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# Effects of Pyramid Viper, *Echis pyramidum* Crude Venom on Hepatic Redox Status and BAX Expression in Rats

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**In the present study, we have investigated the *Echis pyramidum* crude venom induced oxidative stress and apoptosis after 2, 4 and 6 hrs in livers of rats injected with the [$LD_{50}$ and $\mu$ $LD_{50}$. Wistar rats were randomly divided into 3 groups, the control group was intrapretoneal (i.p.) injected with saline while, [$LD_{50}$ and $\mu$ $LD_{50}$ doses envenomed groups i.p. injected with venom at a dose of 0.6325 and 1.265 mg/kg body weight, respectively. Animals were sacrificed after 2, 4 and 6 hrs from the injection. Lipid peroxidation, nitric oxide and glutathione levels as oxidative markers were measured in liver homogenate. In addition, liver functions parameter and activity of catalase were determined. *E. pyramidum* crude venom increased lipid peroxidation and nitric oxide production in liver with concomitant reduction in glutathione, catalase, and total antioxidant capacity. These findings were associated with apoptosis induction in the liver as indicated with the increment in BAX expression. In addition, *E. pyramidum* crude venom caused hepatic injury as indicated by histopathological changes in the liver tissue with an elevation in total bilirubin, serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. On the basis of the present results it can hypothesized that *E. pyramidum* crude venom is a potent toxins-mediated hepatotoxicity associated with apoptosis induction in the hepatic tisue.**

**Key words:** *Echis pyramidum* venom; Hepatotoxicity; Oxidative stress; BAX; Rats.

The venom of most snakes is highly phlogistic in humans. Tissue changes following snake envenomation depend on the species of snake responsible for the bite, the composition of its venom and also the susceptibility of the tissue for a particular component of the venom (Tohamy *et al*., 2014).

Saw-scaled or carpet vipers (Viperidae: *Echis* sp.) reside in West, North and Northeast Africa, Middle East and Central Asia as far as Central and East India, and envenoming by saw-scaled viper species is thought to be responsible for more snakebite deaths worldwide than any other snake genus (Valenta *et al*., 2011; Warrell *et al*., 1977), this including *E. pyramidum*. Envenomed victims typically suffer a combination of systemic and local haemorrhagic symptomatologies and up to 20% mortality rates without antivenom treatment (Casewell *et al*., 2009). The venom components are very similar throughout the *Echis* genus members in that the venom largely consists of enzymes and toxins affecting hemocoagulation, with the envenoming effect chiefly determined by ecarin enzyme that is responsible for a direct activation of prothrombin to a different type of thrombin-meizothrombin that cannot be inhibited by AT-heparin complex (Hasson *et al*., 2003; Valenta *et al*., 2011). Other venom components are desintegrins, presynaptic enzymatic neurotoxins, in case of *E. Pyramidum*,

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and a range of cytotoxic and destructive proteolytic enzymes causing local damage to tissues including formation of necrosis(Valenta *et al*., 2011; Warrell *et al*., 1977).

There are reports showing the effects of various snake venoms on liver tissues in rat that the venom causes damage of the hepatocyes.In the liver, congestion and petechial haemorrhages, microvescicular fatty change, hydropic degeneration as well as necrosis of hepatocytes have been recorded following snake bites (Jarrar, 2011).

Nevertheless, the effect of the venom of *E. pyramidum* was not sufficiently covered in the available literature. Thus, it is of interest to examine the possible damaging effects of [!$LD_{50}$ and µ$LD_{50}$ of the crude venom on liver of rats after 2, 4 and 8hrs of injection, unveiling the role of oxidative stress on the venom induced hepatotoxicityto improve our understanding of snake envenomation in rats.

## MATERIALS AND METHODS

### *Echis pyramidum* venom collection and lethality

The crude venom was obtained by milking of about 30 samples of *E. pyramidum* snakes collected from the south-western region of Saudi Arabia. *E. pyramidum* venom was intraperitoneally injected (in 0.2 ml saline) into Albino rats. Rats of either sex weighing 120-150 g were divided into 6 groups of 6 each and received the doses (0.5,1,3,5,8 & 10 mg/kg) intraperitoneally (i.p.). The results were recorded 72 hrs after the i.p. injection of the venom into rats.The approximate $LD_{50}$ of the crude venom was calculated according to the method of Meier and Theakston (1986) and expressed as mg per kg body weight.

