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Time-dependent effects of the venom of the scorpion *Leiurus q. quinquestriatus* on Na^+, K^+ and Ca^{++} ion concentrations of rabbit's plasma

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ABSTRACT

Many neurotoxic polypeptides (NPs) from venom of the scorpion *Leiurus quinquestriatus* (LqV) have been isolated and functionally characterized that were found to block voltage-activated ion channels in excitable tissues in both mammals and insects. This study aims to reveal the time-dependent alternations of these electrolytes in plasma that induced by (LqV) *in vivo*.

Rabbits were injected subcutaneously with a sub-lethal dose of approximately 0.12 mg/km body weight of crude (LqV). Photometric techniques have been used to monitor changes in the concentrations of Na^+, K^+, and Ca^{++} in plasma six times; the first sample considered as control, the other five samples were collected in 36 minutes interval (after LqV injection). Correlation and regression analysis have shown that potassium and Calcium concentration tended to decrease (r=-0.706) and (r=-0.586) respectively whereas, concentration of Sodium tended to increase (r=0.635). Percentage of concentration change from control showed highly significant fluctuation during the first two hours, sodium was highly decreased by -72.29% below control 36 minutes after injection, for potassium and calcium, concentrations were increased; after 72 minutes potassium raised by 173.76% above control, calcium highest reading observed after 36 minutes (44.03% above control). 108 minutes after injection, percentage of change from control for all the ions was very close to each other (30.54%, 26.31% & 31.67% above control for sodium, potassium and calcium respectively). 144 and 180 minutes after injection, less fluctuation was observed.

Neurotoxic venom of this scorpion was found to change serum concentration of three fundamental electrolytes in nervous system biochemical mechanism. In addition this change may greatly affect toxin binding to ion channels as well as homeostatic balance. We suggest that, fatal symptoms due to scorpion stings are not only limited to direct interactions of PNs with VGSCs, but also toxin-affected neuro-chemical homeostasis could affect the behaviour of VGSCs.

Keywords: LqV, neurotoxins, plasma electrolytes, voltage-gated sodium channels, neural homeostatic plasticity.

INTRODUCTION

Voltage-gated Ion channels are trans-membrane protein complexes that form pores across the cell membrane through which specific ions can diffuse. Each channel is composed of four principal subunits, each on is selective for either Sodium, Potassium, Calcium or Chlorine. These channels are key elements in cellular function since they participate in the generation and propagation of action potentials in neurons and most electrically excitable cells (Dale Purves *et al*., 2001; Yu *et al*., 2003)' (Savio-Galimberti *et al*., 2012)' (Goldin, 2001) (Dale Purves, George J Augustine, David Fitzpatrick, Lawrence C Katz, Anthony-Samuel LaMantia, James O McNamara, 2001). VGSCs are composed of a glycoprotein pore-forming α-subunit which can be associated with up to four different β-subunits(Catterall,

W. A., Goldin, A. L., and Waxman, 2005; Savio-Galimberti *et al*., 2012; Yu & Catterall, 2003).

The α-subunits are organized in four homologous domains (DI-IV)(Guy & Seetharamulut, 1986). The VGSC gene family comprises nine homologous members SCN1A to SCN11A, which encode the sodium selective ion channels NaV1.1 to NaV1.9 (Catterall, W. A., Goldin, A. L., and Waxman, 2005; Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, Noda M, Tamkun MM, Waxman SG, Wood JN, 2000). Hyperpolarization-Activated Cyclic Nucleotide gated channels (HCNs) encoded by four genes HCN1 to HCN4(WN, 2006) (Yu FH, Yarov-Yarovoy V, 2005) are expressed in nervous system(Moosmang S, Biel M, Hofmann F, 1999) (Biel M, Wahl-Schott C, 2009) and heart(Robinson RB, 2003). Their main function is to generate and/or regulate neuronal and cardiac excitability. In general, HCN channels engender and regulate neuronal and cardiac firing rates. Besides acting as a pacemaker, the HCN current also functions as a regulator of resting potential and membrane resistance. The current stabilizes the resting membrane potential because small hyperpolarizations activate the pacemaker channels, whose inward currents depolarize the cell. This depolarization, as a consequence, deactivates the HCN channels, preventing continued departure from the resting potential. The HCNs possess an inherent negative-feedback property. On the contrary, neurotransmitters can influence rhythmic activity in both the heart and the nervous system by either increasing or decreasing the level of cyclic Adenosine monophosphate (cAMP), which in turn directly modulates the activation kinetics and maximal current of HCN channels(Biel M, Wahl-Schott C, 2009).

