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Consequences of *Androctonus mauretanicus* and *Buthus occitanus* scorpion venoms on electrolyte levels in rabbits

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Abstract

Androctonus mauretanicus (*A. mauretanicus*) and *Buthus occitanus* (*B. occitanus*) scorpions, which belong to the *Buthidae* family, are the most venomous scorpions in Morocco. For the first time, we investigated the effects of such scorpion venoms on serum electrolytes in subcutaneously injected rabbits. For this purpose, 3 groups of 6 albinos adult male rabbits (New Zealand) were used in this experiment. Two of the groups were given a single subcutaneous injection of either crude Am venom (5 μg/kg) or Bo venom (8 μg/kg) whereas the third group (control group) only received physiological saline solution (NaCl 0.9%). The blood samples were collected from injected rabbits via the marginal vein at time intervals of 30 min, 1 h, 2 h, 4 h, 6 h and 24 h after venom injection. The concentrations of electrolytes in the serum samples were measured. Our study indicates that scorpion envenomation *in vivo*, rabbit animal model, caused severe and persistent hypomagnesaemia and

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hypochloremia, which are accompanied of hypernatremia, hyperkalemia and hypercalcaemia. The intensity of electrolytes imbalance was clearly superior in the case of *A. mauretanicus* scorpion venom (although a lower quantity of venom was injected). This is coherent with the experimental data which indicate that *A. mauretanicus* venom is more toxic than *B. occitanus* venom.

Keywords: Biochemistry, Toxicology

1. Introduction

Scorpion sting (and resulting envenomation) constitutes a real predicament for healthcare professionals especially in tropical and subtropical zones. Indeed, scorpion envenomation can interfere with the function of the main physiological systems, especially in children [1]. The most severe effects are of cardio-pulmonary and digestive orders [2, 3, 4, 5, 6, 7]. The health services are actually facing difficulties to manage scorpion envenomations due to their high severity and incidence [8, 9, 10].

More than 1500 scorpion species have been identified worldwide, 25 being known to be the most venomous scorpion species mostly part of the *Buthidae* family [11]. The Moroccan black (*Androctonus mauretanicus*) and yellow scorpions (*Buthus occitanus*) are members of this family [12, 13, 14]. They are the major cause of poisoning 30 to 50% of the reported cases [15, 16, 17, 18]. The *Androctonus mauretanicus* scorpion is responsible of the majority of scorpion stings (83% of cases) followed by *Buthus occitanus* (14% of cases) [16, 17, 18].

The effects engendered by the scorpion envenomation are involved in an inflammatory response leading to a multiple organ dysfunctions [19].

The causes behind the differences in severity of intoxication are yet to be determined. However, venom dosage, age, nutritional state, season and geographical area of the scorpion, as well as the age and weight of the victim, site of sting and individual sensitivity are some of the parameters thought to impact the severity of the envenomation [20].

The physiological action of scorpion venom toxins is not entirely known supposedly, it impairs peripheral nervous system inducing an increased release of chemical mediators. Moreover, scorpion venom has been linked to immune system dysfunctions by recruiting leukocytes and other inflammatory cells as well as the expression of adhesion molecules, platelet activating factor, cytokines and immunoglobulins [21].

The scorpion venom is very toxic being composed of neurotoxic proteins that affect ion channels, allergenic compounds (histamine and serotonin), mucopoly-saccharides and enzymes (phospholipases and hyaluronidases) [22, 23, 24].

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The toxicity of scorpion venoms mainly relies on their content in bioactive neurotoxins. The latter are able to block/alter the function(s) of their target ion channels in excitable cells [25, 26, 27], causing multiple physiological events associated with biological, metabolic and electrolyte disturbances [26, 27].

The objective of the present work is to evaluate the toxicity of *A. mauretanicus* and *B. occitanus* scorpion venoms, and their effects on the levels of electrolytes in subcutaneously-injected (New Zealand) rabbits used as animal model.

2. Materials and methods

2.1. Scorpion venoms

The scorpions have been gathered from the regions of Morocco with a higher risk of scorpionism. The raw venoms of both *A. mauretanicus* and *B. occitanus* species were collected by electrical stimulation of the scorpions raised at the Animal Facility of the Pasteur Institute of Morocco (PIM) [16]. The extracted venoms were recovered using cold water and centrifuged down at 10.000-x g. The supernatants were lyophilized and then frozen at −20 °C.

