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EFFECTS OF SCORPION (*HEMISCORPIUS LEPTURUS*) VENOM ON THE LIVER OF ALBINO RATS

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ABSTRACT

Scorpion envenomation is a public health concern due to its influence on human activities. The effects of scorpion (*Hemiscorpius lepturus*) venom on liver enzymes, total bilirubin, and liver histoarchitecture of albino rats were investigated in this study. The venom was extracted from the telson of scorpion, using soxhlet extractor. The LD_{50} of the venom was determined. The animals were divided into three groups and treated as follows; group one received 2.5 mg extract/kg, group two received 5.0 mg extract/kg of the extract, while group three served as control and had distilled water. Blood samples were collected before, and at time intervals after venom inoculation, for estimation of liver enzymes and total bilirubin. The liver was harvested for histological examination. The LD_{50} of the venom was 7.25 mg/kg. The results showed increase in the relative weight of liver tissue, especially in the animals that received the higher dose of the venom. There was significant ($p< 0.01$) increase in the serum levels of alanine aminotransaminase (ALT), and aspartate aminotransaminase (AST), with decrease in alkaline phosphatase (ALP). Total serum bilirubin showed no significant change, ($p > 0.5$). Histological examination of the liver tissues revealed hepatocellular degeneration and aggregation of inflammatory leucocytes after envenomation. The changes in the morphology of the liver (by relative weight) correlated with the histological changes in the tissue, as well as significant changes ($p<0.05$) in liver enzymes. The results suggest possible deleterious effect of scorpion venom on the liver.

KEYWORDS: **Scorpion venom; Envenomation; Liver enzymes; Histology.**

INTRODUCTION

Scorpion envenomation constitute health hazard in many countries of the world, and can cause lethal effects in humans, especially in children [1]. Scorpion stings are common in Iran, particularly in the southern and southwest regions of the country, and in the tropics where they pose serious health challenges [2,3].

Scorpion venoms consist of a complex of several toxins that elicit a wide range of biological actions, as well as toxicity, pharmacokinetics and pharmacodynamic effects [2]. These venoms are associated with high level of morbidity and mortality. *Hemiscorpius lepturus* is the most venomous scorpion and is responsible for 95% of all scorpion sting related mortalities [1]. This scorpion species has been found in tropics [4]. *H. lepturus* is known for its potent cytotoxic venom that can cause cutaneous necrosis and severe systemic pathology which may lead to death [2,5]. About 40, 000 scorpion stings are reported in Tunisia every year [4, 6]. Intensive care admission following scorpion stings in Tunisia generally arises from either *Androctonus australis* or *Buthus occitanus* species envenomation [7,8]. Different species of scorpions are responsible for the different toxicities after

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envenomation in different environments, and at different conditions. As a result, the degree of effects produced after scorpion envenomation is multifactorial, and may depend on the scorpion specie involved, the concentration and composition of the venom, the age of the scorpion, the season of the year, the age of animal beaten and other environmental and physiological factors of the victim. Changes in both biochemical and haematological parameters as well as histo-pathological changes in tissues and organs after envenomation have been reported in experimental animals [9,10]. *H. lepturus* has been reported to have both local and systemic effects in mice, [11-13].

Many studies have reported manifestations caused by venoms of scorpions of the Buthidae family [8,14] but very few have indicated the signs and symptoms as well as the serum biochemical changes induced by *H. lepturus* venom [12]. In an attempt to evaluate the effects of *H. lepturus* venom on organs and biochemical parameters, this study investigated the *in vivo* effects of the venom in albino rats, with regards to changes in the liver enzymes, total bilirubin and histo-architecture of the liver.

MATERIALS AND METHODS

Scorpion collection and identification

Scorpions were collected from Enugu metropolis and Abakaliki in Ebonyi State. Identification of the scorpions was done by a Zoologist, Dr C. Attamah of the Department of Zoology, University of Nigeria, Nsukka, as *Hemiscorpius lepturus* specie. Venom was extracted from a total of twenty-five (25) scorpions.

Animals

Albino rats of both sexes (120 – 150 g) were used for this study. The animals were kept in cages, five animals per cage, in the animal house at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical sciences, Nnamdi Azikiwe University, Agulu, for seven days to acclimatize. During the period of acclimatization, they were adequately fed.

Ethical clearance

Ethical clearance was obtained from the ethical committee of the Faculty of Pharmaceutical sciences, Nnamdi Azikiwe University, Agulu.

