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Mechanisms Underlying Sesamolin-Induced Attenuation of Vascular Dysfunction in Rats With Streptozotocin-Induced Diabetes

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ABSTRACT

Background: Cardiovascular disorders constitute the major causes of morbidity and mortality among diabetic patients.
Objectives: The effect of chronic sesamolin administration was studied on aortic reactivity of rats with streptozotocin (STZ)-induced diabetes
Materials and Methods: One week after induction of diabetes, male rats received sesamolin for 7 weeks. The contractile responses to KCl and phenylephrine (PE) and the relaxation response to acetylcholine (ACh) were measured in aortic rings.
Results: The maximum contractile response to PE of endothelium-intact aortic rings was significantly lower in sesamolin-treated diabetic rats than in untreated diabetic rats. Removal of the endothelium from the aortic rings abolished this difference. The endothelium-dependent relaxation response to ACh was significantly higher in sesamolin-treated diabetic rats than in untreated diabetic rats. Pretreatment of aortic rings with the nitric oxide synthase inhibitor N(G) -nitro-L-arginine methyl ester (L-NAME) significantly attenuated the observed response. A 2-month course of diabetes also resulted in elevated malondialdehyde (MDA) and decreased superoxide dismutase (SOD) activity; sesamolin treatment reversed the increased MDA level and reversed the reduced SOD activity
Conclusions: We conclude that chronic treatment of diabetic rats with sesamolin can prevent abnormal changes in vascular reactivity via nitric oxide regulation and attenuation of oxidative stress in aortic tissue. Furthermore, endothelial integrity is necessary for sesamolin's beneficial effect.

Implication for health policy/practice/research/medical education:
This study has potential application in development of new treatment strategies for attenuation of diabetes-induced vascular complications.

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1. Background

Cardiovascular disorders constitute the major causes of morbidity and mortality among diabetic patients, despite significant achievements in their diagnosis and treatment (1). Some diabetic vascular complications result from changes in vascular responsiveness, as demonstrated by altered responses to vasoconstrictors and vasodilators (2). Most such vascular complications are the result of increased serum glucose and increased generation of reactive oxygen species (ROS), which lead to endothelium dysfunction (3).

There is some evidence that the major lignans in sesame seeds and sesame oil, such as sesamolin, have bene-

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ficial physiological effects by acting as antioxidants (4), anti-carcinogens (5), anti-hypertensives (6, 7), and serum-lipid reducing agents (8). Sesame constituents may also increase plasma levels of α- and γ-tocopherol in rats (9). Recent work has demonstrated that sesame metabolites induce nitric oxide-dependent vasorelaxation in vitro (10), and ingestion of sesame metabolites enhances endothelium-dependent relaxation in rats with deoxycorticosterone acetate (DOCA)-salt hypertension (7).

2. Objectives

The aqueous extract of sesame leaves has been reported to induce dose-dependent vasorelaxation in guinea-pig aortas (11). However, the mechanisms underlying sesamolin's protective effects on the vascular system in vivo are not completely understood. Therefore, this study was designed to assess the beneficial effects of chronic sesamolin treatment on aortic reactivity dysfunction in rats with streptozotocin (STZ)-induced diabetes and to investigate the underlying mechanisms.

3. Materials and Methods

3.1. Animals

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 230–300 g were housed in an air-conditioned colony room at 21± 2 °C and were supplied with a standard pellet diet and tap water ad libitum. Procedures involving animals and their care conformed to the NIH guidelines for the care and use of laboratory animals.

3.2. Experimental Protocol

Rats were rendered diabetic by a single intraperitoneal dose of 60 mg·kg^{-1} STZ freshly dissolved in ice-cold 0.1 M citrate buffer (pH 4.5). Age-matched rats that received an injection of an equivalent volume of buffer comprised the non-diabetic control group. One week after STZ injection, overnight fasting blood samples were collected, and serum glucose concentrations were measured using a glucose oxidation method (Zistchimie, Tehran). Only rats with a serum glucose level higher than 250 mg/dl were considered diabetic. During the following weeks, diabetes was reconfirmed by the presence of polyphagia, polydipsia, polyuria, and weight loss. Normal and hyperglycemic rats (a total of 48) were randomly allocated into 6 groups of 8 rats: vehicle-treated control, sesamolin-treated controls in 2 subgroups, diabetic, and sesamolin-treated diabetics in 2 subgroups. Sesamolin was administered daily p.o. (using a gavage needle) at doses of 10 and 20 mg/kg b.w. dissolved in 0.5% carboxymethylcellulose for 7 weeks. Changes in body weight were regularly recorded throughout the study.

