

## Liver Damage Induced by *Cerastes cerastes* Venom and a Purified Hemorrhagic Toxin

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**ABSTRACT.** Histological alterations produced in the liver tissues of mice intra-muscularly injected with a sublethal dose of the crude venom of the non-horned *Cerastes cerastes* snake were studied after 24h of envenomation. The hepatocytes were noticeably hypertrophied, exhibiting distinct degenerative changes accompanied with disorganization of the hepatic lobular structure. The blood sinusoids were constricted and their endothelial linings were markedly deteriorated. The collagen lying around the blood vessels and the sinusoids have lost their continuity being demolished and dissolved at certain points.

Moreover, the pathogenesis induced by a pure hemorrhagic fraction (HR-1) isolated from the crude venom, on the liver tissue of mouse, was microscopically studied after 24h. of intramuscular injection of a sublethal dose of it. Light microscopic observations revealed cytoplasmic granulation and vacuolation, together with focal areas of cellular degeneration. Hepatic sinusoids were engorged with RBC's and endothelial damage was displayed. Severe degree of collagenolytic activity was also prominent.

It is well known that venoms of Viperidae and Crotalidae snakes exert very strong histolytic consequences (Tu *et al.* 1969), and that hemorrhage is a typical feature detected post the injection of these venoms (Ohsaka 1979), Tu (1977) reported that hemorrhage is not restricted to local effects, but also evident in systemic action in many internal organs. Nikai *et al.* (1984 and 1985) added that systemic hemorrhage was observed in the liver, kidney, lung and heart tissues of animals administered with hemorrhagic toxins isolated from the venom of several snakes.

The aim of the present study is to assess the pathogenesis induced by the crude venom of the non-horned *Cerastes cerastes* snake and its pure isolated hemorrhagic fraction in the liver tissues of mice. This might clarify the mechanisms of action of these toxins in inducing their effect and ascertain whether the isolated hemorrhagic fraction would produce hemorrhage only or the full range of different lesions caused by the whole venom.

### Materials and Methods

The crude venom of the non-horned *Cerastes cerastes* snake was subjected to two steps of fractionation on sephadex G100 (superfine) and DEAE sephadex for the isolation of its hemorrhagic factor "HR-1". The purity of the fraction was examined on polyacrylamide gel electrophoresis (Rahmy *et al.* 1992).

#### Histological preparations

Fifteen adult Swiss mice ( $22 \pm 3$  gm. in weight) were used in the present work being divided into three groups:

The first group included mice injected intramuscularly (i.m.) with 1 ug crude venom/gm body weight, dissolved in 0.1 ml physiological saline solution.

The second group comprised mice i.m. injected with 0.1 saline solution containing 1 ug/gm body weight of the pure isolated hemorrhagic factor.

The third group was left as a control group, i.m. injected with 0.1 saline solution.

All animals were sacrificed after 24h. of the injection and samples were taken from their livers and fixed in Carony's solution then prepared for histological examination (Hx and E) and the demonstration of collagen fibres (Masson trichrome method).

### Results

#### Histological observations

The hepatic tissues of normal mice (Figs. 1 and 2) is composed of a number of polyhedral hepatic lobules attached together by scanty connective tissue elements which contain small portal areas (Fig. 2). The hepatic lobular parenchyma (Fig. 1) consists of radiating plates of polyhedral hepatocytes radially arranged around a central vein. The spaces between the plates are occupied by hepatic sinusoids.