### Experimental animals

Experiments were performed on male albino Wistar rats, weighing 120 -150 g. The animals were obtained from the animal house of National Organization for Drug Control and Research (NODCAR), Giza, Egypt. Animals were kept in wire bottomed cages, in a room under standard condition of illumination with a 12 hrs 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.

### Experimental groups

To study the effect of *E. pyramidum* crude venom, forty two male albino rats were randomly divided into:

Control group (n=6): mice were injected intrapretoneal (i.p.) with 0.2 ml saline /rat, and sacrificed after 6 hrs from injection.

### $LD_{50}$-Envenomed group (n=18)

Rats were injected i.p. with 0.2 ml saline solution containing 0.6325 mg venom/kg body weight. The rats were subdivided to three subgroups (six rats each) sacrificed after 2, 4 and 6 hrs from envenoming respectively.

### $LD_{50}$-Envenomed group (n=18)

were injected i.p. with 0.2 ml saline solution containing 1.265 mg venom/kg body weight. This group was subdivided to three subgroups (six rats each) sacrificed after 2, 4 and 6 hrs from envenoming respectively.

The animals of all groups were sacrificed by cervical dislocation under diethyl ether anaesthesia and blood samples were collected for serum analysis. A few pieces of liver 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, pH 7.4, and finally stored at -20 °C until use in the various biochemical determinations.

### Biochemical studies

### Liver functions tests

Colorimetric determination of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) was estimated by measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4-dinitrophenylhydrazine according to the method of Reitman and Frankel(1957). The color of which was measured at 546 nm. Alkaline phosphatase (ALP) was assayed in serum using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the method that described by Belfield and Goldberg(1971). Also, total bilirubin (TB) in serum was assayed according to the method of Schmidt and Eisenburg(1975).

### Lactate dehydrogenase cytotoxicity assay

Lactate dehydrogenase was determined through spectrophotometry by following the decrease of NADH at 340 nm according, to

Bergmeyer (1983), and was expressed as U/g protein, where protein in the midbrain and striatum homogenates were determined according to Lowry *et al*. (1951).

**Oxidative stress markers**

Homogenates of liver were used to determine lipid peroxidation (LPO) by reaction of thiobarbituric acid (Ohkawa *et al*., 1979). Similarly, nitrite/nitrate (nitric oxide; NO) and glutathione (GSH) were assayed by the methods of Green *et al*. (1982) and Ellman (1959), respectively.

**Enzymatic antioxidant status**

Homogenates of liver were used for the determination of superoxide dismutase (SOD) according to Nishikimi *et al*. (1972) and catalase (CAT) as described by Aebi (1984).

**Histopathological examination**

Small pieces of liver were washed in saline and fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned at 5 µm; and stained with hematoxylin and eosin for light microscopic observations. The preparations obtained were visualized using a Nikon microscopy at a magnification of 400×.

**Immunohistochemical analyses of BAX**

The immunolocalization technique used for BAX was performed on 3-4 µm thickness sections according to Pedrycz and Czerny (2008). For negative controls, the primary antibody was omitted. In brief, mouse anti-BAX (diluted1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), were incubated with sections for 60 minutes. Primary antibodies were diluted in TBS (Tris buffered saline)/1%BSA (bovine serum albumin). Then a biotinylated secondary antibody directed against mouse immunoglobulin (Biotinylated Link Universal–DakoCytomation kit, supplied ready to use) was added and incubated for 15 minutes, followed by horse radish peroxidase conjugated with streptavidin (DakoCytomation kit, supplied ready to use) for an additional 15 minutes of incubation. At the sites of immunolocalization of the primary antibodies, a reddish to brown colour appeared after adding 3-amino-9-ethylcarbasole (AEC) (DakoCytomationkit, supplied ready to use) for 15 minutes. The specimens were counterstained with hematoxylin for 1 minute and mounted using the Aquatex fluid (Merck KGaA, Germany). Next, the staining intensity was grade as very week, week, medium, or strong. All sections were incubated under the same conditions with the same concentration of antibodies and at the same time; in order for the immunostaining to be comparable among the different experimental groups.