2.2. Evaluation of the Median Lethal Dose (LD_{50})

The evaluation of the lethal potency of venoms was conducted according to the LD_{50} value determination method, as recommended by the World Health Organization [16, 28, 29].

Venom doses were first adjusted in NaCl solution (150 mM) then administered in final volumes of 500 µl via intravenous (IV) and intraperitoneal (IP) routes, to populations of six mice per dose of venom.

Mortality rates were recorded 24 h following the injections. The software package GraphPad Prism 5, Inc. was used to determine the median lethal dose according to the supplied algorithm.

The software generated a non-linear curve applying the four-parameter logistical equation with constraints set on minimum (0% mortality) and maximum values (100% mortality).

GraphPad Prism 5 was also utilized to determine the median doses (ANOVA) and the plots were traced by the software Kaleida Graph 4.03 (Synergy Software, Reading, PA, USA) [16].

2.3. Experimental protocol

Adult Albinos rabbits (2–2.5 kg, bred in the animal unit of IPM) were placed in cages and kept at room temperature with access to food and water.

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Three groups of six rabbits were used in the experiment. The first group is a control and was injected by physiological saline solution (NaCl 0.9%). The second group was administered *A. mauretanicus* raw venom (5 μg/kg bodyweight). The third group received an injection of *B. occitanus* raw venom (8 μg/kg bodyweight). All injections were made by subcutaneous route.

The blood samples were collected through the marginal veins of rabbits at the different time points of 30 min, 1 h, 2 h, 4 h, 6 h and 24 h after venom injection. Total blood samples of experimental and control groups were carried out on dry tubes (drawn volume of 3.5 mL) and immediately centrifuged at 3.000 rpm (rotation per minute) for 10 min. Each time, sera were recovered and stored at a temperature of −20 °C until use.

2.4. Biochemical analysis of the serum samples

The levels of electrolytes, i.e. sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}) and chloride (Cl^-), in the various serum samples were determined using a Biochemical Analyzer.

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.

2.5. Ethics committee approval for experimentation in animals

All the testing and procedures involving animals strictly followed the ethical principles in animal research adopted by the World Health Organization [28, 29]. They were approved by a local ethics committee of the Pasteur Institute of Morocco (Casablanca, Morocco). The Ethics Committee of the Pasteur Institute of Morocco, under agreement number 8.3.A-2015, delivered the authorization for animal testing on April 15th, 2015.

3. Results

3.1. Evaluation of the Lethal Median Dose (LD_{50})

Table 1 shows that *A. mauretanicus* venom is more lethal than the *B. occitanus* venom. In addition, the administration route does not have an important effect on the toxicity of the venom.

3.1.1. Effect of A. mauretanicus and B. occitanus scorpion venoms in sodium levels

An injection of either *A. mauretanicus* (5 μg/kg) or *B. occitanus* (8 μg/kg) scorpion venom in rabbits caused a significant increase in serum sodium levels in comparison with the control group at 30 min after injection, with values of 311.1 ± 0.6 mEq/L

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Table 1. Median lethal doses (LD_{50}) of Am, Bo scorpion venoms, as determined using intravenous (IV) and intraperitoneal (IP) injection routes, with 95% confidence intervals and calculated by non-linear regression.

Injection route	LD_{50}	
	***A. mauretanicus* venom**	***B. occitanus* venom**
IV (μg/mouse)	2.4 (2.2 – 2.8)	5.7 (5.3 – 5.9)
IP (μg/mouse)	3.6 (3.4 – 3.9)	6.2 (5.7 – 6.7)

(*A. mauretanicus*) and 202.0 ± 1.2 mEq/L (*B. occitanus*). This increase is slightly attenuated after 1 h and persisted over 24 h (Table 2 and Table 3, Fig. 1 and Fig. 2).

3.1.2. Effect of A. mauretanicus and B. occitanus scorpion venoms in potassium levels

There was a significant and sustained increase in serum potassium levels until 4 h, which slightly diminished thereafter. A significant hyperkalemia persisted over 24 h with 111.7 ± 1.0 and 8.1 ± 1.0 mEq/L for Am and Bo venoms, respectively (Table 2 and Table 3, Fig. 1 and Fig. 2).

3.1.3. Effect of A. mauretanicus and B. occitanus scorpion venoms in magnesium levels

There was a significant decrease in Mg^{2+} serum levels following scorpion venom injection during the whole kinetic of the experiment (30 min, 1 h, 2 h, 4 h, 6 h, 24 h). The lowest values obtained at 24 h post injection were 12.0 ± 0.1 and 19.2 ± 1.2 mg/L for Am and Bo venoms, respectively (Table 2 and Table 3, Fig. 1 and Fig. 2).

