii was collected from Urla, Izmir, Aegean Sea in October 2011 at 1 m depth. The plant was identified at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Ege University, Izmir and specimen vouchers are kept at IZEF Herbarium (IZEF5887). The epiphytes on the leaves were removed with paper towel without damaging the organs. Leaves were dried in shadow and at controlled room conditions (25°C).

**Extraction:** Chopped leaves were infused with aqueous ethanol 50% (v/v) for 3 hours in a water bath at 40°C with a reflux system. Homogenate was filtered and acidified (pH=3) with HCl. Ethanol was evaporated under vacuum at 45°C. Obtained aqueous residue was extracted with ethyl acetate. Water is removed from organic phase with anhydrous sodium sulfate, filtered and evaporated under vacuum. Dry samples were prepared following filtration, evaporation and lyophilization. After 25 repetitions with overall 1.2 kg of plant material 24 g of extract was obtained (Cuny et al., 1995).

**Chemicals:** All drugs were purchased from Sigma (St. Louis, MO, USA). Solvents used in extraction procedure were purchased from Labscan (Dublin, Ireland), while hydrochloric acid and anhydrous sodium sulfate were from Riedel Haen (Germany).

**Animals:** Three-month-old male Wistar rats (200-225 g; n=30; Lemali Ltd, Ankara, Turkey) were used. Animals were maintained under 22 ± 2°C and a 12 hours light-dark cycle day and had unrestricted access to pelleted food and water. The experiments were carried out in accordance with the guidelines of Local Ethics Committee of Animal Experiments, Ege University, Izmir, Turkey (B.30.2.EGE.01.00.01/04.44-215a). At end of the experimental protocol final body weights were recorded and animals were killed by means of an overdose of sodium pentobarbital.

**Glucose tolerance:** Rats were fasted overnight and randomized to three groups (n=6). Control group received 1 mL of distilled water orally (Group I). Z. noltii was administered at concentrations of 100, 500 mg/kg b.wt (p.o, Groups II and III, respectively). Following Z. noltii administration, all groups received glucose (p.o, 2 g/kg b.wt.). Blood samples were taken from the tail vein just prior to and 30, 60, 120 and 240 min after glucose loading. Glucose was assayed by glucose oxidase method (Trinder, 1976). Data obtained from glucose tolerance test were used as a hypothetical reference to determine the dose level which will be used in evaluation of short- and long-term effects of Z. noltii on diabetic rats.

**Antidiabetic effects:** Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (120 mg/ kg) (Cooperstein and Walkins, 1981). After 72 hours, animals with glucose levels higher than 250 mg/dL were considered diabetic (Perfumi and Tacconi, 1996) and randomized to five groups (n=6). Group I (no alloxan treatment) and Group II (diabetic control) rats were given 1 mL of distilled water. Groups III-V received aqueous suspension of Z. noltii on the 3rd day after alloxan administration (respectively, p.o, 50, 100, and 150 mg/kg). Blood samples were taken from the tail vein (fasted rats) just prior to administration of the extract and at 2, 4, 6 and 8 hours intervals. Serum was separated and glucose levels were estimated. These rats were given the same doses of the extract once daily for 15 days in the multidose study. Blood (non-fasted rats) was taken on 6, 9, 12, 15 and 18th day after alloxan administration (Sabu et al., 2002) and serum glucose levels were measured as mentioned above.

**Liver and kidney functions:** Alkaline phosphatase (ALP) (King et al., 1980), glutamate pyruvate transaminase (GPT) (Bergmeyer et al., 1980), blood urea nitrogen (BUN) (Haslam et al., 1966), and creatinine (Brod et al., 1948) were measured. Protein content was determined by the method of (Lowry et al., 1951). Total white blood cell count was determined using a hemocytometer.

**Antioxidant status:** In liver homogenates, reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, and malondialdehyde (MDA levels) were determined spectrophotometrically (Victor III; Perkin Elmer, Turku, Finland) using commercially available assay kits and according to the manufacturer’s instructions (Bioxytech GSH-412, GPx-340, SOD-525, Catalase-520, and MDA-586, respectively; Oxis International, Portland, OR, USA). Total nitrite in
Aortic tissue was estimated colorimetrically using Griess reagent (Guevara et al., 1998). Nitrite concentration in the sample was calculated using sodium nitrite as standard and normalized to the protein content of aorta.

