Hepatic and renal tissue damages induced by *Cerastes cerastes gasperetti* crude venom

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Abstract: Snakebite remains an important medical problem in both developing and developed countries because; it is an important cause of mortality and morbidity. Viper snakes are widely distributed in desert areas; the *Cerastes cerastes gasperetti* is one member of the family Viperidae where their bites are a serious threat to life and since *Cerastes cerastes gasperetti* venom has received little interest, this study was designed to investigate the effect of LD_{50} of *Cerastes cerastes gasperetti* crude venom on histopathological changes as well as on some important serum and tissue homogenates (liver and kidney) parameters. In addition, *Cerastes cerastes gasperetti* crude venom caused hepatic and renal injuries as indicated by histopathological changes in the liver and kidney tissues. Serum ALT, AST activities, total bilirubin, creatinine and blood urea nitrogen levels were all increased. On contrary, serum levels of ALP, γ-GT, albumin, uric acid and glucose were decreased as a result of envenomation. Kidney and liver lipid profile levels, glycogen content in kidney were also decreased, in contrast, glycogen content in liver and LDH activity in renal and hepatic homogenates expressed an increment as a result. On the basis of the above results it can hypothesize that *Cerastes cerastes gasperetti* crude venom is potent toxin-mediated hepatorenal toxicity and causes disturbance in carbohydrates and lipids metabolism.
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1. Introduction

The horned viper *Cerastes cerastes gasperetti* (gasperetti's sand viper) belongs to the family Viperidae which is the most frequent of vipers. Viperidae is a family that has venomous species of medical importance and the clinical toxicology are cytotoxic, haemostatic disorders, neurotoxic. They are responsible for many human snake bites and it is considered as a common cause of life threatening and fatal snake bite in Africa where viperidae snakes are mainly hemotoxic (Gasperetti, 1988; Al-Sadoon, 1989; Masood, 2012; WHO, 2012).

Cerastes cerastes gasperetti venom has received little interest, however, even though this snake is distributed throughout many deserts, particularly in Egypt, Jordan and Saudi Arabia, especially in the central region (Al-Sadoon, 1989). Snake venom is modified saliva that is primarily used for hunting (capturing and digesting the prey) (Egan, 2007).

The venom is mainly made of proteins mostly enzymes besides non-protein components, which are divided into inorganic and organic constituents. All venoms cause severe changes in one or more body organs of the victim (Masood, 2012). Various local tissue alternations accompanying snake bite such as hemorrhage, edema and myonecrosis, may result in tissue loss or organs dysfunction (Gutierrez, 1995). Following envenoming, spread of the various venom components depends to a large extent on their molecular weights. Thus, Reid and Theakston (1983) showed that most constituents of viper venoms have high molecular weights and are slowly absorbed by the lymphatic system to produce maximum serum levels within 6-24 hours (Warrell, 1995).

Snake venom components, especially those of viper venoms, activate, inhibit or liberate enzymes by destroying cellular organelles (Abdel-Nabi et al., 1997; Marsh et al., 1997). Common initial signs of envenoming are hypoglycemia (Abu-Sinna et al., 1993), general metabolic disturbance (Mahmoud, 1983), muscular dystrophy (Mohamed & Khaled, 1966), nephrotoxicity (Ickowicz et al., 1966) and cytotoxicity (Bertke & Atkins, 1961).

Schneemann et al. (2004) reported that there is a small literature on envenoming by desert horned vipers. So, the objective of the present work is to unveil on the histopathological & biochemical changes induced in enovomated mice with *Cerastes cerastes gasperttii* crude venom.

2. Material and Methods

2.1. Snake venom:

2.1.1. Gasperetti's sand viper venom collection and preparations:

Cerastes cerastes gaspartii snakes were collected from the central region of Saudi Arabia. The snakes were kept in a serpentarium in the Zoology Department, College of Science, King Saud

University. The snakes were warmed daily using a 100-watt lamp for nine hours, and water was always available. The snakes were fed purpose-bred mice every 10 to 14 days. The venom was milked from adult snakes, lyophilized and reconstituted in 1X phosphate- buffered saline prior to use.

2.1.2. Determination of LD_{50} dose:

The approximate median lethal dose (LD_{50}) of the crude venom was calculated according to the method described by Meier and Theakston (1986).

