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Histochemical Study of a *Cerastes cerastes* Hemorrhagic Toxin on the Liver*

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ABSTRACT. The effects of an intramuscular injection of the sublethal dose of the venom of the non-horned *Cerastes cerastes* snaks, as well as its purified hemorrhagic toxin "HR-a", on some cellular components of the liver cells of mice were histochemically demonstrated. After 24 h. of injection, depletion of polysaccharide and fat contents were observed in the hepatic cells of mice injected with the hemorrhagic fraction, whereas no changes were recorded in sections obtained from venom-treated animals. On the other hand, depletion of RNA contents was induced in cells of venom-injected animals, but no changes in such contents were detected in cells of HR-a injected animals. The hepatic cell's nuclei were mostly negative for DNA reaction in materials obtained from envenomated mice. However, margination of DNA was prominent due to the injection of the hemorrhagic toxin. A severe degree of protein depletion was also detected in the hepatocytes post-injection with either the venom or the hemorrhagic toxin.

Snake venom is a mixture of compounds, many of which are known to be proteins possessing marked enzymatic activities and lethal toxicities (Marinetti 1965). Hemorrhagic toxins are among the most important protein fractions which have been isolated and characterized by many researchers from the venoms of several snakes (Nikai *et al.* 1984, Bjarnason and Fox 1987, Mori *et al.* 1987). Snake venoms were elucidated by some researchers to induce certain histochemical alterations in the tissues of envenomated animals (Nikolic *et al.* 1963, Mohamed *et al.* 1978).

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The main purpose of the present study is to demonstrate the histochemical disturbances produced by the crude venom of the non-horned *Cerastes cerastes* and its purified hemorrhagic toxin (Rahmy *et al.* 1992), in the hepatic cells of mice. This might clarify whether the action of the venom is due to the presence of such hemorrhagic toxin or to a synergistic effect of several toxins existing together in the venom. It might also indicate the mechanism of action of these toxins within the hepatic cells of the treated animals.

Materials and Methods

The crude venom of the Egyptian sand viper *Cerastes cerastes* (non-horned members) was subjected to two steps of fractionation on sephadex G100 (superfine) and DEAE sephadex for the isolation of its hemorrhagic factor "HR-a" (Rahmy *et al.* 1992).

Fifteen adult Swiss mice (22±3 g in weight) were divided into three groups:

The first group included mice injected i.m. in the medial parts of their left thighs with 1 μg crude venom/g body weight, dissolved in 0.1 ml physiological saline solution (0.85% NaCl).

The second group was also injected in the same way with 0.1 ml saline solution containing 1 μg/g body weight of the purified hemorrhagic toxin.

The third group was left as a control group and comprised mice injected with 0.1 ml saline solution.

All the injected animals were sacrificed after 24 hr of the injection and samples from their livers were removed and fixed in Carnoy's or neutral formalin solution.

The carnoy-fixed materials were used for the following purposes:

1. Demonstration of general polysaccharide contents by Periodic Acid Schiff's reaction "PAS" (Mc-Manus 1946).
2. Illustration of total proteins by the Mercury Bromophenol Blue method (Bonahag 1955).
3. Visualization of both nucleic acids (DNA and RNA) by the Methyl Green Pyronin method of Kurnick (1950).

Formalin-fixed materials were applied post fixation in 1% OsO_4 to show lipid contents of the cell using the technique of Mallory (1961).

Results

Polysaccharide contents:

Polysaccharide contents of normal liver materials were demonstrated as intense red masses localized mainly at one pole of the hepatocytes. This polarization is identified as glycogen flight a phenomenon known to take place under the effect of the penetrating fixative which push the polysaccharide contents at one pole of the cell (Fig. 1). Examination of crude venom- envenomated liver tissues has shown almost the same intense deposits of polysaccharide materials as those located in the normal hepatic cells (Fig. 2). On the other hand, specimens obtained from mice treated with the hemorrhagic fraction have displayed a moderate to weak PAS reactivity, indicating average depletion of polysaccharide contents in this group as compared with the control materials (Fig. 3).

Lipid contents:

Hepatocytes of control animals demonstrated differently sized fat droplets scattered within the cell cytoplasm (Fig. 4). No detectable changes were observed in such contents in the hepatic cells of crude venom-injected animals as compared with control materials (Fig. 5). Hepatic cells obtained from animals injected with the hemorrhagic fraction have shown that lipid contents were differently distributed in the different cells; some cells became almost devoid of any fat contents, while others revealed an accumulation of large-sized fat droplets (Fig. 6).