Effects of extract on vascular responses: Aortic rings (2 mm) were mounted under 2 g resting tension on stainless steel hooks within 25 ml tissue baths and maintained at 37°C in Krebs solution (mM): NaCl, 118; KCl, 4.7; CaCl₂·2H₂O, 2.5; KH₂PO₄, 1.20; MgSO₄·7H₂O, 1.17; Glucose, 11.1; NaHCO₃, 25, gassed with 95% O₂ and 5% CO₂. Tension was recorded isometrically by a data acquisition system (Biopac, MP100) equipped with a Grass FT03 transducer. Vascular reactivity studies were performed as previously described (Gokce and Haznedaroğlu, 2008).

Reactive oxygen species: Levels of superoxide anion and other reactive species in aortic rings were determined according to the method described by Wang et al (2001), with slight modifications. 2 mm cut aortic segments were placed in a 96 well plate containing PBS-HEPES buffer (0.5 M PBS containing 20 mM HEPES, pH 7.4). After addition of chemiluminescence enhancers, lucigenin or luminol (final concentration of 5 µmol/L for each), ROS were quantified using a multi-plate reader (Victor III-1420, Perkin Elmer, Turku, Finland). Results were corrected for wet tissue weight and expressed as relative light units rlu/mg tissue) (Haklar et al., 2003).

Determination of endothelial nitric oxide synthase (eNOS): Endothelial nitric oxide synthase levels in aortic tissue was determined using a commercially available ELISA kit (USCNlife, Missouri, ABD) according to the manufacturer’s instructions. Optical density was measured at 450 nm using a microplate reader (Victor III-1420, Perkin Elmer, Turku, Finland). A standard linear regression curve was generated and the concentration of each sample was calculated by the curve equation.

Statistical analysis: Data are expressed as the mean ± S.E. Student’s t-test for unpaired samples (GraphPad Prism, Version 3.02, San Diego, CA, USA). A value of p< 0.05 was considered significant. Agonist stimulated vasoactivity was evaluated in means of maximal effect (Eₘₐₓ) and pD₂ (negative logarithm of the concentration that produced half of the Eₘₐₓ as a measure of sensitivity) values. Acetylcholine-induced relaxant responses were normalized by the initial phenylephrine contraction. Contractile responses to phenylephrine and 5-HT were normalized with maximal contractions induced by potassium chloride.

Results

Sixty minutes after glucose loading, serum glucose level of 71.7 ± 6.8 mg/dL increased up to 198.0 ± 8.1 mg/dL and returned to baseline at 240 min. Z. noltii extract improved glucose tolerance in a dose-dependent manner (Figure 1, *p<0.01; control vs. Z. noltii-100 and Z. noltii-100 vs. Z. noltii-500). Effect of the extract on glucose tolerance at the dose level of 500 mg/kg remained statistically significant at 120 min (Figure 1, *p<0.05; control vs. Z. noltii-500).

In the single dose study, effects of Z. noltii extract on blood glucose levels were evaluated on the 3rd day after alloxan administration. Z. noltii extract (50 mg/kg) did not reduce serum glucose. At dose levels of 150 and 250 mg/kg, glucose levels were decreased by 24.8% and 29.9% at the 6th hour, respectively. Antidiabetic effect of the extract was slightly decreased at the 8th hour, but remained statistically significant (Table I).

In parallel experiments, Z. noltii extract was administered to diabetic rats for 15 days at aforementioned doses (Table II). Z. noltii extract (50 mg/kg) started to lower serum glucose on the 9th day and an overall reduction of 26% was observed on the 18th day (p<0.01). At dose level of 150 mg/kg, serum glucose was found to decrease by 52.5% on the 18th day (p<0.0001). The effect of the extract was more pronounced at 250 mg/kg, starting on the 6th day with a reduction rate of 18.9% (p<0.01) and finally reaching to its maximum on the 18th day by 61.9%. Between the 12th and 18th days, antidiabetic effect of Z. noltii extract was in a concentration dependent manner (Table II).

