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Biochemical Characterization of Pyramid Viper, *Echis pyramidum*, Venom

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Abstract.- This study was conducted to investigate the toxicological effects of *Echis pyramidum* crude venom and its purified protein fractions in male mice. The crude venom was fractionated and purified into eight fractions by 8% preparative native polyacrylamide gel electrophoresis. The mice were injected interaperitoneally with 50% of the lethal dose of crude venom and its purified fractions to keep 50% population of the injected animals alive to observe the different changes in the blood parameters. A significant increase was in plasma ALT level in animals injected with crude venom and fractions F1, F6 as compared to control group. A significant increase was in plasma AST level by crude venom and fraction F7. Glucose level increased significantly with crude venom whereas decreased significantly by fractions F4 and F5. There was up to 50% decrease in total plasma protein with crude venom and less than 50% decrease with fraction F7. Low plasma cholesterol levels were observed in group injected with crude venom and fraction F7. While fractions F2, F3 and F6 caused significant increase in plasma cholesterol level. Fraction F1 caused significant decrease in plasma triglyceride level whereas there was significant increase by crude venom. Blood urea level was significantly increased by crude venom fractions F2, F3 and F6. The significant increase in plasma creatinine level was produced by crude venom and fractions F1, F5, F7 and F8. Crude venom and fractions F1, F3, F5, F6 and F7 showed significant increase in uric acid level. The results of this study propose that *E. pyramidum* crude venom and its purified protein fractions exhibited potent toxic effects on some biochemical markers in male mice.

Keywords: *Echis pyramidum*, alanine aminotransferase, plasma cholesterol, plasma triglyceride, aspartate aminotransferase.

INTRODUCTION

Snake bites are the source of fatality and morbidity and pose a serious socio-medical dilemma world wide. The pyramid viper *(E. Pyramidum)* is one of the venomous snakes found in Saudi Arabia. It is widely spread in Jazan which according to surveillance, extends from A-Darb city north to Al-Mousam city in the south, near the Saudi-Yemeni borders and from Jazan city west to Fifa and Al-Da'r cities in the east.

The pyramid viper has been related with the majority of envenomation cases in the region. The victims of bites who were examined in the local hospitals were suffering from symptoms of nausea and vomiting (26.53%), swelling at bitten place (24.5%), low blood pressure (14.3%), painful bitten site (10.2%), sweating (10.2%), bleeding in the area of bite (8.16%), nose bleeding (2.0%), fever (2.0%) and unconsciousness (2.0%) (Al-Shammari, 2007). Snake venoms are mixtures of proteins, peptides, carbohydrates, lipids, metal ions and organic compounds. The proteins and peptides account for approximately 90% of the dry weight (Bieber, 1979; Bougis *et al*., 1986; Ownby and Colberg, 1987). The non-protein fraction of snake venom is composed of sodium, potassium, phosphorous, chloride, calcium, magnesium, manganese, zinc and copper. It also contains riboflavin, nucleosides, peptides, amino acids, amides, lipids and some carbohydrates (Bieber, 1979). The snake venom proteins are enzymes, toxins or nerve growth factors. The biological activities are predominantly found in the protein fractions rather than in the non-protein fractions (Tu, 1977).

Efforts have been made to correlate the toxicity of snake venoms to their enzymatic

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activities. Owing to the diverse character of the venom and the antagonistic behavior of its different components, it is advantageous to utilize purified venom fractions in place of crude venom for diverse toxicological and pharmacological studies.

Relatively little work has been done on the toxicological effects of *E. pyramidum* crude venom and purified protein fractions. Okuda *et al*. (2001) purified novel disintegrins, the platelet aggregation inhibitors pyramidin A and B from the venom of *E. pyramidum*. Al-Asmari *et al*. (2006) studied the effect of crude venom on time-course of lipid peroxidation in different organs of mice. Adel Al-Missery *et al*. (2010) investigated the effect of crude venom on the activities of certain serum enzyme of mice. Wahby *et al*. (2012) isolated purified hemorrhagic metalloproteinase enzyme from *E. pyramidum* venom and studied its hemorrhagic activities in the skin of rabbit. Most recently, Conlon *et al*. (2013) isolated [Ser^{49}] phospholipase A_2 from the venom of the saw-scaled vipers *E. ocellatus, E. pyramidum leakeyi, E. carinatus sochureki,* and *E. coloratus* and observed their cytotoxic activity against human non-small cell lung adenocarcinoma A549 cells. So there is a potential to fractionate and purify all the protein fractions of *E. pyramidum* venom and study toxicological effects of these purified venom fractions. We carried out this study with the aim to have better understanding of the role played by different components of venom in envenomation by using the crude venom and its purified protein fractions.