Protein contents:

In sections of control materials treated with bromophenol blue, protein contents were present in the cytoplasm of the hepatic cells exhibiting an intense blue coloration (Fig. 7). The hepatic cells of mice envenomated with the crude venom have revealed a severe degree of protein depletion. The cells contained scattered cytoplasmic granules with faint to moderate stainability, alternating with numerous vacuoles with no protein contents (Fig. 8). Tissues examined from animals injected with the hemorrhagic fraction (HR-a) demonstrated a moderate reactivity for protein contents within the cytoplasmic areas and a still negative representation in the vacuoles (Fig. 9).

Nucleic acids (DNA and RNA):

RNA was detected in the cytoplasm of normal hepatic cells revealing a homogenous strong pink coloration. The nuclei of such cells were stained greenish blue indicating their contents of DNA (Fig. 10).

The hepatic cells of specimens obtained from crude venom-injected animals revealed RNA depletion reflected by a moderate to weak coloration. Numerous cytoplasmic vacuoles were also observed within those cells and had almost revealed no

RNA contents. The nuclei of the hepatic, sinusoidal and inflammatory cells were stained greenish blue indicating normal DNA contents. However, some hepatic nuclei showed negative DNA contents (Fig. 11).

Injection of the hemorrhagic fraction had induced depletion of the hepatic DNA contents which exhibited either a negative or a very faint greenish blue color. Margination of DNA materials was also displayed by some nuclei in this case. On the other hand, no detectable changes were observed in the RNA contents of the hepatic cell cytoplasm as represented in (Fig. 12).

Discussion

It is believed that snake venoms have different effects on the different metabolic pathways within the host (Mohamed *et al.* 1980). Glycogen depletion in liver cells of snake venom-envenomated animals was reported by several investigators (Mohamed *et al.* 1975, 1978 and 1980), and fatty changes were mostly encountered in liver tissues because the cells of such organ are principally involved in fat metabolism and largely dependent upon lipids for energy production (Robbins and Angell 1976). The depletion of both glycogen and fat contents, due to injection of fraction HR-a, could be attributed to the hypoxic state of the hepatic cells at this stage. This hypoxia might be due to the deprivation of aerobic oxidative respiration of the cells due to either mitochondrial alterations (Jennings *et al.* 1969) or insufficient supply of blood oxygen to the liver cells as a result of the severe hemorrhage induced by such fraction (Rahmy *et al.* 1992). Hypoxia induces mobilization of the cell's stored glycogen to be used as a source of energy and consequently mobilization of the fat contents of the cells after the consumption of the stored glycogen contents (Robbins and Angell 1976).

On the other hand, the depletion of protein contents in liver cells of the treated animals could be attributed to the proteolytic activity of both the venom and its hemorrhagic fraction (Rahmy *et al.* 1992).

Moreover, the acidophilia of the hepatic cytoplasm, due to venom injection, could be contributed in part for denaturation of the cytoplasmic protein, and in another part, in activation of acid RNase enzymes which destroy normal basophilic cytoplasmic RNA (Robbins and Angell 1976). The depletion of both RNA and protein contents could be also attributed to the effect of lysosomal RNase and protease enzymes released from the lysosomes as a result of the cytotoxic activity of the venom (Robbins and Angell 1976).

The normal appearance of RNA contents in tissues obtained from HR-a-injected mice might indicate that this fraction does not have RNase activity, or that the cellular degeneration of liver cells induced by such fraction was not severe enough to induce the release of the lysosomal RNase to exert its action.

General polysaccharides (PAS reaction): Counter stained with HX.

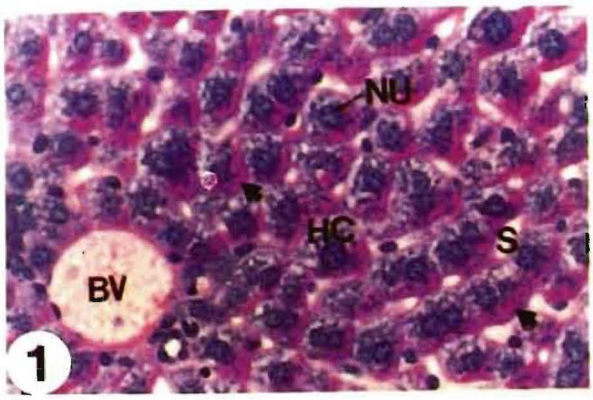

Fig. 1. Liver of control mouse showing intense polysaccharide contents (Arrows).