Table III summarizes the effects of Z. noltii extract on hepatic and renal function in alloxan-diabetic rats. As seen, treatment with 50 mg/kg did affect neither the significantly high levels of ALP, GPT, BUN and creatinine, nor the overall oxidative status. Conversely, rats treated with higher doses of the extract (150 and 250 mg/kg) showed significant improvements in hepatic and renal function (Table III). Additionally, these two
Table I: Effects of Z. noltii extract (single dose) on serum glucose levels in alloxan-induced diabetic rats

| Group | Treatment (mg/kg) | 0 hour | 2 hour | 4 hour | 6 hour | 8 hour |
|-------|------------------|--------|--------|--------|--------|--------|
| I     | Normal           | 71.7 ± 6.8 | 68.4 ± 7.1 | 65.2 ± 8.1 | 68.6 ± 8.3 | 70.3 ± 8.1 |
| II    | Control (aloxan) | 269.1 ± 11.7<sup>a</sup> | 275.3 ± 10.9<sup>b</sup> | 278.2 ± 12.1<sup>c</sup> | 274.3 ± 9.2<sup>d</sup> | 268.6 ± 8.8<sup>e</sup> |
| III   | Z. noltii extract (50) | 262.6 ± 10.8 | 265.6 ± 9.8 | 259.4 ± 9.7 | 256.4 ± 11.1 | 253.7 ± 9.9 |
| IV    | Z. noltii extract (150) | 259.3 ± 9.7 | 254.2 ± 9.3 | 238.6 ± 8.8 | 206.1 ± 10.4<sup>b</sup> | 215.4 ± 10.2<sup>c</sup> |
| V     | Z. noltii extract (250) | 255.1 ± 12.1 | 243.1 ± 11.1 | 231.7 ± 9.1 | 192.4 ± 9.6<sup>b</sup> | 199.5 ± 9.7<sup>c</sup> |

Serum glucose levels were obtained from fasted rats on the 3<sup>rd</sup> day after alloxan administration. Data are expressed as mean ± S.E; (n=6); p<0.0001 (compared to normal group with corresponding hour), p<0.01 and p<0.05 (compared to control group with corresponding hour).

Table II: Effects of Z. noltii extract (daily treatment) on serum glucose levels in alloxan-induced diabetic rats

| Group | Treatment (mg/kg) | 3 days | 6 days | 9 days | 12 days | 15 days | 18 days |
|-------|------------------|--------|--------|--------|--------|--------|--------|
| I     | Normal           | 73.7 ± 6.1 | 71.6 ± 7.5 | 70.1 ± 9.4 | 72.9 ± 7.6 | 69.6 ± 5.6 | 73.8 ± 7.1 |
| II    | Control (aloxan) | 271.2 ± 11.8<sup>a</sup> | 278.2 ± 10.4<sup>b</sup> | 270.3 ± 10.4<sup>c</sup> | 272.2 ± 9.8<sup>d</sup> | 267.4 ± 9.9<sup>e</sup> | 261.8 ± 8.3<sup>f</sup> |
| III   | Z. noltii extract (50) | 268.7 ± 9.7 | 248.4 ± 9.8 | 229.3 ± 8.2<sup>c</sup> | 217.3 ± 7.9<sup>d</sup> | 207.5 ± 8.7<sup>e</sup> | 198.5 ± 7.9<sup>f</sup> |
| IV    | Z. noltii extract (150) | 274.1 ± 8.5 | 230.2 ± 9.3 | 209.9 ± 11.1” | 173.1 ± 8.8”<sup>a</sup> | 149.8 ± 9.5”<sup>b</sup> | 130.2 ± 10.2”<sup>c</sup> |
| V     | Z. noltii extract (250) | 270.3 ± 9.4 | 219.5 ± 8.1” | 165.2 ± 9.9”<sup>a</sup> | 140.2 ± 7.6”<sup>b</sup> | 129.6 ± 8.4”<sup>c</sup> | 103.4 ± 11.1”<sup>d</sup> |

Values are of serum glucose levels obtained from alloxan-induced diabetic rats in the absence and in the presence of 15 day Z. noltii extract treatment (from the 3<sup>rd</sup> to the 18<sup>th</sup> day) and expressed as mean ± S.E; n=6; p<0.0001 (compared to normal group with corresponding day); p<0.001, p<0.01, p<0.001 and p<0.0001 (compared to control group with corresponding day). p<0.05, p<0.01 and p<0.001 (compared to Group III with corresponding day). *p<0.0001 (compared to Group IV with corresponding day).

