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Histopathological Changes Induced by Single Dose of LD_{50} *Naja naja* Snake Venom on the Liver of Male Albino Rats

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Abstract

Background The common sign of snake envenomation is hepatotoxicity or liver injury that is dependent on quality and quantity of venom.

Objective To clarify the effect of intraperitoneal (i.p.) injection of LD50 dose of *Naja naja* snake venom on the hepatic tissues of albino rats after 3 and 24 hr from envenoming respectively.

Methods The rats were divided into 3 groups, the first group served as a control group, while the other groups 2 and 3 were treated with the snake venom (0.05 µg/g body weight i.p) and sacrificed by decapitation after 3 and 24 hours of the snake venom injection respectively. The livers were isolated and histological sections were prepared.

Results Intraperitoneal LD_{50} for Naja *naja* snake cobra was determined in rats to be equal to 0.05 µg/g body weight. Histopathological changes in liver tissues after 3 hr from injection were congestion of the central veins, congested liver sinusoids, leucocytes infiltration, cytoplasmic vacuolization and nuclear pyknosis, cellular swelling and necrosis of some cells. While histopathological changes in liver tissues after 24h from injection were the same signs in addition to cellular swelling, necrosis and damage of the injured hepatocytes with acute inflammation cells infiltration.

Conclusion The injection of $LD50_{dose}$ of *Naja naja* snake venomin rats can induce hepatic damage and hepatotixicity in albino rats.

Keywords *Naja naja,* snake venom, Rats, Liver, Histopathological changes.

Introduction

The venom of most snakes is highly phlogistic in humans [1]. 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 [2]. *Naja naja* (cobra) is one of the most dangerous snake species in the world, where it provokes a high number of human deaths due to envenomations [3]. The main compounds of *Naja naja* venom are complex mixture of biologically active components comprising hydrolytic enzymes that cause several disorders such as hemorrhage, coagulation disturbances, edema and myotoxicity [4]. These enzymes are peptidases [5], phospholipases A_2 [6], metallopeptidases and non-enzymatic proteins/peptides like cardiotoxins, that caused hemolysis, local inflammation, depolarization, and contracture of smooth, skeletal and cardiac muscles [7], and small amounts of organic and inorganic molecules [8]. There are reports showing the effects of various snake venoms on liver tissues in rat that the venom causes damage of the hepatocyes [9-11]. The objective of this study is to

determine the histological alterations in the liver of rats following Naja *naja* envenomation in an attempt to improve our understanding of snake envenomation in rats.

Methods

Venom

Lyophilized crude venom of snake *Naja naja* venom was obtained from India (Sigma loeate Ltd). The crude venom was dissolved in phosphate buffered saline (PBS), pH 7.2. The determination of the median lethal dose LD_{50} of the snake Naja *naja* venom by intraperitoneal (i.p.) injection was carried on 40 adult healthy albino rats. The injected dose was 0.05 µg/g body weight of snake venom calculated according to the method of Meier and Theakston [12]. Results are shown in table (1)

Animals and Experimental design

Healthy adult albino rats of same age group (80±5 days) and weight (190±10 g) were taken from the High Institute for Infertility Diagnosis and Assisted Reproduction Technologies, AL-Nahrain University and the animals were housed in standard condition and fed with normal diet and water *ad libitum*. Animals were divided into three groups of 8 animals each. The first group, control, animals were injected i.p. with 0.1 ml in phosphate buffered saline and sacrificed 24 hr after injection. Groups two and three were injected i.p. with LD50 (0.05 µg/g body weight) of cobra venom and sacrificed at 3 and 24 hr after envenomation respectively. All animals were sacrificed, then liver was isolated and cut to small pieces from each experimental rats then transferred immediately to 10% formalin for 24 hr and dehydrated in ascending grades of ethanol (50-100%). Clearing was done in xylene and embedded in paraffin wax. Sections (4-5 µm thick) were prepared and then stained with hematoxylin and eosin (H & E) and methylene blue stain to be examined with light microscope.