Homeostatic functions of ion channels: Ion channels serve three principal physiological roles, first of all; they set up the resting membrane potentials of all cells. Thus, when open, potassium ion-selective channels and anion channels, they cause the membrane potential to become more negative (hyperpolarize cells), whereas sodium or calcium-selective channels and non-selective cation channels cause the membrane potential to become more positive (depolarize cells). The second function is to flux ions through ion channels since; it contributes to the electrolyte movements required for volume regulation of single cells. And for the net polarized transport of salt across epithelia like gut, kidney, or the choroid plexus (Hille, 2001)' (AF, 1952)

Scorpion venoms: scorpion venoms are highly complex mixtures of enzymes, peptides, nucleotides, lipids, mucoproteins, biogenic amines and other unknown substances, Predictions suggest that close to 100,000 distinct polypeptides are present in all known scorpion species(Possani *et al*., 1999). Neurotoxins present in scorpion venoms have evolved towards a specific bioactivity. Multiple toxins present in the venom of the scorpion *L. quinquestriatus* (LqV) that guarantee efficient immobilizing preys and defense against mammalian predators (Rodríguez De La Vega & Possani, 2005).

Scorpion α-toxins: Electrophysiological studies have shown that the foremost effects of scorpion α-toxins are a remarkable slowing of fast inactivation of VGSCs and minor modifications of the voltage dependence of channel activation(Chen & Heinemann, 2001; Chen *et al*., 2002). Since these toxins prevent a component of outward gating charge movement associated with channel inactivation(Sheets *et al*., 1999), it is likely that they are able to slow inactivation by preventing the outward movement of domain 4 of VGSCs (DIV

S4 segment) a conformational change necessary for fast inactivation(Catterall, 2000). In this sense, scorpion α-toxins can be considered gating-modifier toxins(Bosmans & Tytgat, 2008). A consequence of scorpion α-toxins in vivo is that they prolong the action potentials of excitable cells. As a consequence, these toxins can kill organisms by inducing paralysis and arrhythmia. However, the binding affinity of scorpion α-toxins to mammalian VGSCs is reduced by membrane depolarization and increased by alkaloid binding at Site 2 (Chen & Heinemann, 2001; Chen *et al*., 2002) (Catterall, 2000)' (Conti *et al*., 1976; Strichartz *et al*., 1987). Interaction of several scorpion α-toxins from the scorpion *Leiurus quinquestriatus hebraues* (Lqh II, Lqh III, and LqhαIT) with Nav1.5 toxins removed fast inactivation. However, association and dissociation rates of Lqh III were much slower than those of Lqh II and LqhαIT, to the extent that Lqh III would not dissociate from the channel during a cardiac activation potential. Toxin dissociation remained voltage dependent even at high voltages. Slow inactivation of Nav1.5 was significantly enhanced by Lqh II and Lqh III. The half-maximal voltage of steady-state slow inactivation was shifted to negative values, the voltage dependence was increased and slow inactivation at high voltages became more complete indicating that VGSC slow inactivation is directly modulated by scorpion α-toxins (Chen & Heinemann, 2001).

Scorpion β -toxins: Studies on the molecular mechanism of β-toxins focusing on their ability to open NaV channels at resting voltage have shown that they left-shift the voltage dependence of channel activation. This effect is use dependent because the activation shift is enhanced when channels are pre-activated with a depolarizing pre-pulse. Compared the effects of CssIV (from *Centruroides suffusus suffusus*) on brain-type NaV1.2 and heart-type NaV1.5 channels and found remarkable differences. voltage–sensor trapping model was proposed in which CssIV reduces the activation energy necessary for channel opening by arresting the voltage sensor of domain 2 in an activated position (Sautière *et al*., 1998)' (Catterall *et al*., 2007) (Mantegazza & Cestèle, 2005). Scorpion β-toxins bind to receptor site 4 and show rather complex effects, on the one hand, they induce spontaneous and repetitive firing of action potentials by permitting NaV channels to activate at sub-threshold membrane potentials. On the other hand, they reduce the peak NaV channel current(Catterall *et al*., 2007)'(De La Vega & Possani, 2007) Thus, it appears that scorpion β-toxins have a bimodal function because they can enhance (excitatory mode) and inhibit (depressant mode) the activity of NaV channels and hence the excitability of neurons. Furthermore, β-toxins are subtype specific, as they discriminate between different NaV channel isoforms(Leipold *et al*., 2012). Accordingly, the physiological consequences of a certain β-toxins are hard to predict because they may depend not only on the dominant mode of the toxin but also on the affected channel subtypes. Excitatory insect toxins Lqh IT1-a, b, c and d were found to induce a spastic paralysis. They bind voltage-independently at site-4 of sodium channels and shift the voltage of activation toward more negative potentials thereby affecting sodium channel activation and promoting spontaneous and repetitive firing(Leipold *et al*., 2012).