Table 2. Kinetic of the subcutaneous administration of *A. mauretanicus* venom on serum electrolyte levels: sodium (mEq/L) potassium (mEq/L), magnesium (mg/L), calcium (mg/L) and chloride (mEq/L) in rabbits at 30 min (G1), 1 h (G2), 2 h (G3), 4 h (G4), 6 h (G5) and 24 h (G6) hours post injection of venom.

Groups	Na^+ (mEq/L)	K^+ (mEq/L)	Mg^{2+} (mg/l)	Ca^{2+} (mg/L)	Cl^- (mEq/L)
G0 (control)	141.0 ± 0.6^c	4.3 ± 0.3^c	24.0 ± 0.5^c	151.0 ± 0.2^c	95.0 ± 1.5^b
G1 (30 min)	311.1 ± 0.6^c	9.3 ± 0.9^b	19.6 ± 0.2^c	270.7 ± 0.2^c	62.6 ± 2.1^a
G2 (1 h)	285.2 ± 0.2^c	9.7 ± 0.2^c	18.2 ± 0.5^c	288.5 ± 0.6^c	51.6 ± 2.0^a
G3 (2 h)	271.6 ± 2.0^a	10.3 ± 0.3^c	17.6 ± 0.4^c	287.6 ± 0.1^c	39.4 ± 0.2^c
G4 (4 h)	288.2 ± 0.2^c	11.7 ± 1.0^b	18.9 ± 0.6^c	266.7 ± 0.01^c	42.6 ± 1.3^b
G5 (6 h)	281.2 ± 3.1^a	10.9 ± 0.1^c	12.3 ± 0.5^c	287.5 ± 0.6^b	53.4 ± 0.3^a
G6 (24 h)	289.3 ± 1.5^b	10.7 ± 0.2^c	12.0 ± 0.1^c	285.6 ± 0.5^c	71.0 ± 3.5^a

Data are presented as mean ± SEM. $^aP < 0.005$, $^bP < 0.01$ and $^cP < 0.001$ versus control group using Dunnett's test.

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Table 3. Kinetic of a subcutaneous administration of *B. occitanus* venom on serum electrolyte levels: sodium (mEq/L), potassium (mEq/L), magnesium (mg/L), calcium (mg/L) and chloride (mEq/L) in rabbits at 30 min (G1), 1 h (G2), 2 h (G3), 4 h (G4), 6 h (G5) and 24 h (G6) hours post injection of venom.

Groups	Na^+ (mEq/L)	K^+ (mEq/L)	Mg^{2+} (mEq/L)	Ca^{2+} (mEq/L)	Cl^- (mEq/L)
G0 (control)	141.0 ± 0.6^b	4.3 ± 0.3^c	24.0 ± 0.5^c	151.0 ± 0.2^c	95.0 ± 1.5^b
G1 (30 min)	201.1 ± 1.2^a	6.3 ± 0.1^c	21.9 ± 0.01^c	180.7 ± 1.5^b	72.1 ± 1.1^b
G2 (1 h)	180.6 ± 0.2^c	7.0 ± 0.2^c	21.7 ± 1.1^b	188.4 ± 0.5^c	61.6 ± 1.3^b
G3 (2 h)	172.6 ± 0.6^c	7.6 ± 2.0^a	21.7 ± 0.01^c	179.2 ± 1.1^b	51.6 ± 2.0^a
G4 (4 h)	185.2 ± 0.2^c	8.1 ± 1.0^b	22.1 ± 1.0^b	166.4 ± 0.01^c	58.2 ± 3.0^a
G5 (6 h)	182.4 ± 1.1^a	7.7 ± 0.1^c	20.2 ± 1.5^b	179.7 ± 0.3^c	72.2 ± 0.4^c
G6 (24 h)	187.4 ± 2.0^a	7.4 ± 0.2^c	19.2 ± 1.2^b	181.4 ± 0.3^c	78.5 ± 1.3^b

Data are presented as mean ± SEM. $^aP < 0.005$, $^bP < 0.01$ and $^cP < 0.001$ versus control group using Dunnett's test.