MATERIALS AND METHODS

Venom

Specimens of *E. pyramidum* were captured in the Saudi Arabian desert and maintained at 28±2°C on a diurnal cycle of 7 h light/17 h dark. Each snake was fed one 20-30 g mouse every two weeks with water *ad libitum*. Venom was milked from the snakes as described by Al-Saleh *et al*. (1994) and stored at -20°C until used.

Acrylamide was obtained from Merck, München, Germany. N,N-methylene bisacrylamide and coomassie brilliant blue R250 were purchased from Fluka Chemie AG, Buchs, Switzerland. Ammonium persulphate, sodium dodecyl sulphate (SDS) and Tris (hydroxymethyl) methylamine were from BDH Chemicals, Poole, UK. Standard proteins and ampholine (pH 3.5–10.0) were from Pharmacia Uppsala, Sweden. Glycerol from Win Lab, Ltd., Maidenhead, UK and glycin and N,N,N,N Tetramethylethylene-diamine were from Riedel-dekhen AG, Wunstrofer StraBe, Germany. Protein molecular mass standards (Protein Marker II (6.5-200) pre-stained) were from AppliChem GmbH, Ottoweg 4, D-64291 Darmstadt, Germany. All other chemicals used were of analytical grade.

Fractionation and purification of crude venom

Crude venom of *E. pyramidum* (10 mg) was fractionated using a native polyacrylamide gel electrophoresis (PAGE); 8% acrylamide separating slab gel of pH 8.8 as described by Laemmli (1970). The separated venom fractions were eluted from the gel according to the procedure described by Walker *et al*. (1982). The eluted protein fractions were dialyzed against distilled water, lyophilized and stored at 4°C. The concentration of protein in each fraction thus obtained was determined by the method of Lowry *et al*. (1951). Each fraction was analyzed for purity on analytical native (non-SDS) PAGE as described under the fractionation procedure.

Determination of molecular weight by SDS-PAGE

The molecular weights of purified venom fractions were determined by running the purified venom fractions (20µg) along with crude venom (200µg) and standard proteins on SDS-PAGE at 8% acrylamide concentration according to the method of Laemmli (1970). The samples were boiled with sample buffer in the presence of 0.1% SDS. The proteins used as molecular mass markers were myosin (200 kDa), β-galactosidase b (116 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa) and lysozyme (14.4 kDa) and aprotinin (6.5 kDa).

Isoelectric focusing (IEF)

The isoelectric pH values (pI values) of the purified venom fractions were determined by using an LKB 2117 Multiphor system according to the instructions of the manufacturer.

Determination of median lethal dose (LD_{50}) of crude venom

The lethality of crude venom was assessed in Swiss male mice (30±2 g) obtained from the College animal house and maintained at 28±2°C with free access to a commercial pellet diet and water *ad libitum*. They were divided into six groups and there were 10 mice per group. The mice were injected interaperitoneally with gradually increased doses of crude venom (1.25, 2.5, 5.0, 7.5, 10.0 and 20.0 mg/kg body weight, in a fixed volume of 200 µl and the LD_{50} was calculated according to the arithmetic method of Karber (1931). Control mice were injected with 0.9% NaCl. The observation time limit used was 24 h, post-injection. These experiments were approved by an institutional ethics committee for animal care and use.

Effect of crude venom and its fractions on some blood parameters of normal male mice

In this experiment ten groups, each of 10 male mice; one group for crude venom, another for control, and 8 for 8 purified venom fractions were used. The mice were injected interaperitoneally with 50% of the lethal dose of crude venom and its purified fractions to keep 50% population of the injected animals alive to observe different changes in the blood parameters. Blood was drawn after one hour of the injection because epidemic studies showed that victims of snake bite reached the hospital or healthcare center in half-one hour after the bite time. The blood was drawn from eye capillary tube because the quantity of blood from eye is more than tail. It is difficult to draw blood from heart and there is also the risk of contamination of the blood sample.

The blood was collected in EDTA tubes, mixed and stored in refrigerator for a couple of minutes. Then blood samples were centrifuged at 3000 rpm/minute for 10 minutes, the plasma was separated and kept in freezer at −4°C. The analysis was carried out within one hour of the sampling.

The four parameters; blood glucose, total protein, aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined by using Bayer Health Care, Express Plus Germany, and Kits; Reagents CK, Ref: 114978, Bayer Health Care, NY, USA. The other five parameters *viz.*, blood cholesterol (CHO), triglycerides (TG), creatinine (Crea), blood urea and uric acid were determined by using EKTA Chem. 250 Analyzer, Kodak, NY, USA and kits, Vitros Products Chemistry (Cat. Ref: 145-0261) from Ortho-Clinical Diagnostics, Gohnson and Gohnson Company, NY, USA.