LqV toxic polypeptides: Voltage gated ion channel blockers for Sodium channels were identified (Bosmans *et al*., 2005; Stevens *et al*., 2011) and for Potassium channels (Castle & Strong, 1986; Garcia *et al*., 1994), and for Chloride channels (DeBin *et al*., 1993; Lippens *et al*., 1995) which is being

studied as a new targeting molecule for brain cancer cells (Soroceanu *et al*., 1998; Veiseh *et al*., 2009, 2010).

Pathology of LqV: Intravenous (IV) administration of LqV in anaesthetized dogs was shown to reduce the heart rate by 13%, and evoked some abnormal waveforms in the electrocardiogram (ECG). In isolated atria, LqV (10-2 mg/mL) exposure abolished the sinus rhythm and decreased the spontaneous rate by 38%, and increased the contraction amplitude by 85% and duration of the contractions by 17%, changes were found to be dose dependent. The gross electrical activity of the preparation and the duration of the individual atrial muscle action potential were prolonged by 150% and 186%, respectively. In isolated papillary muscle, LqV evoked irregular contractions, and the duration of the action potential was increased about 15-fold. The effects by LqV in the action potential were present when calcium channels were blocked but not when extracellular sodium was substituted according to this it was shown that the lethal cardio-toxic effects by LqV were mainly due to its direct action in myocardial cells, and partly to an alteration in the autonomous nervous activity(Purali & Yagcioglu, 2002). A study on the hemodynamic effect of IV administration of LqV induced significant combined respiratory and metabolic acidosis (arterial pH progressed from 7.35 ± 0.03 at baseline to 7.10 ± 0.06 at 90 minutes). There were large increases in blood pressure, left ventricular (LV) end systolic pressure, stroke work, and velocity of contraction. Twenty minutes following venom injection, cardiac output (CO) increased by 37% but then declined to 36% below baseline by 90 minutes ($P < .05$). Cerebral blood flow, (CBF) increased significantly in proportion to increased perfusion pressure; hence, there was no change in coronary vascular resistance. There was no evidence of myocardial ischemia or LV dysfunction because there was no change in myocardial pH, percentage fiber shortening, or LV end-diastolic pressure. Despite the fact that some variables returned to baseline at 90 minutes, they did not reach steady state; thus, the preparation would have continued to deteriorate (Tarasiuk *et al.*, 1994). A case of scorpion stung human victim has been reported to have ECG abnormalities that simulate early myocardial infarction, pulmonary edema and congestive heart failure accompanied these ECG changes (Maheshwari & Tanwar, 2012). In addition, cytotoxicity has been reported on 293T and C2C12 eukaryotic cell lines, cell survival highly reduced at the concentration of (50 µg/ml) of LqV. These effects were rapid and observed within 30 minutes. The apparent initial damage to the nucleus and lysis of the plasma lemma and/or organelle membranes, which was evident by a significant increase in cytosolic Lactate dehydrogenase (LDH) release, suggested that this toxin acts at the membrane level (Omran *et al*., 1992) (Fadol *et al*., n.d).

MATERIALS AND METHODS

Ethics Statement

Protocol of the study was approved by zoology departmental board, faculty of Science, University of Khartoum according to the same University Senate Ethical Committee (SEC) under ethical standards of The National Health Research Ethics Committee (NHREC), Sudan that follows international ethical standards of the International Council for Laboratory Animal Science (ICLAS).