2.2. Experimental animals:

Experiments were performed on male albino mice, 6–8 weeks old, weighing 22 ± 5 g. The animals were obtained from from Schistosome Biological Supply Center at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Animals were kept in wire bottomed cages, in a room under standard condition of illumination with a 12 h light-dark cycle at 25 ± 1°C. They were provided with tap water and balanced diet ad libitum. They were acclimatized to the environment for one week prior to experimental use. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

2.3. Experimental groups:

To study the effect of *Cerastes cerastes gasperetti* crude venom, sixty male albino mice were randomly divided into:

Control group (n=10): mice were injected intrapretoneal (i.p.) with 0.2 ml saline solution / mice, and sacrificed after 1st, 3rd and 6th hr from injection.

The LD50 dose envenomed group (n=10): mice were i.p. injected with 0.2 ml saline solution containing 0.978 mg/kg body weight (b.wt.) of the mice, and sacrificed after 1st, 3rd and 6th hr from injection.

2.4. Histopathological studies:

Tissue samples were fixed in 10% neutral formalin for 24 hours and paraffin blocks were obtained and routinely processed for light microscopy. Slices of 4-5 µm were obtained from the prepared blocks and stained with hematoxylin-eosin. The preparations obtained were visualized using a Nikon microscopy at a magnification of 400×.

2.5. Biochemical studies:

Blood was collected from each mouse into plain centrifuge tubes, left for 1hr at room temperature (25ºC ± 2) and serum was separated by centrifugation at 600g for 15 min and analyzed, without delay, for the concentration of total bilirubin, albumin, urea, creatinine, uric acid, glucose. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gammaglutamyl transferase (γ-GT) were determined using Kits purchased from Bio-diagnostic Co., Egypt.

A few pieces of liver and kidney were fixed with 10% neutral buffered formalin for histopathological investigations, whereas the majority of pieces were homogenized in ice-cold medium containing 50 mM Tris–HCl and 300 mM sucrose, pH 7.4, and finally stored at -80 °C until use in the various biochemical determinations.

The homogenate was centrifuged at 500 g for 10 min at 4°C. The supernatant (10%) was used for the determination of cholesterol, triglycerides, phospholipids levels and lactic dehydrogenase (LDH) activity.

2.6. Statistical analysis

The data are presented as means ± S.E. and statistically analyzed using ANOVA test (version 17.00). Significance was set at the level of $P \leq 0.001$ vs control.

3. Results

Lethality test:

Results indicated that the approximate (LD_{50}) of the venom is equal to 0.978 mg/kg body weight (Figure 1).

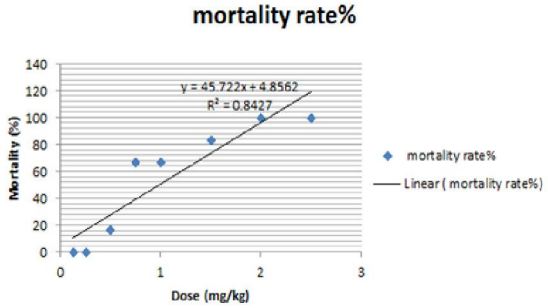

Fig. 1: Twenty four hours dose mortality curve of male albino mice administrated different doses of *Cerastes cerastes gaspartii* venom by i.p. injection.

Histopathologegical results:

Control liver section (Figure 2a) showed central vein and surrounding hepatocytes. Sinusoids lined with van Kupffer cells. While liver sections of mice injected with LD_{50} of *Cerastes cerastes gasperetti* venom showed inflammatory cellular infiltrations around the hepatic vein, dilated blood sinusoids and prominent van Kupffer cells (Figure 2b, c and d). Severe necrosis and apoptosis were also seen after one and three hours of injection (Figure 2b and c).

Microscopic examination of the renal tissue shows that the venom induced a severe glomerular

degeneration and coagulative necrosis. Also, the urinary spaces appeared wider as compared with the control one. Moreover, most of the renal tubules were degenerated and filed with cellular debris (Figures 3b, c and d).

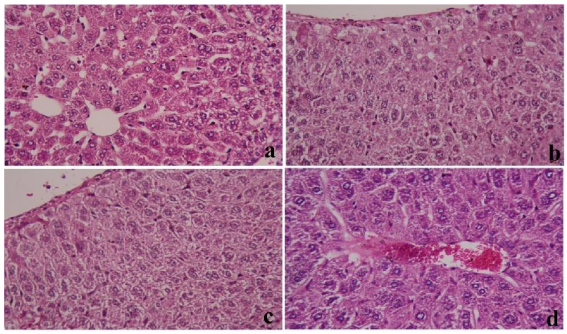

Fig. 2: a) Control liver section shows central vein and surrounding hepatocytes, sinusoids lined with van Kupffer cells, b) *Cerastes cerastes gaspartii* venom injected mice after 1 hour with inflammatory cellular infiltrations around the hepatic vein, dilated blood sinusoids and prominent van Kupffer cells, c) *Cerastes cerastes gaspartii* venom injected mice after 3 hours with inflammatory cellular infiltrations around the hepatic vein, dilated blood sinusoids and prominent van Kupffer cells d) *Cerastes cerastes gaspartii* venom injected mice after 6 hours with sever hemorrhage in the central vein (H&E stain, X 400).