Results

Venom Lethality:

The approximate i.p. LD_{50} for Naja *naja* snake cobra was determined in rats to be equal to 0.05 µg/g body weight, as shown in table 1.

Table 1. Determination of LD_{50} of *Naja naja* snake Cobra venom

Dose µg/g body weight	No. of animals	Survival (S)	Death (D)	% Mortality
0.02	8	8	0	0
0.04	8	5	3	37.5
0.06	8	3	5	62.5
0.08	8	1	7	87.5
0.1	8	0	8	100

LD_{50} = 0.05 µg/g body weight rats

Histological studies:

Light microscopic observation revealed that the control hepatic tissue (group 1) showed normal cells with prominent round nuclei and eosinophilic cytoplasm. The hepatocytes radiated towards a central vein and separated by blood sinusoids (Figures 1 & 2).

In the second group showed some histopathological changes were recorded after 3 hr from envenoming including some scattered of the hepatocytes suffering from cytoplasmic vacuolization, nuclear pyknosis, few cellular rarified of cytoplasmic components and central vein congestion. Congested blood sinusoids and lymphocytic infiltration were also recorded (Figures 3 through 6).

The liver tissues of the third group showed more severe histopathological changes were recorded after 24 hr from envenoming. These changes include extreme extend of the cellular swelling, necrosis and damage. Numerous inflammatory cells and Kupffer cells hyperplasia were noticed in between the necrotic hepatocytes (Figures 7 through 9). The injured hepatocytes were mostly infiltrated with numerous inflammatory cells (Figures 10 through 12).

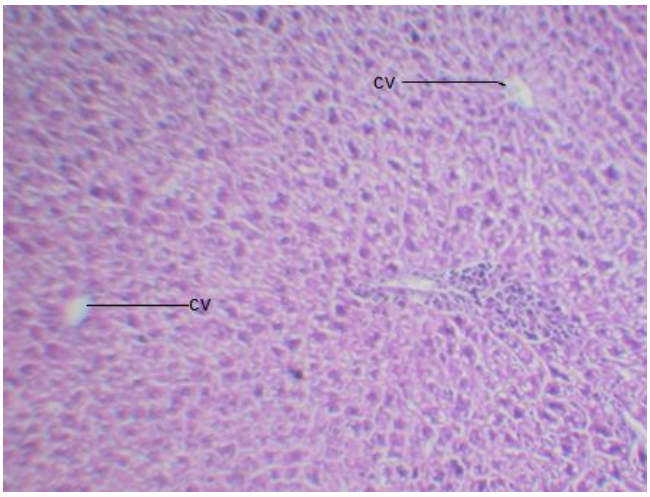

Figure 1. Liver section (from control) showing hepatocytes radiating from the central vein (CV) (H & E, 40X)

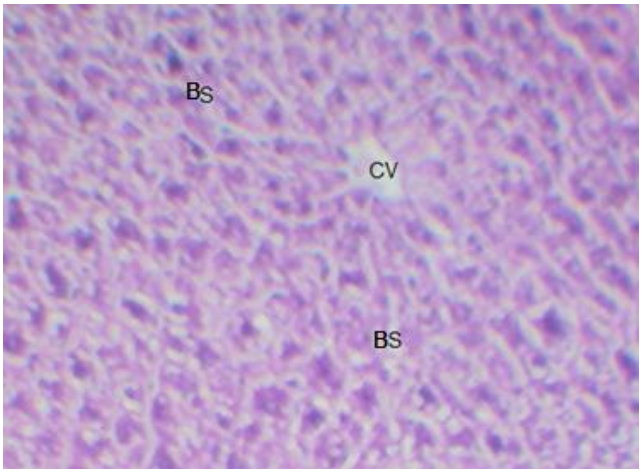

Figure 2. Liver section (from control) showing homogenously stained cytoplasm and normal nuclei, blood sinusoids (BS) and a central vein (CV) (H & E stain, X100)

Discussion

Several researches dealing with the effects of snake venoms in cells or tissues from the organs of rodents, like liver, kidney and muscle showed varying results, depending on the experimental concentrations, exposure time, site of injection, the species of the snake and the composition of the venom [13,14]. Snake venoms comprise complex mixtures of enzymatic and non-enzymatic proteins and small organic compounds.