Colorimetric test kits for Na^+ and K^+ were from HUMAN™ diagnostics, Germany. Ca^{++} test kit was from SPINREACT™, Spain. Spectrophotometer used was JENWAY™ 6305, UK. Optical path of quvette quartz 1cm, temperature 25± 2°C. Each test was done in 250 µl of

plasma; methods are briefly described as follow:

***Collection of samples and venom extraction*:** Five specimens of *L. quinquestriatus* were collected from eastern Khartoum(Abushama, 1968; Cloudsley-Thompson, 1961). Species identified by Sudanese natural history museum. Scorpions were left starved for four days to become acclimated to the lab conditions and to guarantee that the venom has been accumulated and restored after a last kill. Electrical stimulation of the telson has been done using commercial electric adapter adjusted to 10 mVolt, one electrode was connected to steel plate while the other was connected to a steel pin; specimens were put on the plate and telson was stimulated using the pin while the telson was inserted into 1.5 ml tube.

***Dose determination & venom injection*:** this study was designed to exert minimal intoxication symptoms that are not life-threatening, the LD50 of LqV for a mammal is weight dependant and ranges between 0.16 to

0.5 mg/kg body weight, the same authors reported that the average quantity of LqV/telson is 0.225 mg (Hassan, 1984) (Ismail, 1995) (Shaul Hamelech, 1957). In the present work no attempt was done to calculate the quantity of venom/telson and it was assumed that the quantity/telson is 0.225 mg, accordingly; the five telsons of the scorpions were manipulated and diluted with saline solution such that every 0.2 ml contains 0.12 mg LqV. A dose of 0.2 ml of the crude venom was selected to exert the required effects. Four common rabbits *Oryctolagus cuniculus* (0.75 Kg ± 5 g) were injected subcutaneously in the upper region of the left thigh. By the determined dose of the crude LqV extract (0. 12 mg/ kg in 0.2 ml).

***Blood sampling and plasma preparation*:** Blood samples were collected directly from the external jugular vein. About 300 µl of blood was drawn at a time. Samples from all rabbits were pooled. The sample was then immediately centrifuged at 3,000 rpm for Five minutes (to prevent clotting that may affect calcium concentration). From the supernatant (plasma), about 250 µl was removed and placed into three new tubes for spectrophotometry tests. The first blood sample, which is considered the control blood sample, was taken before venom injection. Five more samples spaced by 36 minute intervals were taken after experimental invenomation. The time intervals was chosen as 36 minutes according to the exploratory test which showed that after three hours the treated rabbit showed almost complete recovery, (data not shown) (3hrs x60)/ 5 closes = 36 minutes. All animal models were survived after the test.

Spectrophotometry:

***Determination of Na^+ concentration*:** using Mg-urinylacetate method; Na^+ is precipitated with Mg-urinylacetate, the urinyl ions remains in a suspension form in a yellow brown complex with thioglycolic acid. Absorption measured at 410 nm (Henry, 1974; Trinder, 1951).

***Determination of K^+ concentration*:** using sodium-tetraphenylboron turbidity test; K^+ ions in protein-free alkaline medium react with sodium-tetraphenylboron resulting in turbidity. Absorption measured at 578nm(Tietz & Herstatt, 2006).

***Determination of Ca^{++} concentration*:** using O-Cresolphtalein complex method; Ca^{++} and O-Cresolphtalein in alkaline medium forms a violet complex that can be measured photometricaly at 570nm (W., 1984).

Mathematical analysis: Linear regression analysis was carried out to describe the overall pattern of change regardless of deviation from control. In addition, data were analysed by comparing percentage of concentration change in each time to control

concentration calculated according to the equation:

$$\%Change = \frac{ControlCon. - SampleCon.}{ControlCon.}\%$$

For the first three readings, each sample was compared to the previous one by subtracting each reading from the previous.

RESULTS

Regression analysis: data are represented in Table 1.

Table 1: Time dependent alternations of rabbit's plasma concentrations of Na^+, K^+, & Ca^{++} ions induced by approximately 0.12 mg/Kg crude venom from the scorpion *Leiurus q. quinquestriatus* (LqV) injected subcutaneously: Concentrations measured photometricaly using Mg-urinylacetate method, sodium-tetraphenylboron turbidity test and O-Cresolphtalein complex method for Sodium, potassium and Calcium respectively. Time (0): Control sample taken before LqV injection. R: correlation coefficient between concentration in each reading with time.