**DNA fragmentation assay using agarose gel electrophoresis**

As a measure of apoptotic DNA fragmentation, the presence of DNA ladder was determined according to Wlodek *et al*. (1991). Extraction of DNA was done according to the method of Aljanabi and Martinez(1997). A gel was prepared with 2% agarose containing 0.1% ethidium bromide (200 µg/ml). The DNA samples were mixed with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanole FF and 30% glycerol) and loaded into the wells (20 µl of DNA/lane) with a standard molecular-sized ladder marker (Pharmacia Biotech., USA). The gel was electrophoresed at a current of 50 mA for 1.5 hrs using the submarine gel electrophoresis machine. The DNA was visualized and photographed with illumination under UV light.

**Statistical analysis**

The data are presented as means ± SEM. Data were analyzed in SPSS version 17.0. Unpaired student t-test was used a test of significance. *P* value < 0.001 was taken as the level of significance.

## RESULTS

Changes in levels of serum parameters affected by the single i.p. injection of *E. Pyramidum*crude venom after 2, 4, and 6 hrs are shown in Figure 1. ALT, AST, ALP and total billirubin were significantly increased (*p*Â0.05) when compared to the control rats. Moreover, the effect of µ $LD_{50}$ *of E. Pyramidum* venom was greater *than* $LD_{50}$ in producing the hepatic tissue injures. The leakage of LDH from intracellular to extracellular is an indicator of cell membrane damage, based on the present results (Figure 2), *E. Pyramidum* venom induced hepatic cellular damage as indicated by the elevation LDH activity at all the experimental time when compared with the corresponding control values.Moreover, the elevation in LDH activity was increased with the dose of venom.

We checked the oxidative stress status in the liver tissue in response of *E. Pyramidum*crude venom by measuringthe levels

of LPO, NO and GSH in liver homogenate. Results in Figure 3 showed that, the crude venom of *E. Pyramidum*induced elevation in LPO production and NO generation in liver homogenatewith concomitant reduction in GSH ($p$<0.05) at all the experimental time when compared with the corresponding control value.Again, the effect of µ $LD_{50}$ *of E. Pyramidum* venom was greater *than* [! $LD_{50}$ in producing oxidative stress in hepatic tissue. We also determined some major components involved in the downregulation of substances formed during oxidative stress such as catalase and total antioxidant capacity (Figure 4). Conspicuously, catalase activity was increased significantly ($p$Â0.05) after 2 and 4 hrs. After then, the activity tended to decrease significantly at the same level after 6 hrs of $LD_{50}$*and* µ $LD_{50}$of *E. Pyramidum*crude venom injection. Although, the TAC level were significantly ($p$<0.05)decreased at all the experimental time as compared with the control rats (Figure 4).

Control liver section (Figure 5A) appeared with normal architecture, while liver sections of rats injected with *E. Pyramidum* crude venom showed inflammatory cellular infiltrations around the hepatic vein, dilated blood sinusoids, hepatocytic vacuolations and prominent van Kupffer cells (Figure 5B-5H). Severe necrosis and apoptosis were also seen (Figure 5c).We noticed that injures in the hepatic tissue of rats were increased with the dose *E. Pyramidum* venom and the time after envenomation.

The immunohistochemical investigation for BAX displayed that there were some immunoreactivity in the hepatic tissue of the control group, which indicated the normal life cycle of cells (Fig. 6A). Immunostaining for BAX was increased gradually in the hepatic tissue (Fig. 6B-6H)after 2, 4 and 6 hrs of $LD_{50}$*and* µ $LD_{50}$of *E. Pyramidum* crude venom injection. The expressions of BAX in µ $LD_{50}$were greater than [! $LD_{50}$envenomed rats.

## DISCUSSION

The present investigation showed that the *E. Pyramidum* crude venom augments activity of hepatic enzymes and also produces histological alterations and oxidative stress, suggesting hepatotoxicity.