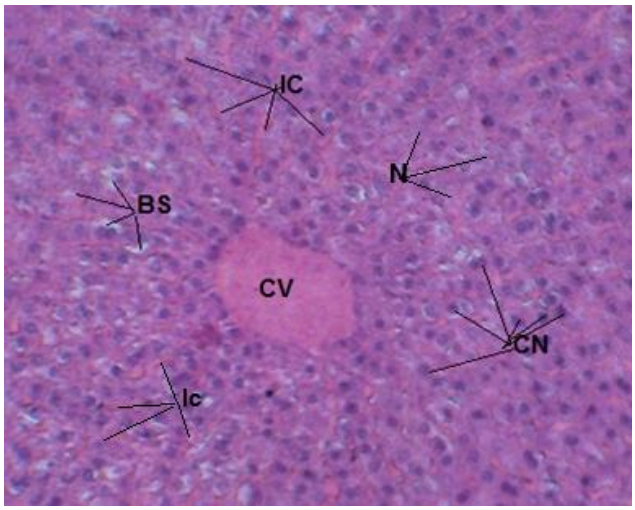

Figure 3. Section of liver tissues of rat after 3h from envenoming with LD50 snake venom showing condensed nuclei (CN) cellular necrosis (N) and number of inflammatory cells (IC), blood sinusoids (BS) and a central vein (CV) congested, (H & E, X100)

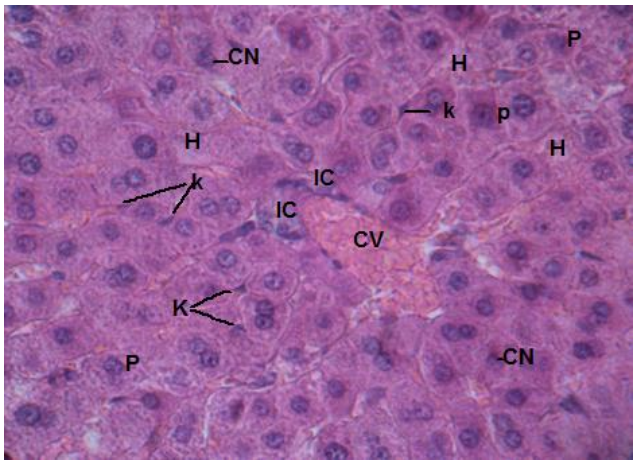

Figure 4. Liver section rat after 3h from envenoming with LD_{50} snake venom showing hydropic degeneration (H), increase number of kupffer cells (K), nuclear condensation (CN) and pyknosis (P), inflammatory cells (IC) and CV: central vein congested, (H & E, X200)

The pathology of envenomation includes both local and systemic effects such as neurotoxicity, myotoxicity, cardiotoxicity, coagulant disorders, hemorrhagic, hemolytic and edema forming activities [15]. Liver is considered as a target organ for envenoming by different types of snake venoms. Liver injury is among the common and most serious symptoms of cobra snake envenoming [16].

In the present work, the livers of rats after 3 and 24h envenomed with the LD_{50} of *Naja naja* snake

venom showed marked histopathological changes. The LD_{50} was selected for studying the histopathological changes associated with snake envenoming. These changes included congestion of intrahepatic blood vessels, increase in number of Kupffer cells, inflammatory cell, hydropic degeneration, variable degrees of cellular swelling, cytoplasmic changes, cellular necrosis and cellular damage.