Scorpion venom extraction

Venom was obtained from the scorpions by grinding the telson using grinder. The ground sample (1.65 g) was soaked in 250 ml 95% methanol and allowed to stand for 30min [15]. Then, this was filtered and the filtrate subjected to soxhlet extraction. The extract obtained was left in open room to concentrate for twenty-four hours, and stored at 4^{o}C until used.

Determination of LD_{50} (median lethal dose)

The LD_{50} was determined using Lorke's method [16].

Administration of venom

The rats were group into three of five animals in each group, and administered as follows;
Group 1 received 2.5 mg/kg of the extract
Group 2 received 5.0 mg/kg of the extract
Group 3 received distilled water (2 ml/kg).

The extract was administered through intramuscular injection.

Collection of blood samples

Blood samples were collected through ocular puncture before envenomation (zero hour), and this served as the baseline and control for the liver enzymes. Samples were then collected at intervals following envenomation after 0.5, 1, and 2 hrs. About 2 ml of sample was collected in each case, into a plane bottle. The blood samples were centrifuged at 1500 revolution per min, for 15min in other to obtain the serum. The serum collected was used to determine the activities of liver enzymes viz; the aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and the total bilirubin concentration.

Analysis of liver enzymes

The activities of liver enzymes and total bilirubin concentration were measured respectively, using their corresponding kits, [17,18].

Histology preparations and analysis of liver specimen

The animals were sacrificed after 2hrs and the liver harvested for histological analysis. The harvested liver was first weighed to obtain the absolute weight of the liver, and the relative weight determined for each animal group, and compared with that of the control, respectively. The relative weight was obtained from percentage weight of liver out of the total body weight of the animal. The tissue was fixed with 10% formalin solution, and immersed in a series of ethanol solutions of increasing concentrations. Then, the tissue was immersed in three different xylene immersions, infiltrated with molten paraffin wax in three different solutions, and allowed to cool.

Sectioning/staining

The tissue was secured on the microtome and cut into sections. The sections were attached to a glass slide, smeared, and allowed to dry. This was stained with hematoxylin and counter stained with eosin dye. The slide was mounted in glycerin jelly and observed at varying magnifications of a light microscope. The histology of the liver in both treatment groups were studied and compared to that of the animals given distilled water (the control).

Statistical analysis

Results were expressed as the mean ± S.E (n=5). Statistical significance of the results was assessed by one-way Analysis of Variance, ANOVA, using the Dunnett's method of comparism. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The percentage yield of scorpion venom was 36.25 % and the venom median lethal dose (LD_{50}) was 7.35 mg/kg. The absolute weight of liver was obtained after venom administration, and their relative weights calculated in percentages from the absolute weight of the liver in relation to the total body weight of the animal (Table 1). There was no significant ($p>0.05$) increase in the relative weight of the liver after evenomation, (Table 1). The percentage weight difference in the liver was not significant ($p > 0.05$). There was significant increase ($p < 0.05$) in the serum alanine aminotransferase levels after 0.5hr and 1hr of envenomation, (Figure 1). There was a time dependent effect of the venom on the activities of alanine aminotransferase (Figure 1). There was no significant ($p > 0.5$) change after 30min of envenomation at low treatment dose, 2.5 mg/kg (Figure 2). However, the serum levels aspartate aminotransferase showed significant increase at time intervals following administration of high dose of venom. There was significant decrease in the serum levels of alkaline phosphatase, ($P < 0.05$), after 0.5hr, 1hr, and 2hr (Figure 3). There was a significant and time dependent decrease in the values of serum alkaline phosphatase after envenomation, (Figure 3). The serum total bilirubin measured at time intervals after envenomation did not significantly change ($p > 0.05$) when compared to the serum levels prior to administration of venom (Figure 4).

A histological slide preparation of the liver tissues of animals given distilled water (the control group) showed the normal hepatic architecture (Figure 5). The histo-architecture of the liver after envenomation showed multi-focal areas of hepatocellular degeneration and necrosis, with aggregation of inflammatory leucocytes around the portal area as shown in the histology plates (Figures 6-8). The sections of the liver here showed mild infiltration of inflammatory leucocytes around the portal area (black arrow) (Figure 8). Also observed was hyperplasia of the kupffer cells.

DISCUSSION

Scorpion venom causes significant cell damage to vital organs in the body, including the liver tissues, and might lead to liver failure and consequent death. Studies have shown that there are increased inflammatory processes on organs such as lungs, kidneys, liver, and heart tissues, after scorpion envenomation in rats [8,12,19].