In this study, the injection of *E. Pyramidum* crude venom exerts possible hepatotoxicity as verified by the increase in serum ALT, AST, ALP activities and TB level. In fact,

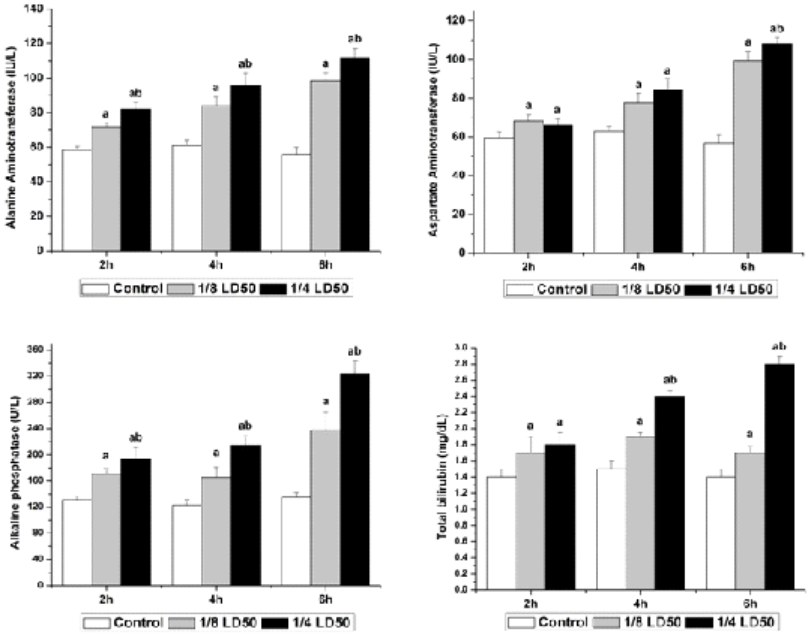


**Fig. 1.** Changes in liver function of rats induced by *E. pyramidum* crude venom after 2, 4 and 6 hrs

these enzymes are known as important markers of hepatocellular damage as affirmed by Abdel Moneim *et al*. (2011).The increased circulating levels of hepatic enzymes observed in the present work are similar to elevated serum activity of the same enzyme after rats and mice envenomations (Al-Sadoon *et al*., 2013; Tohamy *et al*., 2014).

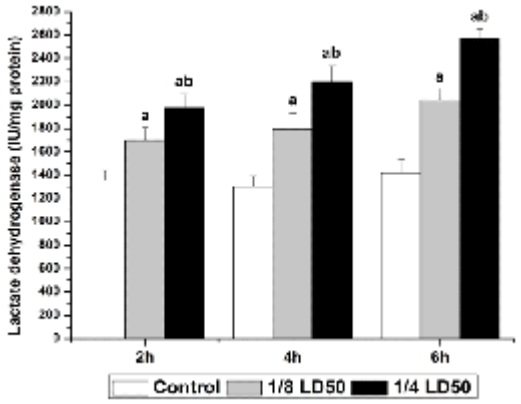


**Fig. 2.** Level of liver lactate dehydrogenase (LDH) of rats induced by *E. Pyramidum* snake venom after 2, 4 and 6 hrs

The venom active compounds responsible for the hepatotoxicity are still undetermined, but they probably involve phospholipases $A_2$ ($PLA_2$)action on cell membrane lipids and mitochondrial respiration (França *et al*., 2009). The secretory $PLA_2$ proteins present in venoms from the Viperidae that includes *Echis*. The $PLA_2$ enzymes were originally identified by their ability to hydrolyze the ester bond but snake venom $PLA_2$ proteins exhibit a wide range of pharmacological properties. These include myonecrosis, neurotoxicity, cardiotoxicity, anti-coagulant, hemorrhagic, hemolytic, and anti-angiogenic activities, and the ability to inhibit platelet aggregation (Conlon *et al*., 2013; Kini, 2005).

