HISTOPATHOLOGICAL CHANGES IN LIVER OF MICE AFTER EXPERIMENTAL ENVENOMATION WITH ANDROCTONUS AMOREUXI SCORPION VENOM

By

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Abstract

A total of 78 adult male Albino mice were divided into thirteen groups (6 mice in each). One served as a control group and the other twelve groups were venom treated groups. The mice of treated groups were injected with 0.1 ml saline solution in which a particular amount of scorpion venom. The first 6 groups were subcutaneously injected with 1/2 LD50 (0.05 µg/g body weight), while the other 6 groups were injected with 1/4 LD 50 (0.025 µg/g body weight) by the same route. The animals from each group were anesthetized with ethyl ether and sacrificed at different time intervals (3, 6, 9, 12 hrs, 4 & 7days post toxin administration).

The microscopic examination of liver tissue obtained from envenomed animals showed variable histopathological changes being severely increased with the time interval of envenoming. The most obvious changes in the liver were acute cellular swelling, hydropic degeneration, congestion of central veins and portal blood vessels. Besides, extramedullary hematopoiesis and invaginations in nuclei of hepatic cells, with formation of intranuclear cytoplasmic inclusions were observed.

Key words: Egypt, Androctonus amoreuxi, venom, histopathology, liver.

Introduction

Scorpions are common venomous animals in temperate desert and tropical habitats of the world. They can be found in the sea, up mountains, in both desert and the tropics, in caves, just above anywhere accept perhaps the Arctic and Antarctic (Fet et al., 2000). Scorpions are a world health problem in tropical and subtropical regions (Abbass et al, 2009). Great attention must be directed towards such animals to avoid or reduce their dangers (Shoukry and Morsy, 2011).

In Egypt, there are about 13 poisonous species of scorpions some of them are deadly poisonous (Abdel-Rahman et al, 2010). Seven genera such as leivrurs, Androctonus (family: Buthidae), Scorpionaurus (family: Scorpionidae) and Nebo uierichonticus (family: Diplocentrida) were collected from St. Catherine and Wadi Feiran regions (Ibrahim and Abdel-Rahman, 2011).
Androctonus amoreuxi scorpion is moderately abundant in many places in South Sinai and highly distributed in Upper Egypt. It is also common on the deserts of North Africa and South West Asia. It usually called fat tail scorpion, which produce one of the most potent and important venom of Buthidae. *A. amoreuxi* scorpion considered among medically important species in the world. It belongs to family Buthidae, genus *Androctanus* and species *amoreuxi* (Fet, 1997). The clinical manifestations of scorpion envenomation vary among different species. The severity of envenomation depends on the sting site as well as species, size and degree of agitation of the scorpion and symptoms only develop after 30 minutes and sometimes only after 4 to 12 hrs, increasing in severity over the following 24 hrs. Most of scorpion sting result in no more than to local pain, lasting to about 4 hours (De Roodt et al, 2001). The distribution of the venom in the body of the envenomed victims (Dosgupta et al, 1991; Revelo et al, 1996) is variable. In general, the target organs of the venom scorpions of Family *Buthidae* are heart, brain, blood, lungs, liver and kidney (Bhatt et al., 1988). Scorpion venom is composed of varying concentration of neurotoxin, cardiotoxin, nephrotoxin, hemolytic toxin and hepatotoxic (Nair et al., 1973; Marie and Ibrahim, 1976; Rochat et al, 1979). Histopathological studies carried out by Correa et al. (1997), Bessa-lem et al. (2003), Naser et al. (1992) and Patel et al. (1992) on another scorpion species, proved the occurrence of circulatory, degenerative and necrotic changes in the liver.

By literatures review, none was published on the hepatic histopathological effects of *Androctonus amoreuxi* scorpion venom. The present study aimed at the evaluation of mice hepatic changes after *Androctonus amoreuxi* envenomation.

**Materials and Methods**

The crude venom was obtained by the electric stimulation of the scorpion telson every week by using electric current according to the method described by (Deodoras and Vad, 1962).

