Time-Course of Lipid Peroxidation in Different Organs of Mice Treated with *Echis pyramidum* Snake Venom

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ABSTRACT: This study examined the effect of *Echis pyramidum* (*EP*) venom on time-course of lipid peroxidation in different vital organs of mice. Adult male Swiss albino mice were injected with *EP* venom (2 mg/kg, i.p.); control mice received vehicle alone (normal saline). Mice were killed at 1, 3, 6, 12, and 24 h postenvenomation. The liver, lung, kidney, heart, and brain (cerebrum and cerebellum) were collected for the estimation of malondialdehyde (MDA), an index of lipid peroxidation. The results of this study showed that a single injection of *EP* venom caused a significant lipid peroxidation in all the organs studied. The onset of lipid peroxidation was as early as 1 h and persisted for several hours, suggesting an important role of oxidative stress in the cytotoxicity of *EP* venom. J Biochem Mol Toxicol 20:93–95, 2006; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jbt.20121

KEYWORDS: **Snake venom; *Echis pyramidum*; Lipid peroxidation; Malondialdehyde; Mice**

Morbidity and mortality due to snakebite is an important sociomedical problem throughout the globe, particularly in the rural areas. There are at least 10 species of terrestrial venomous snakes inhabited in Saudi Arabia [1]. Of these, three species of *Echis* (*Echis pyramidum*, *Echis coloratus*, and *Echis carinatus sochureki*) are distributed throughout the Arabian Peninsula and have been associated with most envenomation cases in this region [2]. The local manifestations caused by *Echis* venoms include edema pain, hemorrhage, and necrosis [1,3,4]. The lethal effects of snake venom are largely attributed to its active ingredient phospholipase A_2 (PLA_2). Phospholipid hydrolysis by PLA_2 enzyme releases arachidonic acid whose metabolism results in the formation of potentially toxic reactive oxygen species (ROS) and lipid peroxides [5,6]. El Asmar et al. [7] have reported that the increase in polyunsaturated fatty acids following envenomation may lead to an increase in the rate of lipid peroxidation, which might be responsible for tissue damage. Several isoforms of PLA_2 have been identified [8–10] that may explain variable toxicities of venoms from different genera of snakes. PLA_2 from snake venom has been implicated in multiple pathologies including neurotoxicity [11], nephrotoxicity [12,13], lung toxicity [14], hepatotoxicity [15], and cardiotoxicity [16,17]. Recently, scorpion envenomation has also been shown to produce significant lipid peroxidation in heart, lung, liver, and kidney of rodents [18]. In the present investigation, we studied the effects of *Echis pyramidum* (*EP*) venom on time-course of lipid peroxidation in different vital organs of mice.

Male adult albino mice weighing 25 ± 5 g were randomly divided into two groups and kept in temperature and humidity controlled room maintained at 12/12 h light–dark cycles. Standard laboratory food and tap water were freely available ad libitum. Animals of group 1 were injected intraperitoneally with *EP* venom (2.0 mg/kg body weight), whereas group 2 served as a control and received an equal volume of physiological saline. The venom used in this study was collected from *Echis pyramidum* snakes, housed and milked by professionals at the Department of Zoology, King Saud University, Riyadh. The fresh venom was filtered and then lyophilized and stored in dark at 4°C. A stock solution of venom (10 mg/mL) was prepared in saline and used in this study. Mice were killed at 1, 3, 6, 12, and 24 h postenvenomation and their brain, liver, heart, lung, and kidney were removed for the estimation of lipid peroxidation. The experimental protocol was approved by the Institutional Research and Ethics Committee.

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TABLE 1. Effect of *Echis pyramidum* Envenomation (2 mg/kg) on Lipid Peroxidation in Various Body Organs of Mice

	MDA (nmol/g Tissue)					
		Time Postenvenomation				
Samples	*Control*	*1 h*	*3 h*	*6 h*	*12 h*	*24 h*
Liver	13.14 ± 0.07	13.82 ± 0.36	14.08 ± 0.16*	14.40 ± 0.24*	15.53 ± 0.65*	16.05 ± 0.45**
Lung	15.07 ± 0.49	16.22 ± 0.76	16.81 ± 0.63*	17.94 ± 0.91*	19.49 ± 1.23*	20.93 ± 1.41*
Kidney	21.62 ± 0.18	24.12 ± 0.22**	25.12 ± 0.48**	25.64 ± 0.49**	26.49 ± 0.35***	28.08 ± 0.87**
Cerebrum	36.79 ± 0.21	39.54 ± 0.40**	41.79 ± 0.51**	42.77 ± 0.77**	44.48 ± 0.81**	51.10 ± 1.07***
Cerebellum	50.33 ± 0.46	55.28 ± 0.85**	55.78 ± 0.91**	59.60 ± 0.99**	60.31 ± 1.10**	61.45 ± 1.37**
Heart	34.30 ± 1.05	41.57 ± 2.83	44.15 ± 3.12*	61.45 ± 5.87*	71.85 ± 5.12**	49.48 ± 2.43**