In the present study, we demonstrated a direct cytotoxic effect of *E. Pyramidum* crude venom on hepatic tissue confirmed by the increment in LDH activity. This elevation in LDH activity suggests a cytotoxic activity of *E.*

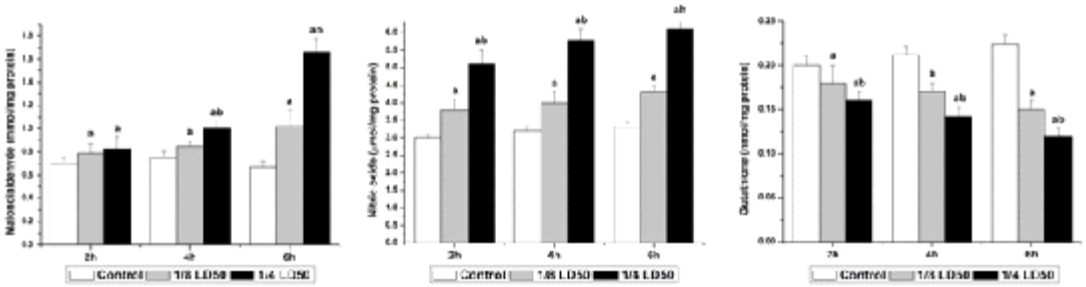

**Fig. 3.** Levels of liver lipid peroxidation (LPO), nitrite/nitrate (NO) and glutathione (GSH) of rats induced by *E. Pyramidum* snake venom after 2, 4 and 6 hrs.

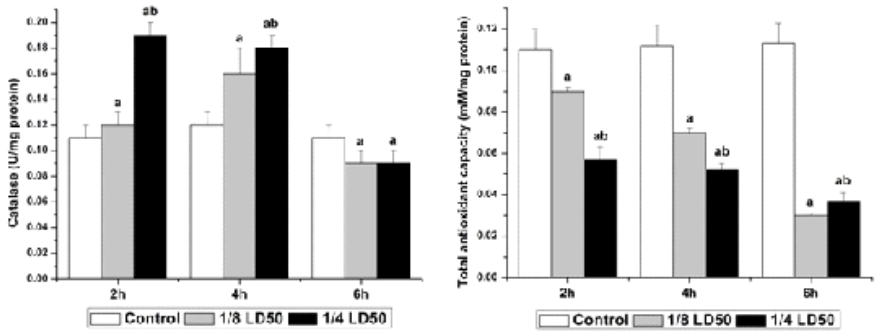


**Fig. 4.** Activity of hepatic catalase and level of total antioxidant capacity of rats induced by *E. Pyramidum* snake venom after 2, 4 and 6 hrs.

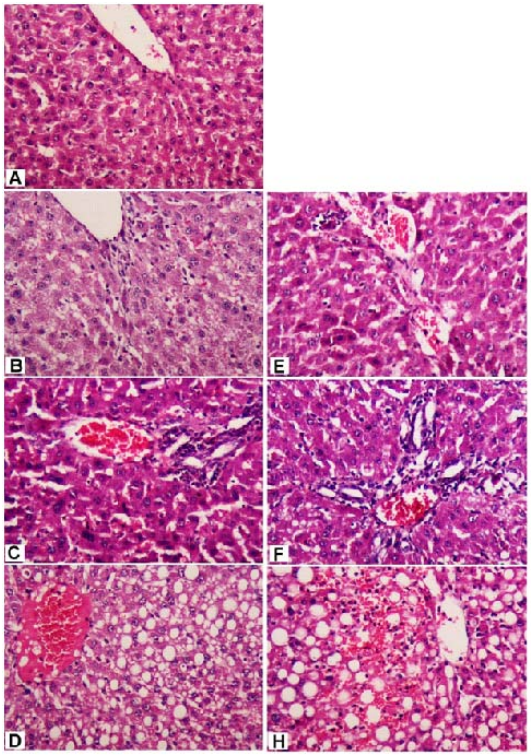


**Fig. 5.** (A): liver section from control showing normal architecture. (B & E): Section of liver tissues of rat after 2 hrs from envenoming with [! and µ$LD_{50}$*E. Pyramidum*snake venom showing prominent inflammation and pyknotic nucleus in hepatocytes. (C & F): Section of liver tissues of rat after 4 hrs from envenoming with [! and µ$LD_{50}$*E. Pyramidum*snake venom showing sever degeneration in hepatocytes, congested central vein, prominent inflammation and pyknotic nucleus in hepatocytes. (D & H): Section of liver tissues of rat after 6 hrs from envenoming with [! and µ$LD_{50}$*E. Pyramidum*snake venom showing sever degeneration in hepatocytes, congested central vein, prominent inflammation, hepatocytic vacuolation and pyknotic nucleus in hepatocytes.(400x).