Histological Observations: (Hx and E) x 40

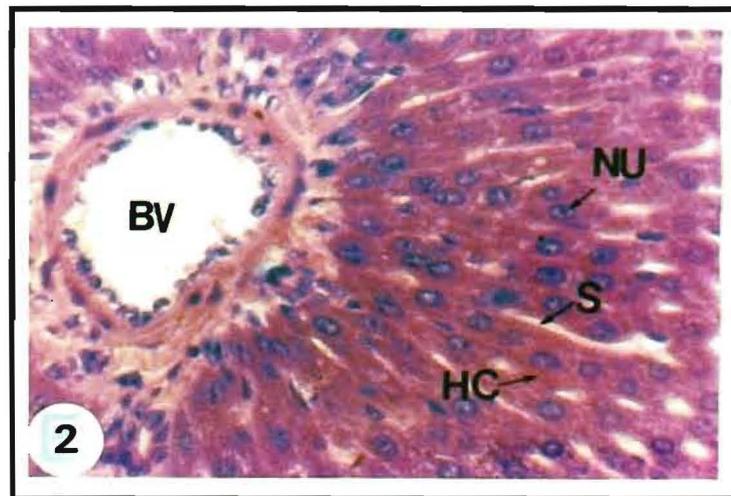
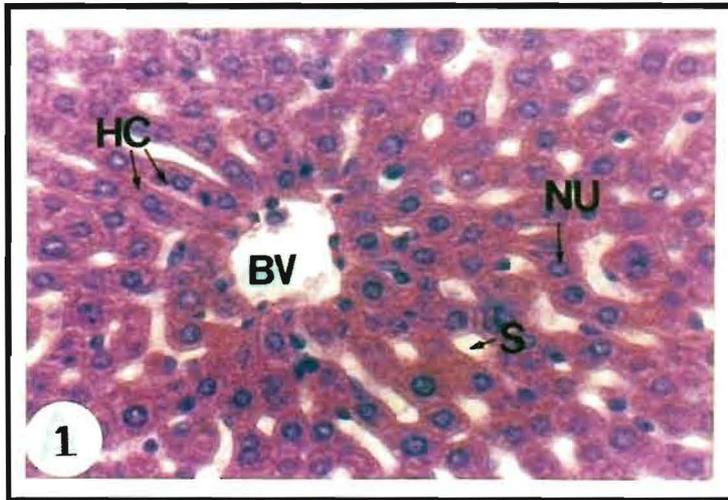
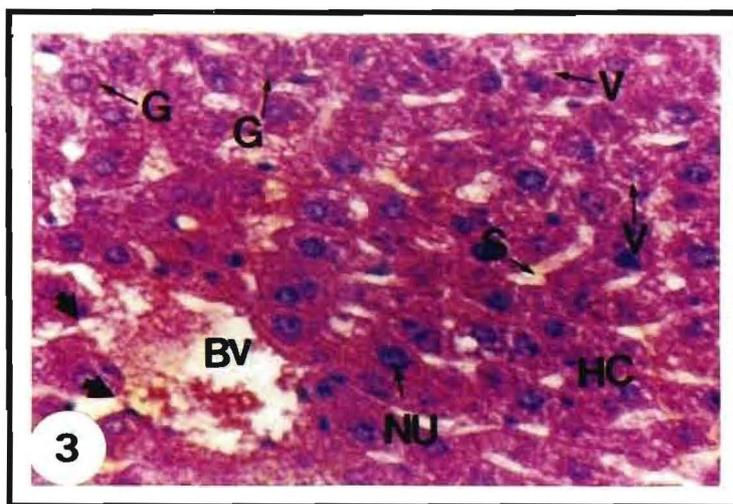
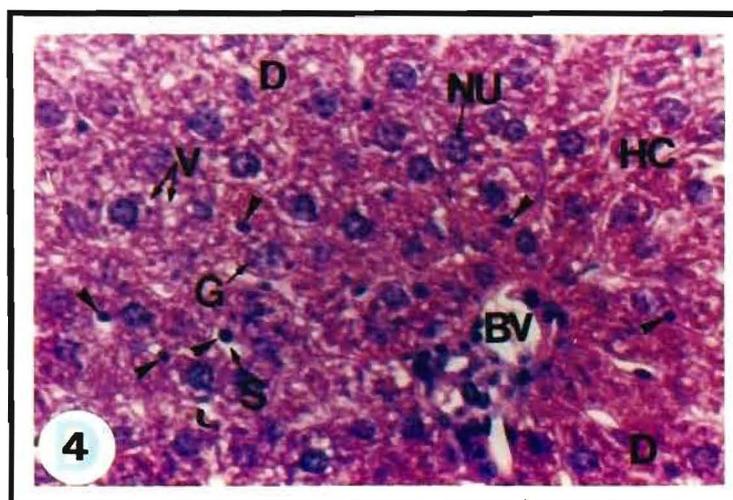


Fig. (1-2). Control liver sections.