Statistical analysis

In order to calculate certain statistical measurements such as the average concentration, changes in blood parameters and standard deviation SPSS program was used. The t-test was also used for comparison between the groups injected with crude venom and purified fractions below the lethal concentration. Also to check the assumption saying that no changes will occur between the crude venom group, its purified fraction group and control group. The final decision about results was based on p-values. When p-values $\leq \alpha$, then we used alternative assumption (5%), if p-values $< \alpha$, we used null hypothesis, it means there are no significant values.

RESULTS AND DISCUSSION

E. pyramidum crude venom was fractionated into eight fractions by preparative native PAGE. The recovery of the protein was 60%. The purity of all fractions was checked by analytical native PAGE. On native PAGE all fractions appeared as a single band (Fig. 1). However, on SDS-PAGE all fractions appeared as a single band except fraction F2 which showed two bands (Fig. 2). The appearance of more than one band with fraction F2 could be explained by assuming that either fraction F2 was oligomeric protein that was dissociated into its subunits by the denaturing agent or that fraction F2 on native PAGE composed of more than one protein, with the same mobility on native PAGE. All fractions exhibited acidic pI except fraction F8 which showed basic pI (Table I).

The median lethal dose for *E. pyramidum* crude venom determined by Karber method was 5.06mg/kg (Table II). Recently Al-Missiry *et al*. (2010) reported LD_{50} 1.4mg/Kg for an Egyptian species of *E. pyramidum* which is almost 3.5 times less as compared to our result.

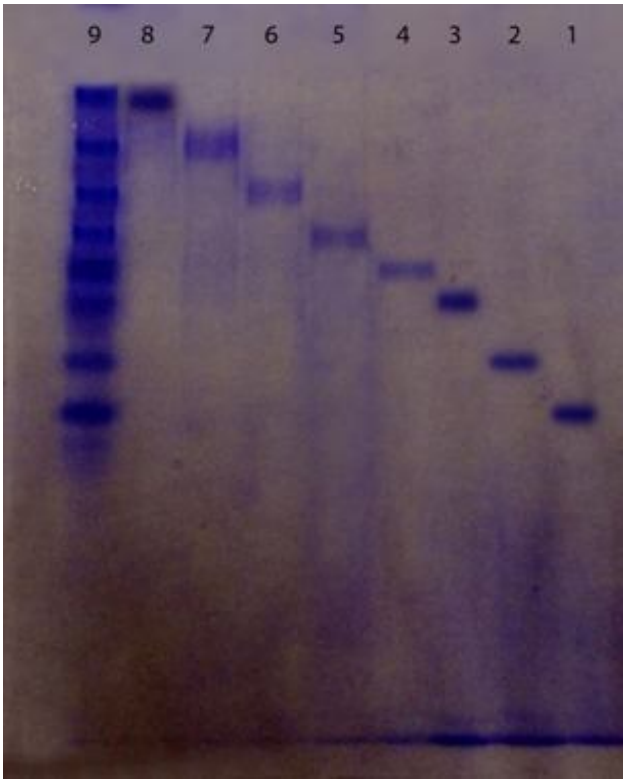

Fig. 1. Analytical native PAGE of *E. pyramidum* venom along with purified fractions (F1-F8); (lane 1: 30 µg F1), (lane 2: 30 µg F2), (lane 3: 30 µg F3) (lane 4: 30 µg F4), (lane 5: 30 µg F5), (lane 6: 30 µg F6), (lane 7: 30 µg F7), (lane 8: 30 µg F8) and crude venom (lane 9, 200 µg).

Table I:- **Molecular weight and isoelectric point of purified fractions of *E. pyramidum* crude venom.**

Fraction No.	Mol. Weight (K Da)	Isoelectric pH(pI)
F1	105	6.3, 4.8
F2	138, 42	5.7
F3	28	5.4
F4	83	6.3
F5	20	5.4
F6	12	3.5, 4.6
F7	10.5	5.7
F8	15	7.1

The study assessed the impact of *E. pyramidum* crude venom and its purified protein fractions on the effects induced by the venom on some biochemical markers. A significant increase