In this study, the serum levels of alanine aminotransaminase (ALT), and aspartate aminotransaminse (AST), enzymes showed significant increase, while alkaline phosphatase (ALP), showed significant decrease after envenomation compared to the levels before venom administration. The increase in ALT and AST enzymes were time dependent and significant ($P< 0.05$) at high treatment dose of venom. The result of this study was similar to the previous findings [12,19,20], that envenomation by different scorpions resulted in increase in the circulating enzyme levels which included Glucose-6-phosphate dehydrogenase (G-6-PD), Lactate dehydrogenase (LDH), Alanine aminotransaminaes, ALT, Aspartate aminitransaminase, AST, creatinine phosphokinase, succinate dehydrogenase.

Changes in the AST and ALT might signify a direct action of the venom on the liver and heart. However, the sustained significant decrease in the level of serum alkaline phosphatase (ALP) after envenomation was similar to the findings from other studies [12,19], which reported inhibition in the ALP, acetyl cholinesterase (AChE) and Na^+K^+-ATPase enzymes activities after scorpion envenomation.

The changes in the levels of ALT, AST, and ALP in envenomed animals were similar to the reports of Costal-Oliveira *et al.,* and others [12,19]. Aspartate aminotransferase is a transferase enzyme distributed to all parts of the body but mostly concentrated in the liver and the heart. Therefore, increase in the level might be attributed to hepatic failure or damage or myocardial infarction. ALT and AST are considered liver specific enzymes. ALT increases more and remains longer than AST during hepatic failure, hepatic inflammation or injury to the liver [12], as observed in our study. These changes in AST, ALT, and ALP levels might suggest a direct

Table 1: The mean absolute weights, the relative weights, and percentage weight differences of the liver for the treatment groups and the control after envenomation.

	Treatment Groups		
	Distilled water (2ml/kg)	Venom 2.5 mg/kg	Venom 5 mg/kg
Mean absolute weight of liver (gram) ± SD	5.03 ± 0.73	4.63 ± 0.18	5.09 ± 0.92
Relative weight of liver (in percentage)	3.00	3.59	3.69
	–	0.59	0.69

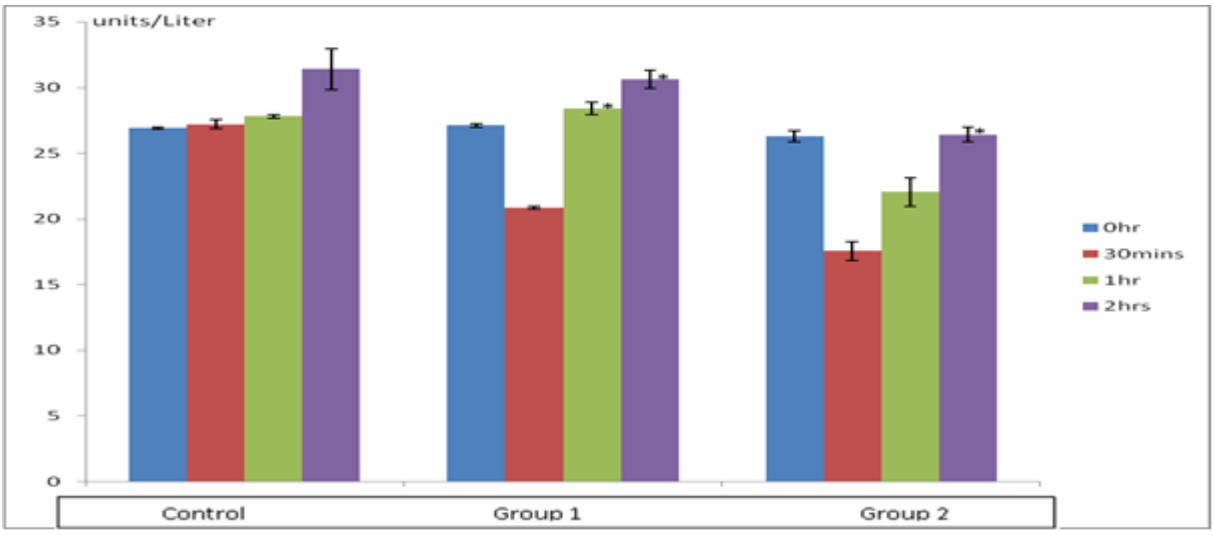

Figure 1: Effect on the serum levels of alanine aminotransaminasse, (ALT). Group 1 – 2.5 mg/kg, Group 2 – 5.0 mg/kg, Control – distilled water (2ml/kg), * – significant values, black arrows – standard error of the mean.