Envenomation with the crude venom caused disorganization of the hepatic lobular structure and alteration of the general radiating architecture of the hepatic parenchyma (Figs. 3 and 4). The hepatocytes were extremely swollen and their cytoplasm exhibited a severe degree of granular degeneration accompanied with the presence of numerous clear vacuoles denoting fatty or hydrobic degeneration (Fig. 4). Areas of cellular necrosis and damage were also noticed containing deteriorated cells with ruptured membranes and disorganized cytoplasm (Fig. 4). The cell cavity at these cells contained pyknotic nuclei embedded in few granular structures and cell debris. The damaged cells expelled their contents at the intracellular spaces (Fig. 4).



**Fig. (3-4).** Swollen hepatocytes of envenomated mice exhibiting cytoplasmic granulation (G) and vacuolization (V). Note disorganization of the hepatic lobular structure and focal areas of cellular damage (D). Note blood vessels with damaged endothelium (Arrows).



The hepatic sinusoids were completely blocked in many areas due to the extreme swelling of the hepatic cells (Fig. 4) and kupffer cells were highly activated and became rounded in shape (Fig. 3). Blood capillaries were engorged with blood and their endothelia showed areas of damage and degeneration (Fig. 3).

Hepatocytes of animals injected with HR-1 were less severely affected as compared to those obtained from crude venom-injected animals. But nonetheless, cytoplasmic granulation with some vacuolization were common (Figs. 5 and 6), but no cellular swelling was detected. Focal areas of cellular degeneration were apparent in which the cell cytoplasm were partially deteriorated and their cell membranes were damaged at certain points (Fig. 5 and 6).

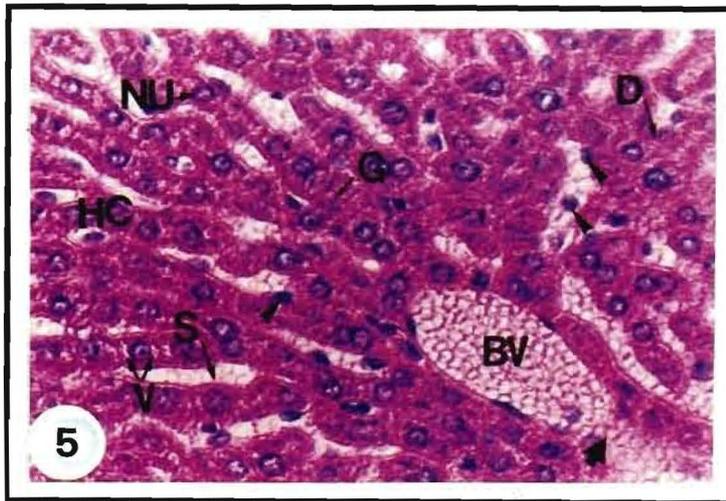
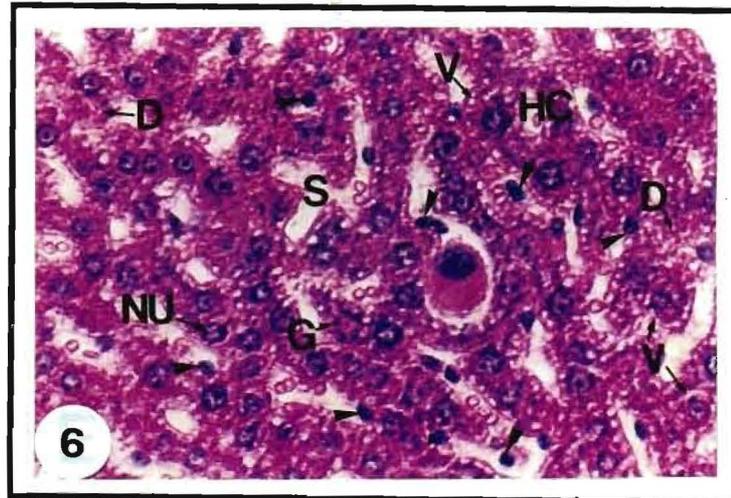


Fig. (5-6). Liver cells of mice treated with HR-1 showing necrobiotic changes (V, G). The blood vessel (BV) is engorged with RBCs and displayed damaged endothelium (Arrow). Note the presence of numerous phagocytes (Arrow heads) in the sinusoidal spaces. Note focal areas of cellular damage (D).



