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Novel Use of Uric Acid and Sodium Arsenite to Induce Vascular Endothelial Dysfunction in Rats

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Abstract: The present study has been designed to investigate the potential of *in-vivo* administration of uric acid and sodium arsenite in the development of vascular endothelial dysfunction (VED) in rats. The uric acid (100, 150, 200 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5, 2 mg kg^{-1} day^{-1}, i.p., 2 weeks) were administered to rats. Vascular endothelial dysfunction was assessed by employing isolated aortic ring preparation, electron microscopy of thoracic aorta and estimating serum concentration of nitrite/nitrate. Further, serum thiobarbituric acid reactive substances (TBARS) and aortic production of superoxide anion were estimated to assess oxidative stress. High dose of uric acid (200 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (2 mg kg^{-1} day^{-1}, i.p., 2 weeks) were noted to produce high mortality rate (>85%) in animals. On the other hand, less mortality rate (<5%) was observed in animals treated with uric acid (100, 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks). Moreover, uric acid (100, 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks) were noted to produce vascular endothelial dysfunction by attenuating acetylcholine-induced endothelium dependent relaxation, impairing the integrity of vascular endothelial lining, decreasing serum nitrite/nitrate concentration and increasing serum TBARS and aortic superoxide anion generation. Hence, it may be concluded that uric acid (100 to 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1 to 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks) may be employed as potential chemically-induced models to produce vascular endothelial dysfunction in rats.

Key words: Vascular endothelial dysfunction, uric acid, sodium arsenite

INTRODUCTION

Endothelium forms an innermost lining of blood vessels (Luscher and Barton, 1997; Endmann and Schiffrin, 2004). Vascular endothelium has anticoagulant and antithrombotic activites in order to ensure free flow of blood through arteries (Bombeli *et al*., 1997). Endothelial dysfunction has been characterized by partial or complete loss of balance between vasorelaxation and vasoconstriction (Vane *et al*., 1990; Masaki, 1995) and thrombosis and thrombolysis (Danon and Skutelsky, 1976). Experimental and clinical evidences suggest that endothelial dysfunction leads to reduced endothelial NO production (Bugiardini *et al*., 2004; Lerman and Zeiher, 2005). Vascular endothelial dysfunction has been associated with various disorders such as hypertension (Sainani and Maru, 2004), coronary artery diseases (Caramori and Zago, 2000), diabetes mellitus (De Vriese *et al*., 2000; Nakagami *et al*., 2005), atherosclerosis (Spieker *et al*., 2001; Bonetti *et al*., 2003) and stroke (Cosentino *et al*., 2001; Faraci and Lentz, 2004). However, the pathophysiology of vascular endothelial dysfunction is poorly

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understood. Numerous animal models have been employed to produce vascular endothelial dysfunction (Balakumar *et al*., 2007). Moreover, developing new models may allow us to understand the complex pathophysiology of vascular endothelial dysfunction and reveal possible therapeutic targets. Gout is a metabolic disorder characterized by increase in uric acid level and it has been shown to produce vascular diseases in humans (Akkasilpa *et al*., 2004; Lin *et al*., 2004). Further, arsenic, a ubiquitous element found in foods and drinking water, is associated with vascular pathogenesis. (Abernathy *et al*., 1999). Therefore, the present study was undertaken to investigate the potential of uric acid and sodium arsenite in the development of vascular endothelial dysfunction in rats.

MATERIALS AND METHODS

The experimental protocol used in the present study was approved by Institutional Animal Ethical Committee. Age matched young male Wistar rats weighing about 200-250 g were employed in the present study. They were fed on standard chow diet (Kisan Feeds Ltd., Chandigarh, India) and water *ad libitum*. They were housed in animal house and were exposed to 12 h light and 12 h dark cycle.

Assessment of Vascular Endothelial Dysfunction

Isolated Rat Aortic Ring Preparation

The rats were decapitated, thoracic aorta was removed, cut into a ring of 4-5 mm length and mounted in 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) 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 (Pieper, 1997) with a force-transducer (Ft-2147) connected to Physiograph (INCO, Ambala, India).