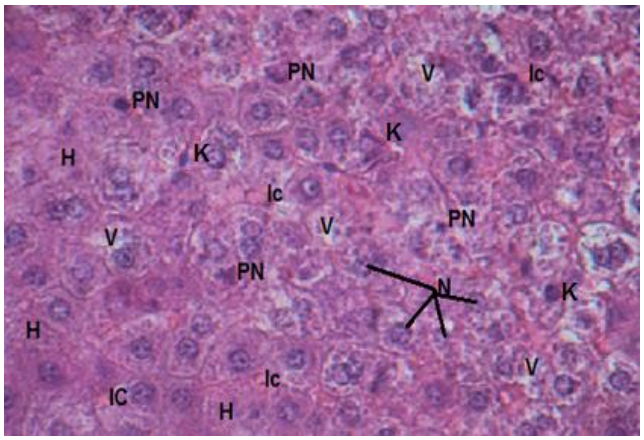

Figure 5. Liver section of rat after 3 hr from injection of snake venom showing hydropic degeneration (H), increase number of kupffer cells (K), variable sized nuclei (N), cytoplasmic vacuolization (v), pyknotic nuclei (PN) and inflammatory cell infilterations (Ic) (H & E stain, X400).

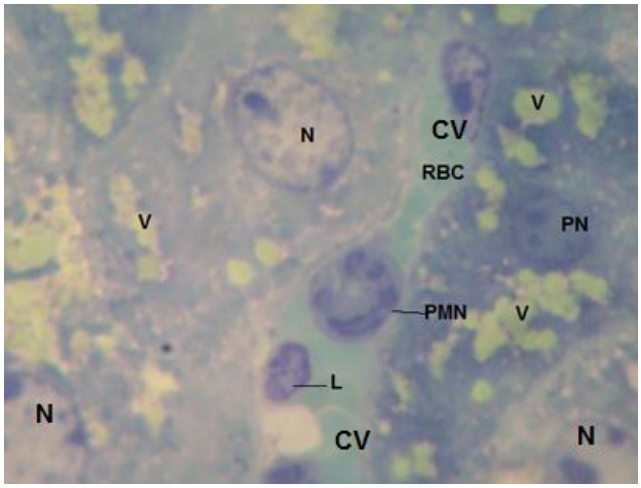

Figure 6. Section of liver tissues of rat after 3 hr from injection of snake venom showing hydropic degeneration (H), variable sized nuclei (N), cytoplasmic vacuolization (v), pyknotic nuclei (PN), lymphocyte (L), polymorph-onuclear cell (PMN) and central vein filled with erythrocytes (RBC) (Mathylene Blue stain, X1000).

The results of the present study were in agreement with those reported by Chethankumar [8] who observed that cellular swelling might be due to the action of *Naja naja* venom phospholipase, which causes disturbance of the cell membrane permeability with, which the Na^+/K^+ ATPase activities and consequent influx of Na^+ and water, induces changes to cellular membranes, especially those related to fatty acid changes in the major membrane phospholipids and eventually lead to cell death.

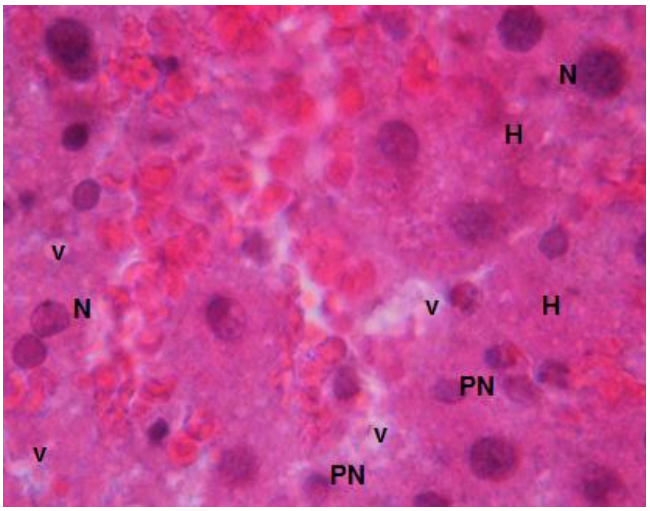

Figure 7. Section of liver tissues of rat after 24 hr from injection of snake venom showing hydropic degeneration (H), variable sized nuclei (N), cytoplasmic vacuolization (v), pyknotic nuclei (PN), central vein filled with erythrocytes and the sinusoids are filled with erythrocytes (H & E stain, X200).