Table III: Effects of Z. noltii extract on liver and kidney functions

| Treatment (mg/kg) | ALP (KA/dL) | GPT (U/mg protein) | BUN (mg/dL) | Creatinine (mg/dL) |
|------------------|-------------|-------------------|-------------|-------------------|
| Normal           | 33.7 ± 2.8  | 353.3 ± 21.9<sup>a</sup> | 9.2 ± 0.6   | 0.7 ± 0.0         |
| Control (aloxan) | 50.3 ± 6.7<sup>b</sup> | 353.3 ± 21.9<sup>a</sup> | 19.3 ± 2.2<sup>c</sup> | 2.1 ± 0.1<sup>d</sup> |
| Z. noltii extract (50) | 42.6 ± 4.8  | 285.6 ± 19.8      | 16.4 ± 11.1 | 1.7 ± 0.1         |
| Z. noltii extract (150) | 35.3 ± 2.7<sup>e</sup> | 242.2 ± 12.3<sup>f</sup> | 13.6 ± 0.7<sup>c</sup> | 1.5 ± 0.0<sup>d</sup> |
| Z. noltii extract (250) | 31.1 ± 12.1<sup>g</sup> | 206.1 ± 9.1<sup>f</sup> | 11.5 ± 0.6<sup>c</sup> | 1.2 ± 0.0<sup>d</sup> |

ALP: alkaline phosphatase; GPT: glutamate pyruvate transaminase; BUN: blood urea nitrogen. Liver and kidney markers were measured on the 18<sup>th</sup> day after alloxan administration. Data are expressed as mean ± S.E; n=6; p<0.001 and p<0.0001; compared to normal group; p<0.05, p<0.01 and p<0.001 (compared to control group).

Table IV: Effects of Z. noltii extract on body weight, total leucocyte count and liver glycogen

| Treatment (mg/kg) | Body Weight | Total leucocyte count (mm<sup>3</sup>) | Liver glycogen (μg/g tissue) |
|------------------|-------------|--------------------------------------|-----------------------------|
|                 | Initial     | Final                                | 12324 ± 4567                | 76.9 ± 4.6                  |
| Normal           | 241.7 ± 7.8 | 259.4 ± 9.1                          | 8567.2 ± 389.2<sup>b</sup>  | 58.1 ± 4.8<sup>d</sup>     |
| Control (aloxan) | 245.3 ± 9.6 | 205.3 ± 21.9<sup>g</sup>             | 9453.6 ± 511.2              | 67.2 ± 4.1                  |
| Z. noltii extract (50) | 249.6 ± 10.1 | 245.6 ± 19.8<sup>b</sup>             | 11036.3 ± 658.1<sup>c</sup> | 74.5 ± 5.1<sup>e</sup>     |
| Z. noltii extract (150) | 243.3 ± 8.1 | 249.2 ± 12.3                          | 13002.5 ± 703.1<sup>c</sup> | 77.6 ± 5.5<sup>f</sup>     |
| Z. noltii extract (250) | 247 ± 12.1 | 263.1 ± 9.1                           | 13002.5 ± 703.1<sup>c</sup> |                              |

Data are expressed as mean ± S.E; n=6; p<0.001 (compared to initial body weight of the same group), p<0.05 (compared to initial body weight of the same group), p<0.0001 and p<0.001 (compared to normal group), p<0.05 and p<0.001 (compared to control group).

dose levels recovered the weight loss and low white blood cell count observed in alloxan-diabetic rats while decreasing liver glycogen (Table IV). Z. noltii extract (150 and 250 mg/kg) also showed a protective effect on liver oxidative status (Table V). Antioxidants namely GSH, GPx, SOD and catalase were increased by Z. noltii extract administration. When compared to alloxan-diabetic rats, MDA formation, as an indirect measure of lipid peroxidation, was found to be significantly low in high dose Z. noltii extract-treated rats (Table V).

In order to evaluate the effects of Z. noltii extract on vascular responses, cumulative concentration-response curves for acetylcholine, phenylephrine and 5-HT were obtained in thoracic aorta. Z. noltii extract treatment, at all tested dose levels, recovered the impaired acetylcholine relaxations observed in alloxan-diabetic rats (Figure 2) and resulted in a concentration-dependent
increase in sensitivity (Table VI). Addition-ally, contractile responses to phenylephrine (Figure 3) and 5-HT (Figure 4) which were significantly augment-ed in hyperglycemic rats were dose-depen-dently aste-nuated by Z. noltii extract treatment (Table VI). These changes in vascular reactivity by Z. noltii extract treatment were accompa nied by significant alterations in ROS production in rat aorta. Lucigenin- and luminal-enhanced chemiluminescences in alloxan-diabetic aorta were approximately 4 and 5 times higher than in those of control tissues, respectively (Figure 5A and 5B). Starting from the lowest dose, Z. noltii extract, signifi-cantly inhibited superoxide anion generation and for-mation of other reactive species (Figure 5A and 5B). More-over, concentrations of eNOS in aorta from Z. noltii extract treated diabetic rats were significantly higher when compared to diabetic control (Figure 6A). On the other hand, total nitrite levels in aorta were found to be similar among experimental groups (Figure 6B).