Blood vessels and hepatic sinusoids were engorged with RBCs and areas of damage was displayed by some vessels (Fig. 5). The blood cells were extravasated through those gaps (Fig. 5). Rounded phagocytes were also seen in the sinusoidal spaces as illustrated in Figure (5).

#### Demonstration of collagen fibers

Collagen fibers of normal hepatic tissues appeared mainly attached to the outer basal lamina of the endothelial cells lining the blood vessels as well as around the hepatic sinusoids (Figs. 7 and 8).

Demonstration of Collagen Fibers: (Masson trichrome) x 40

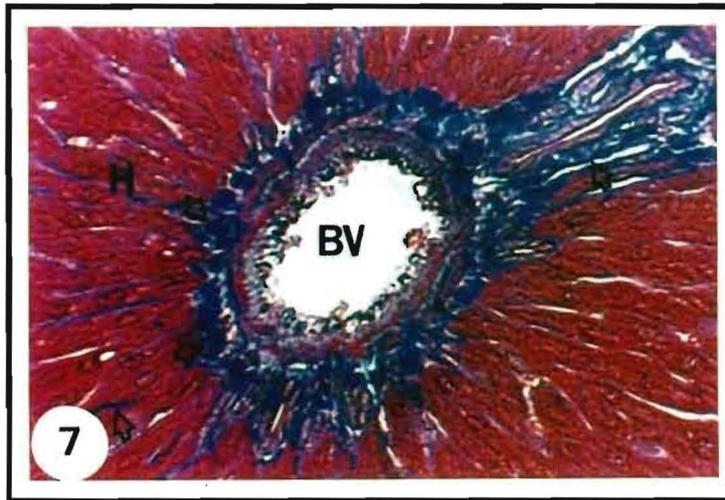
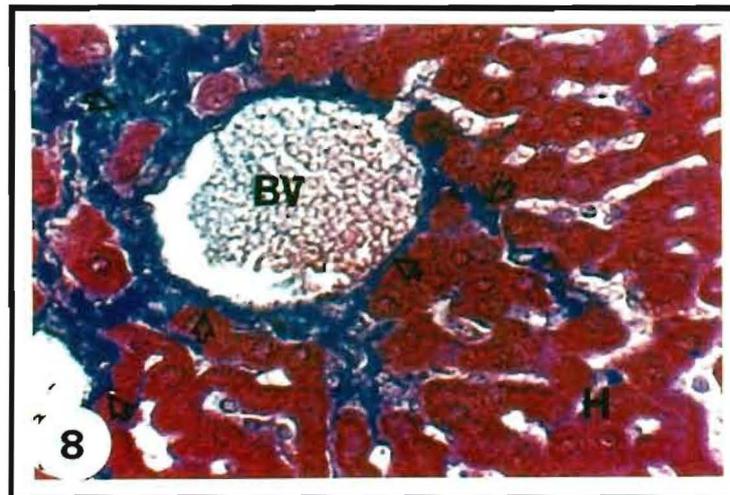


Fig. (7-8). Collagen fibers of normal liver tissues (Arrows).



Hepatic tissues of crude venom envenomated-animals showed identical observations of predominant collagenolysis (Figs. 9 and 10). Collagen fibers lying around the blood vessels have lost their continuity and appeared to be dissolved at certain points at which endothelial damage were presented. They were decreased in intensity and exhibited a faint blue colouration. Collagenolysis was also displayed by fibers coating the sinusoidal spaces.

