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The Defensive Effect of Benfotiamine in Sodium Arsenite-Induced Experimental Vascular Endothelial Dysfunction

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Abstract The present study has been designed to investigate the effect of benfotiamine, a thiamine derivative, in sodium arsenite-induced vascular endothelial dysfunction (VED) in rats. Sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks) was administered in rats to produce VED. The development of VED was assessed by employing isolated aortic ring preparation and estimating the serum and aortic concentrations of nitrite/nitrate. Further, the integrity of vascular endothelium in thoracic aorta was assessed by scanning electron microscopy. Moreover, the oxidative stress was assessed by estimating serum thiobarbituric acid reactive substances (TBARS) and aortic superoxide anion generation. The administration of sodium arsenite markedly produced VED by attenuating acetylcholine-induced endothelium-dependent relaxation, decreasing serum and aortic concentrations of nitrite/nitrate, and impairing the integrity of vascular endothelium. Further, sodium arsenite produced oxidative stress by increasing serum TBARS and aortic superoxide generation. The treatment with benfotiamine (25, 50, and 100 mg^{-1} kg^{-1} day^{-1} p.o.) or atorvastatin (30 mg^{-1} kg^{-1} day^{-1} p.o., a standard agent) prevented sodium arsenite-induced VED and oxidative stress. However, the beneficial effects of benfotiamine in preventing the sodium arsenite-induced VED were attenuated by co-administration with *N*-omega-nitro-L-arginine methyl ester (L-NAME) (25 mg^{-1} kg^{-1} day^{-1}, i.p.), an inhibitor of NOS. Thus, it may be concluded that benfotiamine reduces oxidative stress and activates endothelial nitric oxide synthase to enhance the generation and bioavailability of NO and subsequently improves the integrity of vascular endothelium to prevent sodium arsenite-induced experimental VED.

Keywords Sodium arsenite · Endothelial dysfunction · Benfotiamine · eNOS

Introduction

Endothelium is a monolayer of cells that line the intimal surface of the entire vasculature [1]. Endothelium maintains the balance between the releases of endothelium derived relaxing and contracting factors to regulate vascular homeostasis [2, 3]. A major vasodilatory substance

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released by the endothelium is nitric oxide (NO), which is also known as endothelium-derived relaxing factor or endothelium-derived nitric oxide [4, 5]. Endothelial nitric oxide synthase (eNOS), in the presence of cofactors such as Ca^{2+}/calmodulin, flavin adenine dinucleotide, flavin mononucleotide, and tetrahydrobiopterin (BH_4), catalyzes the conversion of L-arginine to L-citrulline to generate NO [6]. NO has anti-inflammatory and anti-proliferative properties, and it inhibits platelet adhesion and aggregation [7]. Vascular endothelial dysfunction (VED) is an insidious condition during which the generation and bioavailability of NO is decreased due to reduction in the expression and activation of eNOS and excessive generation of reactive oxygen species (ROS) that diminish the vasodilatory, anti-inflammatory, and anti-thrombotic properties of endothelium [8, 9]. VED plays a pathogenic role in atherosclerosis [10], hypertension [11], and coronary artery disease [12]. Arsenic is a ubiquitous element found in several forms of food and environmental media like soil, ground water, and air. The exposure to arsenic plays a detrimental role in the induction of vascular pathogenesis [13]. Arsenic has been reported to increase the expressions of interleukins-1ß, interleukin-6, and monocyte chemoattractant protein (MCP-1), which promotes the attachment, penetration, and migration of leukocytes in vascular smooth muscle cells [14]. Further, arsenic activates nuclear factor kappa-B [15] and upregulates the expression of various proatherogenic genes to induce atherosclerosis [16]. Moreover, sodium arsenite has been reported to inhibit the activity of eNOS and Akt that decrease the amount of NO generation [17], which may lead to VED. In addition, arsenic has been reported to produce oxidative stress by activating NADPH oxidase [18, 19]. Furthermore, we have recently demonstrated that administration of sodium arsenite for 2 weeks produced VED in rats by impairing the integrity of vascular endothelium, reducing the endothelium-dependent vasodilation, and increasing the oxidative stress [20, 21]. Benfotiamine, a lipophilic thiamine derivative, prevents the progression of diabetic complications, probably by inhibiting the formation of advanced glycation products (AGE) through the activation of transketolase [22]. Benfotiamine has been shown to reduce the oxidative stress through the mechanism unrelated to AGE formation [23]. Activation of Akt/protein kinase B has been shown to stimulate eNOS, which generates NO and reduces oxidative stress and thus prevents VED [24–26]. Benfotiamine has been reported to activate Akt/protein kinase B [27, 28]. Recently we have demonstrated that benfotiamine prevented nicotine and uric acid-induced experimental VED by improving the integrity of endothelium, enhancing the endothelial function, and reducing the oxidative stress [29]. The present study investigated the dose-dependent effects of benfotiamine in sodium arsenite-induced VED in rats.

Materials and Methods

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Age-matched young Wistar rats weighing about 200–240 g were employed in the present study. Rats were fed on standard chow diet (Ashirwad Industries, Mohali, India) and water *ad libitum*. They were acclimatized in animal house and were exposed to normal cycles of day and night.

