Int J Clin Exp Med 2015;8(7):11734-11737
www.ijcem.com /ISSN:1940-5901/IJCEM0009824

Original Article

Effect of *Androctonus bicolor* scorpion venom on the activities of serum enzymes in rats

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Received May 2, 2015; Accepted June 23, 2015; Epub July 15, 2015; Published July 30, 2015

Abstract: We studied the effects of black fat-tailed scorpion (*Androctonus bicolor*) venom on the activities of liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatine kinase (CK) in the sera of rats. The animals were subcutaneously injected with a single dose of crude *Androctonus bicolor* venom (200 μg/kg body-weight) and were sacrificed at different time intervals including 30 min, 1 h, 2 h, 4 h, 8 h and 24 h after venom injection. There was no significant change in ALT activity in rats injected with *Androctonus bicolor* venom. Although *Androctonus bicolor* venom did not produce any change in serum AST activity until 1 h post-dosing, it significantly decreased this enzyme activity at 2 h onwards. There were significant decreases in ALP activities throughout the study though mild surges in the enzyme activity were observed at 1 h and 8 h post-dosing. There was a continued significant decrease in serum LDH activity until 8 h after *Androctonus bicolor* venom injection followed by normalization of LDH activity at 24 h. The activities of serum CK and GGT were significantly decreased at all the time points following *Androctonus bicolor* envenomation in rats. In conclusion, *Androctonus bicolor* envenomation in rats significantly reduced the activities of serum enzymes including AST, ALP, LDH, CK and GGT. *Androctonus bicolor* venom induced hypomagnesemia may account for persistently reduced activities of liver enzymes due to the cofactor role of magnesium in enzyme activities.

Keywords: Scorpion venom, *Androctonus bicolor*, liver enzymes, toxicity, rats

Introduction

The black fat-tailed scorpion (*Androctonus bicolor*) of the family Buthidae is considered as one of the most venomous scorpions in the world. These scorpions are black or brown in color, their movement is very fast and their nature is highly aggressive. All the scorpions of this species are dangerous and may cause multisystem manifestations, especially in the young children [1]. Presence of low molecular weight biogenic amines in scorpion venoms causes local reactions due to their action on blood vessels and nerve endings that induces swelling, redness, pain and itching [2]. However, major toxic effects of scorpion venom can be attributed to the presence of larger peptides such as melittin that can cause damage to the cell membrane resulting in cytolysis [3]. Yang et al [4] have recently identified a novel type of venom peptide with six disulfide bridges, referred to as Androcin, from the scorpion *Androctonus bicolor*. Androcin was found to induce severe akinesia and anxiety-like symptoms in mice, thereby providing the scorpion with an effective tool to subdue offending animals [4].

Venoms from species of the *Androctonus* genus are potentially toxic [5]. In a recent experimental study, *Androctonus bicolor* envenomation caused severe and persistent hypomagnesaemia with accompanied hypernatremia, hyperkalemia and hypercalcaemia in rats [6]. However, the effects of *Androctonus bicolor* venom on serum biomarkers of liver function and tissue injury are not known. We therefore investigated the effects of *Androctonus bicolor* venom on activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatine kinase (CK) in rats.