3.1.4. *Effect of A. mauretanicus and B. occitanus scorpion venoms in calcium levels*

We observed a significant increase in serum calcium at all time points with concentrations in electrolyte up to 288.5 ± 0.6 (mg/L) and 188.4 ± 0.5 (mg/L) for Am and Bo, respectively (Table 2 and Table 3, Fig. 1 and Fig. 2).

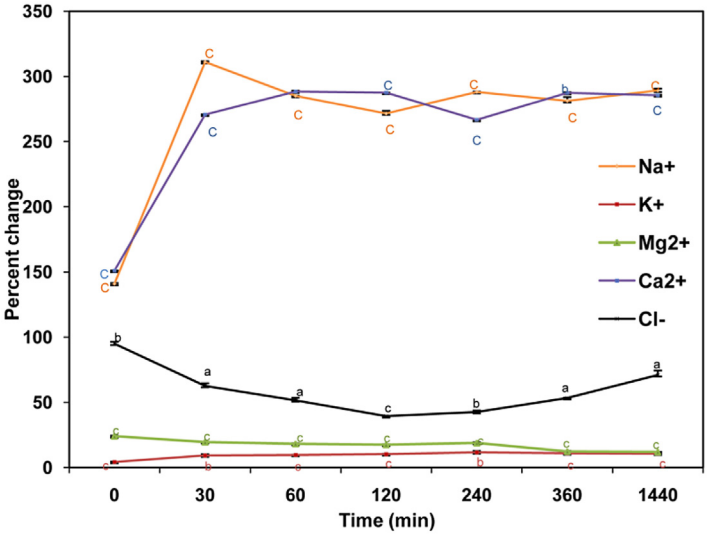

Fig. 1. Percent change in serum electrolyte levels for *A. mauretanicus venom*. Effects of injections of Am venoms (5 μg/kg bodyweight) on serum electrolyte levels for Am venom. The sample bloods were collected at 30 min, 1 h, 2 h, 4 h, 6 h and 24 h post mortem animals. The 'control' animals were injected with the physiological saline solution (NaCl 0.9%).

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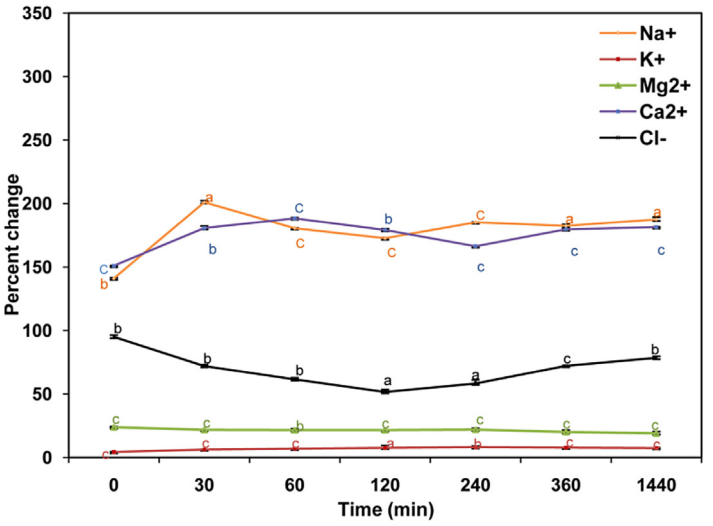

Fig. 2. Percent change in serum electrolyte levels for *B. occitanus* venom. Effects of injections of Bo venoms (8 μg/kg bodyweight) on serum electrolyte levels for Am venom. The sample bloods were collected at 30 min, 1 h, 2 h, 4 h, 6 h and 24 h post mortem animals. The 'control' animals were injected with the physiological saline solution (NaCl 0.9%).

3.1.5. Effect of A. mauretanicus and B. occitanus scorpion venoms in chloride levels

Serum chloride levels were significantly decreased with 39.4 ± 0.2 (mEq/L) for Am and 51.6 ± 2.0 (mEq/L) for Bo (Table 2 and Table 3, Fig. 1 and Fig. 2).

These results show that the imbalance of electrolytes is more important in the case of Am scorpion venom. The maximum post-dosing increases in sodium, potassium and calcium were of 100% (30 min), 100% (4 h) and 30% (2 h), respectively for the Am venom, and 49% (30 min), 35% (4 h) and 25% (1 h), respectively, for the Bo venom. The maximum decreases in serum magnesium and chloride after venom injection were of 60% (4 h), 44% (24 h) for the *A. mauretanicus* venom, and 25% (4 h), 19% (2 h), respectively, for the *B. occitanus* venom (Fig. 1 and Fig. 2).

