Effect of Sublethal Dose of the Viper *Cerastes Cerastes* Crude Venom on Rabbit Biochemistry and Hematology

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ABSTRACT

The objective of this study was to explore the impact of sublethal concentrations of viper *Cerastes cerastes* crude venom on biochemical and hematological parameters in rabbit. Thirty adult male rabbits were divided into three groups (10 individuals per each group). The first group was the control which received physiological saline. The second group was injected interaperitoneally with 0.3µgm/g crude venom and the third group was injected with 0.6 µgm/g crude venom. The results indicated that 0.3 µgm/g crude venom induced significant decreases in total protein, globulin, triglycerides and cholesterol, and significant increases in serum glucose and liver enzymes (ALT and AST). The above mentioned parameters showed highly significant changes when 0.6 µgm/g crude venom was used. Haemoglobin concentration (Hb), red blood cell count (RBCs), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were significantly decreased. Inversely, hematocrit (PCV%), mean corpuscular volume (MCV) and white blood cell count (WBCs) were significantly decreased. In conclusion, sublethal concentrations of viper *Cerastes cerastes* crude venom induced changes on biochemical and haematological parameters of rabbit in a way that could induces animal health disorders.

Keywords: *Cerastes*; venom; rabbit; hematology; biochemistry.

1. INTRODUCTION

Cerastes cerastes and *C. c. gasperettii* (family Viperidae) are more familiar horned vipers of the great deserts of North Africa and the Middle East (Gasperetti, 1988). There are approximately 420 venomous species of snakes living on the earth (Lewis and Gutmann, 2004). Snakes are considered to be exceptional model organisms (Shine and Bonnet, 2000), in part because of their unique adaptations for feeding and foraging (Greene, 1997). Limited ecological studies have been done on snakes and lizards of different geographical regions of Saudi Arabia like Southern Hijaz (Parker, 1938), Eastern Arabia, Northeastern Arabia (Mandeville, 1967) and Central Arabia (Al-Wailly and Al-Uthman, 1971). The viper distributes on a large scale in Africa (Marsh ***et al***., 1997). A poisonous viper, as the name suggests, lives in the sandy deserts of Egypt (Zimmerman ***et al***., 1981). Many studies have been conducted on the metabolism, cardiovascular and hematological effects of snake venom on humans and animals (Abu-Sinna ***et al***., 1993). *C. cerastes* venom is toxic, primarily due to the activity of various proteins and enzymes that it contains (Oukkache ***et al***., 2012). The venom also displays a range of biological activities including anti-angiogenic, antimicrobial, antibacterial, and antagonist effects (Hanane-Fadila and Fatima, 2014).

The significance of the biochemical blood parameters for the objective assessment of health disorders and monitoring stress factors in pre-clinical stages is necessary (Hinton ***et al***., 1982). Such biochemical and

physiological values can be used as indicators of care in breeding rabbits (Hoy and Verga, 2006). These parameters are also important and reliable to monitor health and nutrition in animals (Gupta ***et al***., 2007). Blood composition may be affected by some factors such as nutrition, management, growth, sex and age diseases (Ewuola ***et al***., 2004). The study is designed to investigate the effects of two doses of the *C. cerastes* crude venom on the serum biochemical and hematological parameters of rabbits.

2. MATERIALS AND METHODS

2.1 Collection of snake venom

Horned viper *C. cerastes* (n = 10), were donated from private pet shop in Cairo of Egypt. The viper individuals were identified and the venom was collected by manual milking and then all the individuals were returned to pet shop. Venoms were taken to the clinical laboratory in the national Research Center of Egypt in which all experimental procedures have been conducted. Venom was freeze-dried and stored at -20 °C until use. LD_{50} with a dose of 0.66 µg/g of crude venom was used according to Meier and Theakston (Meier and Theakston, 1986).

2.2 Experimental design

Thirty male rabbits were used with an average body weight of 801 ± 28g. Healthy rabbits were selected from the animal house at the Faculty of Agriculture, University of Cairo. These rabbits were placed in the measuring 45 × 30 × 42 cm rods in a standard case and are provided daily with the experimental diets in the form of mash. *Ad libitium* and clean drinking water was provided throughout the experimental period. The animals were adapted under room temperature and humidity with normal light / dark cycle for two weeks and had free access to water and a standard pure powder diet.