Finally, the rats were anesthetized with diethyl ether and decapitated. Through opening the abdomen, the descending thoracic aorta was carefully excised and placed in a petri dish filled with cold Krebs solution containing 118.5 mM NaCl, 4.7 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 11 mM glucose. The aorta was cleaned of excess connective tissue and fat and cut into rings of approximately 4 mm in length. Aortic rings were suspended between the bases of two triangular-shaped wires. One wire was attached to a fixed tissue support in a 50-ml isolated tissue bath containing Krebs solution (pH 7.4) maintained at 37°C and continuously aerated with a mixture of 5% CO_2 and 95% O_2. The other end of each wire was attached by a cotton thread to a F60 isometric force transducer (Narco Biosystems, USA) connected to a computer. In all experiments, special care was taken to avoid damaging the luminal surface of endothelium. Aortic rings were equilibrated at a resting tension of 1.5 g for at least 45 min. In some experiments, the endothelium was mechanically removed by gently rubbing the internal surface with filter paper. Isometric contractions were induced by the addition of phenylephrine (PE, 1 μM); once the contraction stabilized, a single concentration of acetylcholine (ACh, 1 μM) was added to the bath in order to assess the endothelial integrity of the preparations. The endothelium was considered to be intact if ACh elicited a vasorelaxation of ≥50% of the maximal contraction obtained in aortic rings pre-contracted with PE. The absence of Ach's relaxant action in an aortic ring indicated the total removal of endothelial cells. After assessing the integrity of the endothelium, the aortic rings were allowed to recuperate for at least 30 min.

At the end of the equilibration period, dose-response curves with KCl (10–50 mM) and PE (10^{-10}–10^{-5} M) were cumulatively obtained in aortic rings with and without endothelium. To evaluate ACh (10^{-9}–10^{-4} M)-induced vasodilatation in aortic rings with endothelium, the aortic rings were pre-contracted with a submaximal concentration of PE (10^{-6} M) that produced 70–80% of the maximal response. The sensitivity to ACh was evaluated as pD2, which is the negative logarithm of the drug concentration required to produce 50% of the maximum response.

To determine the role of NO, rings were incubated with 100 μM L-NAME, a non-selective NOS inhibitor, 30 min before the experiment. To determine the role of endothelial vasodilator factors in response to ACh, aortic rings were incubated with 10 μM indomethacin (INDO), an inhibitor of COX-derived prostanoid synthesis, 30 min before application of ACh.

After each vasoreactivity experiment, aortic rings were blotted and weighed, and the cross-sectional area (csa) was calculated using the following formula: csa (mm^2) = weight (mg) × [length (mm) × density (mg/mm^3)]$^{-1}$. The density of the preparation was assumed to be 1.05 mg/mm^3.

3.3. Determination of MDA Concentration in Aortic Rings

After dissecting the aortic segments and removing extra tissues, they were blotted dry and weighed, then made into a 5% tissue homogenate in ice-cold 0.9% saline solution. A supernatant was obtained from the tis-

sue homogenate by centrifugation (1000 × *g*, 4°C, 5 min). The MDA concentration (thiobarbituric acid reactive substances, TBARS) in the supernatant was measured as previously described (12). Briefly, trichloroacetic acid and TBARS reagent were added to the supernatant, mixed, and incubated at 100°C for 80 min. After cooling on ice, the samples were centrifuged at 1000 × *g* for 20 min, and the absorbance of the supernatant was read at 532 nm. The TBARS results were expressed as MDA equivalents using tetraethoxypropane as a standard.

3.4. Measurement of SOD Activity in Aortic Rings

Supernatants from the tissue homogenate were obtained as previously described (13). Briefly, the supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37°C) for 40 min, and NBT was added. Blue color formation was then monitored spectrophotometrically at 550 nm. The amount of protein that inhibited NBT reduction to 50% of maximum was defined as 1 nitrite unit (NU) of SOD activity.

3.5. Drugs

PE, sesamolin, STZ, ACh, INDO, and L-NAME were purchased from Sigma Chemical (St. Louis, MO, USA). All other chemicals were purchased from Merck (Germany) and Temad (Iran). The INDO solution was prepared in ethanol so that the ethanol concentration of the medium was less than 0.001% (v/v).