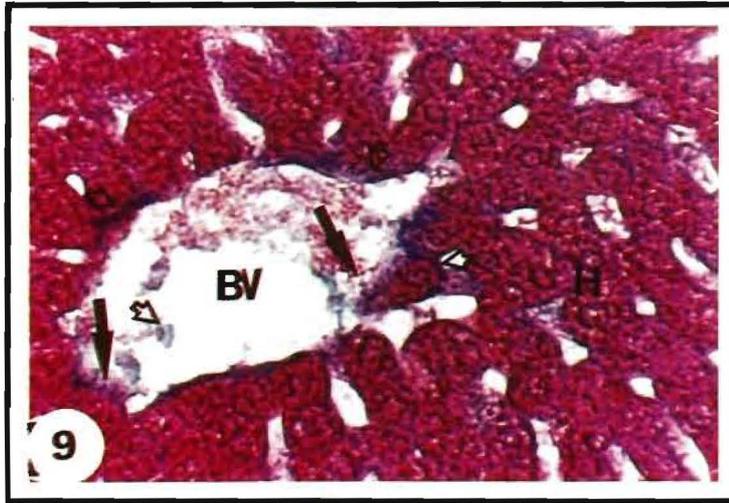
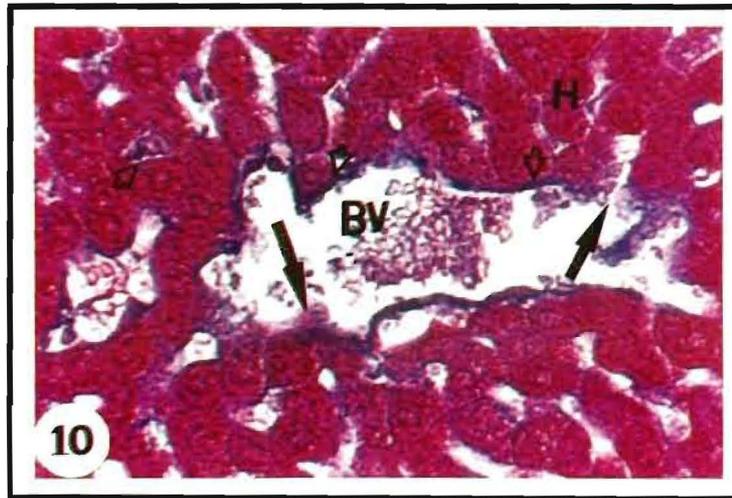


Fig. (9-10). Liver section of envenomated mice showing areas of collagen dissolution (Arrows) around the blood vessels (BV).



The purified toxin showed severe signs of collagenolytic activity. Remnants of dissolved fibers, with faint blue stainability were the only structures that remained around the blood vessels and in between the hepatocytes (Figs. 11 and 12). The fibers have lost their normal arrangement and disappeared at different areas.

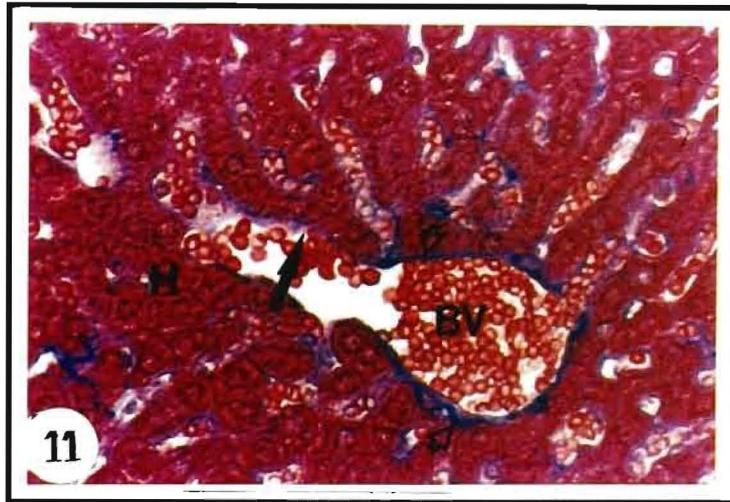
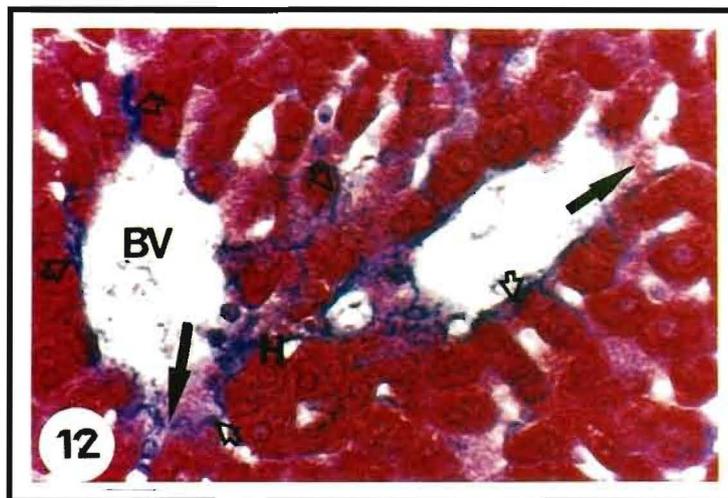