Animals were divided into three groups. The first group (control, n=10) was injected interaperitoneally (i.p.) with 100 µL physiological saline (0.9 % NaCl). The second group (n=10) had received a single 0.3µgm/g body weight dose of crude venom in 100 µL saline solution interaperitoneally. The third group (n=10) had received a single 0.6 µgm/g body weight dose of crude venom in 100 µL saline solution interaperitoneally. After 24 hours of the injection, blood samples were randomly collected intravenously from rabbit ear vein using a disposable needle and syringe. Each blood sample was divided into two tubes; the first tube contained a potassium dioxide salt of acetylene diphenyl acetate acid (EDTA-K2) as an anticoagulant for hematology. The second tube was without an anticoagulant for biochemistry tests. Biochemical tests were conducted in the National Research Center in Cairo, Egypt.

Sera were separated by centrifuging the blood samples at 3000 g for 30 min. Kits for the biochemical parameters were purchased from Spinreact, S. A. Ctra. Santa Coloma, Spain. Glucose was measured as described by the method of Trinder (1969). Total serum protein was determined according to Peters (1968). Serum albumin was determined according to Doumas ***et al***. (1972). Globulin was measured as the difference between total protein and albumin. Cholesterol (Young ***et al***., 1972), triglycerides (Hare, 1950), creatinine (Patton and Crouch, 1977), urea (Fossati, et al., 1980), uric acid (Reitman and Frankel, 1957) were determined according to kit guidelines. Alanine transaminase (ALT) and aspartate transaminase (AST) activities were also measured (EL-Asmar ***et al***., 1979). Alikaline phosphatase (ALP) was measured according to El-Aaser and El-Merzabani (1975).

The haematological parameters were analyzed through standard procedures. WBCs and RBCs counts have been obtained by the haemmocytometern method, while the differential counting was performed according to Schalm ***et al***. (1975). The packed cell volume (PCV) was determined by the microhymatocrit method (Steel and Torrie, 1980). Hemoglobin (Hb), mean hemoglobin (Mesh), average particle size (mKV) and mean surgical hemoglobin concentration were obtained measured and calculated according to Thrall and Weiser (2002). Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) had been obtained from the calculation according to the standard equations.

2.3 Statistical Analysis

Data have been statistically analyzed by Analysis of Variance (ANOVA) (Al-Jammaz ***et al***., 1999). Means were compared using Duncan multiple range test. Percentage changes were calculated as follow: % decrease = decrease ÷ original number × 100, % increase= increase ÷ original number × 100 (https://www.skillsyouneed.com/num/percent-change.html).

3. RESULTS

Significant reduction in total protein, globulin ($p<0.05$) , cholesterol and triglycerides ($p<0.01$) have been recorded in male rabbits after 24 hours injection with a single dose of 0.3µgm/g bw crude venom compared to the control (Table 1). These decreases showed percentage change of -20.53%, -29.90%, -31.2% and -35%, respectively. Meanwhile, injection of a single dose 6 µgm/g bw of crude venom for 24 hours induced highly significant decreases in the abovementioned parameters. The percentages of reduction change were -28.46%,-33.55%, -38.5 % and -47 %, respectively (Table 1). On the other hand, 0.3 µgm/g bw crude venom led to increases ($p <0.01$) in creatinine, urea and glucose at 0.3 µgm/g bw and this increase was highly significant ($p <0.001$) when 0.6 µgm/g bw was used as compared to the control rabbits. This elevation exhibited % changes of 87.8%, 95.3% and 60%, respectively for the high dose (Table 1).

The data (Table 2) showed that significant increase in ALT ($p< 0.01$), AST, ALP ($p< 0.05$) and ACP ($p< 0.01$) at the single dose of 0.3 µgm/g bw crude venom for 24 hours and highly significant ($p <0.001$) elevations in these enzymes were shown when 0.6 µgm/g bw crude venom were used as compared to those of the control rabbits. These increases exhibited 81%, 34.4%, 44.51% and 73.3% percentage changes, respectively.