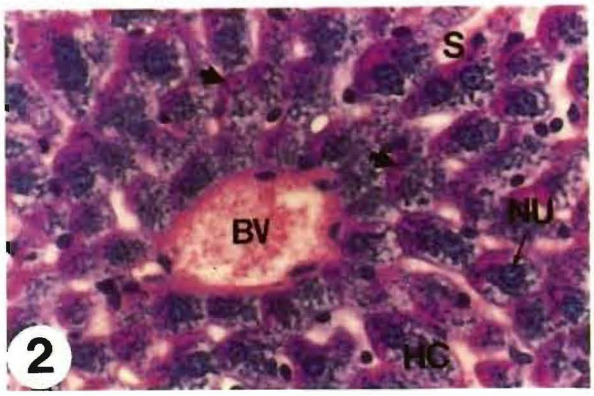

Fig. 2. Hepatocytes of crude-venom envenomated mice still exhibiting intense PAS reactivity (Arrows).

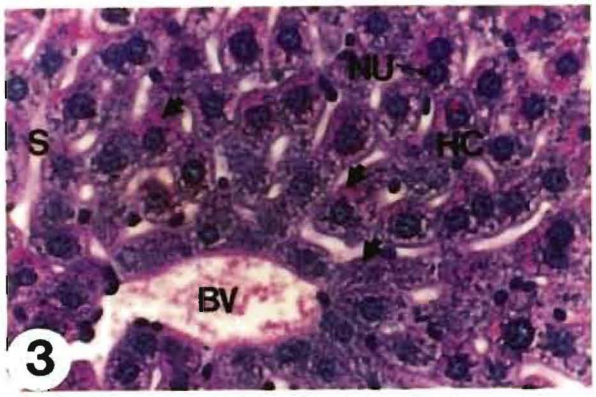

Fig. 3. Glycogen depletion (Arrows) in liver cells of mouse injected with HR-a.

Lipid contents: (OsO_4-post-fixation).

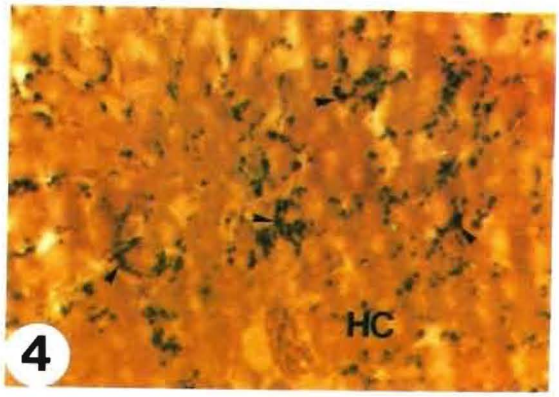

Fig. 4. Lipid inclusions in control liver cells (Arrow heads).

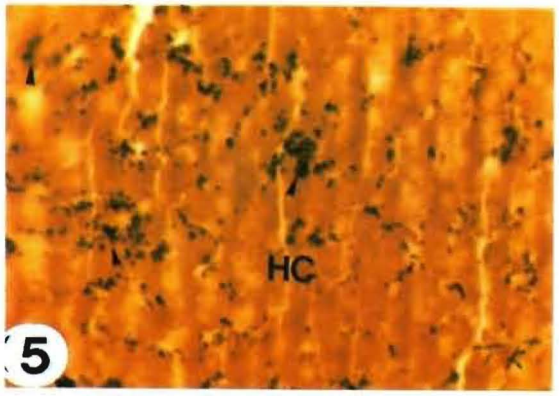

Fig. 5. No apparent changes in lipid inclusions (Arrow heads) of hepatocytes obtained from envenomated mice.

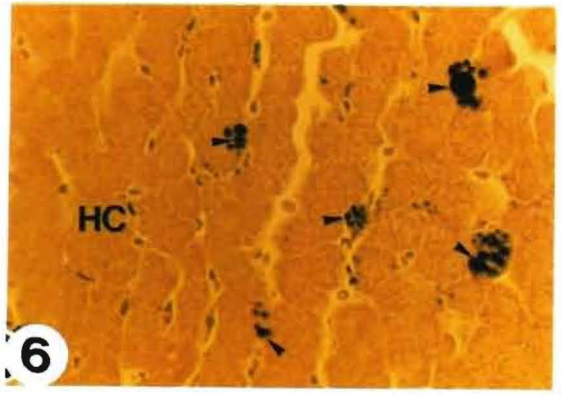

Fig. 6. Diminution of fat contents in liver cells of animals injected with HR-a. Note the presence of large-sized lipid vacuoles (Arrow heads).