Clean laboratory bred adult male Albino mice weighting 20±3.9 gm. were purchased from Theodor Bilharz Research Institute, Giza, Egypt. They were transferred to wire-bottomed cages at the Animal house of Zoology Department, Faculty of Science, Suez Canal University. The animals were kept at an optimal temperature and fed on a special rodent diet supplied by Medical Professions for the Veterinary Products and Fodders Additions Company (MUVCO). The mice were given fresh water through a glass bottle with a capillary dropper fixed to the cage wall in an available a position. The water was changed and the cages were cleaned every day. The mice were weighted just before the beginning of each experiment.

Determination of the LD50 of the Crude Venom: The LD$_{50}$ of the crude venom was calculated according to Meier and Theakston (1986). The lyophilized crude venom was reconstituted with physiological saline solution
(1mg venom/5ml physiological saline solution; 0.85% NaCl) then diluted into the following concentration: 0.12, 0.16, 0.2, 0.24, 0.28, 0.32, 0.36, and 0.4 μg/g body weight. 0.1 ml from each concentration was subcutaneously (SC) injected into adult male Albino mouse (20±3g in weight) respectively. After injection, the survival time (T) of each injected mouse (D) was recorded. Regression line was plotted by using the values of D/T versus D. The constant a (Slope) and b (LD50) of the regression line was calculated.

Experimental groups: a- Control group: Six mice were subcutaneously injected with 0.1 ml saline solution to be used as control animals. b- Envenomed group: Seventy two mice were divided into twelve groups (6 mice in each). Mice of all subgroups were injected with 0.1 ml saline solution in which a particular amount of scorpion venom, 6 group were subcutaneously injected with 1/2 LD50 (0.05 μg/g body weight), while the other 6 groups were injected with 1/4 LD 50 (0.025 μg/g body weight) by the same route. Animals from each group were anesthetized with ethyl ether and sacrificed at different time intervals (3, 6, 9, 12 hrs, 4 & 7 days post-envenomation, and compared with control ones to delineate the sequence of events caused by the venom at different time intervals of envenoming.

Light microscopic examination of the liver tissues obtained from envenomed animals showed variable histopathological changes being severely increased with the time interval of envenoming (Tab. 1).

Discussion
Gross pathological examination revealed that the body organs of the envenomed animals varied according to the time interval between venom injection and observation. The observed hepatic congestion after 3- 6 hrs from envenoming with different doses of Androctonus amoreuxi venom was previously observed by Correa et al. (1997) due to injection of Tityus serrulatus scorpion venom.

The present study clarified that injection of A. amoreuxi scorpion venom induced several serious histopathological alternations in the hepatic tissue of envenomed mice. The severity and degree of extent of these alterations were dose and time dependent.

Results
The histopathological changes induced in liver were recorded in mice envenomed with 1/4 LD50 and 1/2 LD50 doses of the crude venom. The effects were studied after 3, 6, 9, 12 hrs, 4 & 7 days post-envenomation, and compared with control ones to delineate the sequence of events caused by the venom at different time intervals of envenoming.

Histopathological examination: After scarification, liver was collected from all animals fixed in neutral buffered formalin 10% for 3 days at room temperature, dehydrated in different grades of ethanol, cleared in zylol, embedded in paraffin wax, and then sectioned at 4microns. The sections were stained with hematoxylin and eosin (Drury and Wallington, 1980).
Fig. 1: Histopathological changes of mice liver. (A): Control liver section showed cords of hepatocytes surrounding central veins (CV), and hepatic sinusoids (HS). (B): 6 hrs post-injection (PI) with 1/4 LD$_{50}$ of venom showing congestion of blood vessels (CB). (C): After 4 days (PI) with 1/4 LD$_{50}$ showed hydropic degeneration (HD), some nuclei of hepatocytes display karyolysis (KL) and karyorhexis (KH). (D): After 9 hrs (PI) with 1/2 LD$_{50}$ showed extramedullary hematopoiesis (arrows) and dilatation of hepatic sinusoids (DS). (E): After 4 days (PI) with 1/2 LD$_{50}$ showed dilated blood vessels (DB) with fibrinoid degeneration (FD) and deposition of faint basophilic homogenous material in the portal area (F): After 7 days (PI) with (PI) with 1/4 LD$_{50}$ showing hepatocyte- and karyomegally (arrows) (H&E, X200)
Fig. 2: (A): liver section of mouse after 12 hrs of subcutaneous injection of *Androctonos amorenxi* venom (1/2 LD$_{50}$) showed convoluted nuclei (CN) of some hepatocytes and loss of chromatin in others i.e. necrotic cells (NC) besides some hepatocytes display karyorhexis (KR) and karyolysis (KH). (B): After 9 hours of subcutaneous injection of *Androctonos amorenxi* venom (1/2 LD$_{50}$) showing necrotic cells (NC), nuclear invagination (NI) and apoptic bodies (arrows). (C): After 12 hrs of subcutaneous injection of *A. amorenxi* venom (1/2 LD$_{50}$) showed convoluted nuclei of some hepatocytes with intranuclear cytoplasmic inclusios (CI), besides loss of chromatin in others i.e. necrotic cells. (H&E, 400 X)
Alterations included variable degrees of cellular swelling, cytoplasmic changes, cellular necrosis and cellular damage accompanied with loss of common architecture the hepatic parenchyma at different stages of the envenoming. Cytoplasmic granularity and vacuolization of hepatocytes with any doses after 3-6 hrs agreed with (Omran and Abdel-Rahman, 1992). They revealed cytoplasmic granulation and vacuolization suggesting hydropic degeneration. Cytoplasmic vacuolization was in hepatocytes of animals envenomed with T. discrepans venom (D'Suze et al, 2004).