The aortic ring preparation was primed with 80 mM KCl to check its functional integrity and to improve its contractility. The cumulative dose responses of acetylcholine (Ach; 10^{-8}-10^{-4} M) or sodium nitroprusside (SNP; 10^{-8}-10^{-4} M) were recorded in phenylephrine (3×10^{-6} M) precontracted preparation with intact or denuded endothelium, respectively (Mittra and Singh, 1998). The intimal layer of aortic ring was rubbed gently with a moistened filter paper for 30 sec to obtain endothelium free preparation (Ignarro *et al*., 1988). Loss of Ach (1×10^{-6} M)-induced relaxation confirmed the absence of vascular endothelium.

Electron Microscopic Study

Three to four millimeter longitudinal strips of thoracic aorta were fixed in 3% glutaraldehyde phosphate buffer (pH 7.4) and subsequently dehydrated in a series of alcohol and acetone concentrations. The tissue was embedded in CY 212 araldite and ultra thin sections of 60-80 nm thickness were prepared using an ultracut E (Reichert Jung, Vienna, Austria). The sections were examined using Morgagni 268(D) electron microscope (FEI Company, OR, USA) attached with image analyzer. Electron micrographs were critically examined for the integrity of vascular endothelium (David *et al*., 1973; Schiller *et al*., 1999; Shah and Singh, 2006).

Estimation of Serum Nitrite/Nitrate Concentration

Four hundred microliter of carbonate buffer (pH 9.0) was added to 100 μL of serum sample followed by the 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 sodium hydroxide. Following this, 400 μL of zinc sulfate solution (120 mM) was added to deproteinate the serum samples. The samples were allowed to stand for 10 min and then

centrifuged at 4000 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 the clear supernatant and serum nitrite/nitrate was measured spectrophotometrically (DU 640B Spectrophotometer, Beckman Coulter Inc., CA, USA) at 545 nm (Sastry *et al*., 2002).

Estimation of Serum Thiobarbituric Acid Reactive Substances (TBARS)

One milliliter of 20% trichloroacetic acid was added to 100 μL of serum and then 1% TBARS reagent (1.0 mL) was added, mixed and incubated at 100°C for 30 min. After cooling on ice, samples were centrifuged at 1000 g for 20 min. Serum concentration of TBARS was measured spectrophotometrically (DU 640B Spectrophotometer, Beckman Coulter Inc., CA, USA) at 532 nm (Ma *et al*., 2003).

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 100 μM of nitroblutetrazolium (NBT) and incubated at 37°C for 1.5 h. 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% SDS in water containing 40 mg L^{-1} di-ethylene triamine pentaacetic acid (DTPA). The mixture was centrifuged at 20000 g for 20 min and the resultant pellet were resuspended in 1.5 mL of pyridine and kept at 80°C for 1.5 h to extract formazon. The mixture was centrifuged at 10000 g for 10 min and the absorbance of formazon was determined spectrophotometrically (DU 640 B Spectrophotometer, Beckman Coulter Inc., CA, USA) at 540 nm (Wang *et al*., 1998). The amount of reduced NBT was calculated using the following formula: Amount of reduced NBT = $A \bullet V/(T \bullet Wt \bullet \varepsilon \bullet l)$, where A is absorbance, V is volume of solution (1.5 mL), T is time for which the rings were incubated with NBT (90 min), Wt is blotted wet weight of the aortic rings, ε is extinction coefficient (0.72 L $mmol^{-1}$ mm^{-1}) and *l* is the length of light path (10 mm).