Total proteins (Mercury Bromophenol Blue reaction).

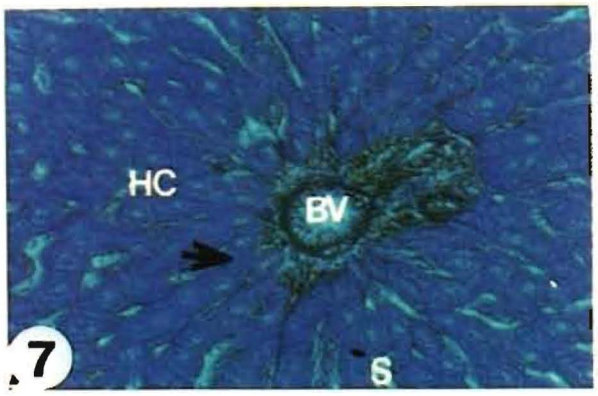

Fig. 7. Intense protein contents in liver cells of normal mice (Arrow).

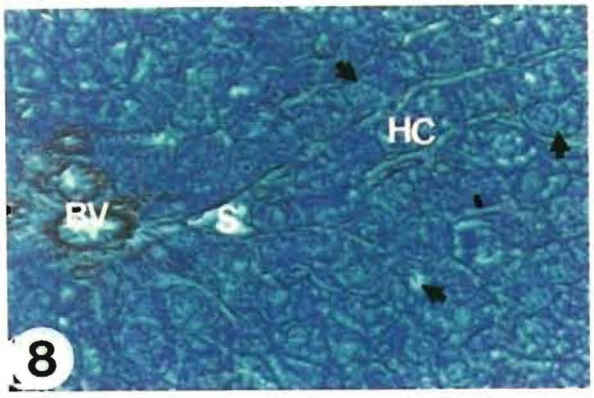

Fig. 8. Severe protein depletion (Arrows) in hepatocytes of crude venom-injected mice.

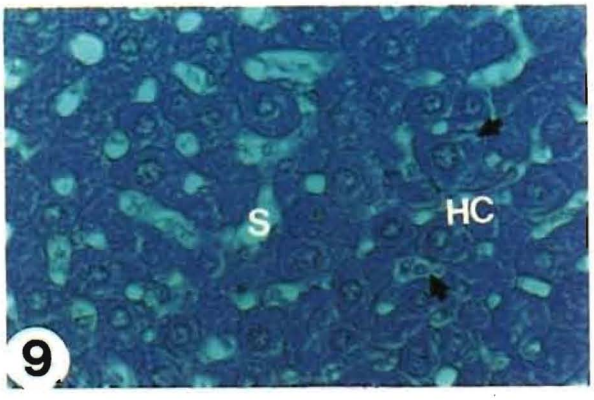

Fig. 9. Hepatic cells of mice injected with HR-a showing moderate protein contents. Note sites of protein depletion (Arrows).

Nucleic acids (Methyl Green Pyronine reaction).

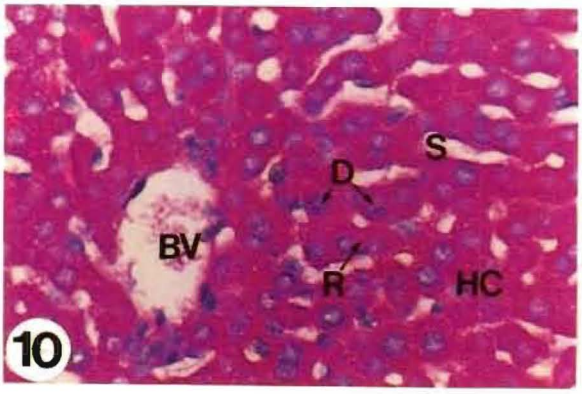

Fig. 10. Control hepatocytes showing strong pink coloration of RNA contents (R) in the cytoplasm, and greenish blue coloration of DNA contents (D) in the nuclei.