The present results (Table 3) showed that RBCs count, Hb concentration, MCH and MCHC were significantly decreased ($p<0.05$) at 0.3 µgm/g bw crude venom while more significant reduction in these parameters at 0.6 µgm/g bw crude venom were shown as compared to those of the control animals. These reductions showed - 38.5%, - 30%, - 19%, and - 44.3% percentage changes, respectively. On the other hand, the data had shown significant increases ($p<0.05$; $p<0.01$) in WBCs, PCV% and MCV at 0.3 µgm/g bw single dose. This elevation was highly significant ($p<0.01$; $p<0.001$) at 0.6 µgm/g bw as compared to those of the controls. The % changes of these increases were 47.1%, 32.4% and 74.6%, respectively.

4. DISCUSSION

The present data showed reduction in total protein and globulin in rabbits after injection with two doses of crude venom for 24 hours and these results were supported by the renal function disorders and bleeding in some internal organs. The elevation in vascular permeability and bleeding in vital organs is due to toxic effect of different snake toxins as discussed by Meyer and Stoke (1991) and March ***et al***. (1997). Moreover, the snake bites cause a toxic impact on the victims due to the presence of lipolytic and proteolytic enzymes in their toxins. It is worthy to mention that several studies have been conducted on the metabolic and hematological effects of snake venom on humans and experimental animals (Tan and Ponnudurai, 1990; Al-Jammaz ***et al***., 1999) and found that various snake venoms cause alterations in rat metabolism (Tan and Ponnudurai, 1990).

In this study, high levels of urea and creatinine in the serum of rabbits indicated the weakness in the kidney function. Similar observations had been reported in mice treated with various venoms (Soslau ***et al***., 1988).

Table 1. The impact of two doses of viper *Cerastes cerastes* on some biochemical parameters in Rabbits after crude venom injection for 24 hours (mg/dl).

	Control	(0.3µgm/g)		(0. 6µgm/g)	
	Means ± S.E.	Means± S.E.	Change%	Means ± S.E	Change%
Total protein	6.43±0.42	5.11±0.34*	- 20.53%	4.6±0.34*	- 28.46%
Albumin	3.41±0.24	3.00±0.21	- 12.02%	2.6±0.22*	- 23.75%
Globulin	3.01±0.121	2.11±0.111*	- 29.90%	2.2±0.23**	- 33.55%
Creatinine	0.49 ±0. 12	0.72 ±0.11**	46.9%**	0.92 ±0.13***	87.8%
Urea	38.6 ± 1.8	61.44 ± 2.4**	59.2%	75.4 ± 3.3***	95.3$
Uric acid	2.5± 0.13	1.8 ± 0.21	- 28%	1.1± 0.16	- 56%
Glucose	85.3±3.12	112.3±3.5**	31.7 %	136.4±2.5***	60%
Cholesterol	92.2±4.21	63.4±1.2**	-31.2%	56.7±8.13**	- 38.5%
Triglycerides	85.6±2.4	55.6±3.3**	-35 %	45.34±3.3**	- 47 %

* p<0.05, ** p<0.01, *** p<0.001.

Table 2. The impact of two doses of viper *Cerastes cerastes* on liver function, enzyme in rabbits after crude venom injection for 24 hours (u/l).

Parameter	Control	(0.3µgm/g)		(0. 6µgm/g)	
	Means ± S.E.	Means ± S.E.	Change%	Means ± S.E.	Change%
ALT	79.8±3.6	112.5±7.21**	40%	144.5±3.7***	81%
AST	125.41±6.6	153.87±8.70*	22.7%	168.5±6.4**	34.4%
ALP	188.5±7.14	233.4±2.4*	23.8%	272.4±2.6**	44. 51 %
ACP	122.4±1.3	182.3±3.2**	49%	212.1±1.6***	73. 3 %

Table 3. The impact of two doses of viper *Cerastes cerastes* on haematological parameters in rabbits after crude venom injection for 24 hours.