3.6. Data and Statistical Analysis

All values are given as mean ± SEM. The contractile response to PE is expressed as grams of tension per csa of tissue. The relaxation response for ACh is expressed as a percentage decrease of the maximum contractile response induced by PE. Statistical analysis was carried out using repeated-measures ANOVA and 1-way ANOVA followed by Tukey post-hoc tests. A *P*-value less than 0.05 was considered statistically significant.

4. Results

After 8 weeks, vehicle-treated diabetic rats weighed significantly less than non-diabetic controls ($P < 0.005$). Sesamolin treatment at both doses, but especially at 20 mg/kg, caused a non-significant decrease in weight loss in diabetic rats as compared to diabetic rats treated with vehicle. Vehicle-treated diabetic rats had also an elevated serum glucose level compared to non-diabetic rats ($P < 0.0005$); treatment of diabetic rats with sesamolin, especially at a dose of 20 mg/kg, caused a non-significant decrease in serum glucose compared to untreated diabetic rats. In addition, sesamolin treatment of control rats did not significantly change serum glucose levels. The cumulative addition of KCl (10–50 mM) and PE (10^{-10}–10^{-5} M) resulted in concentration-dependent contraction of the aortic rings from all groups (*Figures 1, 2*). The

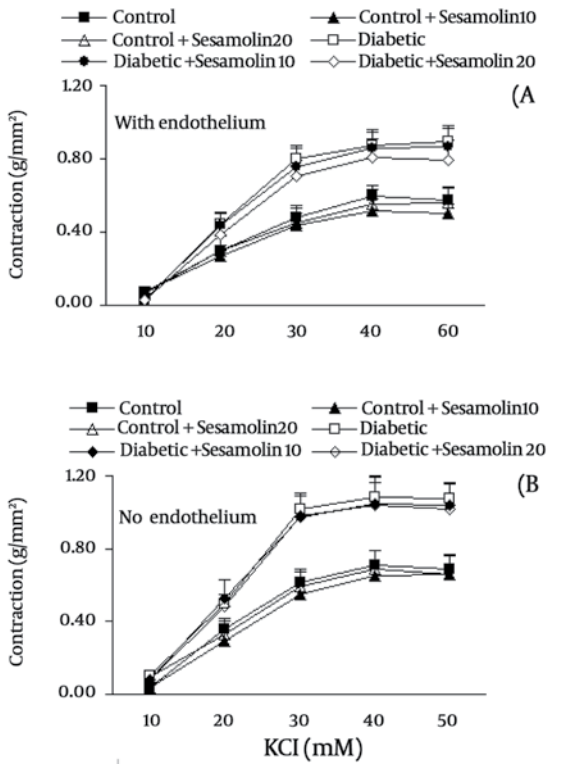

Figure 1. Cumulative Concentration-Response Curves for KCl in Aortic Rings 8 Weeks after the Experiment in the Presence (A) and Absence (B) of Endothelium (Mean ± S.E.M).

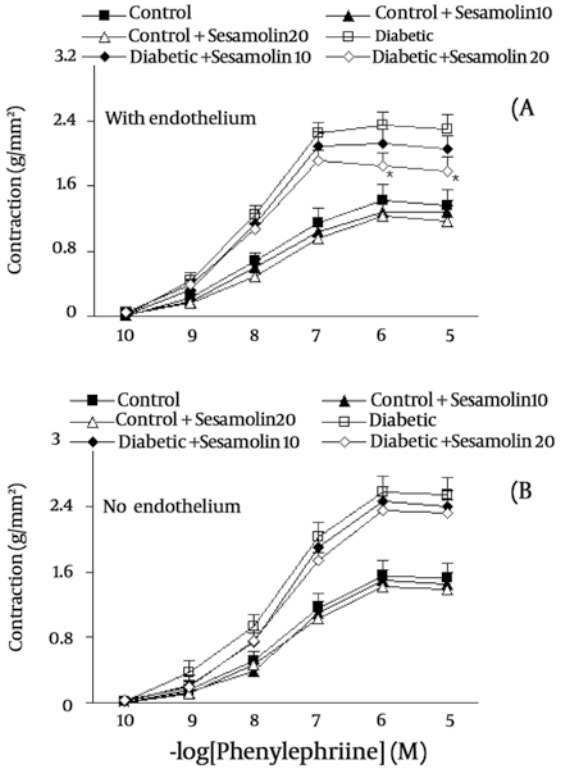

Figure 2. Cumulative Concentration-Response Curves for PE in Aortic Preparations 8 Weeks after the Experiment in the Presence (A) and Absence (B) of Endothelium (Mean ± S.E.M).