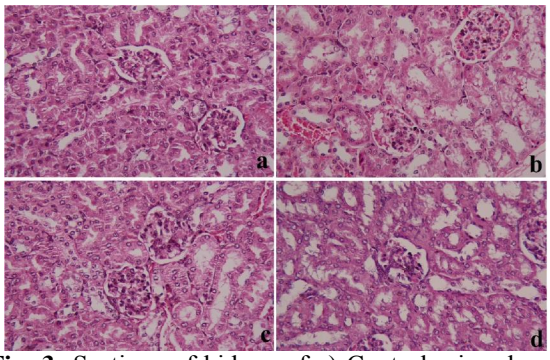

Fig. 3: Sections of kidney of a) Control mice shows intact architecture of the glomerulus and renal tubules, b) *Cerastes cerastes gaspartii* venom injected mice after 1 hour with swelling glomerulus, wide urinary space, and degenerative renal tubules, c) *Cerastes cerastes gaspartii* venom injected mice after 3 hours with swelling glomerulus, wide urinary space, and degenerative renal tubules and, d) *Cerastes cerastes gaspartii* venom injected mice after 6 hours with shrunken glomerulus, wide urinary space, and degenerative renal tubules (H&E X 400).

Biochemichal results:

In the present work, mice envenomation with *Cerastes cerastes gasperetti* crude venom at LD50 dose for the 1st, 3rd and 6th hr. caused different changes of the selected biochemical parameters. Data represented in Table (1) shows that *Cerastes cerastes gasperetti* crude venom induced a highly significant elevation in serum ALT, AST activities, total bilirubin, creatinine, blood urea nitrogen levels at the 6th hr at $P \leq 0.001$ as compared to the control group. While, it caused a highly significant reduction in the activities of ALP & γ-GT at 6th hr, meanwhile a non-significant decrease was recorded in albumin and uric acid levels.

The level of serum glucose of envenomated mice was highly significant decreased at the 1st, 3rd and 6th hr as compared to the control group (Table 2). In addition, the envenomation changed the contents of glycogen significantly in hepatic and renal tissues. Glycogen content was significantly increased in hepatic tissue. On contrast, it was significantly decreased in renal tissue.

Table (3) showed that the i.p injection of the crude *Cerastes cerastes gasperetti* venom induced a highly significant reduction in the levels of hepatic cholesterol, triglycerides and phospholipids. The level of renal cholesterol was non-significantly decreased while the triglycerides and phospholipids levels were significantly decreased as a result of LD_{50} envenomation.

The crude *Cerastes cerastes gasperetti* venom injection resulted in a highly significant elevation in LDH activity of both hepatic and renal tissues as shown in Figure (4).

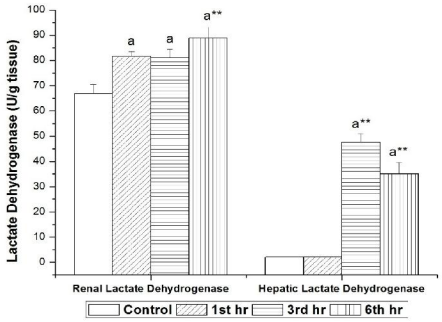

Figure 4. Crude *Cerastes cerastes gasperetti* venom injection

4. Discussions

Measurement of biological parameters in serum and vital organs (liver and kidney) is of an importance in the assessment of the pathophysiological state of snake bite victims (Al-Jammaz et al., 2002). So, this work was undertaken

to study the effect of *Cerastes cerastes gasperetti* crude venom on histopathological changes and on serum biochemical, renal, hepatic homogenates parameters.

The envenomated mice with *Cerastes cerastes gasperetti* crude venom for 1, 3 and 6 hr showed a highly significant elevation in serum ALT, AST activities, total bilirubin, urea, creatinine levels on contrary, a reduction in ALP, γ-GT activities, uric acid and albumin levels. These results go hand in hand with that of (Maxine & Benjamine, 1985; Al-Jammaz et al., 1998; 1999; Fahim, 1998; Al-Jammaz, 2001; Shaban & Hafez, 2003; Salman, 2009; Al-Saadon et al., 2012).

