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Evaluation of oxidant/antioxidant status in serum of sheep experimentally envenomated with *Hemiscorpius lepturus* scorpion venom

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Abstract

Scorpion envenomation is a main general health problem in developing countries, especially in tropical and subtropical regions. *Hemiscorpius lepturus* as a member of the Hemiscorpiidae family is cause of the most scorpion sting lethality in Iran. In the present study, the oxidative stress and antioxidant defense in serum of envenomated sheep with the venom of *Hemiscorpius lepturus* were investigated. Nine sheep were randomly divided into three groups (three in each). Groups A, B and C received 0.10, 0.05 and 0.01 mg kg^{-1} of *H. lepturus* venom subcutaneously, respectively. Blood sampling were performed 30 min before envenomation (control) and 30 min, 1, 2, 3 and 6 hr after envenomation and serum levels of total antioxidant capacity (TAC), malonedialdehyde (MDA) and protein carbonyl (PCO) were determined. The TAC was significantly increased at the doses of 0.10 mg kg^{-1} (at 3 hr) and 0.05 mg kg^{-1} (at 6 hr) compared to pre-injection time. However, no significant differences were observed in serum levels of MDA and PCO in different groups. It can be concluded that the dose of 0.01 mg kg^{-1} of venom had no effect on stress factors of serum, but according to increased level of TAC at the doses of 0.05 and 0.10 and no significant changes in serum levels of MDA and PCO, the oxidative damage has been prevented by the antioxidant defense system response.

بررسی وضعیت اکسیدانت/آنتی اکسیدانت در سرم گوسفند متعاقب مسمومیت تجربی با سم عقرب همی *اسکورپیوس لپتوروس*

چکیده

در کشورهای در حال توسعه به ویژه در مناطق گرمسیری و نیمه گرمسیری عقرب گزیدگی مشکل عمده بهداشت عمومی محسوب می‌شود. عقرب همی *اسکورپیوس لپتوروس* به عنوان یکی از اعضای خانواده همی اسکورپیده عامل اکثر موارد مرگ و میر ناشی از نیش عقرب در ایران می‌باشد. در این مطالعه تنش اکسیداتیو و دفاع آنتی اکسیدانی در سرم گوسفندان مسموم شده با سم عقرب همی *اسکورپیوس لپتوروس* مورد ارزیابی قرارگرفتند. نُه رأس گوسفند به صورت تصادفی به سه گروه سه رأسی تقسیم شدند. گروه‌های A، B و C به ترتیب ۰/۱۰، ۰/۰۵ و ۰/۰۱ میلی گرم بر کیلوگرم از سم عقرب همی *اسکورپیوس لپتوروس* را به صورت زیر جلدی دریافت نمودند. خون گیری ۳۰ دقیقه قبل (شاهد) و ۳۰ دقیقه، یک، دو، سه و شش ساعت پس از تزریق سم انجام گردید و سطوح سرمی ظرفیت آنتی اکسیدانتی تام (TAC)، مالون دی‌آلدئید (MDA) و پروتئین کربونیل (PCO) تعیین گردید. در دوزهای ۰/۱۰ میلی گرم بر کیلوگرم (در ساعت ۳) و ۰/۰۵ میلی گرم بر کیلوگرم (در ساعت ۶) TAC در مقایسه با زمان قبل از تزریق به شکل معنی‌داری افزایش یافت . با این وجود، هیچگونه تغییر معنی‌داری در سطوح سرمی MDA و PCO در گروه‌های مختلف مشاهده نشد. می توان چنین نتیجه گیری نمود که دوز ۰/۰۱ میلی گرم بر کیلوگرم از سم تأثیری بر فاکتورهای تنش سرمی نداشت، اما بر اساس افزایش سطح TAC در دوزهای ۰/۰۵ و ۰/۱۰ میلی گرم بر کیلوگرم و عدم وجود تغییرات محسوس در سطوح سرمی MDA و PCO، آسیب اکسیداتیو توسط پاسخ دستگاه دفاع آنتی اکسیدانتی مهار گردیده است.