Experimental Protocol

Eight groups were employed in the present study and each group comprising of 8-10 animals. The uric acid was dissolved in carboxy methyl cellulose (CMC) of 0.5% w/v. The sodium arsenite is dissolved in double distilled water. Group I (Control), rats were maintained on standard food and water and no treatment was given. Group II (CMC *per se*), rats were administered 1 mL of CMC (0.5% w/v, i.p.) for 3 weeks. Group III (Uric acid 100 mg kg^{-1} treated), rats were treated uric acid (100 mg kg^{-1} day^{-1}, i.p.) for 3 weeks. Group IV (Uric acid 150 mg kg^{-1} treated), rats were treated uric acid (150 mg kg^{-1} day^{-1}, i.p.) for 3 weeks. Group V (Uric acid 200 mg kg^{-1} treated), rats were treated uric acid (200 mg kg^{-1} day^{-1}, i.p.) for 3 weeks. Group VI (Sodium arsenite 1 mg kg^{-1} treated), rats were treated sodium arsenite (1 mg kg^{-1} day^{-1}, i.p.) for 2 weeks. Group VII (Sodium arsenite 1.5 mg kg^{-1} treated), rats were treated sodium arsenite (1.5 mg kg^{-1} day^{-1}, i.p.) for 2 weeks. Groups VIII (Sodium arsenite 2 mg kg^{-1} treated), rats were treated sodium arsenite (2 mg kg^{-1} day^{-1}, i.p.) for 2 weeks.

Statistical Analysis

All values were expressed as mean±SEM. Data for isolated aortic ring preparation were statistically analysed using two way ANOVA. The data for serum levels of nitrite/nitrate and TBARS and aortic superoxide anion generation were statistically analysed using one way ANOVA followed by Tukey's multiple range test. A p-value<0.05 was considered to be statistically significant.

Drugs and Chemicals

L-phenylephrine was obtained from Sigma-Aldrich Ltd., St. Louis, MO, USA. Acetylcholine hydrochloride, sodium nitroprusside, nitroblue tetrazolium, DTPA, 1, 1, 3, 3 tetra methoxypropane

and carboxymethyl cellulose were purchased from HIMEDIA, Mumbai, India. The uric acid, sodium arsenite, thiobarbituric acid and glutaraldehyde were purchased from Loba Chemie, Mumbai, India. All other chemicals used in the present study were of analytical grade.

RESULTS

CMC (0.5% w/v, 1 mL, i.p., 3 weeks) used as vehicle has not produced any significant effect on various parameters employed in the present study. High dose of uric acid (200 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (2 mg kg^{-1} day^{-1}, i.p., 2 weeks) were noted to produce high mortality rate (>85%) in animals. On the other hand, no or less mortality rate (<5%) was observed in animals treated with uric acid (100, 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks). Ach and SNP were noted to produce endothelium dependent and independent relaxation, respectively in phenylephrine (3×10^{-6} M) precontracted isolated rat aortic ring

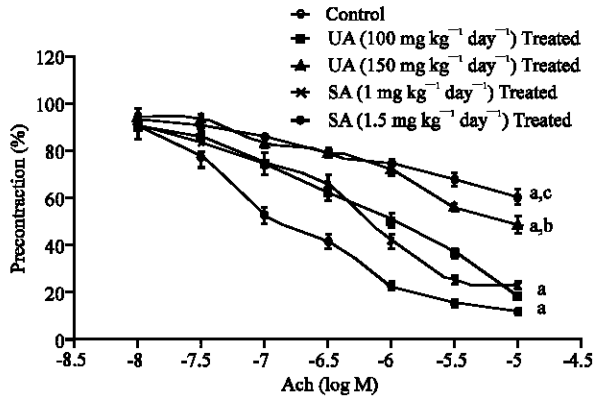

Fig. 1: Effect of Uric acid (UA) and sodium arsenite (SA) on acetylcholine-induced endothelium dependent relaxation. Responses are expressed as percentage contraction induced by phenylepherine (3×10^{-6} M). All values represent mean±SEM. a = $p<0.05$ vs Control; b = $p<0.05$ vs UA (100 mg kg^{-1} day^{-1}) treated; C = $p<0.05$ vs SA (1 mg kg^{-1} day^{-1}) treated