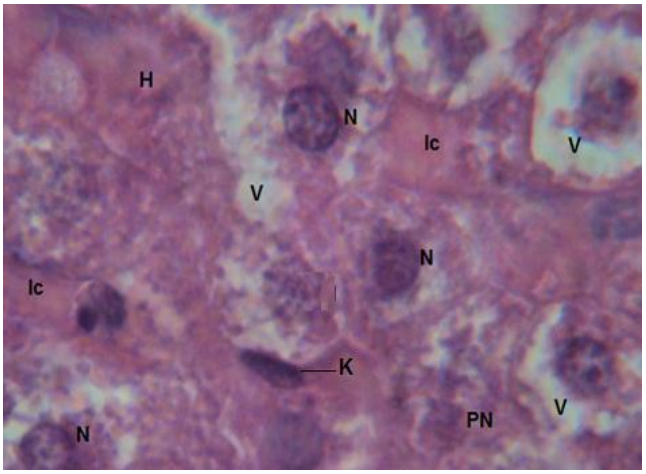

Figure 8. Section of liver tissues of rat after24 hr from injection of snake venom showing hydropic degeneration (H), kupffer cell (K), variable sized nuclei (N), cytoplasmic vacuolization (v), pyknotic nuclei (PN) and inflammatory cell infilterations (Ic) (H & E stain, X600).

In addition, Rahmy and Hemmaid [17] reported that snake (*Naja haje*) envenoming causes cellular swelling, cytoplasmic granulation and vacuolization in addition to intrahepatic hemorrhage, liver necrosis and activation and hyperplasia of the Kupffer cells. This activation of these cells might represent a defense mechanism of detoxification induced by the venom correlated with the degree of injury to the hepatic tissue which increases autophagy throughout the hepatic tissue.

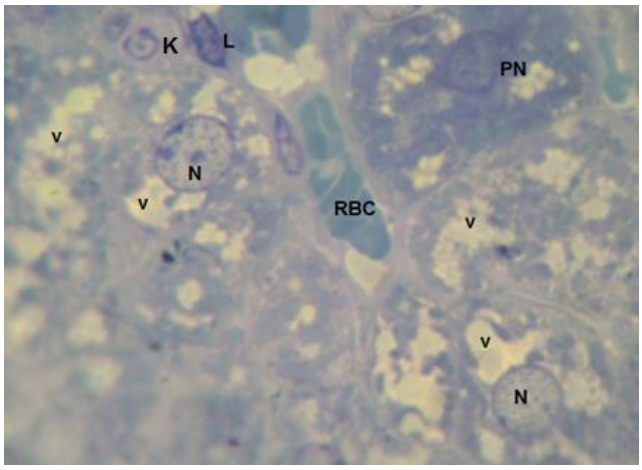

Figure 9. Section of liver tissues of rat after 24 hr from injection of snake venom showing hydropic degeneration (H), hypertrophy of kupffer cell (K), variable sized nuclei (N), cytoplasmic vacuolization (v), pyknotic nuclei (PN), leucocyte (L) and central vein with erythrocytes (RBC) (Mathylene Blue stain, X1000).

