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The effect of ascorbic acid on *Bitis arietans* venom induced toxicity in rats

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Abstract Ascorbic acid (AsAc) was tested to evaluate its ability to reverse the oxidative stress induced by envenoming. Test groups of rats were envenomed with sub-lethal doses (4.0 mg/kg s.c.) of *Bitis arietans* venom (BaV) whilst, single doses (500 mg/kg, orally) of AsAc were pre-administered in half of them. Blood samples were collected within three periods and levels of lipid peroxidation (LPO) and total-SH increased significantly, whilst, the 'Venom + AsAc' groups were significantly less than both, the respective 'Venom' groups and controls, at different periods. The antioxidant, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzyme level changes were trivial at the three periods, whilst, there were no changes in the 'Venom + AsAc' groups, compared with controls, except SOD which, became significant after 24 h. SCr and BUN levels were significantly higher than the controls within the three periods with variable degrees, whilst, the 'Venom + AsAc' group level changes were insignificant compared with controls and their respective 'Venom' groups at all periods. Blood urea Nitrogen (BUN), became significantly lower after 24 h. After 6 and 24 h AST levels were significantly higher than controls, whilst, ALT was not. Level changes of both AST and ALT 'Venom + AsAc' groups were insignificant, compared with controls at all periods. It is concluded that oxidative stress due to envenoming by BaV induced variable levels of significant changes in levels of nephrotoxic, hepatotoxic markers and antioxidant enzyme parameters. Administration of AsAc relatively adjusted these changes with different degrees, at variable periods of time that demands further deeper research in beneficiary mechanisms of antioxidants.

1. Introduction

Snakebites are serious socio-medical problem that lead to morbid and mortal impact on victims, and in Saudi Arabia alone, there are more than 10 terrestrial snake species that are highly venomous (Al-Asmari and Debboun, 2013). The puff adder

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Bitis arietans, and the other vipers have similar serious effects on their victims (Al-Jammaz, 2001), attributed to the complex toxins, enzymes and protein combinations that affect the hepatic, renal, metabolic, cardiovascular and haematological systems, clinically and experimentally (Al-Jammaz, 2001; Fernandez et al., 2014). Prompt antivenom treatment is essential and crucial to avoid poor quality of life and mortality (Lavonas et al., 2002).

The oxidative stress status, which ensues due to snake bite envenoming is another dimension of kidney impairment and acute renal failure (Yamasaki et al., 2008), associated with the antioxidant defence system, that could be subject for countering by antioxidant therapy (Al-Asmari et al., 2014). Reactive oxygen species (ROS) are involved in the inflammatory reactions, thereby affecting the cellular physiology and play a significant role in the pathological conditions (Carroll et al., 2007). These free radical ROS, apart from being involved in damaging cellular components, do play a significant role in venom induced toxicity, as had been observed in experimenting with envenomed mice (Dousset et al., 2005). AsAc is an antioxidant that has beneficial effects on several types of cancer (Domitrović, 2006) and could be involved in amelioration of ROS cellular damage, generated during metabolism and exposure to toxins and carcinogens (Banerjee et al., 2009), in addition to enhancement of protease inhibitor effects involved in preventing organ functional damage (Fatani et al., 2006). In this present study, our aim was to investigate the beneficiary effects of AsAc on hepatorenal toxicity and oxidative stress caused by *B. arietans* venom in albino rats.

2. Materials and methods

2.1. Materials (chemicals, reagents and animals)

The reagents, Hydrogen peroxide, Succinic acid, Pyrogallol, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), Tris-base, Potassium phosphate dibasic salt, Potassium phosphate monobasic salt, Hydrochloric acid, Methanol and AsAc were obtained from Sigma, USA. Ready kits for the quantification of serum enzymes (AST and ALT), BUN and Creatinine were obtained from United Diagnostics Industry, Dammam, Saudi Arabia.