Assessment of Vascular Endothelial Dysfunction

Isolated Rat Aortic Ring Preparation

The development of VED was assessed by determining the acetylcholine (Ach)-induced endothelium-dependent relaxation using isolated aortic rings. The rat was decapitated, and

the thoracic aorta was removed, cut into rings of 3–4 mm in length, and mounted in an organ bath containing Krebs–Henseleit solution (NaCl, 119 mM; KCl, 4.7 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 1.0 mM; glucose, 11.1 mM; KH_2PO_4, 1.2 mM; and $CaCl_2$, 2.5 mM) of pH 7.4, bubbled with carbonated oxygen (95% O_2 and 5% CO_2), and maintained at 37°C. The preparation was allowed to equilibrate for 90 min under 1.5 g tension. The isometric contractions were recorded using a force-transducer (Ft-2040) connected to Physiograph (INCO, Ambala, India). The aortic ring preparation was primed with 80 mM of KCl to check its functional integrity and to improve its contractility. The cumulative dose responses of Ach (10^{-8} to 10^{-4} M) or sodium nitroprusside (SNP; 10^{-8} to 10^{-4} M) were recorded in phenylephrine (3×10^{-6} M)–precontracted preparation with intact or denuded endothelium, respectively [30]. The intimal layer of the aortic ring was rubbed gently with a moistened filter paper for 30 s to obtain endothelium-free preparation [31]. Loss of Ach (1×10^{-6} M)-induced relaxation confirmed the absence of vascular endothelium.

Scanning Electron Microscopy

The scanning electron microscopy study was carried out in the Central Instrument Laboratory, Panjab University, Chandigarh. The scanning electron microscopic study was performed to examine the integrity of vascular endothelium [32, 33]. The longitudinal strips of thoracic aorta (3–4 mm) were fixed in 3% glutaraldehyde phosphate buffer (pH 7.4) and subsequently dehydrated in a series of acetone solution (50% for 20 min, 70% for 20 min, 80% for 20 min, 90% for 20 min, and 100% for 50 min), followed by isoamylacetate (100%) and acetone (100%) solution in the ratio of 1:1 for 20 min, and followed by isoamylacetate (100%) alone for 20 min. Arterial segments were further dried using four flushes of liquid CO_2 with 100-psi pressure in critical point drier. The segments were then mounted on aluminum stubs and coated with gold palladium (JFC-1100) and were viewed using JOEL JSM 6100 scanning electron microscope to observe the integrity of vascular endothelium.

Estimation of Serum and Aortic Nitrite/Nitrate Concentration

The aortic tissue was homogenized in phosphate-buffered saline (pH 7.4) and centrifuged at 10,000×*g* for 20 min. The supernatant was used to estimate the aortic concentration of nitrite/nitrate and protein content. Carbonate buffer (400 μL; equal volumes of 500 mM sodium bicarbonate and 50 mM sodium carbonate were mixed to obtain 50 mM carbonate buffer; pH 9.0) was added to 100 μL of serum sample or 100 μL of supernatant from homogenized aortic samples, followed by addition of small amount (0.15 g) of copper–cadmium alloy. The tubes were incubated at room temperature for 1 h to reduce nitrate to nitrite. The reaction was stopped by adding 100 μL of 0.35 M NaOH. Following this, 400 μL of zinc sulfate solution (120 mM) was added to deproteinate the samples. The samples were allowed to stand for 10 min and then centrifuged at 4,000×*g* for 10 min. Greiss reagent (250 μL of 1.0% sulfanilamide and 250 μL of 0.1% *N*-naphthylethylenediamine) was added to aliquots (500 μL) of clear supernatant and nitrite/nitrate concentration was measured spectrophotometrically (Thermo Double Beam Spectrophotometer, Thermo Electron Corporation, UK) at 545 nm. The standard graph of sodium nitrite was plotted to calculate the concentration of serum nitrite/nitrate (μM/L) and aortic nitrite/nitrate (μM/mg of protein) [34, 35]. The protein concentration in homogenized aortic preparation was estimated by Lowry's method [36].

Assessment of Oxidative Stress

The oxidative stress was assessed by estimating serum thiobarbituric acid reactive substances (TBARS) and aortic superoxide anion generation.

Estimation of TBARS

One milliliter of 20% trichloroacetic acid was added to 100 μL of serum and 1 ml of thiobarbituric acid reagent (1%), which were mixed and incubated at 100°C for 30 min. After cooling on ice, samples were centrifuged at 1,000×*g* for 20 min. Serum concentration of TBARS was measured spectrophotometrically at 532 nm. A standard graph using 1, 1, 3, 3 tetramethoxypropane was plotted to calculate the concentration of TBARS [37].

Estimation of Superoxide Anion

Aorta was cut into transverse rings of 6 mm in length and placed in 5 ml of Krebs–Henseleit solution buffer containing nitroblutetrazolium (NBT; 100 μM/L) and incubated at 37°C for 90 min. The NBT reduction was stopped by adding 5 ml of 0.5 N HCl. The rings were minced and homogenized in a mixture of 0.1 N NaOH and 0.1% sodium dodecyl sulfate in water containing 40 mg/L of diethylenetriamine pentaacetic acid. The mixture was centrifuged at 20,000×*g* for 20 min, and the resultant pellets were re-suspended in 1.5 ml of pyridine and kept at 80°C for 90 min to extract formazan. The mixture was centrifuged at 10,000×*g* for 10 min, and the absorbance of formazan was determined spectrophotometrically at 540 nm [38]. The amount of reduced NBT was calculated using the following formula: Amount of reduced NBT=$A \cdot V/(T \cdot \mathrm{Wt} \cdot \varepsilon \cdot l)$, where A is absorbance, V is volume of solution (1.5 ml), T is time for which aortic rings were incubated with NBT (90 min), Wt is blotted wet weight of aortic rings, ε is extinction coefficient (0.72 l mmol^{-1} mm^{-1}), and l is length of light path (10 mm).