Cytoplasmic granularity and vacuolization were accompanied by swelling of the hepatocytes after injection of ¼ and ½ LD50 doses. Similar results were reported (Correa et al, 1997) in rats injected with fractions from the venom of Tityus serrulatus scorpion. Bessalem et al. (2003) illustrated the degenerative changes and enlargement of most of the hepatic cells in mice envenomed with Androctonus australis hector scorpion. Enlargement or swelling of the hepatocytes could be the main cause of constriction of the hepatic sinusoids and the disturbance of the architecture of the hepatic parenchyma. Similar observations were recorded in liver tissues of animals envenomed with Androctonus crassicauda (Jacobson and Jacobs, 1992) as well as Leiurus quinquestriatus (Omran and Abdel-Rahman, 1992) scorpions. Cellular swelling might be due to the action of venom phospholipase, which causes disturbance in cell membrane permeability with consequent influx of Na and water (Segelke et al, 1998). The enlargement of hepatocytes may be due to hypertrophy of heptocellular smooth endoplasmic reticulum. The dilatation of cisternae of the smooth endoplasmic reticulum was reported (Anderson and Ownby 1997). Dilatation of the endoplasmic reticulum could be attributed to the accumulation of positive sodium ions within the cells as well as entrance of extra-cellular water to the cell due disturbance of the cell membrane permeability induced by the venom (Krimm et al, 1999). Also, the present study showed obvious increase in size of some hepatocytes and their nuclei, megalocytosis, (cytomegaly and karyomegaly). Megalocytosis characterized by large number of swollen hepatocytes with abundant granular eosinophilic cytoplasm and occasional large nuclei (Kwon et al, 2007).

The karyolysis and karyorrhexis of the nuclei of envenomed mice could represent an advanced stage of nuclear changes, which may be attributed to activation of endonucleases enzymes located within the nuclei of the injured cells (Williams et al, 2000).

One of the obvious alterations of scorpion envenomation in the present study was presence of extramedullary haematopoiesis in the liver. Demasi et al. (2003) stated that the preferential uptake of zinc by edema fluid proteins lead to increase in zinc metabolism and activated macrophages. Activated macrophages release cytokines which in turn stimulates of pro-inflammatory peptides which increase vascular permeability. Extramedullary haematopoiesis in the liver was one anatomical marker of hypoxemia the function of
activated macrophage with cytokines regulation. The present study showed that the liver of envenomed mice suffered from many abnormal mitotic figures. There is either clumping or dispersal of chromatin, and these appear to be mitotic onset or late metaphase. The interpretation of mitotic disturbance (Maxie, 2007) after pyrrolizidine alkaloids which inhibit DNA synthesis and mitosis in the hepatocytes, but some are able to replicate their DNA without under-going mitosis, resulting in greatly enlarged hepatocytes with large convoluted nuclei. Some enlarged nuclei have cytoplasmic invaginations that can become entrapped as intra-nuclear inclusions. However, many hepatocytes in an affected liver do not become megalocytic. Those completely inhibited do not replicate DNA at all, whereas those that are more resistant can replicate more normally and give rise to nodular population of smaller, more normal hepatocytes. Inhibited hepatocytes and megalocytes are long-lived but eventually many undergo apoptosis. The present study showed presence of intranuclear inclusions spherical, retractile eosinophilic structures resulted in chronic injured liver under effect of hypoxia or intoxications. Interpreted of such phenomena that hepatocytes during autolysis can imbibe plasma that forms similar round but the less eosinophilic, and non-membrane-bound cytoplasmic inclusions. The hepatotoxicity develops more slowly than in response to toxicants that induce membrane per-oxidation, as many hepatocytes with DNA damage undergo apoptosis, and some megalocytosis, nuclear vesicular and nucleolar prominence, cytoplasmic intra-nuclear inclusions and cytoplasmic bile accumulations (Jessen et al, 2003). In this study, the fibrinoid degeneration and hyperplasia of endothelial cell of portal blood vessels was detected. Fibrinoid degeneration tissues accumulate deposits of an acidophilic homogeneous material that resembles the fibrin when stained. Fibrinoid material does usually contain fibrin and tends to be eosinophilic (staining with acidic dye eosin).