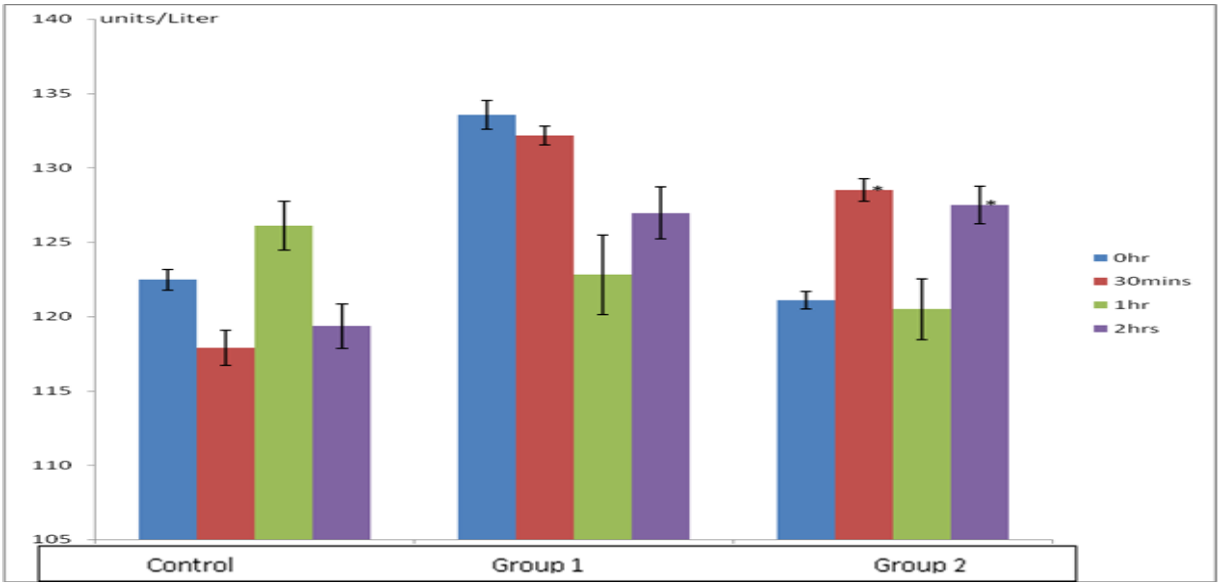

Figure 2: Effect on the levels of serum aspartate transaminase (AST).