Experimental Protocol

Nine groups were employed in the present study and each comprised seven rats. The benfotiamine and atorvastatin were suspended in 0.5% of carboxymethylcellulose. Group 1 (normal control): Rats were maintained on standard food and water and no treatments were given. Group II (sodium arsenite control): Rats were administered with sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1} i.p.) for 2 weeks. Group III (benfotiamine per se): Rats were administered with benfotiamine (100 mg^{-1} kg^{-1} day^{-1} p.o.) for 2 weeks. Group IV (benfotiamine 25 mg^{-1} kg^{-1} day^{-1} treated group): Rats administered with sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks) were treated with benfotiamine (25 mg^{-1} kg^{-1} day^{-1} p.o.), and the treatment was started 3 days before the administration of sodium arsenite and continued for 2 weeks from the day of administration of sodium arsenite. Group V (benfotiamine 50 mg^{-1} kg^{-1} day^{-1} treated group): Rats administered with sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks) were treated with benfotiamine (50 mg^{-1} kg^{-1} day^{-1} p.o.) as mentioned in group IV. Group VI (benfotiamine 100 mg^{-1} kg^{-1} day^{-1} treated group): Rats administered with sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks) were treated with benfotiamine (100 mg^{-1} kg^{-1} day^{-1} p.o.) as mentioned in group IV. Group VII (atorvastatin-treated group): Rats administered with sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks) were treated with atorvastatin (30 mg^{-1} kg^{-1} day^{-1} p.o.) as mentioned in group IV. Group VIII (benfotiamine and *N*-omega-nitro-L-arginine methyl ester (L-NAME)

treated sodium arsenite group): Rats administered with sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks) were subjected for treatment with benfotiamine (100 mg^{-1} kg^{-1} day^{-1} p.o.) as mentioned in group IV and L-NAME (25 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks). The L-NAME treatment was started from the day of administration of sodium arsenite and continued for 2 weeks. Group IX (atorvastatin and L-NAME-treated sodium arsenite group): Rats administered with sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks) were subjected for treatment with atorvastatin (30 mg^{-1} kg^{-1} day^{-1} p.o.) as mentioned in group IV and L-NAME (25 mg^{-1} kg^{-1} day^{-1} i.p., 2 weeks). The L-NAME treatment was started from the day of administration of sodium arsenite and continued for 2 weeks.

Statistical Analysis

All values were expressed as mean ± SEM. Data for isolated aortic ring preparation were statistically analyzed using repeated measures of ANOVA followed by Newman Keul's test. The data for serum and aortic levels of nitrite/nitrate, serum TBARS, and aortic superoxide anion generation were statistically analyzed using one-way ANOVA followed by Tukey's multiple comparison test. The $p<0.05$ was considered to be statistically significant.

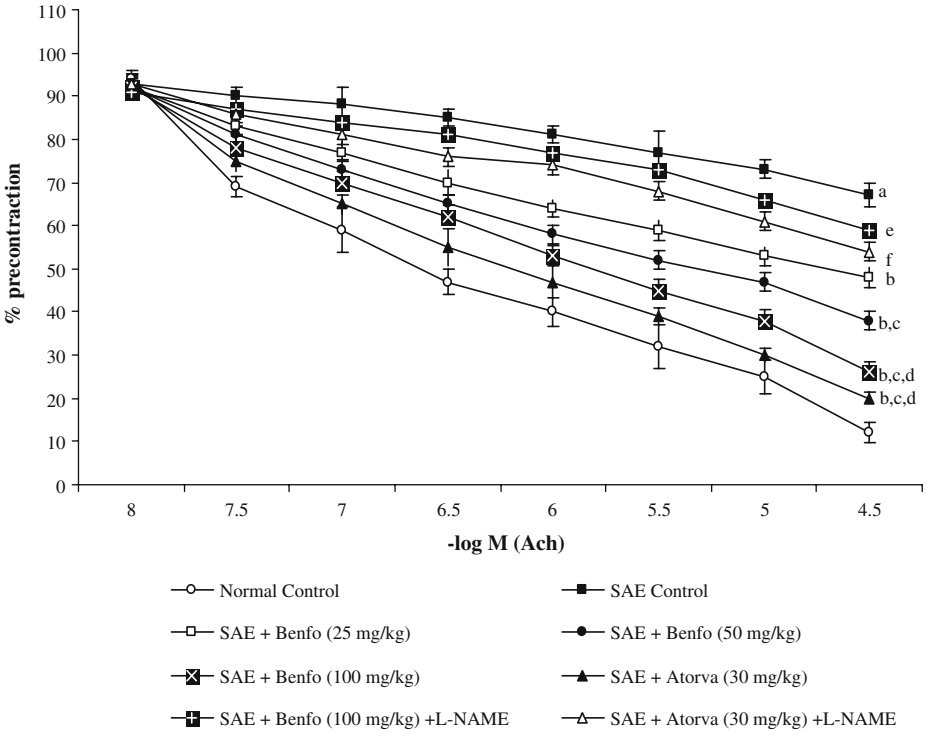

Fig. 1 Effect of benfotiamine on sodium arsenite-provoked attenuation of Ach-induced endothelium-dependent relaxation. Responses are expressed as percentage of maximum contraction induced by phenylepherine (3×10^{-6} M). All values are represented as mean ± SEM. *SAE* sodium arsenite, *Benfo* benfotiamine, *Atorva* atorvastatin in all figures. [a]$p<0.05$ vs normal control; [b]$p<0.05$ vs SAE control; [c]$p<0.05$ vs SAE+Benfo (25 mg/kg); [d]$p<0.05$ vs SAE+Benfo (50 mg/kg); [e]$p<0.05$ vs SAE+Benfo (100 mg/kg); [f]$p<0.05$ vs SAE+Atorva (30 mg/kg)