Con. / Time (m)	Na^+ (mmole/L)	K^+ (mmole/L)	Ca^+ (mmole/L)
0 (Control)	406	13.57	16.67
36	112.5	25.72	24.01
72	562.5	37.15	17.88
108	530	17.14	21.95
144	400	10.35	19.46
180	587	15.71	18.46
R	+0.6352	-0.7064	-0.5857

Sodium concentration: There was a moderate positive correlation (+ 0.6352) between time elapsed since injection and the concentration of sodium in the sense that as time passes the sodium concentration tended to increase. After three hours the concentration was slightly less than 1. *5* fold of the control concentration. However, the first reading was significantly lower than either of the other four which were not significant to one another Fig. (1).

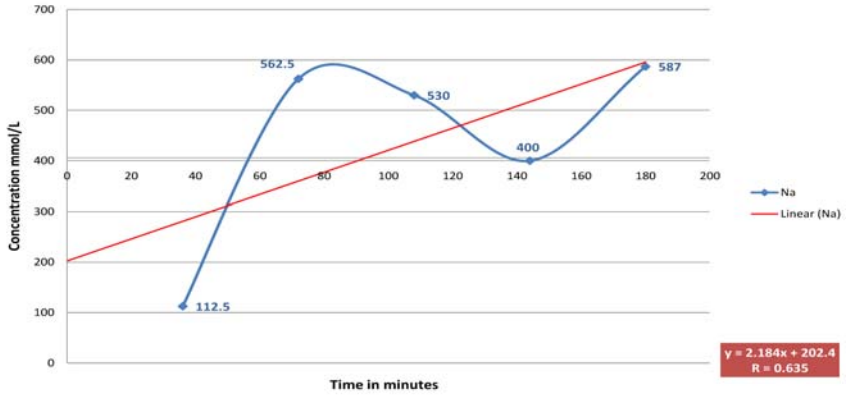

Fig. 1: Time dependent effect of approximately 0.12mg/kg crude venom from the scorpion *Leiurus q. quinquestriatus* (LqV) injected subcutaneously on Sodium concentration in Rabbit's plasma: readings were measured photometricaly using Mg-urinylacetate method (optical density at 410 nm); blood samples were collected in 36 minutes intervals. X-axis value was set to correspond 406 mmol/L which was the control ion concentration (before venom injection). Numbers corresponding each reading represents concentration measured at that time. Equation (at right bottom side) expresses the linear relationship between times elapsed after injection and concentrations measured in corresponding time. R: correlation coefficient.

Potassium concentration: There was a strong negative correlation (- 0.7064) between time elapsed since injection and the concentration of potassium, in the

sense that as time passes; concentration tended to decrease. However, there was a significant increase of potassium at the end of the first hour and concentration was stabilized at 17mmol/L which was significantly above control value Fig. (2).

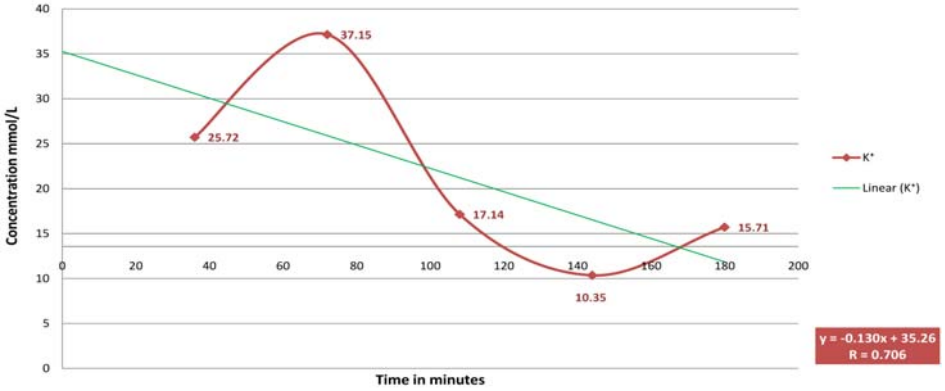

Fig. 2: Time dependent effect of approximately 0.12mg/kg crude venom from the scorpion *Leiurus q. quinquestriatus* LqV injected subcutaneously on Potassium concentration in Rabbit's plasma: readings were measured photometricaly using sodium-tetraphenylboron turbidity test (optical density at 578 nm); blood samples were collected in 36 minutes intervals. X-axis value was set to correspond 13.57 mmol/L which was the control ion concentration (before venom injection). Numbers corresponding each reading represents concentration measured at that time. Equation (at right bottom side) expresses the linear relationship between times elapsed after injection and concentrations measured in corresponding time. R: correlation coefficient.