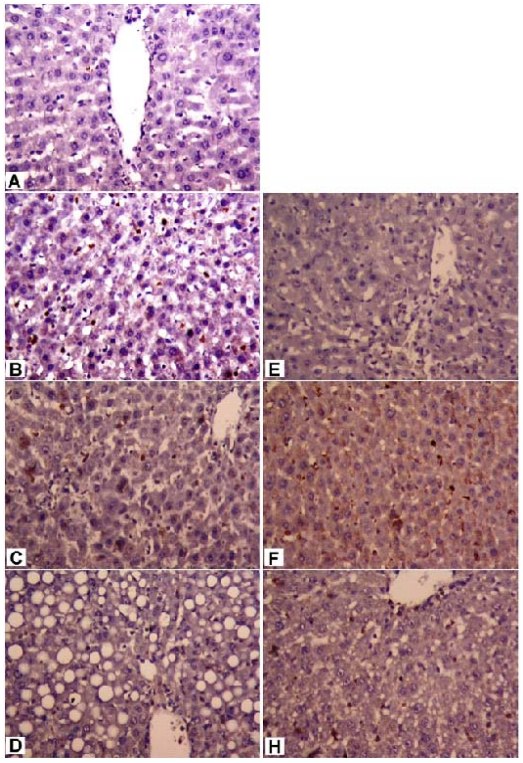


**Fig. 6.** Immunohistochemical localization of BAX antigen in liver tissue of rats. (A): liver section from control showing no staining of BAX. (B-H) liver section from [! and µ $LD_{50}$*E. Pyramidum*snake venom injected rats showing gradual increase in BAX staining intensity. (400x).

*Pyramidum*venom *in vivo*. Our observations fully agree with experimental evidence of rapid and drastic degenerative events that occur in liver after venom injection.

To evaluate the ability of *E. Pyramidum* crude venom to generate an oxidative stress status in hepatic tissue, we choose to monitor one of the earliest responses of oxidative stress which is the increase in oxidative stress markers in the liver homogenates. To further demonstrate the implication of oxidative stress in venom induced toxicity, LPO was monitored. Lipid peroxidation is one of the suggested cytotoxic mechanisms of different venoms. The MDA is an end product of lipid peroxidation, considered as a late biomarker of oxidative stress and cellular damage. It is generally considered as an excellent index of lipid peroxidation (Othman *et al*., 2014). In the present study, injection of *E. Pyramidum* crude venom induced an increment in LPO, confirming an increase of free radicals production. This fact emphasizes that the oxidative damage is induced by the venom in the liver.

Many studies have described the involvement of cytokines in envenomation, including snake, scorpion, and spider venom models. To identify the impact of snake venom in terms of inflammatory activity, we analyzed the NO production in hepatic tissue after *E. Pyramidum* crude venom injection. Higher NO production

observed in hepatic tissue may be a consequenceof characteristics induced by this venom in host tissue, such as damage, prominent necrosis, hemorrhage, and edema, which are characteristics of *Echis* envenomation(Jarrar, 2011; Kumar *et al*., 2006). In fact, NO is also produced during the host response evoked by *E. Pyramidum*venom. This venom induced the synthesis of NO following intraperitoneal injections in rats, mainly through induction of expression of the inducible nitric oxide synthase (iNOS)(Luna *et al*., 2011).

Several works demonstrated that crude Viperidae venom induces apoptosis in different organs (Al-Sadoon *et al*., 2013; Tohamy *et al*., 2014) or isolated enzymes, such as $PLA_2$ (Cummings *et al*., 2000) and metalloproteases (De Moraes *et al*., 2008). Cell death is currently the subject of a considerable number of investigations. This interest stems, in part, from the potential of understanding oncogenesis and from the possibility of exploiting cell death program for therapeutic purposes (Bustillo *et al*., 2009; Yang *et al*., 2005).