Drugs and Chemicals

Acetylcholine hydrochloride and L-phenylephrine were purchased from Sigma-Aldrich Ltd., St. Louis, Mo, USA. Diethyl triamine pentaacetic acid and nitroblue tetrazolium were purchased from Sanjay Biologicals Amritsar, India. 1,1,3,3 tetramethoxypropane and carboxymethyl cellulose were purchased from V. K Chemicals, India. Benfotiamine was obtained from Orchid Healthcare Ltd, Chennai. Atorvastatin was obtained from Dr. Reddy's Laboratory Ltd., Hyderabad, India. Sodium arsenite was purchased from Loba chemie, Mumbai.

Results

The treatment with benfotiamine (100 mg^{-1} kg^{-1} day^{-1}, p.o.) to normal rats did not produce any significant per se effects on various parameters performed in the present study.

Effect of Pharmacological Interventions on Endothelium-Dependent and Endothelium-Independent Relaxation

Ach and SNP were noted to produce endothelium-dependent and endothelium-independent relaxation, respectively, in phenylephrine (3×10^{-6} M) precontracted isolated

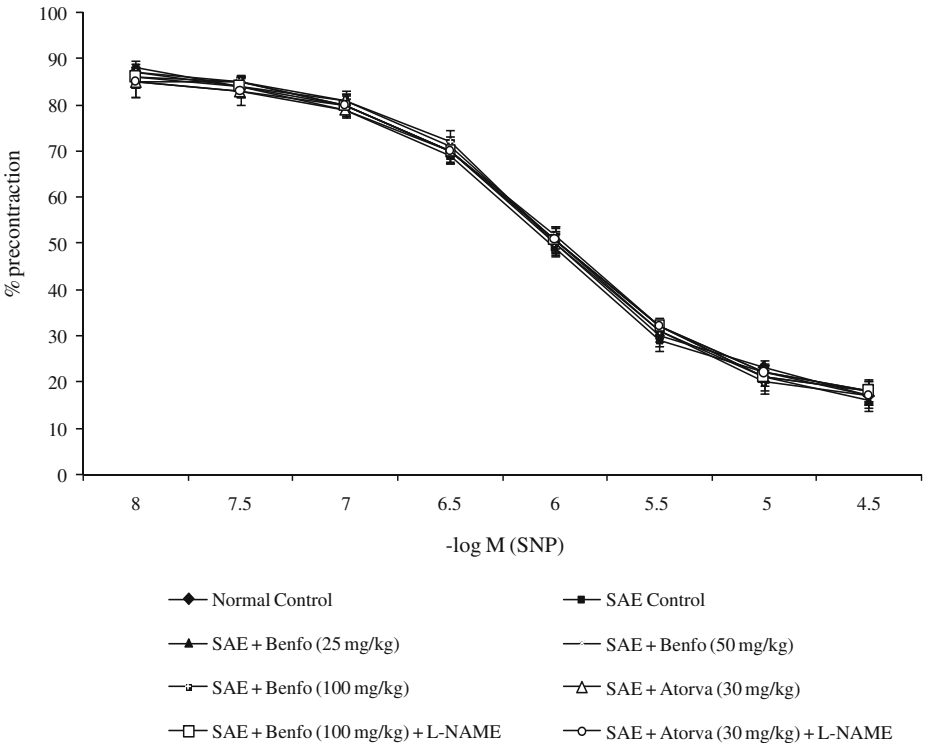

Fig. 2 Effect of benfotiamine on sodium nitroprusside-induced endothelium-independent relaxation. Responses are expressed as percentage of maximum contraction induced by phenylephrine (3×10^{-6} M). All values are represented as mean ± SEM

rat aortic ring preparation in a dose-dependent manner. Administration of sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1}, i.p., 2 weeks) significantly attenuated Ach-induced endothelium-dependent relaxation, but its administration did not affect SNP-induced endothelium-independent relaxation. The treatment with benfotiamine (25, 50, 100 mg^{-1} kg^{-1} day^{-1} p.o.; dose dependently) or atorvastatin (30 mg^{-1} kg^{-1} day^{-1} p.o.) significantly prevented sodium arsenite-provoked attenuation of Ach-induced endothelium-dependent relaxation. However, these ameliorative effects of benfotiamine (100 mg^{-1} kg^{-1} day^{-1}, p.o.) and atorvastatin (30 mg^{-1} kg^{-1} day^{-1} p.o.) have been significantly prevented by co-administration with L-NAME (25 mg kg^{-1} day^{-1} i.p.; Figs. 1, 2).