Calcium concentration: There was a more or less weak negative correlation (-0.5857) between time elapsed since injection and the concentration of calcium, in the sense that as time passes the concentration tended to decrease smoothly. All the readings were significantly increased from the control value. However, the second reading (one hour after injection) showed more significant drop than the others which became more or less stabilized around 21mmol/L Fig. (3).

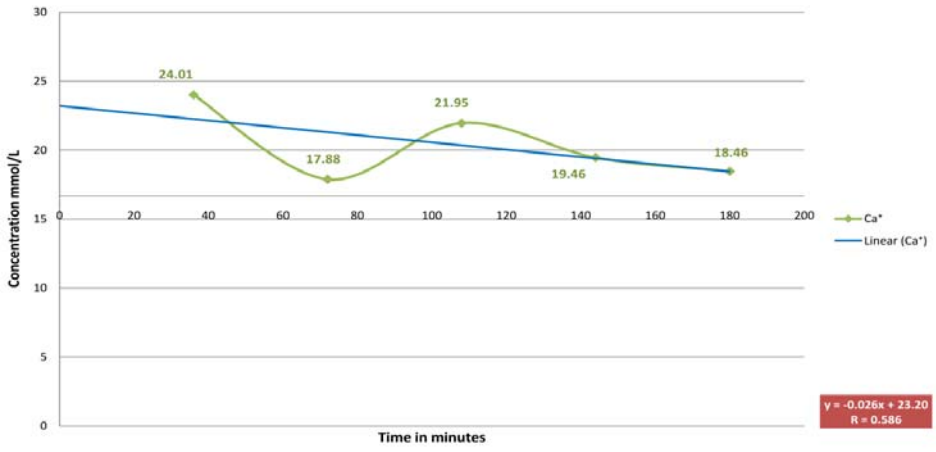

Fig. 3: Time dependent effect of approximately 0.12mg/kg crude venom from the scorpion *Leiurus q. quinquestriatus* LqV injected subcutaneously on Calcium concentration in Rabbit's plasma: readings were measured photometricaly using O-Cresolphtalein complex method (optical density at 570 nm); blood samples were collected in 36 minutes intervals. X-axis value was set to correspond 16.67 mmol/L which was the control ion concentration (before venom injection). Numbers corresponding each reading represents concentration measured at that time. Equation (at right bottom side) expresses the linear relationship between times elapsed after injection and concentrations measured in corresponding time. R: correlation coefficient.

Deviation from control: data are summarized in **Table (2)** and **Fig. (4)**.

Table 2: Percentage of concentration change of rabbit's plasma Sodium, potassium and Calcium ions induced by sub-lethal dose of approximately 0.12mg/kg crude venom from the scorpion *Leiurus q. quinquestriatus* LqV injected subcutaneously, compared to each ion's control concentration: calculations were made according to the equation: (*%age of change=Control con. – Sample con./control con %*). Concentrations measured photometricaly using Mg-urinylacetate method, sodium-tetraphenylboron turbidity test and O-Cresolphtalein complex method for Sodium, potassium and Calcium respectively.

Ion control Con. mmol//L	% of concentration change with time (in minutes)				
	36	72	108	144	180
Na^{+} (406)	-72.29	+38.42	+30.54	-1.48	+44.58
K^{+}(13.57)	+89.53	+173.76	+26.31	-23.73	+15.77
Ca^{++}(16.67)	+44.03	+7.26	+31.67	+16.74	+10.74