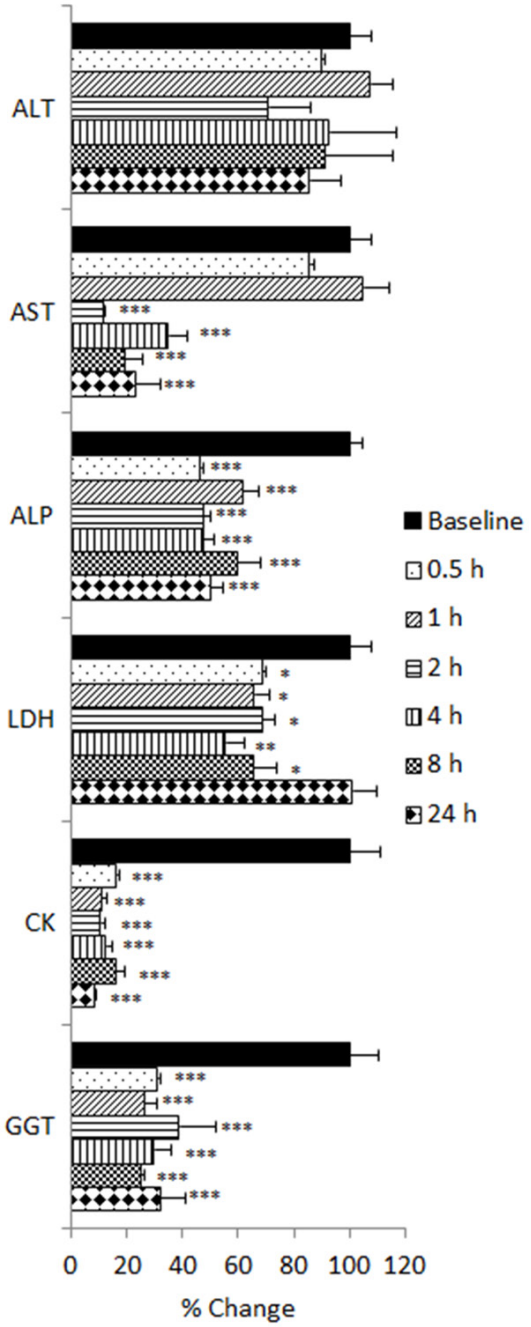

Figure 1. Percent change in liver enzyme activities at different times post-dosing of *Androctonus bicolor* venom (ABV). Baseline (control) values were set as 100. *P<0.05, **P<0.01 and ***P<0.001 versus control group using Dunnett's test.

Materials and methods

We collected *Androctonus bicolor* scorpions from the Riyadh region and kept them in plastic boxes at room temperature. The scorpions were fed with mealworms and received water daily. For venom collection, the scorpions were milked by electrical stimulation and the ejected venom was stored at -80°C.

For testing the acute effects of *Androctonus bicolor* scorpions, we used male Wistar rats (200 ± 20 g bodyweight), grown in our animal care facility. The groups of rats were placed in polycarbonate cages, kept in a room maintained at 23 ± 1°C with 12 h light-dark cycles. The animals were provided free access to standard laboratory food and tap water. The experimental protocol was approved by our Institutional Research and Ethics Committee. The animals were randomly divided into 7 groups of 5 animals each. Group 1 served as control and received vehicle (physiological saline) only. Animals in the remaining groups received a single subcutaneous injection of *Androctonus bicolor* venom (200 µg/kg bodyweight) and sacrificed at different time intervals as follows: 0.5 h, 1 h, 2 h, 4 h, 8 h and 24 h after venom injection. Blood samples were collected by cardiac puncture, sera separated and kept refrigerated until analyzed.

The enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK) and gamma glutamyl transferase (GGT) in sera of rats were determined by commercially available kits (United Diagnostic Industries, Dammam, Saudi Arabia) according to manufacturer's instructions.

The data were analyzed by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using the SPSS statistical package. *P* values <0.05 were considered as statistically significant.

Results

There was no significant change in serum ALT activity in rats injected with *Androctonus bicolor* venom (**Figure 1**). Although a single subcutaneous injection of *Androctonus bicolor* venom (200 µg/kg) in rats did not produce any change in serum AST activity until 1 h post-dosing, it significantly decreased this enzyme activity after 2 h and thereafter (ANOVA F=40.06, P<0.001; **Table 1**). There were significant decreases in ALP activities throughout the study, though mild surges in the enzyme activi-

Table 1. Time course effects of *Androctonus bicolor* venom (ABV) on serum enzymes in rats

Groups	AST (U/L)	ALP (U/L)	LDH (U/L)	CK (U/L)	GGT (U/L)
Control	45.76 ± 3.53	169.2 ± 7.83	120.7 ± 9.76	50.8 ± 5.68	34.52 ± 3.72
ABV (0.5 h)	39.38 ± 4.16	79.14 ± 4.12^{c}	83.20 ± 4.86^{a}	8.36 ± 0.53^{c}	10.78 ± 3.80^{c}
ABV (1 h)	48.18 ± 4.03	104.7 ± 9.56^{c}	79.66 ± 6.15^{a}	5.60 ± 0.86^{c}	9.18 ± 1.52^{c}
ABV (2 h)	5.34 ± 0.31^{c}	80.44 ± 4.13^{c}	83.04 ± 5.42^{a}	5.40 ± 0.91^{c}	13.46 ± 4.58^{c}
ABV (4 h)	16.10 ± 3.16^{c}	80.90 ± 6.25^{c}	67.28 ± 8.11^{b}	6.44 ± 0.93^{c}	10.40 ± 1.98^{c}
ABV (8 h)	8.88 ± 3.09^{c}	101.3 ± 14.3^{c}	79.32 ± 9.61^{a}	8.24 ± 1.49^{c}	8.76 ± 0.44^{c}
ABV (24 h)	10.60 ± 4.01^{c}	85.18 ± 6.93^{c}	122.5 ± 10.1	4.38 ± 0.23^{c}	11.18 ± 2.98^{c}