* $P < 0.05$ as compared to diabetic rats.

maximum contractile responses to KCl and PE of endothelium-intact aortic rings were significantly greater in vehicle-treated diabetic rats than in vehicle-treated control rats ($P < 0.01$–0.005). The concentration-response curves to PE (but not to KCl) of endothelium-intact aortas from was significantly attenuated in diabetic rats treated with 20 mg/kg sesamolin compared to diabetic rats treated with vehicle ($P < 0.05$). Endothelium-denuded aortic rings from all groups showed a higher contractile response to KCl and PE; however, after endothelium removal, the differences between sesamolin- and vehicle-treated diabetic rats were attenuated. This indicates that the endothelium is necessary for sesamolin's beneficial vascular effects. In addition, the contractile response to KCl and PE of endothelium-intact aortic rings was non-significantly reduced in the sesamolin-treated control group compared to the vehicle-treated control group. There were no significant differences in pD2 between the groups (data not shown), indicating that there were no differences in the sensitivity of aortic rings. ACh addition resulted in concentration-dependent relaxation in all aortic rings pre-contracted with PE (*Figure 3*). As expected, the endothelium-dependent relaxation induced by ACh was significantly lower in vehicle-treated diabetic rats than in vehicle-treated non-diabetic rats ($P < 0.05$–0.005). Meanwhile, diabetic rats treated with 20 mg/kg sesamolin and diabetic rats treated with vehicle significantly differed in relaxation response ($P < 0.05$) only at ACh concentrations higher than 10^{-5} M. Sesamolin treatment non-significantly increased the relaxation response of non-diabetic rats.

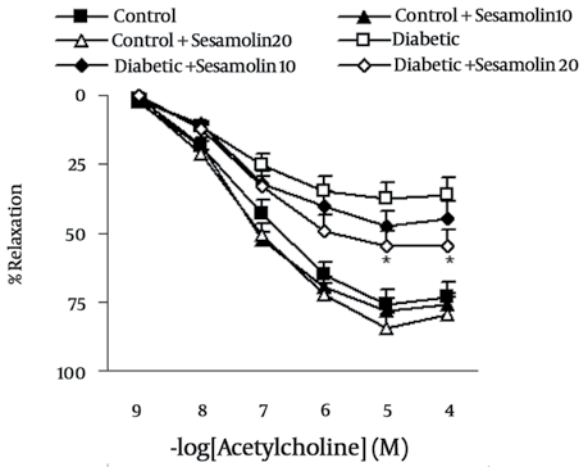

Figure 3. Cumulative Concentration-Response Curves for ACh in Endothelium-Intact Aortic rings Pre-contracted with PE 8 Weeks after the Experiment. Relaxation responses are expressed as a percentage of the submaximal contraction induced by PE that produced 70–80% of the maximal response (mean ± SEM).
* $P < 0.05$ as compared to diabetic rats.

Next, we assessed the relaxation response to ACh. When aortic rings from diabetic rats treated with 20 mg/kg sesamolin were pre-incubated with L-NAME, there was an almost complete abolition of ACh's vasodilator effect, indicating the importance of endothelium-derived NO in sesamolin's vascular effect (*Figure 4*). When aortic rings from diabetic rats treated with 20 mg/kg sesamolin were pre-incubated with INDO, there was a partial, non-significant decrease in the endothelial vasodilator response to ACh (*Figure 5*).

Finally, we assessed aortic lipid peroxidation markers (*Figure 6*). STZ-induced diabetes resulted in an elevation of MDA content and a decrease in SOD activity ($P < 0.005$–0.001) in aortic tissue. Chronic treatment of diabetic rats