Another interesting finding in this study was the observation of increased positivity of the pro-apoptotic protein, BAX following *E. Pyramidum* crude venom injection as shown in Figures 6. This protein is considered to be a key factor regulating apoptosis (Abdel Moneim, 2012). The expressions of BAX protein played a pivotal role in the regulation of apoptotic cell death and inhibited production of free radicals and oxidative stress-induced cell death. The expression ofBAX protein was increased significantly in rats injected with *E. Pyramidum*venom, which indicated that the cellular injury may be the result of the elevation of BAX expression. According to Chien *et al*. (2008) apoptosis was induced by activation of both ER pathway and mitochondrial death pathway which results in increased level of $Ca^{2+}$ and glucose-related protein 78.

In conclusion, the present study provides evidence that *E. Pyramidum* crude venom induces hepatotoxicity in rats, by increasing blood liver enzymes and oxidative stress levels and inducing apoptosis in hepatic tissue.These results provide valuable insight into the toxicity of *E. Pyramidum*. However, a deficit in our study is the usage of crude venom so we don't know which component leads to our results.

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**REFERENCES**

1. Abdel-Moneim, A.E., Dkhil, M.A., Al-Quraishy, S., The redox status in rats treated with flaxseed oil and lead-induced hepatotoxicity. *Biol Trace Elem Res* 2011; **143**: 457-467.
2. Abdel Moneim, A.E., Evaluating the potential role of pomegranate peel in aluminum-induced oxidative stress and histopathological alterations in brain of female rats. *Biol Trace Elem Res* 2012; **150**: 328-336.
3. Aebi, H., *Catalase in vitro. Methods Enzymol* 1984; **105**: 121-126.
4. Al-Sadoon, M.K., Abdel Moneim, A.E., Diab, M.M., Bauomy, A.A., Hepatic and renal tissue damages induced by Cerastes cerastes gasperetti crude venom. *Life Sci J* 2013; **10**: 191-197.
5. Aljanabi, S.M., Martinez, I., Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res* 1997; **25**: 4692-4693.
6. Belfield, A., Goldberg, D.M., Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. *Enzyme* 1971; **12**: 561-573.
7. Bergmeyer, H.U., Bergmeyer, J., Grassl, M., Methods of enzymatic analysis, 3rd ed. Verlag Chemie, Weinheim ; Deerfield Beach, Fla, 1983.
8. Bustillo, S., Lucero, H., Leiva, L., Acosta, O., Kier Joffé, E., Gorodner, J., Cytotoxicity and morphological analysis of cell death induced by Bothrops venoms from the northeast of Argentina. *J Venom Animals Toxins Trop Dis.* 2009; **15**: 28-42.
9. Casewell, N.R., Harrison, R.A., Wuster, W., Wagstaff, S.C., Comparative venom gland transcriptome surveys of the saw-scaled vipers (Viperidae: Echis) reveal substantial intra-family gene diversity and novel venom transcripts. *BMC Genom.* 2009; **10**: 564.
10. Chien, C.M., Yang, S.H., Chang, L.S., Lin, S.R., Involvement of both endoplasmic reticulum- and mitochondria-dependent pathways in cardiotoxin III-induced apoptosis in HL-60 cells. *Clin Exp Pharmacol Physiol* 2008; **35**: 1059-1064.
11. Conlon, J.M., Attoub, S., Arafat, H.,

Mechkarska, M., Casewell, N.R., Harrison, R.A., Calvete, J.J., 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* 2013; **71**: 96-104.