Fig. (11-12). High degree of collagenolysis (Arrows) in hepatic tissues of mice treated with HR-1. Open arrows indicate collagen fibers. HC: Hepatic cells  
BV: Blood vessel  
NU: Nucleus  
S: Sinusoid



## Discussion

In the present study, hemorrhage was the most prominent feature displayed by the hepatic tissues of animals injected with either the crude venom or the hemorrhagic fraction. These findings receive good support from the observations reported by Tu and Homma (1970), Vest (1981), Kishida *et al.* (1985), Nikai *et al.* (1984, 1985 and 1986) and Maung (1985) using different types of snake venoms and some of their isolated hemorrhagic toxins. The dissolution of the collagen fibres located around the blood capillaries - induced by venom intoxication - might suggest that collagen fibres are the first target to be attacked by the venom, or the used fraction for inducing their hemorrhagic activities. It might also indicate that the collagenolytic activity of the toxin plays a major role in its mechanism of action (Rahmy 1989).

Kaiser and Raab (1967) concluded that all Viperidae venoms possess a collagenolytic action which might play an important role in inducing hemorrhage. Moreover, Fox and Bjarnason (1983) reported the collagenolytic specificity of the hemorrhagic toxins on the pericellular collagenous basement membranes and other connective tissue collagens.

On the other hand, Hong (1982) mentioned that the collagenolytic activity is not directly responsible for the hemorrhagic effect observed in snake envenomation. Nevertheless, it is possible that the hemorrhagic action of the venom is not only due to its collagenolytic specificity, but it might also be due to other specificities that could be studied in the future such as elastase action for example.

From another angle, the disorganization of hepatic lobular structure and alteration of its general architecture which were observed after 24h. of venom injection were also reported before in case of *Vipera ammodytes* (Edlinger and Dietal 1959) and *Naja nigricollis* (Mohamed *et al.* 1974a) envenomation and was attributed to the cytotoxic properties of the venom.

The vacuolization and granular degeneration of swollen hepatocytes due to crude venom injection, were also referred to in case of *Walterinnesia aegyptia* (Gitter *et al.* 1962), *Naja nigricollis* (Mohamed *et al.* 1974b), *Naja naja* (Mohamed *et al.* 1978) and *Pseudocerastes fieldi* (Hassan *et al.* 1986) envenomation. The swelling of the hepatocytes could be attributed to an alteration in the permeability of the cell membranes leading to an increase of embition water which is then followed by cellular degeneration.

In a publication issued by Robbins and Angell (1976) they postulated that cellular swelling is virtually the standard primary morphologic response to all forms

of cell injury. It implies loss of intracellular reserves of energy or increased permeability of cell membranes leading to an increase of intracellular water. As water sufficiently accumulates within the cells, it produces clear cytoplasmic vacuoles indicating hydropic degeneration (Rahmy 1985).

Cytoplasmic vacuolization might also indicate fatty degeneration which is the most aminous of the cellular degenerations (Robbins and Angell, 1976). The clear appearance of these fatty vacuoles could be attributed to the dissolution of its fat contents by the chemicals used during the histological processing steps.

Moreover, it was added by Porras (1970) that snake venoms contain components such as phospholipase, protease and hemorrhagic toxins which could damage the membranes and result in loss of volume control. It might also increase the availability of the reducing agent glutathione for membrane protection, and thus cause rupturing of the cell membrane (Ehrich 1982).