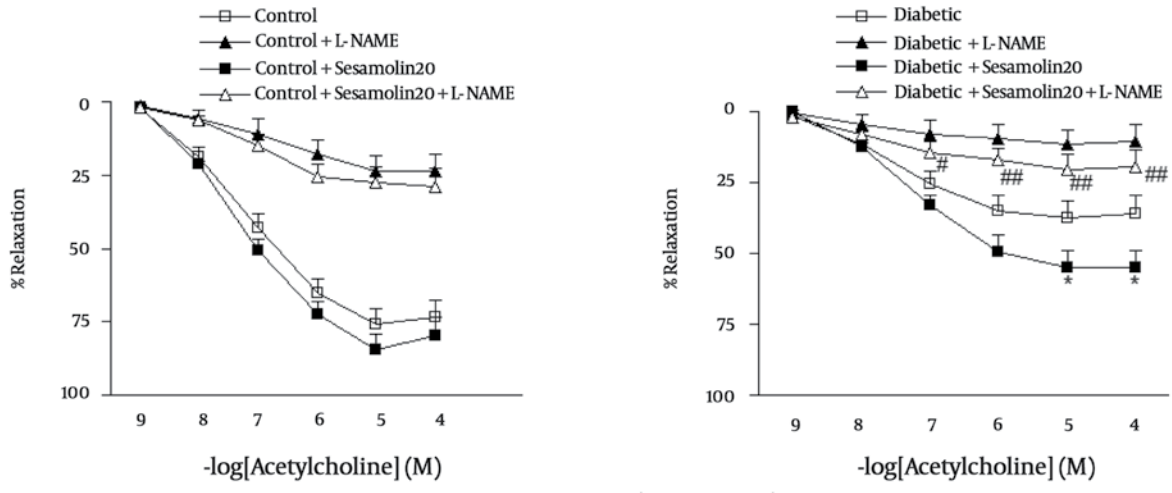

Figure 4. Cumulative Concentration-Response Curves for ACh in Endothelium-Intact Aortic Rings Pre-Contracted With PE in the Presence and Absence of L-NAME 8 Weeks After the Experiment in Control and Diabetic Rats. Relaxation responses are expressed as a percentage of the submaximal contraction induced by PE that produced 70–80% of the maximal response (mean ± SEM). L-NAME, N (omega)-L-arginine methyl ester
* $P < 0.05$ as compared to diabetic rats; # $P < 0.01$; ## $P < 0.005$ as compared to diabetic rats treated with 20 mg/kg sesamolin.

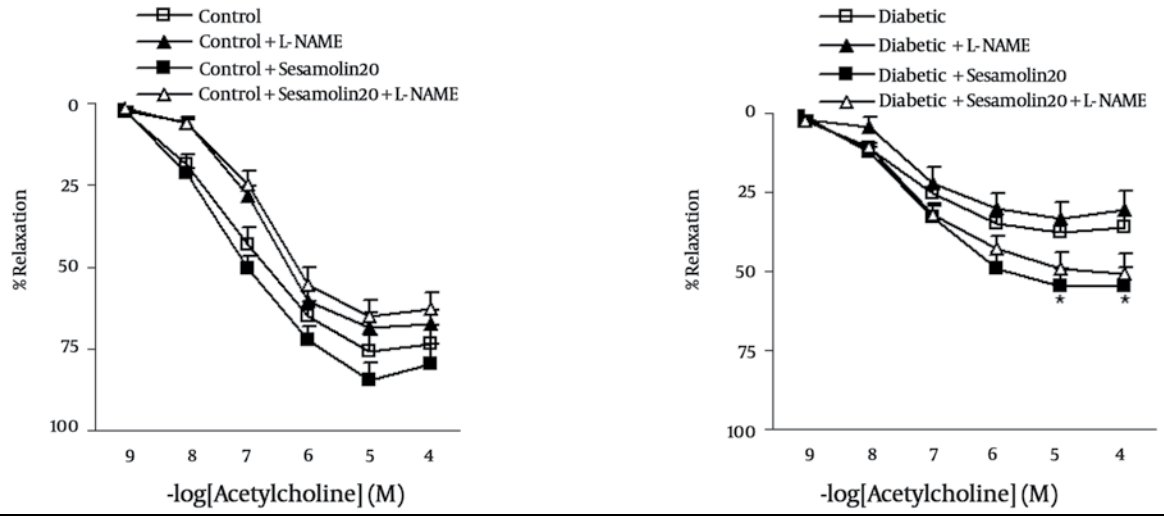

Figure 5. Maximum Relaxation Response to ACh in Aortic Rings Pre-Contracted With PE in the Presence and Absence of INDO 8 Weeks After the Experiment in Control and Diabetic Rats. Relaxation responses are expressed as a percentage (Mean ± SEM).
$^{*}P < 0.05$ as compared to diabetic rats.