4. Discussion

In Morocco, scorpion stings are the major cause of envenomation and a serious medical emergency. The composition of scorpion venoms is very complex and includes a mixture of molecules such as neurotoxic peptides that target ion channels. Toxins modulate the membrane conductance of the Na^+, K^+, Ca^{2+} and Cl^- channels leading to a disturbance in the nervous influx causing physiopathological disorders.

The *Androctonus* and *Buthus* are the most dangerous scorpion genus in Morocco and are incriminated in many cases of evenomation accidents.

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The *A. mauretanicus* and *B. occitanus* venoms have been reported to cause cytotoxicity, tissue necrosis, inflammation, and muscle paralysis. Indeed, myotoxicity is a cause of mortality, which is associated with neurotoxic effects [17].

Our work on the consequences of *A. mauretanicus* venom on architectural structures on brain, heart, lungs, liver and kidneys parenchyma's revealed that the venom displays a fast diffusion kinetic from blood to tissue compartment.

This leads to a serious tissue damage showed by hemorrhages, edema and the recruiting of the inflammatory cells. The physiopathogenic mechanisms explaining these changes tissue are an etiology very complex [6, 7].

In this work, we evaluated the Lethal Median Dose (LD_{50}) mean values of venoms by two injection routes: intravenous and intra-peritoneal using male Swiss mice (20 ± 2 g).

IV venom injection is the route that allows the toxins to penetrate in the blood with a complete bioavailability leading to a better expression of the envenomation effects [29].

The low molecular weight of the toxins (less than 7 kDa) favors rapid circulation of the molecules in the bloodstream. We have reported that the ratio between the values (IP versus IV) is from 1.1 to 1.3, suggesting that the absorption and biodistribution of toxins are almost complete and rapid in the case of IV route [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]

The data presented in this study showed the peculiar time-course pattern of serum electrolytes in rabbits injected with a sublethal dose of the *A. mauretanicus* and *B. occitanus* scorpion venoms. Our findings indicate there were increasing trends in serum sodium, potassium and calcium levels whereas decreasing trends in serum magnesium and chloride levels were observed.

When compared to the previously reported data on changes in serum electrolytes following envenomation with different scorpion venoms, the effects of *A. mauretanicus* and *B. occitanus* venoms are similar to those reported by A.Al-Asmari et al., 2015. The results of the latter have described that a single subcutaneous injection of *Androctonus bicolor* venom (200 μg/kg) in rats caused some significant increases in serum concentrations of sodium, potassium and calcium, whereas they have measured a significant decrease in serum concentrations of magnesium and chloride electrolytes [22].

El Khalil et al., 2013 showed that a sublethal dose of *Leiurus quinquestriatus quinquestriatus* venom, injected subcutaneously into rabbits, causes hypernatremia, hyperkalemia and hypercalcaemia [31].

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Cusinato et al. showed that, following experimental envenomation of rats by the venom of the most dangerous scorpion in Brazil, i.e. *Tityus serrulatus* species, no significant modifications were observed in serum concentrations of Na^+ and Ca^{2+} while a significant augmentation in serum concentrations of both K^+ and Mg^{2+} was observed [26, 32].

O. Ozkan et al., 2006 demonstrated that the *Androctonus crassicauda* scorpion venom causes a significant diminution in serum Na^+ as well as Cl^- and an elevation in serum concentrations of K^+ and Ca^{2+} [33].

Despite the toxicity observed in mice, *A. mauretanicus* venom showed neurotoxic and myotoxic effects higher than those observed with *B. occitanus* venom [30]. Our results clearly indicate that *A. mauretanicus* and *B. occitanus* envenomations caused severe and persistent hypomagnesaemia and hypochloremia, which were accompanied by hypernatremia, hyperkalemia and hypercalcaemia. (a) The hypomagnesaemia can be attributed to the poor intestinal absorption of rabbits; (b) The hypernatremia can be induced following dehydration and increased renal resorption; (c) the hyperkalemia can be caused by potassium retention of rabbit kidneys causing a series of diarrheas followed by dehydration; (d) the hypocalcaemia can be inferred to the stimulation of the opening of the voltage-gated Ca^{2+} channels following the depolarization of synaptic terminals which causes a massive Ca^{2+} release, and (e) the hypochloremia may be attributed to a decrease in renal resorption causing vomiting and cramps.