واژه های کلیدی: ظرفیت آنتی اکسیدانتی، عقرب، گوسفند، مالون دی‌آلدئید

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Introduction

Scorpion envenomation is a common medical problem in many countries and is an important cause of morbidity and mortality.[1] The most important Iranian scorpion fauna consists of Buthidae, Scorpionidae and Hemiscorpiidae that some of these species such as *Hemiscorpius lepturus*, *Androctonus crassicauda* and *Mesobuthus eupeus* are found in Khuzestan province. *Hemiscorpius lepturus* is a member of the Hemiscorpiidae family and a statistical study has reported that 12.00% of all scorpion stings in Khuzestan province are due to *Hemiscorpius lepturus* and it is responsible for more than 95.00% of the mortalities.[2,3] Therefore, it can be said that this scorpion is the most dangerous scorpion in Iran.

The reactive oxygen species generation and oxidative stress have been implicated in the development of many diverse diseases including hypertension, cardiac dysrhythmia and myocardial damage[4] that all of which are present in scorpion envenomation.[5] *Hemiscorpius lepturus* venom can make a great catecholamines release due to over-stimulation of the autonomic nervous system and catecholamines can induce the free radicals production and participate in the oxidative stress.[6,7]

Although there is a lot of information about scorpion envenomation especially complications of *Hemiscorpius lepturus* in humans, the researches done so far on livestock are very limited.[8,9] Due to the nomadic sheep breeding system in Khuzestan province, especially in the eastern parts, which is considered as one of the main habitats of *Hemiscorpius lepturus* scorpion, it is expected that scorpion sting be important among herds in this region. However, due to lack of noticeable pain at the site of bite,[2] it remains hidden in most cases. Limited evidence indicates that free radical generation occurs during scorpion envenomation, especially in *Hemiscorpius lepturus*. Regarding the breeding of sheep being constantly exposed to scorpion bite, similarity of *Hemiscorpius lepturus* biting clinical signs to the other diseases such as babesiosis causing hematuria or hemoglobinuria and lack of paraclinical information on scorpion bites in sheep, as a preliminary study, we sought to find one of the paraclinical indicator which can help to identify pathophysiological mechanisms and treatments of scorpion sting in sheep. Thus, the aim of the present study was to investigate the oxidative stress and antioxidant defense for assessment of the pathophysiological mechanisms in envenomated sheep with the venom of *Hemiscorpius lepturus*.

Materials and Methods

The study was conducted on nine native healthy male lambs, aged about six months and weighing 18.00 - 22.00 kg, in the Teaching Veterinary Hospital and approved by the Department of Clinical Sciences at Shahid Chamran University of Ahvaz, Ahvaz, Iran (N0.92.6.23.886703). Two weeks before the commencement of the experiment, all the animals were examined clinically and received the antiparasitic agent (0.20 mg kg^{-1} ivermectin; Razak Co., Tehran, Iran). The animals were fed alfalfa, barely, bran and wheat straw during the study. At the experiment time, the animals were randomly divided into three groups (three animals in each). The experimental groups A, B and C received 0.10, 0.05 and 0.01 mg kg^{-1} of *Hemiscorpius lepturus* venom (Razi institute, Karaj, Iran) subcutaneously in the groin region, respectively.[10] The calculated doses were dissolved in 1 mL saline and injected subcutaneously at zero time. Blood sampling was performed 30 min before (control) and 30 min, 1, 2, 3 and 6 hr after envenomation. The samples were taken from jugular vein into plain vacutainers. Separated sera were stored at – 70 ˚C for the oxidative stress parameters measurement.

Malondialdehyde (MDA) as an end product of lipid peroxidation was estimated by thiobarbituric acid reactive substances method modified by Satoh(TBA; Merck, Darmstadt, Germany).[11,12] In this method, 1.00 mL of TBA-trichloroacetic acid (TCA) solution was added to 0.50 mL of each specimen in test tube and then it was placed in a boiling water bath for 15 min. After cooling in tap water, the tube was centrifuged at 1000 *g* for 10 min and the absorbance of the supernatant was measured spectrophotometrically (PowerWave S2; Biotek, Winooski, USA) at 532 nm. The concentration of MDA was determined using 1, 1, 3, 3-tetraetoxypropane (Sigma-Aldrich Chemie GmbH, Taufkrichen, Germany) at concentrations of 2.00 - 30.00 µmol L^{-1} as a standard and the MDA concentration in terms of µmol L^{-1} was calculated.