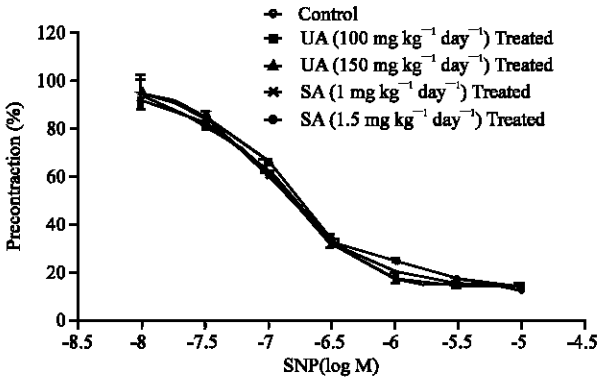

Fig. 2: Effect of uric acid (UA) and sodium arsenite (SA) on sodium nitroprusside (SNP)-induced endothelium independent relaxation. Responses are expressed as percentage of maximum contraction induced by phenylepherine (3×10^{-6} M). All values represent mean±SEM

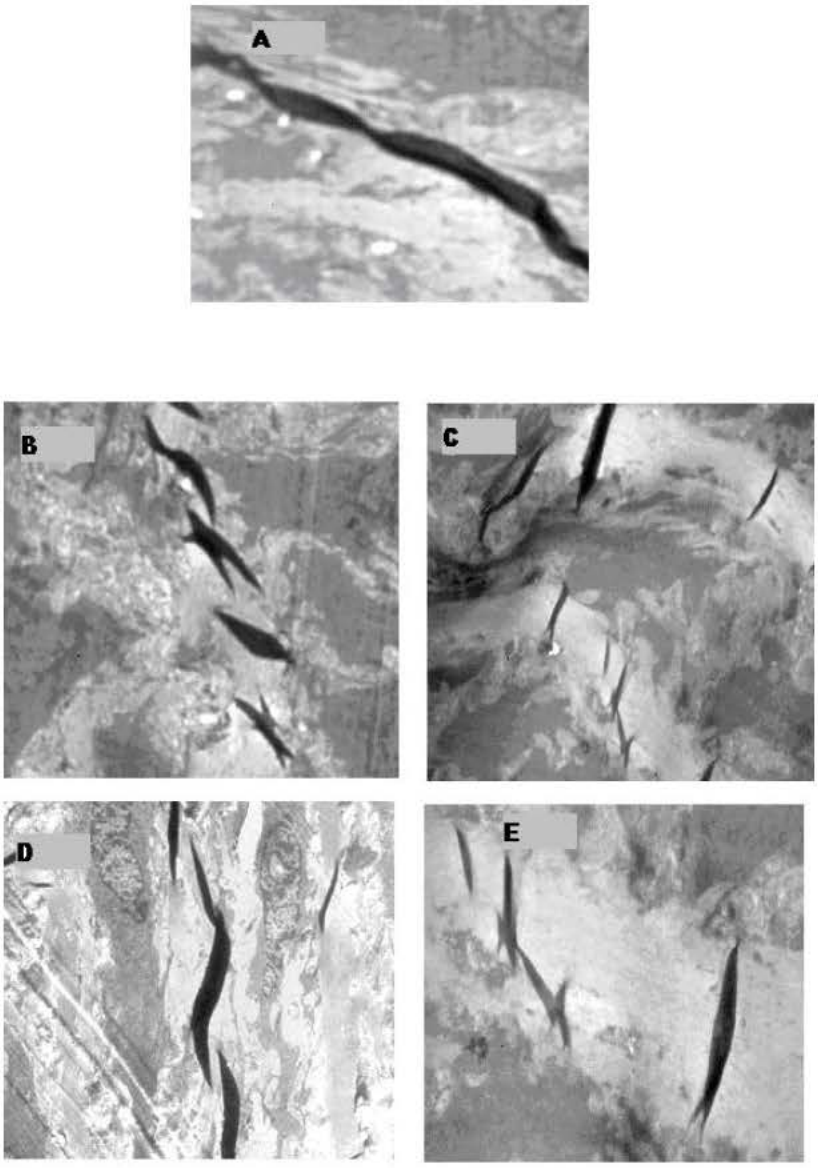

Fig. 3: Effect of uric acid (UA) and sodium arsenite (SA) on the integrity of vascular endothelial lining. A. represents control, B represents uric acid (100 mg kg^{-1} treated), C represents uric acid (150 mg kg^{-1} treated), D represents sodium arsenite (1 mg kg^{-1} treated) and E represents sodium arsenite (1.5 mg kg^{-1} treated)