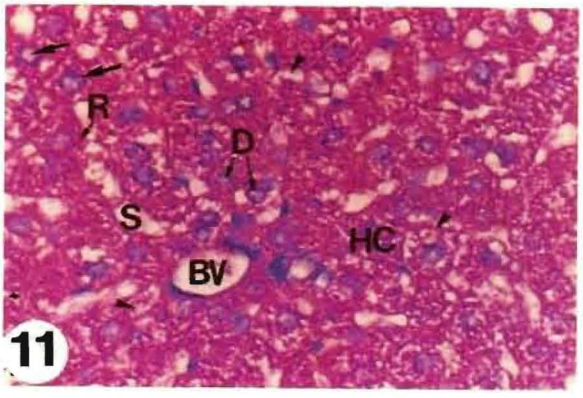

Fig. 11. Highly depleted RNA contents (Arrow heads) together with moderately depleted DNA contents (Long arrows) in hepatocytes of crude venom-injected mice.

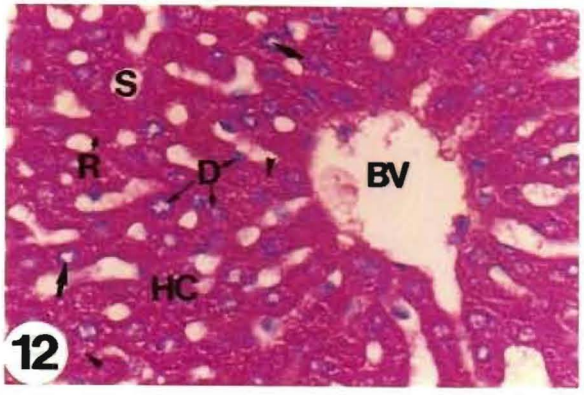

Fig. 12. Rather normal contents of RNA (Arrow heads) with depletion and margination of DNA materials (Long arrows) in liver cells of mice treated with HR-a.

HC: Hepatic cell
BV: Blood vessels
S: Sinusoids
R: RNA contents
D: DNA contents

Depletion of DNA contents in the nuclei of some hepatic cells due to venom injection might be due to either the direct action of the venom, or due to necrotic changes that occurred following cellular injury of the cytoplasm (Rahmy *et al.* 1992). Coagulative clumping and margination of chromatin in liver cells of hemorrhagic fraction-injected mice indicate that those cells are going through a necrotic process (Robbins and Angell 1976).

In conclusion, it could be suggested that the histochemical alterations induced by the venom or its hemorrhagic fraction on liver tissues might be explained in view that this tissue is involved in the degradation of such toxin as a main detoxifying system of the animal body. It could be also marked that the effects induced by the crude venom on the hepatic cell contents of the mice is not due to direct effect of the hemorrhagic toxin, but could be attributed to the action of several factors acting together synergistically.

Table 1. Intensity of cellular contents in liver tissues of control and treated mice

Reaction / group	Poly saccharides	Lipids	Proteins	Nucleic Acids	
				RNA	DNA
Control group	+++	+++	+++	+++	+++
Crude venom treated group	+++	+++	+	+±	++
Hemorrhagic Toxin treated group	±	+	++	+++	+

+++: Intense content ++: Moderate content +: Weak content

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دراسة هستوكيميائية على تأثير الجزيء المسبب للنزيف من سُم الأفعى المقرنة على الكبد

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تم هستوكيميائيا دراسة تأثير حقن الجرعة الأقل من المميتة من سم الأفعى المقرنة وكذلك تأثير حقن الجزيء المفصول منها والمسبب للنزيف على مكونات خلايا الكبد في فئران التجارب بعد ٢٤ ساعة من الحقن. وقد أظهرت النتائج حدوث نقصان في محتوى السكريات العديدة والدهون في أنسجة الكبد المأخوذة من الفئران المحقونة بالجزيء المسبب للنزيف. من ناحية أخرى ظهر نقصان في مضمون حمض الريبونيكليك في الأنسجة الكبدية للفئران المعاملة بالسم وقد اختفى حمض الديوكسي ريبونيكليك من معظم نوايا الخلايا الكبدية للفئران المحقونة بالسم. أما نوايا الخلايا الكبدية للحيوانات المعاملة بالجزيء المسبب للنزيف فقد أظهرت أيضاً اختفاء لذلك الحمض بالإضافة إلى حدوث تجمع له على الحواف الداخلية لأنوية بعض الخلايا.

بالإضافة إلى ما سبق فقد شوهد تناقص حاد في المحتوى البروتيني للخلايا الكبدية سواء في الفئران المحقونة بالسم أو الأخرى المحقونة بالجزيء المفصول منه.