**Discussion**

In the present study, we for the first time demonstrate that Z. noltii extract lowers blood glucose and protects vascular endothelium from the harmful effects of hyperglycemia in alloxan-diabetic rats. As is known, alloxan increases production of reactive oxygen species (ROS) leading to cytotoxicity in pancreatic β-cells, and thereby inhibits insulin activity while affecting major organs and haemopoietic system (Sakurai et al., 2001; Sabu et al., 2002). Additionally, alloxan impairs endothelial dependent vasorelaxation and increases contractile responses to agonists such as phenylephrine and 5-HT (Gokce and Haznedaroglu, 2008). Our results indica

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**Table V: Effects of Z. noltii extract on oxidative status**

| Parameter                  | Normal        | Control (alloxan) | Z. noltii extract (50 mg) | Z. noltii extract (150 mg) | Z. noltii extract (250 mg) |
|----------------------------|---------------|-------------------|--------------------------|---------------------------|---------------------------|
| GSH (nmol/mg protein)      | 7.39±0.41     | 4.16±0.19         | 4.51±0.33                | 5.87±0.43                 | 6.31±0.42                 |
| GSSG (nmol/mg protein)     | 0.25±0.03     | 0.91±0.06         | 0.71±0.05                | 0.62±0.08                 | 0.32±0.07                 |
| GPx (U/mg protein)         | 0.18±0.04     | 0.06±0.01         | 0.09±0.02                | 0.14±0.03                 | 0.15±0.05                 |
| MDA (nmol/mg tissue)       | 354.2±10.2    | 442.2±14.9b       | 422.3±13.1               | 390.2±23.2                | 355.6±11.6                |
| SOD (U/mg protein)         | 6.31±0.41     | 3.26±0.27         | 3.55±0.31                | 4.41±0.55                 | 5.56±0.47                 |
| Catalase (U/mg protein)    | 153.6±6.72    | 98.3±7.01b        | 119.2±8.32               | 126.2±7.54                | 142.7±10.6                |

GSH: reduced glutathione, GSSG: oxidized glutathione, GPx: glutathione peroxidase, MDA: malondialdehyde, SOD: superoxide dismutase. Data are expressed as mean ± S.E; n=6; *p<0.01, **p<0.001, ***p<0.0001 and "p<0.01 (compared to normal group); "p<0.05 and ""p<0.001 (compared to control group).

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**Table VI: Effects of Z. noltii extract on acetylcholine, phenylephrine and 5-HT-induced vascular responses**

| Group | Treatment (mg/kg) | Acetylcholine | Phenylephrine | 5-HT |
|-------|-------------------|---------------|---------------|------|
|       | E_{max} (max)     | pD2           | E_{max}       | pD2  | E_{max}       | pD2  |
| I     | Normal (1)        | 85.1 ± 7.3    | 6.9 ± 0.08    | 146.1 ± 12.1 | 6.2 ± 0.06 | 126.1 ± 8.1 | 5.9 ± 0.04 |
| II    | Control (alloxan) | 21.2 ± 4.2a   | 5.9 ± 0.05a   | 370.2 ± 24.2a | 6.8 ± 0.06a | 280.2 ± 14.2a | 6.6 ± 0.05a |
| III   | Z. noltii extract (50) | 38.5 ± 5.1a  | 6.3 ± 0.06a   | 278.3 ± 15.2a | 6.5 ± 0.05a | 227.3 ± 10.2a | 6.5 ± 0.03 |
| IV    | Z. noltii extract (150) | 55.2 ± 6.2a   | 6.5 ± 0.05a   | 226.3 ± 11.4a | 6.4 ± 0.03a | 180.4 ± 9.1a | 6.3 ± 0.04 |
| V     | Z. noltii extract (250) | 69.3 ± 6.5a   | 6.8 ± 0.04a   | 177.4 ± 8.5a | 6.3 ± 0.03 | 130.2 ± 6.3a | 6.2 ± 0.05 |