Table 1: The effect of LD50 of crude *Cerastes cerastes gasperetti* i.p injection at the 1st, 3rd and 6th hr on liver and kidney functions in serum of mice.

Group	Control	1st hour	3rd hour	6th hour
ALT (U/ml)	44.40±0.82	44.31±0.97	41.87±1.02	66.78±1.47 a**
AST (U/ml)	52.82±0.65	52.11±0.38	61.20±1.46 a**	57.39±1.78 a
Total Bilurobin (mg/dl)	3.45324±0.17	3.57908±0.13	4.17±0.10 a*	4.85±0.14 a**
ALP (IU/l)	59.30±1.46	70.05±1.66 a**	52.08±1.42 a	35.18±2.33 a**
γ GT (U/l)	2.86±0.16	4.74±0.13 a**	5.08±0.08 a**	2.02±0.11 a**
Albumin (g/dl)	3.51±0.09	3.34±0.08	3.33±0.04	3.35±0.03
Creatinine (mg/dl)	0.23±0.01	0.59±0.02 a**	0.96±0.03 a**	0.97±0.03 a**
BUN (mg/dl)	25.20±1.82	44.38±1.58 a**	39.09±1.66 a**	49.89±1.34 a**
Uric acid (mg/dl)	2.07±0.11	2.83±0.14 a*	1.71±0.20	1.86±0.14

Values are means ± SE. a: Significant against vehicle control group at P≤0.05, * Significant at P≤0.01 and ** Significant at P≤0.001, n=6.

Table 2: The effect of LD50 of crude *Cerastes cerastes gasperetti* i.p injection at the 1st, 3rd and 6th hr on serum glucose, kidney and liver glycogen contents in tissue homogenates of mice.

Group	Control	1st hour	3rd hour	6th hour
Serum Glucose (mg/dl)	127.5±1.96	99.33±2.71 a**	47.26±2.33 a**	109.8±2.90 a**
Hepatic glycogen (mg/g tissue)	4.36±0.09	5.78±0.48 a	4.50±0.59	5.34±0.39 a
Renal glycogen (mg/g tissue)	7.97±0.30	5.68±0.55 a	6.61±0.73 a	6.48±0.51 a

Values are means ± SE. a: Significant against vehicle control group at P≤0.05, * Significant at P≤0.01 and ** Significant at P≤0.001, n=6.

Several investigators reported that, crude viper snake venom induced an increase in serum ALT activity, similarly, activity of serum AST increased significantly at different time intervals (Al-Jammaz, 2001; Shaban & Hafez, 2003; Al-Saadon et al., 2012).

In this respect, Tietz (1983) & Al-Jammaz (2001) mentioned that although the tissues of the kidney, heart and skeletal muscles have significant amounts of ALT, liver damage can be fairly assumed when serum ALT levels increase because it is more specific to liver cells. Shaban & Hafez (2003) deduced the rapid rise in ALT and AST activities recorded 24 hr after venom envenomation may be attributed to severe injuries and necrosis of hepatocytes as well as to a nephrotoxic action of the venom as reported by Abdel-Nabi (1993). Organ damage is followed by an increase in levels of ALT and AST (Omran et al. 1997). Measurement of enzyme activity in serum is of importance since it helps to assess the state of the liver and other organs. Normally serum transaminase levels are low, but after extensive tissue injury, these enzymes are liberated into the serum and the levels of serum transaminase, were reported to be increased following damage to skeletal muscles, myocardial muscles and liver (Harper et al. 1977). Mohamed et al. (1981) indicated that, the reduction of ALP, γ-GT activities could be due to renal damage as well as to the inhibition of their activities caused by the venom.

In addition, Al- Saadon et al. (2012) reported that crude snake venom injection increased the serum level of creatinine significantly in rats. Al-Jammaz (2001); Salman (2009) found that the envenomation of experimental animals with a viper snake venom increased the levels of serum urea and creatinine while the level of serum uric acid decreased. Likewise, several investigators noticed a reduction in laboratory animals exposed to viper snake venoms (Abdel-Nabi et al., 1997; Al-Jammaz et al., 1998; Fahim, 1998; Al-Jammaz et al., 1999; Schneemann et al., 2004).