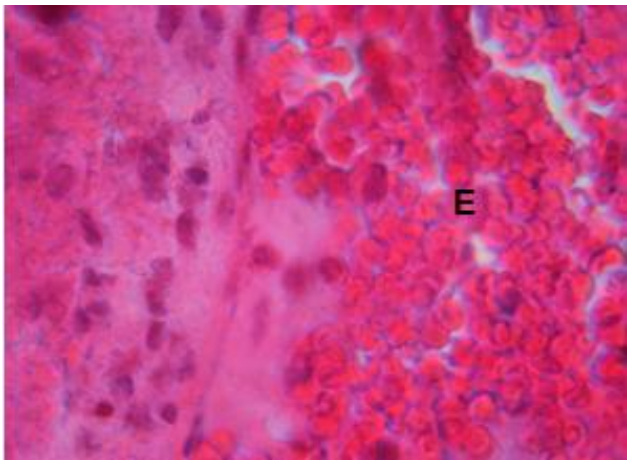

Figure 10. Section of liver tissues of rat after 24 hr from injection of snake venom showing erythrocytes (E) (H & E stain, X200).

Hanafy et al in 1999 [18] explained that *Cerastes cerastes* envenoming causes cellular swelling, cellular necrosis, nuclear pyknosis and presence of foci of damaged hepatic cells invaded with inflammatory cells. The appearance of vacuoles within the hepatocytes of the envenomed rats might indicate venom interference with mitochondrial and microsomal function that leads to disruption of lipoprotein and lipids accumulation. Similar findings were obtained by Abdel Ghani et al in 2009 [19] who attributed these changes to a hepatotoxic effect of the Naja Nigricollis venom and it is more likely to be described as cytoplasmic changes of some snake toxins.

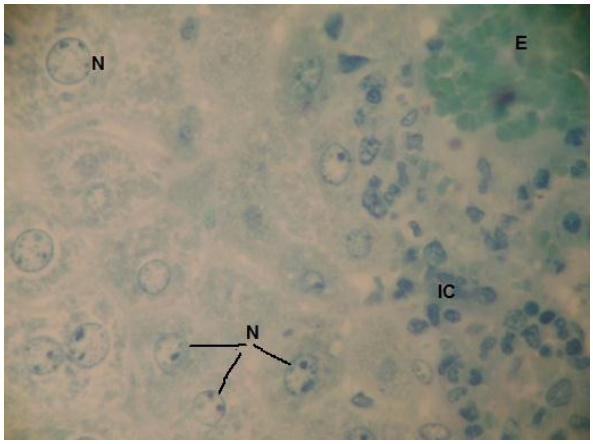

Figure 11. Section of liver tissues of rat after 24 hr from injection of snake venom showing erythrocytes (E), variable sized nuclei (N) and inflammatory cell infilterations (Ic) (Mathylene Blue stain, X100).

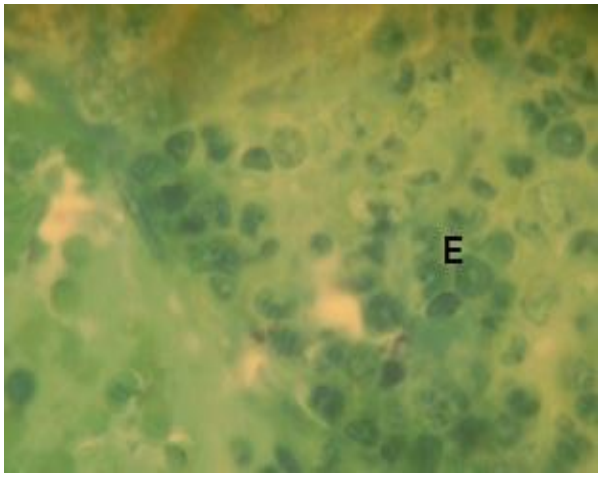

Figure 12. Section of liver tissues of rat after 24 hr from injection of snake venom showing erythrocytes (E) (Mathylene Blue stain, X400).

The present study has indicated that *Naja naja* envenomation causes acute toxic insult to the envenomated rats as a result of metabolic disturbance. More work is needed to illustrate the histochemical and ultrastructural alterations induced by *Naja naja* venom in the liver and other vital organs.

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