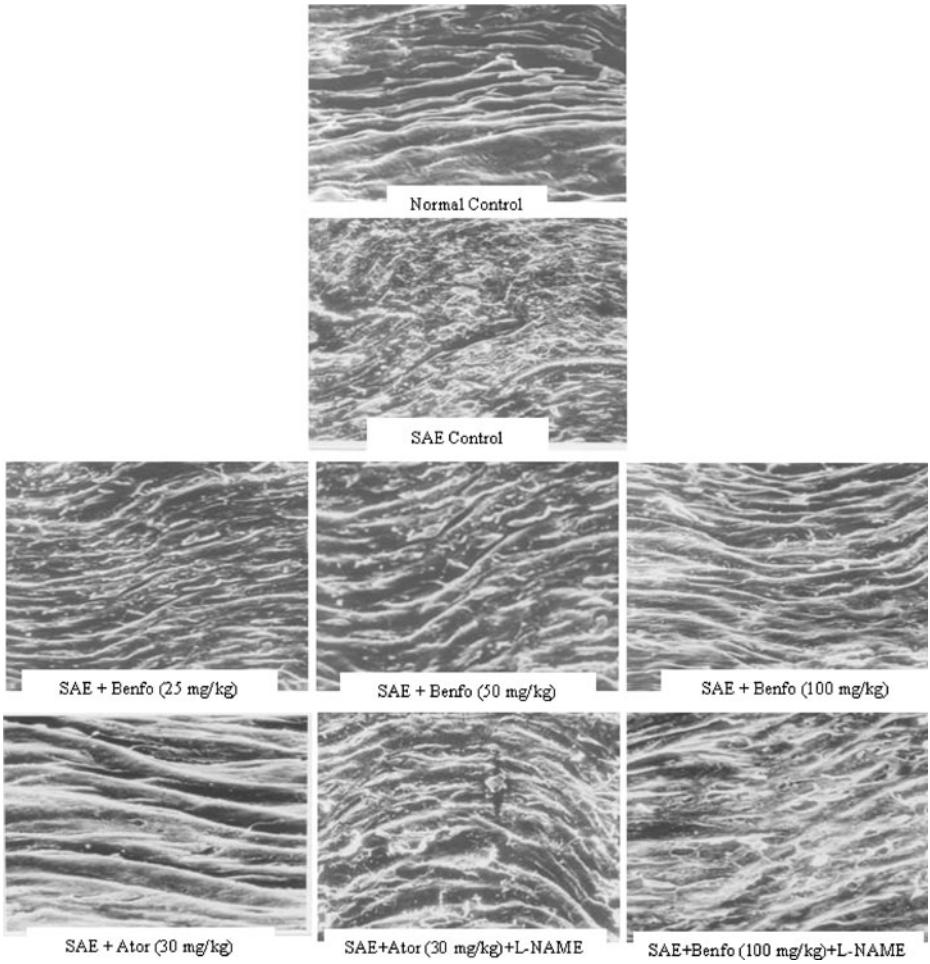

Fig. 3 Effect of benfotiamine on the integrity of vascular endothelium. The scanning electron microscopic study was performed to examine the integrity of vascular endothelium using JOEL JSM 6100 scanning electron microscope (×800). The impairment in integrity of vascular endothelium was noted in aorta of sodium arsenite-administered rats. The treatment with benfotiamine or atorvastatin markedly prevented sodium arsenite-induced impairment in integrity of vascular endothelium

Effect of Pharmacological Interventions on Integrity of Vascular Endothelium

The integrity of vascular endothelium was noted to be impaired in sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1}, i.p., 2 weeks) administered rats. However, treatment with both benfotiamine (25, 50, 100 mg^{-1} kg^{-1} day^{-1}, p.o.) and atorvastatin (30 mg^{-1} kg^{-1} day^{-1}, p.o.) markedly improved the integrity of vascular endothelium in sodium arsenite-administered rats. But the ameliorative effects of benfotiamine (100 mg^{-1} kg^{-1} day^{-1} p.o.) and atorvastatin (30 mg^{-1} kg^{-1} day^{-1}, p.o.) in improving the integrity of vascular endothelium have been significantly prevented by co-administration with L-NAME (25 mg^{-1} kg^{-1} day^{-1} i.p.; Fig. 3).

Effect of Pharmacological Interventions on Serum and Aortic Nitrite/Nitrate Concentration

The serum and aortic concentrations of nitrite/nitrate were noted to be reduced in sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1}, i.p., 2 weeks) administered rats as compared to normal rats. However, treatment with benfotiamine (25, 50, and 100 mg^{-1} kg^{-1} day^{-1} p.o.; dose dependently) or atorvastatin (30 mg^{-1} kg^{-1} day^{-1} p.o.) significantly attenuated sodium arsenite-induced decrease in serum and aortic nitrite/nitrate concentration. However, these ameliorative effects of benfotiamine (100 mg^{-1} kg^{-1} day^{-1} p.o.) and atorvastatin (30 mg^{-1} kg^{-1} day^{-1}, p.o.) were significantly prevented by co-administration with L-NAME (25 mg kg^{-1} day^{-1} i.p.; Figs. 4, 5).