B. arietans venom was obtained by manual milking of snakes (both sexes) that were collected from the South Western Regions of Saudi Arabia and maintained in the animal house facility of the Research Center, Prince Sultan Military Medical City (PSMMC), Saudi Arabia. The crude venom was either lyophilized and stored in the fridge at 4 °C or diluted in distilled water and kept frozen in aliquots at -80 °C. Stock solutions of venom were reconstituted in sterile 0.9% NaCl immediately before use.

Male Wistar albino rats, weighing 150–200 g were used in the study. They were obtained and maintained in the animal house facility of the Research Center, PSMMC, Saudi Arabia. They were acclimatized for a week prior to the experiment on standard rat pellet diet with free access to water. Body weights of rats were recorded at the start and completion of the procedure.

2.2. Methods (experimental design and Biochemical analysis)

After acclimatization (7 days) the animals were divided into three different treatment groups (a, b and c) for each test parameter: (a) normal controls (5 rats), (b) venom alone; three groups (according to the injection period of 3 h, 6 h and 24 h) of five rats (15 rats), which were injected with 200 µl/rat venom (4.0 mg/kg s.c.), (c) venom with administration of AsAc; three groups (according to the injection period of 3 h, 6 h and 24 h) of five rats (15 rats), which were injected with 200 µl/rat venom (4.0 mg/kg s.c.). AsAc was administered (500 mg/kg, orally) before venom administration, referring to previous that employed high doses of ascorbic acid (Klenner, 1971; Elshama et al., 2013).

After the completion of treatment schedule and before sacrifice, the rats were anesthetized with anaesthetic ether. Blood was withdrawn from the heart with the help of disposable syringes. Serum was obtained by centrifugation at 3000 rpm for 10 min.

Liver was carefully separated from the treated and control animals, and homogenized according to several current methods (Doss and Anand, 2012) in 0.1 M Tris–HCl buffer pH 7.5 by a glass-Teflon homogenizer (Thomas PA, USA) by passing 5 pulses; at 4 °C to make a 10% w/v homogenate. The homogenate was then subjected to high speed Ultra-Turrex Kunkel homogenizer (Type T-25, Janke & Kunkel GMBH & Co. KG. Staufen). Homogenate was centrifuged at 3000 rpm at 4 °C for 10 min in Beckman J2-M1 (Beckman instruments, Inc Palo Alto, C.A. USA) high-speed refrigerated centrifuge to remove the cell debris. The supernatant was saved in aliquots and stored at -20 °C for assay analyses of enzymatic antioxidant parameters, as described below.

All serum parameters i.e. AST, ALT, BUN and SCr were done using commercially available kits from United Diagnostics Industry, Dammam, K.S.A. Readings were done spectrophotometrically and data were collected in duplicate, according to manufacturer's procedure.

The activities of the three key antioxidant enzymes of the defence system i.e. superoxide dismutase, catalase and glutathione peroxidase were measured simultaneously in the liver homogenate extracts under similar conditions using same solutions to avoid day-to-day experimental variations.

Superoxide dismutase (SOD) was assayed by the method of Marklund and Marklund (1974). To 0.05 ml of supernatant 2.85 ml of 0.05 mM Tris-buffer, pH 8.2 was added, mixed well and incubated at 25 °C for 20 min. The reaction was started by adding 0.1 ml of 8 mM pyrogallol solution. Change in absorbance per minute was immediately recorded for the initial 3 min at 420 nm. A reference set, containing 0.08 ml distilled water instead of supernatant solution, was also run simultaneously.

Catalase (CAT) was assayed according to the method of Claiborne (1985) as described by Giri et al. (1996). The assay mixture consisted of 1.95 ml of 0.05 M potassium phosphate buffer pH 7.0, 1 ml of 0.019 M hydrogen peroxide and 0.05 ml homogenate (50–100 µg protein) in a final volume of 3 ml. The decrease in absorbance at 240 nm was immediately noted after every 30 s for 3 min. Enzyme activity was calculated using the molar extinction coefficient of H_2O_2 (436 M^{-1}-cm^{-1} at 240 nm).

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