E_{max}: maximal effect; pD_{2}: negative logarithm of the concentration that produced half of the E_{max}. Data are expressed as mean ± S.E; n=6; *p<0.001; compared to normal group; "p<0.05, ""p<0.01, """"p<0.001 and """"""p<0.0001; compared to control group); "p<0.05; compared to Group III; "p<0.05 (compared to Group IV).
that administration of Z. noltii extract to alloxan diabetic rats lowers blood glucose and recovers vascular endothelial function in a dose-dependent manner. Also, at dose levels of 150 and 250 mg/kg, Z. noltii extract improves oxidant status and inhibits lipid peroxidation. Since pancreatic tissue damage mediated by lipoxygenase-derived peroxides is closely related with insulin secretion (Metz, 1984; Walsh and Pek, 1984), we firstly hypothesized that glucose lowering activity of Z. noltii extract would be related to its antioxidant effects. However, Z. noltii extract 50 mg/kg, while showing significant antidiabetic activity, failed to preserve the levels of antioxidants and did not alter lipid peroxidation. Taking the above debate into consideration, it is conceivable that antidiabetic effects of Z. noltii extract may possibly not only be related to its antioxidant properties.

Endothelial dysfunction which can be defined as loss of the balance between vasoconstrictors and vasodilators, is a major complication of diabetes and a well-documented phenomenon in various experimental models of hyperglycemia (Oyama et al., 1986; Meraji et al., 1987; Shukla et al., 2004; Gokce and Haznedaroglu, 2008). Increased superoxide anion (O₂⁻) generation and hydrogen peroxide (H₂O₂) accumulation have been...
demonstrated to decrease agonist-stimulated activity of nitric oxide (NO) in diabetic aorta (Karasu, 2000). In the present study, Z. noltii extract, at all tested dose levels, restored acetylcholine relaxations and increased pD$_2$ values in a concentration-dependent manner. This recovery by Z. noltii extract was completely inhibited by the NO synthase inhibitor L-NAME (data not shown), ruling out the possibility that Z. noltii extract causes vasodilation by directly affecting vascular smooth muscle. These findings have given rises to the question whether protective effect of Z. noltii extract on endothelium-dependent relaxation may be related to alterations in NO bioavailability and/or eNOS synthesis. It has been previously shown that polyphenolic compounds in red wine activates eNOS via PI3K pathway (Ndiaye et al., 2005). Also, Hancornia speciosa extract which is rich in polyphenolics was shown to produce NO-dependent vasorelaxation in rat aorta (Ferreira et al., 2007). Moreover, Posidonia oceania, another seagrass widely allocated in the Mediterranean Sea, has been shown to lower blood glucose and prevent hyperglycemia-induced endothelial dysfunction in a similar pattern (Gokce and Haznedaroglu, 2008). Taking into consideration that phosphatidylinositol 3-kinase (PI3K) pathway is crucial to many of the effects of insulin (Epstein, 1999), we have therefore measured eNOS and total nitrite (NOx) levels in aortic tissue. While not affecting NOx, Z. noltii extract elevated eNOS levels in a dose-dependent manner.

On the other hand, our results indicated that increased contractile responses to phenylephrine and 5-HT were normalized by Z. noltii extract treatment with an accompanying reduction in sensitivity. Impaired NO synthesis is known to increase vasocontractility in diabetic animals (Benter et al., 2005). However, in our study, inhibition of NO synthesis by L-NAME did not result in further increments in phenylephrine and 5-HT contractions. Thus, we have questioned the role of ROS production in the contractile responses. Indeed, as reflected by lucigenin and luminol chemiluminescence, Z. noltii extract, inhibited the production of O$_2^{-}$ and other reactive species. Since ROS, especially O$_2^{-}$ are regarded as endothelium-derived contracting factors which play major roles in the regulation of arterial tone (Katusic and Vanhoutte, 1989), free radical scavenging activity of the extract may therefore account for the attenuated contractile responses observed in Z. noltii extract-treated alloxan-diabetic animals.

In conclusion, Z. noltii extract is shown to lower blood glucose and prevent hyperglycemia-induced endothelial dysfunction. Antioxidant/free radical scavenging properties of the extract are unlikely to be the only mechanisms underlying its antidiabetic action. Studies addressing the effects of Z. noltii extract on eNOS-PI3K pathway may provide new insights into mechanisms underlying diabetes and endothelial dysfunction.

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