The intensity of electrolytes imbalance was clearly superior in the case of *A. mauretanicus* scorpion venom (although a lower quantity of venom was injected), in association with a greater toxicity of Am venom as compared to Bo venom.

Magnesium plays a key role in cellular cytoskeleton contraction and at the myoneural junction; it can therefore impair skeletal and cardiac muscle functions. Magnesium is the second most abundant intracellular cation, with 67% of total body stores found in bone, 31% intracellular, and only 2% extracellular (measurable space). It is an essential component with regard to cardiac and vascular functions. Magnesium (i) regulates contractile proteins, (ii) modulates transmembrane transport of calcium, sodium and potassium, (iii) acts as a co-factor in the activation of ATPase, (iv) controls the regulation of energy-dependent cytoplasmic and mitochondrial metabolisms, and (v) influences DNA and protein syntheses at the subcellular level [33, 34, 35, 36, 37]. Magnesium is reportedly involved in over 300 enzymatic reactions. It is required in (i) the energy metabolism, (ii) glucose utilization, (iii) protein synthesis, (iv) fatty acid synthesis and breakdown, (v) muscle contraction, (vi) ATPase functions, (vii) hormonal reactions, and (viii) maintenance of cellular ionic balance [33, 34, 35, 36, 37].

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The deficit in magnesium inhibits the functioning of Na^+/K^+ pumps, resulting in a decrease of intracellular levels of both potassium and sodium leading to an ion imbalance on either side of the cell membrane.

In our study, serum potassium and sodium levels were significantly increased by the scorpion envenomation in rabbits.

The leak of potassium should be a consequence of magnesium deficit; therefore, patients suffering of scorpion envenomation should be treated with an exogenous supply of Mg^{2+}.

We reported that serum magnesium levels are inversely proportional to serum calcium levels. Magnesium is known to affect calcium pump functioning. In fact, lower concentrations of intracellular magnesium stimulate the calcium transport into the cell, whereas an increase in magnesium level results in a decrease of the intracellular calcium levels [33, 34, 35, 36, 37].

We have already reported that the lethality of *A. mauretanicus* and *B. occitanus* scorpion venoms could be linked to myotoxic and neurotoxic activities and it is well known that scorpion envenomations associated with cardiorespiratory alterations may complicate into heart failure and pulmonary edema [5, 6, 7, 30]. Sharon et al. (2008) have reported that hypomagnesemia affects muscle weakness, vascular smooth muscle tone, ventricular arrhythmias and myocardial contractility. Therefore, our study indicates that scorpion envenomation *in vivo* – in the rabbit animal model – caused severe and persistent hypomagnesaemia and hypochloremia, which are accompanied of hypernatremia, hyperkalemia and hypercalcaemia [37]. If the same occurs in humans, this highlights the importance to monitor both magnesium and chloride levels in sera of victims of scorpion envenomation, and possibly treat the patients with such deficiencies in electrolytes. Na^+ channels are essential elements in cardiac excitability and function. It is expected that changes in properties of the cardiac sodium channels might result in some important cardiac malfunctions like arrhythmia. Indeed, various forms of arrhythmia have been reported.

5. Conclusion

Our study is the first report evaluating the effects on serum electrolytes of injections of *A. mauretanicus* and *B. occitanus* scorpion venoms in rabbits. The results clearly indicate that *A. mauretanicus* and *B. occitanus* envenomations are responsible for an important and persistent hypomagnesaemia which were accompanied with hypernatremia, hyperkalemia, and hypocalcaemia. The intensity of electrolytes imbalance was clearly superior in the case of *A. mauretanicus* scorpion venom (although a lower quantity of venom was injected), suggesting some venom-specific/dependent effects. Imbalance in the magnesium homeostasis

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can induce serious pathologies/troubles such as arrhythmias, hypertension and other disorders some of which are only managed by magnesium treatment. It appears to be important to add the dosage of serum magnesium in the routine serum electrolytes test (sodium, potassium, calcium, and chloride). Indeed, we recommend that experimentally envenomed animals and envenomed patients with severe hypomagnesaemia be treated accordingly (with a Mg^{2+} perfusion).

Declarations

Author contribution statement

Khadija Daoudi, Fatima Chgoury: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Myriam Rezzak, Oussama Bourouah, Abdelaziz Soukri: Analyzed and interpreted the data.

Lotfi Boussadda: Contributed reagents, materials, analysis tools or data.

Jean-Marc Sabatier: Wrote the paper.

Naoual Oukkache: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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