12. Cummings, B.S., McHowat, J., Schnellmann, R.G., Phospholipase A2s in Cell Injury and Death. *J Pharmacol Exper Therapeut.* 2000; **294**: 793-799.
13. De Moraes, C.K., Fritzen, M., Chudzinski-Tavassi, A.M., Selistre-de-Araújo, H.S., rACLF, a recombinant snake venom metalloprotease, activates endothelial cells in vitro. *J Venom Animals Toxins Trop Dis* 2008; **14**: 113-127.
14. Ellman, G.L., Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; **82**: 70-77.
15. França, R., Vieira, R., Ferrari, E., Souza, R., Osorio, R., Prianti-Jr, A., Hyslop, S., Zamuner, S., Cogo, J., Ribeiro, W., Acute hepatotoxicity of Crotalus durissus terrificus (South American rattlesnake) venom in rats. *J Venom Animals Toxins Trop Dis* 2009; **15**: 61-78.
16. Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal Biochem* 1982; **126**: 131-138.
17. Hasson, S.S., Theakston, R.D., Harrison, R.A., Cloning of a prothrombin activator-like metallo-proteinase from the West African saw-scaled viper, *E. ocellatus. Toxicon* 2003; **42**: 629-634.
18. Jarrar, B.M., Histological alterations and biochemical changes in the liver of sheep following *Echis coloratus* envenomation. *Saudi J Biol Sci* 2011; **18**: 169-174.
19. Kini, R.M., Structure-function relationships and mechanism of anticoagulant phospholipase A2 enzymes from snake venoms. *Toxicon* 2005; **45**: 1147-1161.
20. Kumar, V., Maheshwari, R., Verma, H.K., Toxicity and symptomatic identification of species involved in snakebites in the Indian subcontinent. *J Venom Animals Toxins Trop Dis* 2006; **12**: 3-18.
21. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275.
22. Luna, K.P., Melo, C.M., Magalhães, V.P., Martins-Filho, O.A., Pereira, V.R., Bothrops erythromelas snake venom induces a proinflammatory response in mice splenocytes. *Int J Infereron Cytokine Mediator Res* 2011; **3**: 9-18.
23. Meier, J., Theakston, R.D., Approximate LD50 determinations of snake venoms using eight to ten experimental animals. *Toxicon* 1986; **24**: 395-401.
24. Nishikimi, M., Appaji, N., Yagi, K., The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun* 1972; **46**: 849-854.
25. Ohkawa, H., Ohishi, N., Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**: 351-358.
26. Othman, M.S., Safwat, G., Aboulkhair, M., Abdel Moneim, A.E., The potential effect of berberine in mercury-induced hepatorenal toxicity in albino rats. *Food and Chemical Toxicology* 2014; **69**: 175-181.
27. Pedrycz, A., Czerny, K., Immunohistochemical study of proteins linked to apoptosis in rat fetal kidney cells following prepregnancy adriamycin administration in the mother. *Acta Histochem* 2008; **110**: 519-523.
28. Reitman, S., Frankel, S., A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; **28**: 56-63.
29. Schmidt, M., Eisenburg, J., Serum bilirubin determination in newborn infants. A new micromethod for the determination of serum of plasma bilirubin in newborn infants. *Fortschr Med* 1975; **93**: 1461-1466.
30. Tohamy, A.A., Mohamed, A.F., Abdel Moneim, A.E., Diab, M.S.M., Biological effects of *Naja haje* crude venom on the hepatic and renal tissues of mice. *J King Saud Uni - Sci* 2014; **26**: 205-212.
31. Valenta, J., Stach, Z., Kolar, M., Envenoming after a snakebite from the Northeast African saw-scaled viper *Echis pyramidum*: prolonged therapy upon failed treatment by antivenom. *Prague Med Rep* 2011; **112**: 226-235.
32. Warrell, D.A., Davidson, N., Greenwood, B.M., Ormerod, L.D., Pope, H.M., Watkins, B.J., Prentice, C.R., Poisoning by bites of the saw-scaled or carpet viper (*Echis carinatus*) in Nigeria. *Q J Med* 1977; **46**: 33-62.
33. Wlodek, D., Banath, J., Olive, P.L., Comparison between pulsed-field and constant-field gel electrophoresis for measurement of DNA double-strand breaks in irradiated Chinese hamster ovary cells. *Int J Radiat Biol* 1991; **60**: 779-790.
34. Yang, R.-S., Tang, C.-H., Chuang, W.-J., Huang, T.-H., Peng, H.-C., Huang, T.-F., Fu, W.-M., Inhibition of tumor formation by snake venom disintegrin. *Toxicon* 2005; **45**: 661-669.