The cellular necrotic lesions detectable in the liver cells due to venom injection could be an extension of the cell injury also observed previously by Robbins and Angell (1976). Liver cell necrotic lesions were similarly observed by Preston *et al.* (1988) due to injection of Australian snake venom. These lesions contained hepatocytes with ruptured membranes and highly deteriorated cytoplasm which might be due to the progressive degradative action of intracellular enzymes on the injured cells (Robbins and Angell 1976). This might be also due to sufficient accumulation of intracellular water which may distend the cell until it ruptures the plasma membranes and coalesces with adjacent cells (Robbins and Angell 1976).

The pathogenesis induced in liver cells of animals injected with the hemorrhagic fraction supports those elucidated by Aswell *et al.* (1979), Willkins *et al.* (1980) and Ehrich (1982) using different types of hemorrhagic fractions isolated from different venoms.

The activation of sinusoidal cells, and the predominance of lymphocyt and phagocytes in the hepatic sinusoids due to injection of the venom or its fraction might indicate that the observable hepatic lesions was caused by lymphocytic attack against hepatocytes (Dienes *et al.* 1982). This result again suggests that the necrotic effect revealed by the hepatic cells is secondary to the endothelial damage and the consequent hemorrhagic and inflammatory actions.

In conclusion, it could be suggested that the pathogenic effects induced by the tested venom or its hemorrhagic fraction on the liver cells might be explained on the basis that these cells are involved in the degradation of such toxins (Robbins and

Angell 1976). It is also suggested that these pathogenesis might be due to either hypoxia, which occurs as a result of loss of blood supply, or deprivation of aerobic oxidative respiration or due to the accumulation of the toxin which may damage the osmotic environment of the cells, thus causing injury or cell death. Lastly, whatever might be the cause of the hepatic cell's alterations, the hemorrhagic toxins exert a direct lytic action on the endothelial cells of the hepatic sinusoids to induce their hemorrhagic actions.

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(Received 20/01/1993;  
in revised form 27/04/1994)

## تحطم الأنسجة الكبدية نتيجة لحقن سم الأفعى المقرنة والجزئ النقي المفصول منها والمسبب للنزيف

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تضمنت الدراسة الحالية التغيرات الهيستولوجية التي تحدث في أنسجة كبد الفئران المعاملة بالجرعة الأقل من المميتة من سم الأفعى المقرنة بعد ٢٤ ساعة من الحقن في العضلات . وقد انعكست هذه التغيرات على هيئة ظهور انتفاخ في الخلايا الكبدية مصحوباً بتغيرات انحلالية في هذه الخلايا بالإضافة إلى حدوث اضطراب في التنظيم المميز للفصوص الكبدية .

وقد ظهرت الجيوب الكبدية ضيقة ومبطنة بخلايا اندوسيلية (بطانية) محطمة وفقدت ألياف الكولاجين المحيطة بالأوعية الدموية والجيوب الكبدية استمراريته وقد ذابت هذه الألياف في بعض المناطق المحددة .

من ناحية أخرى تم في هذه الدراسة فصل الجزئ المسبب للنزيف من السم في صورة نقية ، واطلق عليه اسم (HR-1) وقد تم أيضاً متابعة التغيرات المرضية التي يحدثها هذا الجزئ في أنسجة الكبد في الفئران المحقونة بهذا الجزئ بعد ٢٤ ساعة من الحقن في العضلات . وقد أظهرت الفحوص الميكروسكوبية حدوث تحجب وتحوصل في سيتوبلازم الخلايا الكبدية بالإضافة إلى ظهور مناطق للإنحلال الخلوي الموضوعي في هذه الخلايا وقد كانت الجيوب الكبدية محتقنة بخلايا الدم وأظهرت

تكسير في الخلايا الإندوسيلية المبطنة لها . وبجانب ذلك ظهر نشاط تحللي حاد في ألياف الكولاجين المحيطة بالجيوب الكبدية والأوعية الدموية في تلك الأنسجة الكبدية .