The method of protein carbonyl (PCO) detection depends on the formation of a Schiff base from the reaction of dinitrophenylhydrazine (DNPH, Sigma-Aldrich, St. Louis, USA) with PCOs to form protein hydrazons being measured spectrophotometrically. Briefly, after precipitation of protein with an equal volume of 1.00% TCA (Merck), the pellet was re-suspended in 10.00 mmol L^{-1} DNPH plus 2 N hydrochloric acid (Merck) as a control blank. Next, after the washing procedure with 1:1 ethanol-ethylacetate (Merck) the final plette was dissolved in 6.00 mol L^{-1} guanidine (Merck). The carbonyl group was determined from the absorbance at 370 nm. The carbonyl content was calculated in terms of nmol mg^{-1} protein.[13]

Determination of total antioxidant capacity (TAC) is based on reduction of Fe^{3+} to Fe^{2+} and the reaction of Fe^{2+} with 2,4,6-tri(2-pyridyl)-s-triazine (Sigma-Aldrich) produces blue complex being measured spectro-photometrically at 593 nm.[14]

Statistical analysis. Numeric variables are expressed as arithmetic mean ± standard error of the mean. Oxidative stress data were analyzed by repeated measure and two-way ANOVA with SAS software (version 9.1.3; SAS Institute, Cary, USA).

The effects of different doses of the toxin on serum parameters were analyzed by GLM. Means were compared with the PDIFF test and values of $p < 0.05$ were considered significant.

Results

Evaluation of the oxidative stress state indicated that *Hemiscorpius lepturus* venom was capable of significantly elevating of TAC (Table 1). These serum changes were significant in different groups and times ($p < 0.0001$). In this study, serum MDA levels were fluctuating at different doses and showed significant increase at some times ($p < 0.05$) representing free radicals increase and lipid peroxidation, but general pattern of changes was similar between the different groups in different times (Table 2; $p > 0.05$). The serum levels of PCO in different groups possessed no significant difference (Table 3; $p > 0.05$). This parameter was not affected by the sampling time and injected dose of venom ($p > 0.05$).

Discussion

Limited studies in large animal field have shown hemoglobinuria and fatality of envenomated sheep with *Hemiscorpius lepturus*.[8,9] The results of this study showed that the *Hemiscorpius lepturus* venom can cause dose-dependent increase of serum TAC. In the other words, the amount of injected venom and the exposure duration have been two important factors in stimulating stress and antioxidant defenses. Since antioxidant enzymes are not consumed in the reaction, incremental changes in the TAC in this study indicate that antioxidant enzyme levels are the first line of defense. Oxidative stress increases non-enzymatic antioxidants consumption, so, the TAC should be logically reduced, but here the oxidative damage was not enough to cause complete consumption of the non-enzymatic antioxidants.[15] Non-enzymatic antioxidants prevent free radicals formation and do not allow the formation of end metabolites like MDA and PCO.

In this study, serum MDA levels were fluctuating at different doses and showed significant increase at some times representing free radicals increase and lipid peroxidation. Then, they rapidly declined due to increase of serum antioxidant enzymes. Malondialdehyde as one of the oxidative stress markers is a product of lipid peroxidation that significantly increases by scorpion venom prohibiting the activity of glutathione, superoxide dismutase and catalase and increasing MDA level significantly.[16] In a study conducted in rats, significant differences in serum MDA levels were not observed after injection of *Androctonus australis hector* venom.[17] It has been shown that 30 min after the injection of *Androctonus australis hector* venom in mice, serum MDA levels were increased significantly and returned to normal level within 24 hr, but the MDA level did not show significant difference compared to than the control group when the mice received *Citrullus colocynthis* before venom injection, which actually can be due to its antioxidant properties.[16] In a previous study, significant increases in serum levels of MDA were observed in albino rats intraperitoneally injected by venom of *Leiurus quinquestriatus* scorpion and

Table 1. Serum level of total antioxidant capacity (μmol L^{-1}) in experimentally envenomated sheep (n = 3) at different doses and times.