However, the precise mechanisms whereby the venoms cause reduction of serum uric acid level are not fully known. Many reports attributed the rise in serum urea to an increase of nitrogen retention, and

the rise in serum creatinine level to impairment of renal function, several disturbances in the kidney and nephrotoxicity which lead to impairment of the excretory function of the kidney as well as haemorrhages in some internal organs (Maxine & Benjamine, 1985; Abdel-Nabi, 1993; Rahmy et al., 1995; Abdel-Nabi et al., 1997; Omran et al., 1997; Al-Jammaz, 2001; Salman, 2009). In addition, Meier & Stocker (1991) suggested that viper bites lead to acute nephropathy, where, Ismail et al. (1996) speculated that the tissue distribution of the venom showed the highest uptake in the kidney. Such increased vascular permeability, together with, renal damage would further aggravate the accompanying hypoproteinemia and hypoalbuminaemia (Salman, 2009). Moreover, Tilbury et al. (1987) reported that acute renal failure characterized by vascular lesions and tubular necrosis in the renal cortex following various snake bites.

Table 3: The influence of crude *Cerastes cerastes gasperetti* venom (LD50) on hepatic and renal lipid profiles in mice.

Group	Control	1st hour	3rd hour	6th hour
Hepatic cholesterol (mg/g tissue)	50.00±3.98	134.0±4.64 a**	32.48±2.56 a*	27.39±2.33 a**
Hepatic triglycerides (mg/g tissue)	672.5±24.0	560.2±26.6 a*	349.3±14.9 a**	273.4±19.5 a**
Hepatic phospholipids (mg/g tissue)	0.40±0.08	0.31±0.001	0.11±0.005 a**	0.14±0.007 a*
Renal cholesterol (mg/g tissue)	49.58±6.59	62.91±5.06a*	53.99±3.56	40.78±10.34
Renal triglycerides (mg/g tissue)	582.3±33.2	626.9±40.0	412.4±36.5 a*	428.3±22.5 a*
Renal phospholipids (mg/g tissue)	1.93±0.30	2.28±0.36	0.73±0.12 a*	0.37±0.08 a**

Values are means ± SE. a: Significant against vehicle control group at P≤0.05, * Significant at P≤0.01 and ** Significant at P≤0.001, n=6.

In our results, hypoglycemia was observed at all experimental time intervals which were accompanied with an increment of hepatic glycogen content and a reduction of renal glycogen content at all time intervals. These results are in agreement with (Al-Shammary et al., 1992; Abu-Sinna et al., 1993; Al-Jammaz, 2002).

Al-Shammary et al. (1992) studied the effect of *Cerastes cerastes gasperetti* crude venom on the activity of key metabolic enzymes in cultured human fibroblast. A dramatic reduction in the specific activities of glucose and glycogen degradative enzymes was observed. In addition, some reports cleared that the venom affect on glycogen metabolism in the hepatocytes, muscle fibers and medullary catecholamines which stimulates glycogenolysis and gluconeogenesis in those tissues (Ohhira et al., 1991, Abdul-Nabi et al., 1997 and March et al., 1997).

This reduction of blood glucose level and the glycogen content reduction reflect a disturbance in carbohydrate metabolism which could be attributed to some endogenous insulin-releasing effect of the venom components. The stress caused by venom administration might be another factor in reducing the blood glucose level. Such stress might have occurred as direct effect of the venom enhancing the release of insulin, or indirectly by inhibiting catecholamine release due to exhaustion or blocking of the adrenal sympathetic supply. It is then possible that there is an endogenous insulin releasing effect of one or more venom components; in addition, it is possible that venom components themselves may possess insulin - like effect as proposed by Abu-Sinna et al. (1993).

The envenomated mice in the present work induced a reduction in triglycerides and phospholipids levels were in hepatic and renal tissues, as well as, cholesterol level in hepatic tissue. Al-Jammaz (2001) and Salman (2009) observed that the envenomated rats, guinea pigs showed an increase in serum cholesterol levels and triglycerides, in addition, El-Asmar et al. (1979) attributed the increment to the hepatocytes damage rendering them unable to phosphorylate the increasing amounts of fatty acids, hence leading to fatty liver and alteration of cell membranes of tissues.

LDH activity in the present investigation was a highly significant increased in both renal and hepatocyte homogenates as a result of *Cerastes cerastes gasperetti* crude venom injection in mice. Shaban & Hafez (2003) reported that LDH is one of the enzyme markers of such injured tissues by a snake envenomation also, the authors deduced that the intraperitoneal injected snake venom caused a marked decrease of serum LDH activity, attributed the reduction of LDH activity to renal damage as well as to the inhibition of their activities caused by the venom as had been suggested by Mohamed et al. 1981.

In conclusion, the data presented herein suggested that the injected LD_{50} of crude *Cerastes cerastes gasperetti* venom induced hepatorenal toxicity associated with histopathological alternation in mice.

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