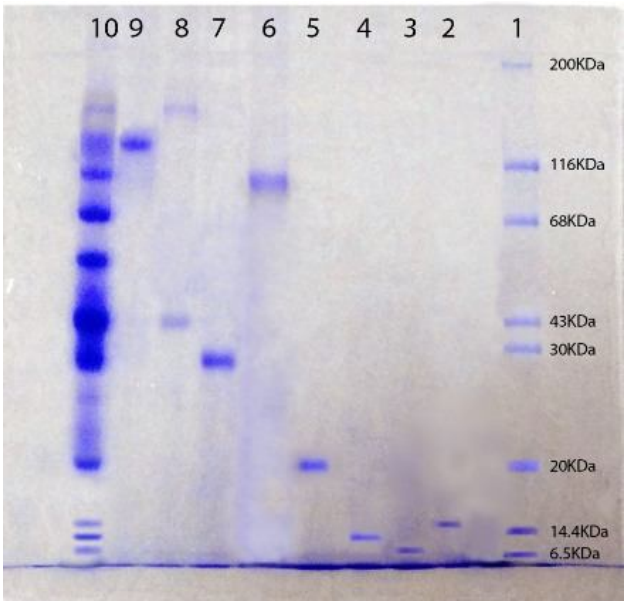

Fig. 2. SDS-PAGE of *E. pyramidum* venom along with purified fractions (F1-F8) and protein molecular mass markers : (lane 1: protein molecular mass markers), (lane 2: 30 µg F8), (lane 3: 30 µg F7), (lane 4: 30 µg F6), (lane 5: 30 µg F5), (lane 6: 30 µg F4), (lane 7: 30 µg F3), (lane 8: 30 µg F2) (lane 9, 30 µg F1) and crude venom (lane 10, 200 µg).
The proteins used as molecular mass markers were myosin (200 kDa), β-galactosidase (116 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa), lysozyme (14.4 kDa) and aprotinin (6.5 kDa).

was noticed in plasma ALT level in the animals injected with crude venom ($p<0.05$, 132±2.28) compared to non-envenomated control group (13.4±0.73) as mentioned in Table III. There was significant but comparatively less increase in plasma.

ALT level by the fractions F1 (43.2±1.5, $p<0.05$) and F6 (55.4±1.67, $p<0.05$). In case of AST, there was significant increase in plasma level in group injected with crude venom (189±3.75, $p<0.05$) about four times as compared to control group (45.7±2.49). The increase produced by fraction F7 was about three times in comparison to control (133.5±2.02, $p<0.05$) (Table III). The results are concurrent with the preceding reports. Sant *et al.* (1974) and Tembe *et al*. (1975) by using *E. carinatus* snake venom found that there were

Table II.- Determination of LD_{50} of *E. pyramidum* crude venom.

Group No.	Dose (mg/kg) I.P.	Dose difference	Mortality	Mortality (Mean X)	Dose difference X Mortality mean
1	1.25		0		
2	2.5	1.25	1	0.5	1.25x0.5 = 0.625
3	5	2.5	5	3	2.5x3 = 7.5
4	7.5	2.5	9	7	2.5x7 = 17.5
5	10	2.5	10	9.5	2.5x9.5 = 23.75
6	20	10	10		
Total					49.375

LD_{50} = Minimum dose that kills all animals − (Total of (dose difference x Mortality mean)
The number of a single group

LD_{50} = 10 − (49.375)/10

LD_{50} = 5.06mg/Kg

Table III.- Effect of *E. pyramidum* crude venom and its purified protein fractions on the level of plasma aspirate aminotransferase (AST) and alanine aminotransferase (ALT).

Enzyme activity (U/L) (Mean ± S.E)	Cont.	Crude venom	F1	F2	F3	F4	F5	F6	F7	F8
ALT	13.4± 0.73	132.5± 2.28b	43.2± 1.5b	25.1± 1.71b	15.8± 1.5	16.6± 1.2	9.7± 0.7	55.4± 1.67b	9.2 ± 0.57	11.8± 0.77
AST	45.7± 2.49	189.6± 3.75b	43.2± 0.97	57.2± 1.1a	36.9± 1.43	53.9± 0.91a	55.9± 2.14a	47.8± 2.23b	133.5± 2.02	53.8± 2.72a

Data are expressed as means ± S.E. (n = 8). [a]P< 0.05; [b]P< 0.001.

elevations in the serum concentration of AST, ALT and ALP in animals four hours post envenomation compared with the control group. Al-Saleh *et al.* (2002) reported that incubation of cultured fibroblast with *E. coloratus* crude venom and some of its purified protein fractions resulted in similar significant stimulatory effects on AST and ALT activity. It is also reported by Abdel-Nabi and Rahmy (1992) using *E. carinatus* venom, the injection of sub-lethal dose caused a significant rise in serum AST, ALT and ALP in rats accompanied with disturbances in the hepatic and renal functions of the envenomated animals through severe hepatocellular injuries, necrosis of hepatocytes and kidney tubules as well as nephrotoxic action. Recently El-Missiry *et al.* (2010) reported the similar results for the elevation of serum AST and ALT level by *E. pyramidum* crude venom in mice. The fast increase in ALT and AST activities recorded in the present study one hour after venom envenomation may be ascribed to brutal injuries and necrosis of hepatocytes as well as to a nephrotoxic action of the venom as reported by Abdel Nabi (1993). The elevation in AST and ALT, gives evidence about the destruction of the liver and heart tissues as a result of venom injection.