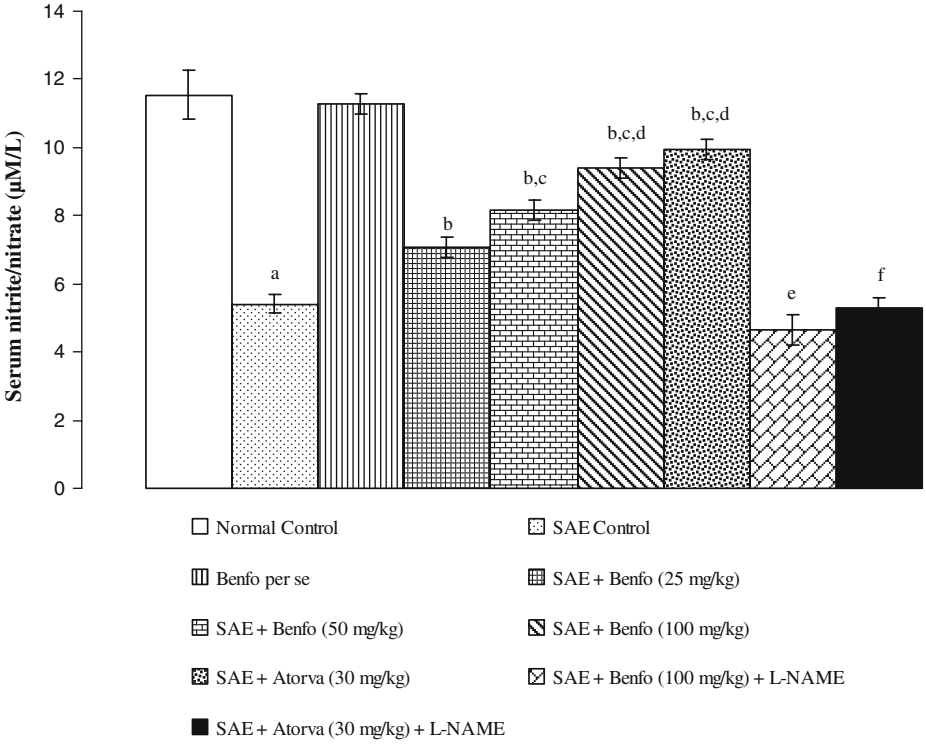

Fig. 4 Effect of benfotiamine on sodium arsenite-induced decrease in serum concentration of nitrite/nitrate (μM/L). All values are represented as mean ± SEM. [a]$p<0.05$ vs normal control; [b]$p<0.05$ vs SAE control; [c]$p<0.05$ vs SAE+Benfo (25 mg/kg); [d]$p<0.05$ vs SAE+Benfo (50 mg/kg); [e]$p<0.05$ vs SAE+Benfo (100 mg/kg); [f]$p<0.05$ vs SAE+Atorva (30 mg/kg)

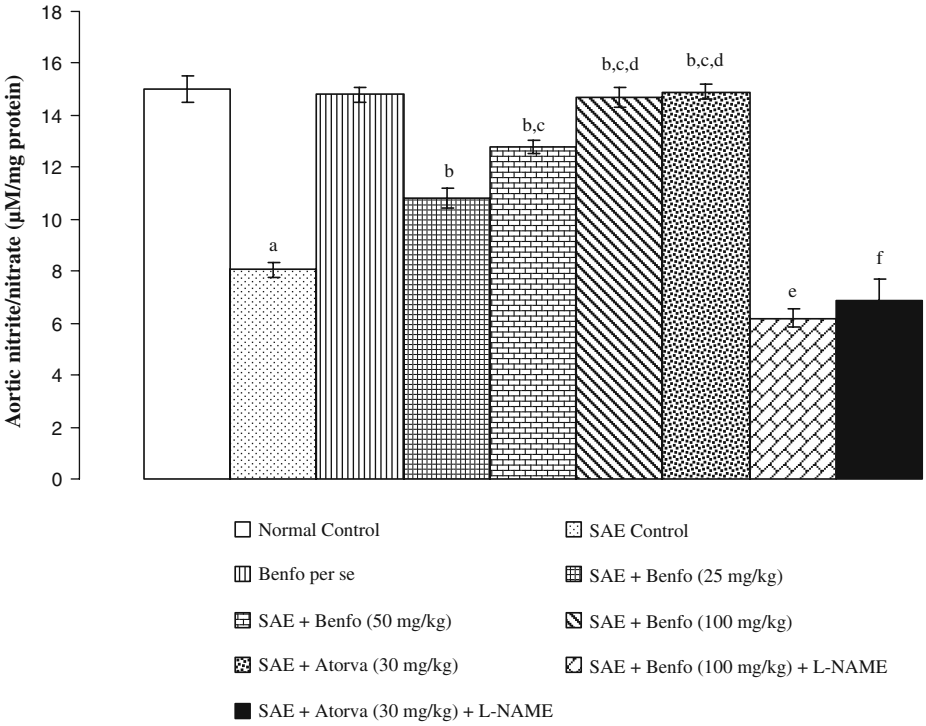

Fig. 5 Effect of benfotiamine on sodium arsenite-induced decrease in aortic concentration of nitrite/nitrate (μM/mg of protein). All values are represented as mean ± SEM. [a]$p<0.05$ vs normal control; [b]$p<0.05$ vs SAE control; [c]$p<0.05$ vs SAE+Benfo (25 mg/kg); [d]$p<0.05$ vs SAE+Benfo (50 mg/kg); [e]$p<0.05$vs SAE+Benfo (100 mg/kg); [f]$p<0.05$vs SAE+Atorva (30 mg/kg)

Effect of Pharmacological Interventions on Serum TBARS and Aortic Superoxide Anion Generation

The lipid peroxidation as assessed in terms of measuring TBARS and aortic superoxide anion generation as measured in terms of reduced NBT were noted to be increased in rats administered with sodium arsenite (1.5 mg^{-1} kg^{-1} day^{-1}, i.p., 2 weeks). However, treatment with benfotiamine (25, 50, and 100 mg^{-1} kg^{-1} day^{-1} p.o.) or atorvastatin (30 mg^{-1} kg^{-1} day^{-1} p.o.) significantly attenuated sodium arsenite-induced increase in serum TBARS and aortic superoxide anion generation. The ameliorative effects of benfotiamine (100 mg^{-1} kg^{-1} day^{-1} p.o.) and atorvastatin (30 mg^{-1} kg^{-1} day^{-1} p.o.) in reducing the oxidative stress were significantly prevented by co-administration with L-NAME (25 mg^{-1} kg^{-1} day^{-1} i.p.; Figs. 6, 7).