Values are mean ± standard error of the mean.
* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

The level of malondialdehyde (MDA), an index of lipid peroxidation, was analyzed spectrophotometrically as described earlier [19]. Tissues were weighed and homogenized (10% w/v) in ice-cold 0.15 M potassium chloride in a potter homogenizer using a motor-driven Teflon pestle. Tissue homogenate (1 mL) was incubated at 37°C in a metabolic shaker for 2 h. One milliliter of 10% (w/v) trichloroacetic acid was mixed with homogenate followed by centrifugation at 3000 rpm for 10 min. Aliquots (1 mL) of the clear supernatant were mixed with 1 mL of 0.67% (w/v) 2-thiobarbituric acid and placed in a boiling water bath for 10 min, cooled and diluted with 1 mL distilled water. The absorbance of the solution was recorded at 535 nm, and the concentration of MDA was calculated using tetraethoxypropane as an external standard. Data were analyzed by Student's *t*-test, and *P* values less than 0.05 were considered as statistically significant.

The results of this study clearly demonstrated that a single injection of *EP* venom at a dose of 2 mg/kg body weight caused a significant and persistent increase in lipid peroxidation in all the organs studied (Table 1). These findings are supported by earlier reports [13,20]. A significant increase in lipid peroxidation was observed within 1 h of venom injection in kidney, cerebrum, and cerebellum, whereas the significant changes in liver, lung, and heart occurred at 3 h. There was a time-course gradual increase in lipid peroxidation in all the organs studied except heart where a steep rise in lipid peroxidation was observed after 3 h, peaking at 12 h, followed by a sharp decline at 24 h (Figure 1). Increase in lipid peroxidation in various organs following venom administration might be due to increased availability of fatty acids, which are mobilized from adipose tissue [7,21]. In vitro studies have shown a direct correlation between degree of lipid peroxidation and PLA_2-induced phospholipids hydrolysis [22]. On the other hand, inhibition of PLA_2 significantly reduced lipid peroxidation [23]. Agents with antioxidant properties have been shown to attenuate viper venom-induced cellular damage by inhibiting the oxidative cascade and improving membrane stabilization [15,24]. Besides a definite role of PLA_2 in snake venom-induced tissue injury, the exact mechanism(s) of venom toxicity is far from clear, and possibly involve multiple pathways. Recently, a large number of genes, mostly involved in inflammation, apoptosis, ion transport, and energy metabolism have been found to be overexpressed in various organs of mice treated with snake venom; in heart alone 50% of these genes were differentially expressed [17].

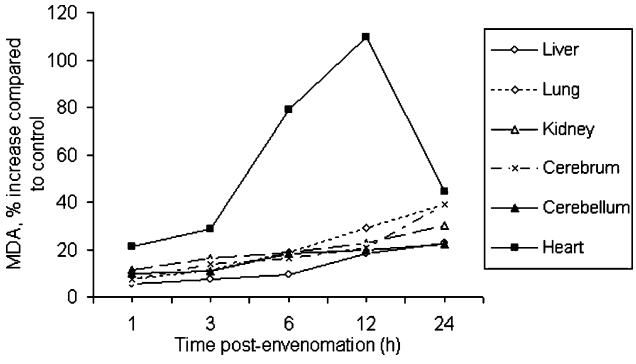

FIGURE 1. Time-course presentation of snake venom-induced lipid peroxidation in different organs of mice shown as percent of control values (mice without envenomation).

In conclusion, this preliminary study clearly demonstrated that systemic injection of *EP* venom produced a significant increase in MDA levels in all the vital organs of mice. The onset of oxidative stress was as early as 1 h that persisted for several hours. Further studies are warranted to study the efficacy of an early-stage intervention with antioxidants in conjunction with antivenoms for the treatment of snakebite victims.

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