The current effort described the effects of *E. pyramidum* venom on some biochemical parameters. It was observed that glucose level increased in test group injected with crude venom ($p<0.05$) as compared to control group. Whereas there was decrease in glucose level in the test groups injected with fractions F1, F3, F4, F5, F7 and F8 but most significant ($p<0.05$) decrease was caused by fractions F4 and F5. Fractions F2 and F6 had no significant effect on plasma glucose level ($p>0.1$) (Table IV). Al-Saleh (2002) also reported that *E. carinatus* crude venom caused noteworthy hyperglyce-mia which started as early as 15 minutes after envenomation and remained evident after 24 hours. It is suggested that hyperglycemic effect produced is due to mobilization of glycogen in the liver which may be due to direct inhibition of glucokinase or indirectly by stimulating the release

Table IV.- Effect of *E. pyramidum* crude venom and its purified protein fractions on some blood parameters of male mice.

Parameter (mg/dl)	Cont.	Crude venom	F1	F2	F3	F4	F5	F6	F7	F8
Glucose	63±1.9	72±3.0a	29± 1.5b	67± 1.5	40±1.3b	53± 2.3a	56±2a	63± 2.9	35± 1.0b	42± 1.3b
Total protein	1.4±0.24	0.7±0.08a	1.8± 0.16	2.1± 0.18	1.5± 0.16	1.9± 0.22	2.2± 0.31	1.5± 0.27	0.9± 0.17	1.3± 0.14
Cholesterol	47.2±1.1	8.7± 0.37 b	48.8± 1.9	62.8± 2.6b	59.5± 1.3b	39.4±2. 5a	48± 1.95	49.1± 1.6	23.2± 1.2b	61± 2.25b
Triglycerides	92±2.2	119±4.9b	67± 2.1b	45± 2.8b	68± 3.4b	50± 1.1b	51± 0.7b	58± 4.3b	38± 2.3b	32± 0.7b
Urea	6±0.7	12±1.0b	7±0.7	8±0.7	8±0.7	7±0.7	6±0.7	9±1.0a	7±0.7	5±0.7
Creatinine	0.1±0.02	1.4±0.09b	1.3± 0.13b	0.9± 0.05b	0.16± 0.03	0.1± 0.02	0.27± 0.02b	0.23± 0.01b	0.18± 0.02b	0.8± 0.05b
Uric acid	0.2±0.01	0.3±0.02b	2.4± 0.18b	0.2± 0.02	0.3± 0.02a	0.2± 0.02	0.5± 0.08b	0.5± 0.08b	0.4± 0.04b	0.2± 0.02

Data are expressed as means ± S.E. (n = 8). * P< 0.05; * * P< 0.001.

of adrenaline (Mohammad *et al.*, 1980; Al-Saleh, 2002). It is also mentioned by Al-Saleh (2002) that the purified fractions F2 and F6 of *E. carinatus* exhibited the highest increase in plasma glucose level after 12 hours of envenomation which is contrary in our report.

There was up to 50% decrease in total plasma protein with crude venom ($p<0.05$, 0.7±0.08) and less than 50% decrease with fraction F7 ($p>0.1$, 0.9±0.17) as compared to control group (1.4±0.24) (Table IV). The decrease in total plasma protein may be attributed to the presence proteolytic enzymes (proteases) in viper venoms. Al-Saleh (1997) reported that the crude *Bitis arietans* and three of its purified protein fractions showed caseinolytic activity. Most recently Wahby *et al.* (2012) isolated and purified hemorrhagic metalloproteinase from three viper venom including *E. pyramidum* showing strong proteolytic activity. The reduced levels of total protein could be due to disturbances in renal functions as well as haemorrhages in some internal organs. In addition, increasing in vascular permeability and haemorrhages in vital organs due to the toxic action of various snake venoms (Meier and Stocker 1991; Marsh *et al.*, 1997).