Discussion

The present study investigated the effect of benfotiamine in sodium arsenite-induced experimental VED. We observed in the present study that the treatment with benfotiamine markedly prevented sodium arsenite-induced VED in rats.

The exposure of arsenic through drinking water has been implicated in the pathogenesis of cardiovascular disorders [39]. Arsenic has been documented to produce vascular

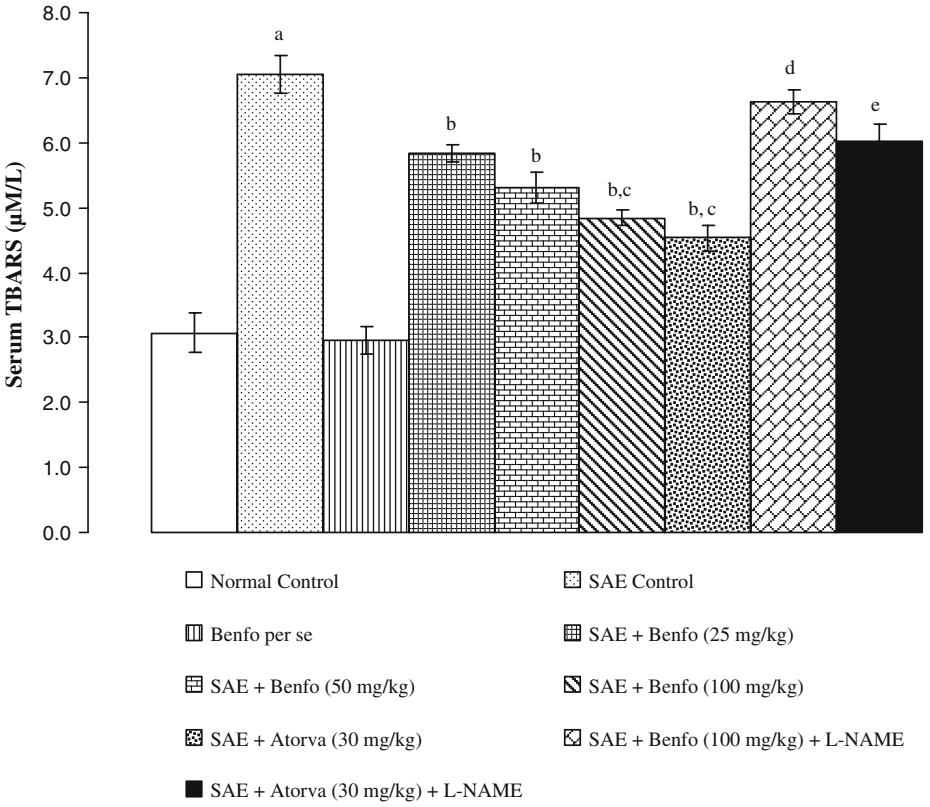

Fig. 6 Effect of benfotiamine on sodium arsenite (*SAE*)-induced increase in serum TBARS (µM/L). [a]$p<0.05$ vs normal control; [b]$p<0.05$ vs SAE control; [c]$p<0.05$ vs SAE+Benfo (25 mg/kg); [d]$p<0.05$ vs SAE+Benfo (100 mg/kg); [e]$p<0.05$ vs SAE+Atorva (30 mg/kg)

pathogenesis by interfering with vascular functions [17]. The VED has been implicated in the pathogenesis of various cardiovascular disorders such as of atherosclerosis, hypertension, and coronary artery disease [10–12]. The impairment in the integrity of vascular endothelium, decrease in serum and aortic nitrite/nitrate concentration, and reduction in Ach-induced endothelium-dependent relaxation have been documented to be an index of experimental VED [29, 31]. In the present study, the administration of sodium arsenite for 2 weeks in rats decreased the serum and aortic concentrations of nitrite/nitrate and reduced the Ach-induced endothelium-dependent relaxation and impaired the integrity of vascular endothelium of thoracic aorta. It suggests that sodium arsenite produced VED, which is consistent with our recent study [21]. On the other hand, administration of sodium arsenite did not alter the sodium nitroprusside-induced endothelium-independent relaxation. The exposure of isolated porcine aortic endothelial cells (PAECS) with sodium arsenite caused downregulation of eNOS and AKT protein levels [17]. Further sodium arsenite has been shown to impair the activity of eNOS possibly via decreasing the levels of BH_4, a cofactor necessary for eNOS activation and subsequently stimulating the process of eNOS uncoupling [40]. Thus, the sodium arsenite-induced VED observed in present study may be due to inactivation of eNOS and consequent reduction in the generation and bioavailability of NO in the vessel wall. This contention is supported by the results