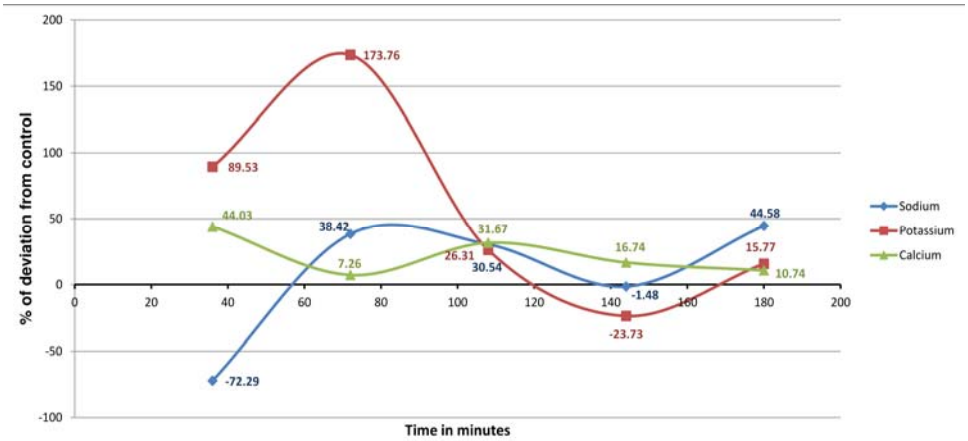

Fig. 4: Percentage of concentration change of rabbit's plasma Sodium, potassium and Calcium ions induced by sub-lethal dose of approximately 0.12mg/kg crude venom from the scorpion *Leiurus q. quinquestriatus* LqV injected subcutaneously, compared to each ion's control concentration: calculations were made according to the equation: (*%age of change=Control con. – Sample con./control con %*). Concentrations measured photometricaly using Mg-urinylacetate method, sodium-tetraphenylboron turbidity test and O-Cresolphtalein complex method for Sodium, potassium and Calcium respectively. Numbers corresponding each reading represent percentage of concentration change.

36 minutes after crude (LqV) injection, sodium and calcium have shown the highest deviation from their control concentrations, sodium was decreased by 293.5mmol/L (-72.29% compared to control) while Potassium concentration was increased by 12.15mmol/L (83.53% above control) calcium was increased by 7.34mmol/L(44.03% above control).

72 minutes after injection; sodium was increased from the first reading by 450mmol/L hence, 38.42% increase above control concentration. Potassium continued to increase and reached the highest reading observed 37.15 mmol/L (173.76% above control) and 11.43mmol/L increase from the first reading. Calcium was decreased by 6.13mmol/L from the previous reading, also it was the lowest reading observed but it is still higher above control by 7.26%.

108 minutes after injection, sodium concentration was slightly decreased compared to second reading by

32.5mmol/L and 417.5mmol/L compared to the first reading, but it is still above control by 30.54%. Potassium continued to decrease compared to both second and first readings by 20.01mmol/L and 8.58mmol/L respectively, but it is still above control by 26.31%. Calcium concentration was increased by 4.07mmol/L compared to second reading which represents 31.67% above control, compared to first reading it was lower by 2.06mmol/L.

In the last two readings (144 and 108 minutes after injection) less fluctuation was observed; sodium at 144 m was very close to control -1.48% below control but it raised above control by 44.58% compared to control 108 minutes after injection. Potassium continued to decrease by 23.73% below control 144 minutes after injection, but 108 minutes after injection it raised above control again by 15.77%. Calcium was decreased to 16.74% above control 144 minutes after injection, 108 minutes after injection calcium continued to decrease to 10.74% above control**.**

DISCUSSION

Neuro-toxinology of venoms and neural homeostatic plasticity are being studied almost separately; since, neuro-toxinology focused on toxin binding to different ion channels. While, homeostatic neurochemistry has intensively studied the roles of different ion channels in both generation of action potentials and the mechanisms of ion homeostasis in the nervous system. But the impacts of neurotoxins on homeostatic neuro-chemical parameters were poorly considered by research. In this work we tried to reveal the impact of the intensively studied neurotoxic venom (LqV) on sodium, potassium and calcium ion concentrations in plasma as a possible indicator for neuro-chemical abnormalities .Despite the fact that the fatalities due to envenomations of some snakes, scorpions and other animals were known to induce neurological symptoms. We describe here a distinct pattern of change characterized by high potassium level (hyperkaliemia) and slightly high calcium level (hypercalcimia) and low sodium level (hyponatriemia) observed during the first two hours. All these symptoms have shown signs of recovery after 108 minutes that Potassium and Calcium decreased with time, while Sodium tended to increase. These findings may further enrich our knowledge about patho-physiology of neurotoxic venoms as a whole.

CONCLUSION

Neurotoxic venom of this scorpion was found to change serum concentration of three electrolytes which are critical for homeostasis, this change may greatly affect toxin binding to ion channels as well as homeostatic balance. We recommend further research in this field.

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