Plasma cholesterol low level were observed in the group injected with crude venom (8.7±0.37, $p<0.05$) and fraction F7 (23.2±1.15, $p<0.05$) as compared to control group. This agrees with a previous report about the viper *Naja haje* venom. Abd El-Aal and Ezzat (1997) found that a sub-lethal dose of *Naja haje* venom decreased serum total cholesterol in rabbits. These authors explained their findings that by the activation of the pituitary-adrenal axis resulting in increased circulatory levels of ACTH and cortisol. ACTH induces specific membrane receptors in the cells of the adrenal cortex to increase serum cholesterol uptake. The increased level of T4 from the thyroid gland following administration of *Naja haje* venom fractions may also explain the reason for lowering serum cholesterol level (Ezzat, 1993). An inverse correlation between serum thyroid hormones and cholesterol levels has been reported by several workers (Devlin, 1993; Ganong, 1991). On the other hand fractions F2, F3 and F6 caused significant ($p<0.05$) increase in plasma cholesterol level (Table IV). Muhammad (2009) reported that *i.p*. injection of *E. coloratus* crude venom induced a significant increase in plasma cholesterol level in rabbits after 1, 2 and 4 hours. Most recently Onyeama *et al.* (2012) reported that the Carpet viper (*Echis ocellatus*) crude venom resulted in significant increase ($p<0.05$) in the serum total cholesterol.

The fraction F1 caused significant decrease ($p<0.05$) in plasma triglyceride level whereas there was significant increase (119±4.85, $p<0.05$) in plasma triglyceride level in animals injected with crude venom (Table IV). El-Aal and Ezzat (1997)

reported that F1 of viper *Naja haji* venom significantly decreased the levels of serum triglycerides after 1 and 2 hours, while F2 significantly increased the level of serum triglycerides after 3 and 4 hours. F3 induced an initial significant increase after 1 hour followed by a significant decrease after 2, 3 and 4 hours. The decrease in serum triglyceride levels by some fractions whereas the opposite effect by crude venom or other fractions might be related to the relative action of each fraction on the hepatic synthesis of triglycerides or to the activity of lipoprotein lipase which regulates tissue uptake of triglycerides (Devlin, 1993). Moreover the increases in serum cholesterol and triglycerides levels in envenomated animals observed in the present study (Table IV) could be due 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 (El-Asmar *et al.*, 1979).

There was significant increase (12±1.0, $p<0.05$) in blood urea level in the group of animals injected with crude venom as compared with the control group. There was significant increase in blood urea level by fractions F2, F3 and F6 but less than caused by crude venom (Table IV). There was an increase in plasma Creatinine level produced by crude venom and some venom fractions (Table IV). The highest increase was caused by fractions F1, F5, F7 and F8. The crude venom and all fractions except fractions F2, F4 and F8 exhibited significant increase in uric acid level. The highest increase was exhibited by fraction F1 (2.4±0.18, $p<0.05$) as compared to control group (0.2±0.01) (Table IV). Abdel Nabi (1993) has reported that a sub-lethal dose of both crude viper *Cerastes cerastes* venom and its B fraction showed a significant rise in blood urea nitrogen and this was parallel to a significant increase in serum creatinine levels as well. Shaban and Hafez (2003) reported that intra-peritoneal injection of a sub-lethal dose of viper *Naja haje* venom (0.2mg/kg) in rats induced a significant elevation in the activities of urea and creatinine as compared to normal control. Recently El-Missiry *et al.* (2010) reported that LD_{50} of *Echis pyramidum* venom induced a highly significant increase in urea and creatinine concentrations compared to the normal control. Also, Tu (1991) and Merchant *et al*. (1989) reported that renal diseases caused by various snake venoms were characterized by raised urea, creatinine and potassium in oliguric patients. The increase in these values is used as an indicator of renal failure. This significant rise was ascribed to the nephrotoxic effect of venom. This was in agreement with the results of Rahmy *et al*. (1992, 1995b) and Yaguchi *et al.* (1996). As they mentioned that grave renal complications in case of *Naja haje* and *Cerastes cerastes* envenomation escort to impairment of the excretory function of the kidney.

It may be concluded from the present study that the *E. pyramidum* crude venom and its purified protein fractions exerted potent toxic effects on some biochemical markers and parameters in male mice. There is a potential to characterize the purified protein fractions completely using advanced techniques, then to correlate their effects to their structure and further manipulation of these proteins to find their potential use in diagnostics, therapeutics and biological research.

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REFERENCES

EL-AAL, ABD. AND EZZAT, A.R., 1997. Effects of three fractions obtained from *Naja haje* venom on hemolysis and lipid metabolism in rabbits. *J. Venom. Anim. Toxins,* **3**: 1-5.

ABDEL-NABI, I.M. AND RAHMY, T.R., 1992. Influence of the venom of snake *Echis carinatus* on the structure and function of the hepatic tissues of white rat. *J. Egypt. Ger. Soc. Zool (C).*, 171-187.

ABDEL-NABI, I.M., 1993. Effect of crude *Cerastes cerastes* venom and fraction B on the clinical parameters of white rat. *J. Egypt. Ger. Soc. Zool.,* **10(A)**:315-326.