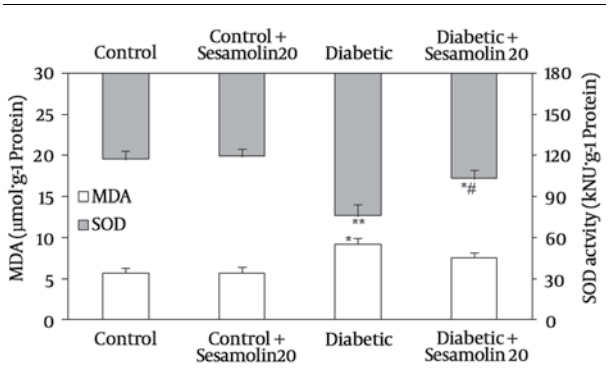

Figure 6. Malondialdehyde level and Superoxide Dismutase Activity in Aortic Tissue
$^{*}P < 0.005$, $^{**}P < 0.001$ vs. control group; $^{\#}P < 0.05$ *vs.* diabetic group

with 20 mg/kg sesamolin non-significantly reversed the increased MDA content and significantly reversed the reduction of SOD activity ($P < 0.05$).

5. Discussion

In this study, sesamolin administration for 7 weeks did not have a significant hypoglycemic effect; however, it reduced enhanced aortic contractility to PE and increased aortic relaxation to ACh. These effects likely involved the NO pathway, since relaxation was blocked in the presence of L-NAME. In the presence of INDO, the relaxation response to ACh was non-significantly, partly attenuated. In addition, endothelium removal affected KCl- and PE-induced contraction in sesamolin-treated diabetic rats. Regarding oxidative stress markers, sesamolin treatment attenuated the increased MDA content and reduced SOD activity. Vascular dysfunction is a major complication of diabetes in humans and in experimental diabetes models. Hyperglycemia is the primary cause of micro- and macrovascular complications in diabetes (14). Compared to aortic rings from non-diabetic rats, those from diabetic rats showed a significant increase in contraction to KCl and PE, consistent with previous studies (12); chronic sesamolin treatment attenuated this change only for PE-induced contractions. The increased aortic contractile responses of diabetic rats may be due to impaired endothelial function (15), enhanced sensitivity of calcium channels (16), increased vasoconstrictor prostanoids due to increased superoxide anions, and increased sensitivity to adrenergic agonists (17); sesamolin treatment may have improved these parameters. In the endothelial cells of most vascular beds, ACh stimulates production and release of endothelial-derived relaxing factors, including NO, prostacyclin, and endothelium-derived hyperpolarizing factor. Therefore, ACh relaxes vascular smooth muscle in an endothelium-dependent manner (18-20), and ACh-induced relaxation is considered endothelium-dependent and NO-mediated (12). Results of the present work revealed that the STZ-induced diabetic reduced the endothelium-dependent relaxation response; this reduced relaxation was partially recovered by sesamolin treatment. Although it has been suggested that sensitivity to ACh decreases in diabetes (17), the results of this study and others (21) demonstrate that long-term diabetes decreases only the maximum response to ACh, but not the sensitivity (pD2). Impaired endothelium-dependent relaxation in rats with STZ-induced diabetes might be caused by increased blood glucose and decreased blood insulin levels. Indeed, hyperglycemia has been shown to damage tissue via several mechanisms, including advanced glycation end-product (AGE) formation, increased polyol pathway flux, apoptosis, and reactive oxygen species (ROS) formation (22). In the present study, sesamolin treatment did not significantly reduce blood glucose levels in STZ-induced diabetic rats; therefore, its beneficial effect on aortic tissue is likely due to mechanisms other than a hypoglycemic effect. The damaging ef-

fects of diabetes on vascular tissue is believed to be due to enhanced oxidative stress, as measured by enhanced MDA and decreased activity of defensive enzymes like SOD (13), as was observed in this study. Enhanced oxidative stress can also lead to diabetes-induced functional changes in vascular endothelial cells and the development of altered endothelium-dependent vasoreactivity. The results of the present study showed that chronic sesamolin treatment decreased MDA content and significantly enhanced SOD activity in aortic tissue of diabetic rats, indicating that sesamolin may improve vascular responsiveness partly by ameliorating lipid peroxidation and oxidative injury.

In conclusion, to the best of our knowledge, this is the first study to demonstrate that chronic treatment of diabetic rats with sesamolin dose-dependently prevented diabetes-related functional changes in vascular reactivity through nitric oxide- but not prostaglandin-dependent pathways and through attenuation of aortic lipid peroxidation. Our data may pave the way for the development of new plant-based drugs that can improve endothelial function and prevent cardiovascular disease.

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