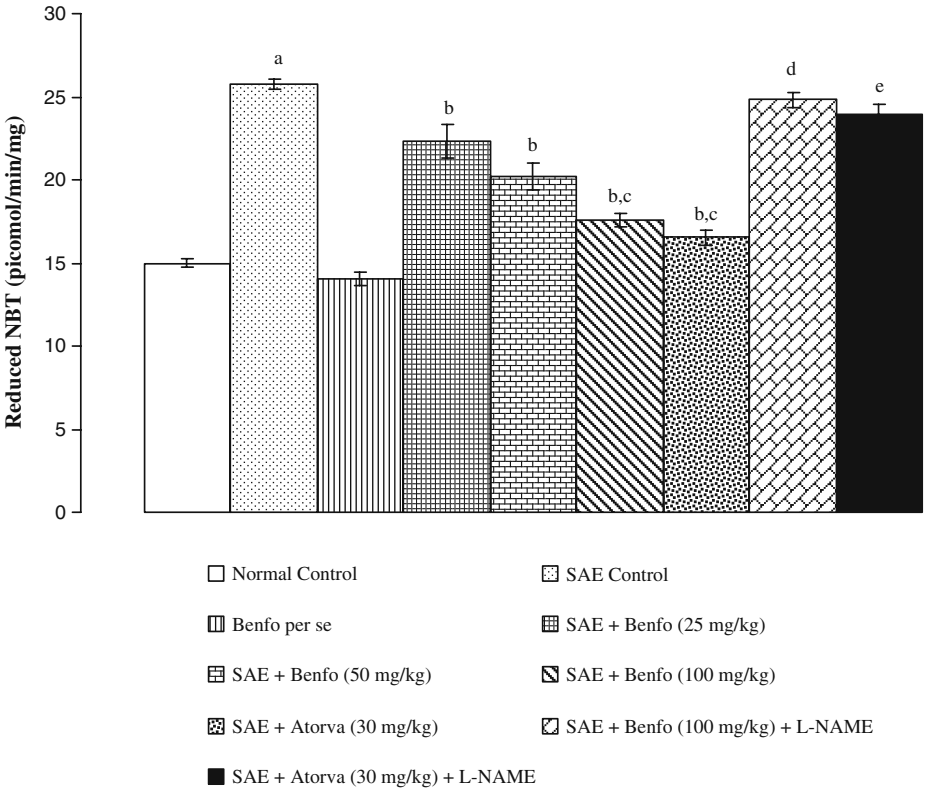

Fig. 7 Effect of benfotiamine on sodium arsenite-induced increase in superoxide anion generation as assessed by estimating NBT ($pmol^{-1}$ min^{-1} mg^{-1}). All values are represented as mean ± SEM. $^{a}p<0.05$ vs normal control; $^{b}p<0.05$ vs SAE control; $^{c}p<0.05$ vs SAE+Benfo (25 mg/kg); $^{d}p<0.05$ vs SAE+Benfo (100 mg/kg); $^{e}p<0.05$ vs SAE+Atorva (30 mg/kg)

obtained in the present study that administration of sodium arsenite significantly reduced the serum and aortic nitrite/nitrate levels and diminished the Ach-induced endothelium-dependent relaxation.

In the present study, rats administered with sodium arsenite for 2 weeks produced a marked rise in oxidative stress. The high oxidative stress has been documented to play a key role in the progression of endothelial dysfunction [41]. Elevated levels of serum TBARS and aortic superoxide generation are considered to be an index of development of oxidative stress [21]. Administration of sodium arsenite caused a significant rise in serum TBARS and aortic superoxide anion generation, which indicate the development of oxidative stress. Arsenic has been reported to generate ROS through activation of NADPH oxidase [18] and uncoupling of eNOS to generate superoxide anion [40]. Thus, the noted VED in sodium arsenite-administered rats may be due to the development of high oxidative stress and consequent inactivation of NO. This contention is strongly supported by the results of the present study that sodium arsenite-administered rats showed high oxidative stress and consequent reduction in serum nitrite/nitrate concentration.

In the present study, the pharmacological treatment with benfotiamine has been noted to prevent sodium arsenite-induced VED by improving the integrity of vascular endothelium, increasing the serum and aortic nitrite/nitrate levels, and enhancing the Ach-induced

endothelium-dependent relaxation. In addition, in the present study, benfotiamine treatment markedly reduced the oxidative stress in sodium arsenite-administered rats. Earlier studies demonstrated that benfotiamine activates PI3k/Akt/Protein kinase B pathways [27, 28]. Activation of Akt/protein kinase B has been shown to further activate eNOS and reduce oxidative stress [24, 25]. Thus, the observed favorable effect of benfotiamine in preventing sodium arsenite-induced experimental VED in the present study may be attributed to activation of eNOS and subsequent enhancement in the synthesis and bioavailability of NO. This contention is strongly supported by the results obtained in the present study that the ameliorative effects of benfotiamine in preventing sodium arsenite-induced VED have been markedly attenuated by co-administration with L-NAME, a NOS inhibitor. Atorvastatin has been well reported to improve the function of endothelium by activating eNOS, enhancing the generation and bioavailability of NO, and reducing the oxidative stress [21, 25]. Therefore, in the present study, atorvastatin has been employed as a standard drug to compare the beneficial effects of benfotiamine in preventing VED in rats administered with sodium arsenite.

On the basis of the above discussion, it may be concluded that benfotiamine reduces oxidative stress and activates eNOS to enhance the generation and bioavailability of NO and subsequently improves the integrity of vascular endothelium to prevent sodium arsenite-induced experimental VED.

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