AL-ASMARI, A., AL-MOUTAERY, K., MANTHARI, R.A AND KHAN, H. A., 2006. Time-course of lipid peroxidation in different organs of mice treated with *Echis pyramidum* snake venom. *J. Biochem. molec. Toxicol*., **20**: 93-95.

AL-SALEH, S.S., NAYYAR, R., AL-SADOON, M.K., AL-JAFARI, A. AND DUHIMAN, A.S., 1994. A rapid fractionation method for the desert cobra venom (*Walterinnesia aegyptia). Med. Sci. Res*., **22**: 659-660.

AL-SALEH, S.S., GHNEIM, H.K., HADDAD, H.Y. AND KHAN, S.U., 2002. Separation and purification of *Echis coloratus* venom and some biological and biochemical effects of the proteins**.** *Cell Biochem. Funct*.**, 20**: 153-162.

Al-SALEH, S.S., 2002. The effect of *Echis carinatus* crude venom and protein fractions on carbohydrate metabolism in rats. *Cell Biochem. Funct*., **20**: 1-10.

Al-SALEH, S.S., 1997. Fractionation and purification of *Bitis arietans* crude venom by gel electrophoresis, and characterisation of some enzymatic and biological activities. *Med. Sci. Res*., **25**: 85-87.

Al-SHAMMARI, A. M., 2007. *Ecological, eco-physiological and toxicological aspects of the pyramid viper (*Echis pyramidum*) in Jazan province, Saudi Arabia*, Ph.D. thesis, King Saud University, Saudi Arabia.

BIEBER, A.L., 1979. Metal and non-protein constituents in snake venom. In: *Handbook of experimental pharmacology* (ed. C.Y. Lee), vol. 52. Springer-Verlag. Berlin, pp. 295-308.

BOUGIS, P. E., MARCHOL, P. AND ROCHAT, H., 1986. Characterization of Elapidae snake venom components using optimized reverse – phase high performance liquid chromatographic conditions and screening assays for neurotoxin and phospholipase A_2 activities. *Biochemistry*, **25**: 7235-7243.

CONLON, J.M, ATTOUB, S, ARAFAT, H, MECHKARSKA, M, CASEWELL, N.R, HARRISON, R.A. AND CALVETE, J.J., 2013. Cytotoxic activities of [Ser49]phospholipase A2 from the venom of the saw-scaled vipers *Echis ocellatus, Echis pyramidum leakeyi, Echis carinatus sochureki* and *Echis coloratus*. *Toxicon,* **71**: 96–104.

DEVLIN, T.M., 1993. Biochemistry with clinical correlations. In: *Biochemistry of hormones I: Polypeptide hormones.* Third eds. John Wiley, New York, pp. 500-600.

EL-AMAR, M. F., FARAG, R. M., SHOUKRY, S. AND EL-SHIMI, I. R., 1979. Effect of scorpion *Leivrus quinquestriatus* H & E venom on lipid metabolism. *Toxicon*, **17**: 279-283.

El-MISSIRY, A.G., SHABAN, E.A., MOHAMED, R.M., AHMED, A. A., ABDALLAH, N.M. AND MOUSTAFA, M.I., 2010. Influence of ionizing radiation on *Echis pyramidium* snake venom: biochemical and immunological aspects. *Egyt. J. Hosp. Med.,* **40:** 314-334.

EZZAT, A.R. AND ABD EL-AALA, A., 1989. Effect of cobra *Naja haje* venom on the adrenal activity in rabbits. *Qatar Univ. Bull.*, **9**: 169-76.

EZZAT, A.R., 1993. Changes in rabbit serum glucose, insulin, T4 and T3 in response to three ionopheretic fractions of *Naja haje* venom. *J. Egypt. Soc. Toxicol.*, **11:** 31-37.

GANONG, W.F., 1991. *Review of medical physiology*. 15eds. Com: Appleton & Lange, Norwalk, pp. 296-311.

GWYNNE, J.T. AND HESS, B., 1978. Binding and degradation of human HDL by rat adrenocortical cells. *Metabolism*, **27**: 1593-1600.

GWYNNE, J.T., HUGHES, T., ROUNDTREE, R. AND HESS, B., 1979. ACTH regulation of the HDL receptors of the rat adrenal cortex. In: *Contemporary endocrinology* (ed. S.H. INGBAR), Plenum Medical Book, New York, pp. 196-203

KARBER, G., 1931. Beitrag zur kollektiven behandlung pharmakologischer Reihenvershuche. *Arch. exp. Pathol. Pharmakol.*, **162**: 480-483.