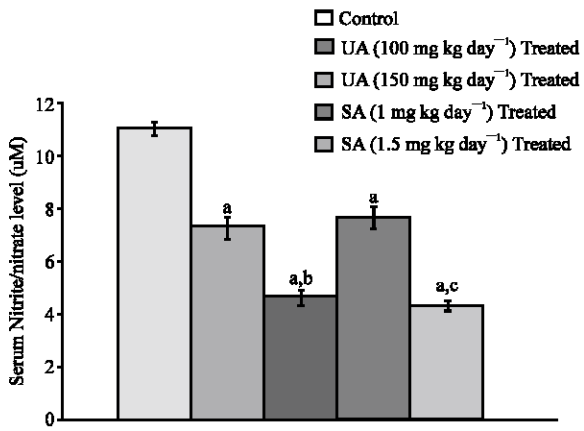

Fig. 4: Effect of uric acid (UA) and sodium arsenite (SA) on serum concentration of nitrite/nitrate in rats. All values represent mean±SEM. a = $p<0.05$ vs Control; b = $p<0.05$ vs UA (100 mg kg^{-1} day^{-1}) treated; C = $p<0.05$ vs SA (1 mg kg^{-1} day^{-1}) treated.

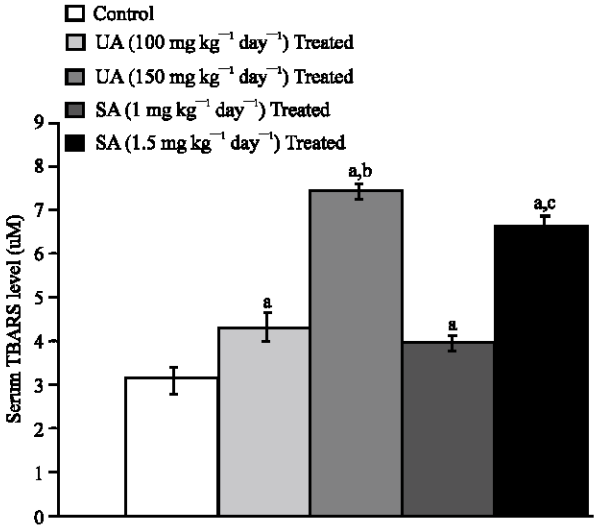

Fig. 5: Effect of uric acid (UA) and sodium arsenite (SA) on serum thiobarbituric acid reactive substances (TBARS) level in rats All values represent mean±SEM. a = $p<0.05$ vs control; b = $p<0.05$ vs UA (100 mg kg^{-1} day^{-1}) treated; C = $p<0.05$ vs SA (1 mg kg^{-1} day^{-1}) treated

preparation in a dose dependent manner (Fig. 1 and 2). Uric acid (100, 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks) significantly attenuated Ach-induced endothelium dependent relaxation (Fig. 1); but they did not affect SNP-induced endothelium independent relaxation (Fig. 2). Further, uric acid (100, 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks) were observed to impair the integrity of vascular endothelial lining of thoracic aorta (Fig. 3). Moreover, uric acid (100, 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks) significantly reduced serum concentration of nitrite/nitrate (Fig. 4). Furthermore, the increase in serum TBARS level and aortic superoxide anion generation were noted in animals administered to uric acid (100, 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks) (Fig. 5and 6).