Parameters	Control	(0.3µgm/g)		(0. 6µgm/g)	
	Means ± S.E.	Means ± S.E.	Change%	Means ± S.E.	Change%
RBCs x 10^6/*u*l	5.2 ± 0.26	4.2+ 0.26*	- 19.2%	3.2+0.26**	- 38.5%
WBCs x 10^3/*u*l	5.1+ 0.42	6.4+ 0.21*	25.1%	7.5+0.45**	47.1%
PCV%	31.2 ± 0.27	37.2 ± 0.48*	19.2%	41.3±0.72**	32.4%
Hb g/dl	13.8 ± 0.61	11.6 ±0.58*	- 16%	9.7 ±0.72**	- 30%
MCH pg/cell	28.7 ± 1.13	25.0 ± 1.12	- 13%	23.2 ± 1.6*	- 19%
MCV	70.12 ± 3.13	91.2 ± 1.18**	30%	122.4±1.3***	74.6%
MCHC g/dl	43.6 ± 1.13	31.2 ± 1.2*	- 28.4%	24.3 ± 1.2**	- 44.3%

Venoms increase blood vessel permeability, along with renal damage which exacerbates the lack of blood proteins and hyponatremia. Moreover, the elevation of urea and creatinine associated with the reducing of uric acid, in the current study, supports the proposed weakness of kidney function. Similar results were reported for rats subjected to various viper venoms (Rahmy ***et al***., 1995). In the current study, viper venom caused an elevation in blood glucose level as venoms produce hyperglyceamia in rats and mice (Sant and Purandare, 1972). It can be attributed that increases in blood glucose level affects the metabolism of glycogen in the liver cells, muscle fibers and medullary catecholamines which stimulate the dissolution of glycogen and gluconeogenesis in those tissues (Mohamed ***et al***., 1981). The present work had indicated reduces in cholesterol and triglyceride. These results are consistent with other investigators who indicated decline in these parameters in the blood of laboratory animals injected with snake venom. They suggested that the snake venom may mobilize lipid level from adipose and other tissues (Ohhira ***et al***., 1991).

In this study liver cells could be exposed to damage by the viper venom which makes liver accumulates fatty acids, thus becoming fatty liver and altering cell membranes and tissue permeability for electrolytes (Al-Jammaz, 2002). Such electrolyte disturbances have been reported in serum of rats after injection by various snakes' venom. Furthermore, Meier and Stocker (Al-Jammaz ***et al***., 1999) indicated that, these disorders may be due to acute nephropathy following snake bites. El-Asmar ***et al***. (1979) predicted that this effect was induced by the stimulation of adrenal cortex leading to the secretion of aldosterone. The present elevation in ALT, AST, ALP and ACP activity indicated that viper venom caused damage of liver, heart and other organs. Such findings are in agreement with previous reports on venoms of other snake species (Sant and Purandre, 1972; Mohamed ***et al***., 1980). It can be concluded that sublethal concentrations of viper *C. cerastes* crude venom caused changes on biochemical and haematological parameters of rabbit.

The reduction in RBCs count may be due to macrocytic or normocytic anemia (Al-jammaz ***et al***., 1992) and also due to a common effect of toxicity with various types of pollutants (Tuschiya, 1979). It was found that snake venom causes dissolution of the blood and the division of red blood cells, which represent a potential source of magnification error in RBCs count due to the presence of hemolytic factors found in snake venom such as Vsvoulibaz- A2 and phospholipase (Borgeest ***et al***., 2004). Jiang ***et al***. (1988) showed the blood dissolution by snake venom depends on the age of RBC and blood viscosity. These results agreed with those obtained by Berberian and Enan (1989) who studied the effects of some antimoulting compounds on the haematological picture in male rats and found significant reductions in Hb, RBCs, MCH and MCHC.

Other investigators related RBCs changes to calcium concentration and pH level as the increase in the pH causes a significant increase in blood dissolution (Bultron ***et al***., 1993). WBCs count was significantly increased in rabbit injected with two doses of crude venom for 24 hours and the hematocrit PCV% increased too. These findings agreed with those obtained by Guilhermino ***et al***. (1998) which indicated that when rats were exposed to cadmium the number of WBCs was elevated. The increase in the haematocrit values may be due to the reduction in RBCs and their swelling as evidenced by the significant increase in MCV.

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