However, in some cases the fibrinoid deposits may contain the significant amounts of the nuclear debris, including acidic DNA and may be hematoxyphilic (staining blue with the basic dye hematoxylin) and these can be explain the presence of deposition of homologous basophilic materials in the portal area, around bile ducts and in sub-endothelium of the blood vessels. Fibrin indicates that the nearby blood vessels have become highly permeable and often destroyed (Kumar et al, 2005).

El Nasr et al. (1992) reported that the main toxic effect of B. quinquestriatus scorpion venom was primarily on the liver blood vessels of as manifested by dilatation of branches of the hepatic arteries and the portal veins together with the occurrence of signs of hemorrhage. Many other authors ensured the presence of hemorrhage in case of envenoming by different types of scorpions (Mohamed et al, 1978; Patel et al, 1992; Zare et al, 1994). Light microscopy of liver sections did not show hemorrhage.
References

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<th>dose</th>
<th>3 hrs.</th>
<th>6 hrs.</th>
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<td>1/4 LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>- Congestion of central veins and portal blood vessels. - Acute cellular swelling of hepatocytes with granular cytoplasm. - Clumping of nuclear chromatin in most hepatocytes - Karyomegaly with marked hyperchromacia of nuclei in numerous cells.</td>
<td>- Congestion of central veins (F. 1, B). - Acute cellular swelling of hepatocytes with granular cytoplasm. - Karyomegaly with marked hyperchromacia of few cells.</td>
<td>- Moderate congestion of central vein - Megalocytosis of hepatocytes - Karyomegaly of nuclei of numerous cells. - Necrobiotic changes indicated by clumping of nuclear chromatin.</td>
<td>- Liver tissue exhibited diffuse hydropic degeneration (F. 1, c), with occasional pyknotic nuclei.</td>
<td>- Megalocytes with karyomegaly (F. 1, F) - congestion - necrobiotic changes in hepatocytes.</td>
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<tr>
<td>1/2 LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>- Congestion of central veins and portal blood vessels. - Dissociation of hepatocytes. - Cytomegaly of some cells, mostly subcapsular. - Karyomegaly with marked hyperchromacia of nuclei in numerous cells. - Atypical mitotic figures</td>
<td>- Same as in 3 hrs group.</td>
<td>- Extramedullary hematopoeisis. (F. 1, D) - Degeneration of liver cells with granularity of cytoplasm. - Nuclear invaginations with intranuclear cytoplasmic inclusions (F. 2, B)</td>
<td>- Mild congestion of central veins. - Hepatocytomegally with obliteration of sinusoids. - Hyper-chromatic nuclei - Nuclear invaginations with intranuclear cytoplasmic inclusions (F. 2, A &amp;C).</td>
<td>- Fibrinoid degeneration &amp; deposition of homogenous basophilic materials in portal area, around bile ducts &amp; in subendothelium of blood vessels (F. 1- E) - Marked swelling in endothelial of blood vessels. - Some nuclei showed nuclear invaginations &amp; others showed convoluted contour. - Extramedullary hematopoiesis.</td>
<td>- Same as in 4 days group &amp; detection of apoptotic bodies &amp; some necrotic cells.</td>
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