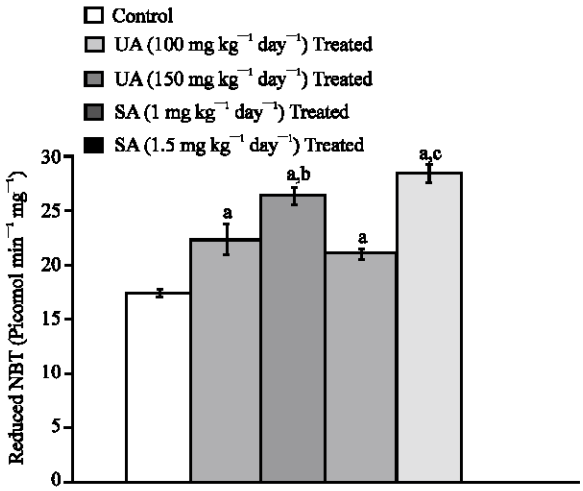

Fig. 6: Effect of uric acid (UA) and sodium arsenite (SA) on superoxide anion generation in rats. All values (n = 6) represent mean±SEM. a = $p<0.05$ vs control; b = $p<0.05$ vs UA (100 mg kg^{-1} day^{-1}) treated; C = $p<0.05$ vs SA (1 mg kg^{-1} day^{-1}) treated

DISCUSSION

Vascular Endothelial Dysfunction (VED) has been reported to be associated with reduction in Ach-induced endothelium dependent relaxation (Feletou and Vanhoutte, 2006) and decrease in serum nitrite/nitrate level (Furchgott and Zawadzki, 1980; Guerci *et al*., 2001). Further, the integrity of endothelial lining has been shown to be impaired as a result of VED (Shah and Singh, 2006). In the present study, uric acid and sodium arsenite have been noted to produce VED assessed in terms of decrease in Ach-induced endothelium dependent relaxation and serum nitrite/nitrate level. Further, uric acid and sodium arsenite were observed to impair the integrity of vascular endothelial lining assessed by electron microscopic study, which suggest the development of VED.

Oxidative stress has been demonstrated to play a major role in the pathogenesis of VED (Cai and Harrison, 2000; Madamanchi *et al*., 2005; Yang and Ming, 2006). Increase in serum TBARS level (Ma *et al*., 2003) and superoxide anion generation have been documented as an index of oxidative stress (Harrison, 1997; Hamilton *et al*., 2001). In the present study, uric acid and sodium arsenite were noted to increase serum TBARS level and superoxide anion generation in thoracic aorta, which suggest the development of oxidative stress and consequently VED.

Uric acid has been documented to impair the endothelial production and release of NO (Kanellis and Kang, 2005; Corry and Tuck, 2006). Further, uric acid has been shown to inactivate NO produced by endothelium (Sanchez-Lozada *et al*., 2006). Moreover, uric acid has been demonstrated to increase ROS (Khosla *et al*., 2005). Therefore, it is suggested that the uric acid-induced VED in the present study may be due to generation of ROS and consequent inactivation of endothelial NO. This contention is further supported by the fact that, in the present study uric acid has increased TBARS level and superoxide anion generation. *in-vitro* studies showed that sodium arsenite inactivates Akt/PkB (Protein kinase B) and thus downregulates vascular eNOS activity (Tsou *et al*., 2005). Moreover, sodium arsenite has been demonstrated to increase the generation of ROS in vascular endothelial cells mainly via activation of NADH/NADPH oxidase (Lynn *et al*., 2000). Therefore, it is suggested that sodium arsenite-induced VED in the present study may be due to downregulation

of eNOS and induction of NADPH oxidase mediated ROS generation. High dose of uric acid (200 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (2 mg kg^{-1} day^{-1}, i.p., 2 weeks) used in the present study were noted to produce high mortality rate (>85%) in rats. On the other hand, no or less mortality rate (<5%) was observed in animals treated with uric acid (100, 150 mg kg^{-1} day^{-1}, i.p., 3 weeks) and sodium arsenite (1, 1.5 mg kg^{-1} day^{-1}, i.p., 2 weeks).

CONCLUSION

On the basis of above discussion, it is concluded that uric acid (100 to 150 mg kg^{-1} day^{-1}, i.p.) for 3 weeks and sodium arsenite (1 to 1.5 mg kg^{-1} day^{-1}, i.p.) for 2 weeks could be employed as potential chemically-induced models to produce experimental vascular endothelial dysfunction.

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