LAEMMLI, U.K., 1970. Cleavage of structural protein during the assembly of the head of the bacteriophage T4. *Nature*, **227**: 680-685.

LOWRY, O.H., ROSEBROUGH, N.J., FAAR, A.L. AND RANDALL, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**: 265-275.

MARSH, N., GATTULLO, D., PAGLIARO, P. AND LOSANO, G. 1997. The *Gaboon viper* and *Bitis gabonica*: hemorrhagic, metabolic, cardiovascular and clinical effects of the venom. *Life Sci.*, **61**: 763-769.

MERCHANT, M.R., KHANNA, U.B., ALMEDIA, A.F., ACHARYA, V.N. AND MITTAL, B.V., 1989. Clinicopathological study of acute renal failure following viperine snake bite. *Assoc. Physicians India*, **37**: 430-433.

MEIER, J. AND STOCKER, K., 1991. Effect of snake venoms on homeostasis. *Toxicology,* **21**: 1711-1820.

MOHAMMAD, A.H., FOUAD, S. AND ABBAS, F. 1980. Metabolic studies of the Egyptian and Allied African snake venoms. *Toxicon,* **18**: 381.

MUHAMMAD, M.A., 2009. Physiological effects of envenomation by two different doses of the viper *Echis coloratus*is crude venom on bio-chemical parameters in serum of Guinea pigs at different times. *Egypt. Acad. J. biol. Sci.*, **1**: 21-31.

OKUDA, D, NOZAKI, C, SEKIYA, F. AND MORITA, T., 2001 Comparative biochemistry of disintegrins isolated from snake venom: consideration of the taxonomy and geographical distribution of snakes in the genus *Echis. J. Biochem.*, **129**:615-20.

ONYEAMA, H.P., AHMED, M.S., OFEMILE, P.Y., IBEKWE, H.A. AND NWANKPA, P., 2012. Effects of *Calliandra portoricensis* extracts on the lipid profile of wistar rats challenged with venom of carpet viper. *J. med. Sci.*, **3**: 674-678.

OWNBY, C.L. AND COLBERG, T.R., 1987. Characterization of the biological and immunological properties of fractions of Prairie rattle snake (*Crotalus viridis viridis*) venom. *Toxicon,* **25:** 329-1342.

RAHMY, T.R., TU, A.T., EL-BANHAWY, M., EL-ASMAR,

M.F. AND HASSAN, F.M., 1992. Cytopathological effect of *Cerastes cerastes* (Egyptian sand viper) venom and its hemorrhagic toxin on liver and kidney. An electron microscopic study. *J. Nat. Toxins,* **1:** 45-58.

RAHMY, T.R., RAMADAN, R.A., FARID, T.M. AND El-ASMAR, M.F., 1995b. Renal lesions induced by cobra envenomation. *J. Egypt. Ger. Soc. Zool*., **17**: 251-271.

SANT, S.M., TEMBE, V.S., SALGANOKAR, D.S. AND PURANDARE, N.M., 1974. Lesions produced by *Echis carinatus* venom in experimental animals. *Postgrad. Med*., **21**: 36-47.

SHABAN, E.A. AND HAFEZ, M.N., 2003. Ability of gamma-irradiated polyvalent antivenin to neutralize the toxicity of the Egyptian Cobra *(Naja haje)* venom. *Egypt. J. Hosp. Med*., **3**: 135–152.

TEMBE, V.S., SANT, S.M. AND PURANDARE, N.M., 1975. A clino-pathologic study of snake bite cases. *Postgrad. Med.*, **21**: 36-47.

TU, A.T., 1977. In: *Venoms: Chemistry and molecular biology* (ed. A. T. Tu), Wiley & Sons, New York, pp. 2-400.

TU, A.T., 1991. Tissue damaging effects by snake venom hemorrhage and myonecrosis. In: *Handbook of natural toxins*, 5, (ed. A. T. Tu,), Dekker., New York, pp. 297-347.

WAHBY, A.F., ABDEL-ATY, A.M. AND EL-KADY, E.M., 2012. Purification of hemorrhagic SVMPs from venoms of three vipers of Egypt. *Toxicon,* **59**: 329–337.

WALKER, J.E., AUFFERT, A.C. AND CARNE, A., 1982. Solid phase sequence analysis of polypeptides eluted from polyacrylamide gels. *Eur. J. Biochem*., **123**: 253-260.

YAGUCHI, T, UEDA, Y. AND ISHITOBI, F., 1996. Clincopathological study of the renal damage caused by mamushi snake (*Agkistrodan biomhoffi*) venom and effects of cepharanthin against it in mice. *Dokkyo. Igakks Zasshi*., **11**: 127-141.

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