Data are presented as mean ± SEM. $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$ versus control group using Dunnett's test.

ty were observed at 1 h and 8 h post-dosing (ANOVA F=15.13, P<0.001). There was a continued significant decrease in serum LDH activity until 8 h after *Androctonus bicolor* venom injection followed by normalization of LDH activity at 24 h (ANOVA F=4.52, P<0.01) (**Table 1**; **Figure 1**). The activities of serum CK were persistently and profoundly decreased at all the time points following *Androctonus bicolor* envenomation in rats (ANOVA F=53.30, P< 0.001). There were significant decreases in serum GGT activities following scorpion venom injection, during the entire course of study (ANOVA F=9.07, P<0.001). The percent time-course changes in serum enzyme activities following *Androctonus bicolor* scorpion venom envenomation are shown in **Figure 1**.

Discussion

The results of this study showed unaltered ALT activity in the serum whereas the activities of AST, ALP, CK and GGT were significantly and persistently decreased (**Table 1**). Both ALT and AST enzymes are associated with liver parenchymal cells. However, ALT is predominantly located in the liver while AST is found in liver, heart muscle, skeletal muscle, kidneys, brain and red blood cells. Hence, ALT is a more specific indicator of liver inflammation than AST.

Although the activity of serum LDH was also significantly decreased until 8 h post-envenomation, it recovered to normal levels after 24 hours (**Figure 1**). Liver is a vital organ which helps in blood purification, detoxification, digestion, excretion, and metabolism. Liver enzymes play an important role in catalyzing chemical reactions involved in normal liver function. Insufficient amount of liver enzymes may be a sign of liver impairment or damaged liver cells which can deteriorate liver function and affect the overall health.

The venom of Asian black scorpion, *Heterometrus fastigiousus*, significantly increased serum ALP and LDH, after 4 h post-dosing in mice [7]. Significant increases in AST, ALT, CK and LDH in rat serum were observed following envenomation with Egyptian scorpion *Leiurus quinquestriatus* [8]. Intravenous injection of sublethal doses of *Tityus serrulatus* scorpion venom significantly increased the levels of AST, CK and LDH in rat serum [9]. Envenomation of dogs by *Tityus serrulatus* venom caused discreet increases in ALT, AST and CK, but no alterations were found in LDH [10]. The scorpion venom *Heterometrus fulvipes* was able to uncouple the respiration of rat liver mitochondria by inhibiting mitochondrial succinate and glutamate dehydrogenases [11]. Elevated liver enzymes indicate inflammation or cellular damage in the liver because inflamed or injured liver cells leak higher than normal amounts of liver enzymes into the bloodstream.

An acute phase decrease in the activities of serum enzymes, as observed in this study, could be specific to *Androctonus bicolor* scorpion venom. However, the exact cause of persistently diminished activities of serum enzymes in rat envenomated with *Androctonus bicolor* is not clear. Recently we have shown severe and persistent hypomagnesemia accompanied with severe hyperkalemia in rats injected with *Androctonus bicolor* venom [6]. Magnesium is involved in over 300 enzymatic reactions including the reactions catalyzed by carboxylases, phosphatases and kinases. Magnesium is also required for the release and proper action of parathyroid hormone [12]. Magnesium's usual role in the sodium-potassium ATPase pump and calcium-blocking activity is impaired by hypomagnesaemia leading to membrane destabilization and hyperexcitability [13]. Administration of magnesium has been

found to be useful for the treatment of the arrhythmias, hypertension, and other disorders associated with Buthinae scorpion envenomation [14]. It is more likely that reduced activity of serum enzymes is related to hypomagnesemia in rats injected with *Androctonus bicolor* venom.

In conclusion, *Androctonus bicolor* envenomation in rats significantly reduced the activities of serum enzymes including AST, ALP, LDH, CK and GGT. *Androctonus bicolor* venom induced hypomagnesemia may account for persistently reduced activities of liver enzymes due to the cofactor role of magnesium in enzyme activities. Further studies are warranted for testing the pharmacological potential of magnesium in protecting rats against *Androctonus bicolor* venom toxicity.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University, Riyadh, Saudi Arabia for funding the work through the Research Group No. RGP-009.

Disclosure of conflict of interest

None.

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