Groups	Time					
	– 30 min	30 min	1 hr	2 hr	3 hr	6 hr
A (0.10 mg kg^{-1})	300.27 ± 1.86^{a}	286.27 ± 17.95^{a}	292.60 ± 38.31^{a}	347.60 ± 63.53^{a}	496.66 ± 90.00^{bB}	742.60 ± 18.36^{cC}
B (0.05 mg kg^{-1})	332.60 ± 20.84^{a}	323.60 ± 20.30^{a}	333.27 ± 47.36^{a}	334.27 ± 9.02^{a}	306.60 ± 26.63^{aA}	462.86 ± 22.53^{bB}
C (0.01 mg kg^{-1})	309.27 ± 3.38^{a}	277.93 ± 7.54^{a}	283.93 ± 2.33^{a}	283.20 ± 14.92^{a}	301.60 ± 24.19^{aA}	279.60 ± 26.29^{aA}

^{abc} Values with different small letters within rows significantly differ ($p < 0.05$).
^{ABC} Values with different capital letters within columns significantly differ ($p < 0.05$).

Table 2. Serum level of malondialdehyde (μmol L^{-1}) in experimentally envenomated sheep (n = 3) at different doses and times.

Groups	Time					
	– 30 min	30 min	1 hr	2 hr	3 hr	6 hr
A (0.10 mg kg^{-1})	2.87 ± 0.29^{a}	3.47 ± 0.56^{ab}	3.15 ± 0.39^{ab}	4.09 ± 0.36^{b}	3.08 ± 0.22^{ab}	3.05 ± 0.14^{ab}
B (0.05 mg kg^{-1})	3.05 ± 0.31^{a}	3.02 ± 0.22^{a}	3.65 ± 0.52^{ab}	3.31 ± 0.47^{ab}	3.45 ± 0.15^{ab}	4.10 ± 0.43^{b}
C (0.01 mg kg^{-1})	2.77 ± 0.34^{a}	2.79 ± 0.38^{a}	3.43 ± 0.54^{ab}	3.02 ± 0.19^{ab}	4.06 ± 0.61^{b}	3.39 ± 0.58^{ab}

^{abc} Values with different small letters within rows significantly differ ($p < 0.05$).
^{ABC} Values with different capital letters within columns significantly differ ($p < 0.05$).

Table 3. Serum level of carbonyl protein (nmol mL^{-1}) in experimentally envenomated sheep (n = 3) at different doses and times.

Groups	Time					
	– 30 min	30 min	1 hr	2 hr	3 hr	6 hr
A (0.10 mg kg^{-1})	15.28 ± 2.41	15.11 ± 2.19	15.68 ± 2.63	15.22 ± 1.14	14.72 ± 1.84	NA
B (0.05 mg kg^{-1})	15.69 ± 1.90	16.33 ± 2.21	15.54 ± 2.32	17.21 ± 2.53	15.67 ± 1.18	15.28 ± 2.09
C (0.01 mg kg^{-1})	14.69 ± 2.31	14.28 ± 2.07	14.23 ± 2.20	15.39 ± 2.22	14.84 ± 2.33	15.00 ± 2.61

No statistically significant differences were found in rows and columns ($p > 0.05$).
NA: not available.

it has been concluded that *Leiurus quinquestriatus* crude venom causes oxidative stress and alterations in the investigated biochemical parameters.[1]

The protein oxidation, similar to lipid peroxidation, is a major component of oxidative damage and one of the primary indicators of oxidative stress. Oxidative stress, an imbalance toward the pro-oxidant side of the pro-oxidant/antioxidant homeostasis, occurs in several human diseases. High levels of PCO groups have been observed among diseases including Alzheimer, rheumatoid arthritis, diabetes, sepsis, chronic renal failure and respiratory distress syndrome.[18] However, in this study, serum levels of PCO were not significantly different compared to control group may be due to acute toxicity of venom and insufficient time for protein oxidation.

In this study, the dose of 0.01 mg kg^{-1} of venom had no effect on stress factors of serum, but according to increased level of TAC at doses of 0.05 and 0.10 mg kg^{-1} and no significant changes in serum levels of MDA and PCO, it can be concluded that the antioxidant defense system response has prevented the oxidative damage. Lipid and protein oxidations likely need more time to increase or the levels quickly return to the normal values due to the antioxidants presense. Due to the death of sheep, it was not possible to continue the examination. Therefore, more studies are needed in this regard.

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Conflict of interest

The authors declare that they have no conflicts of interest regarding this paper.

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