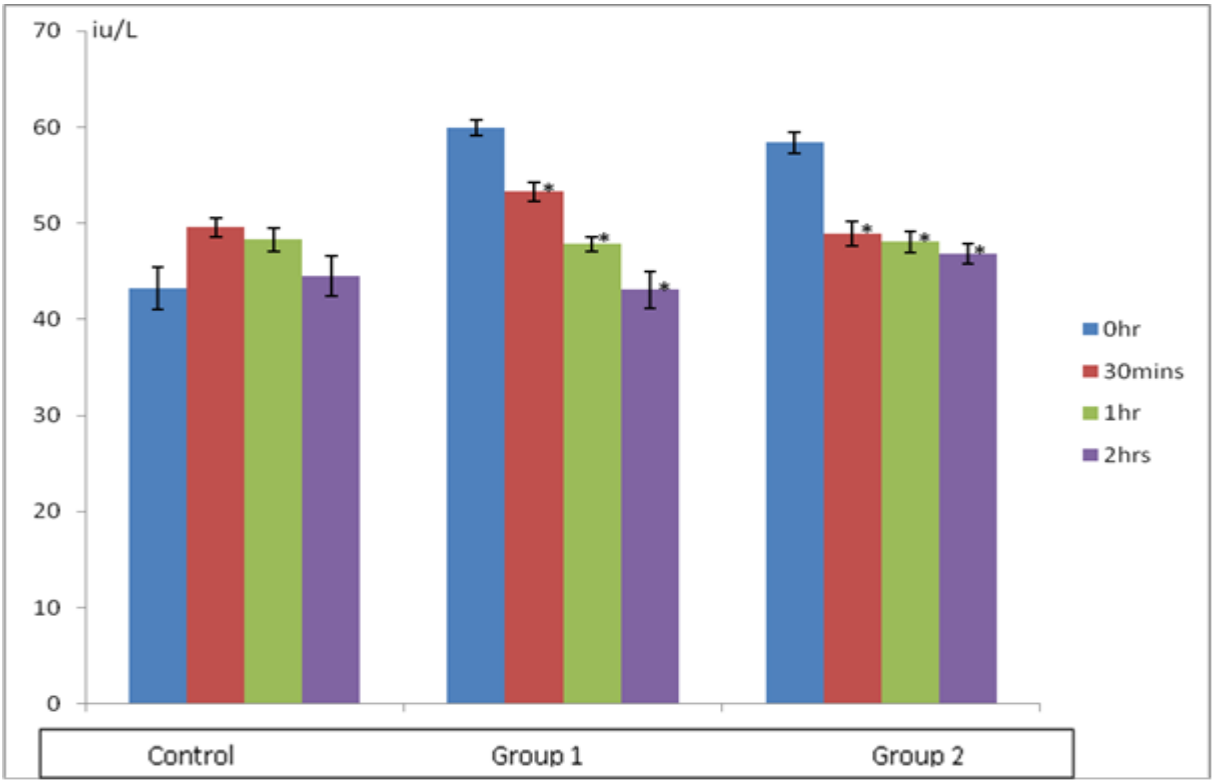

Figure 3: Effect on levels of serum alkaline phosphatase (ALP).

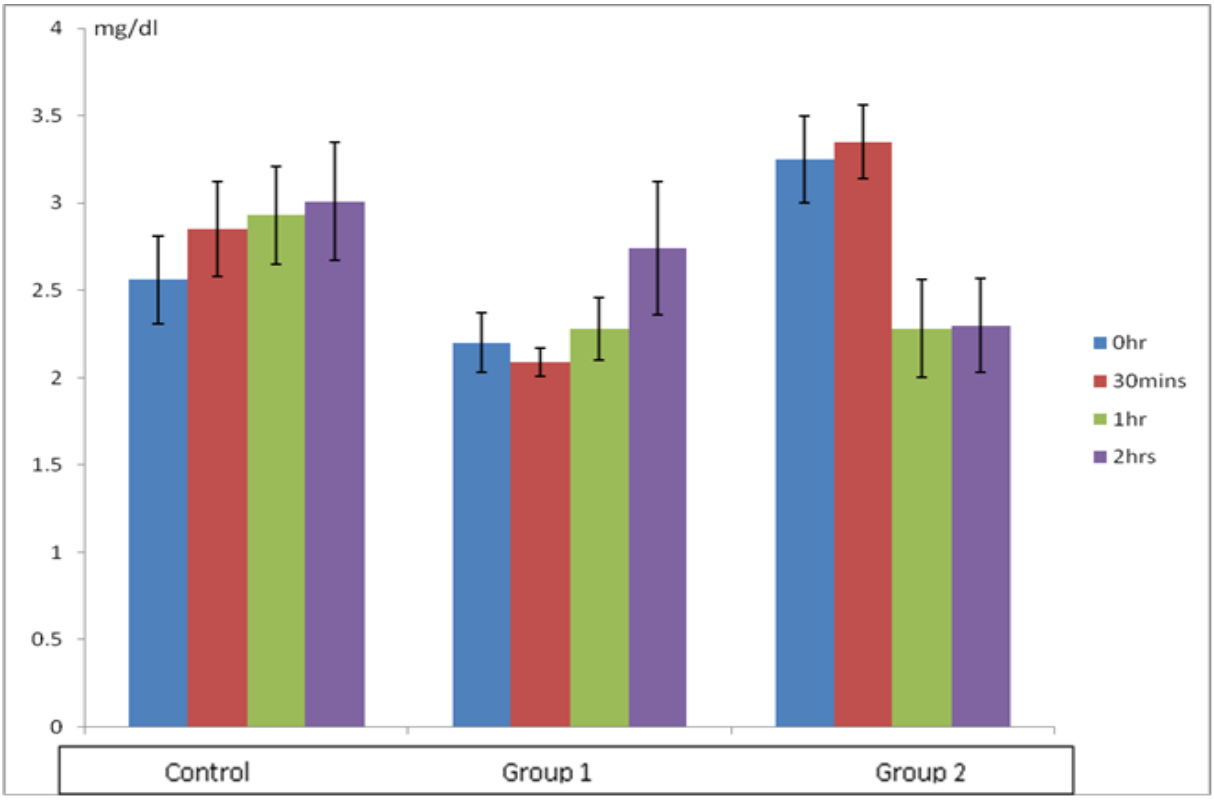

Figure 4: Effect on levels of total serum bilirubin.

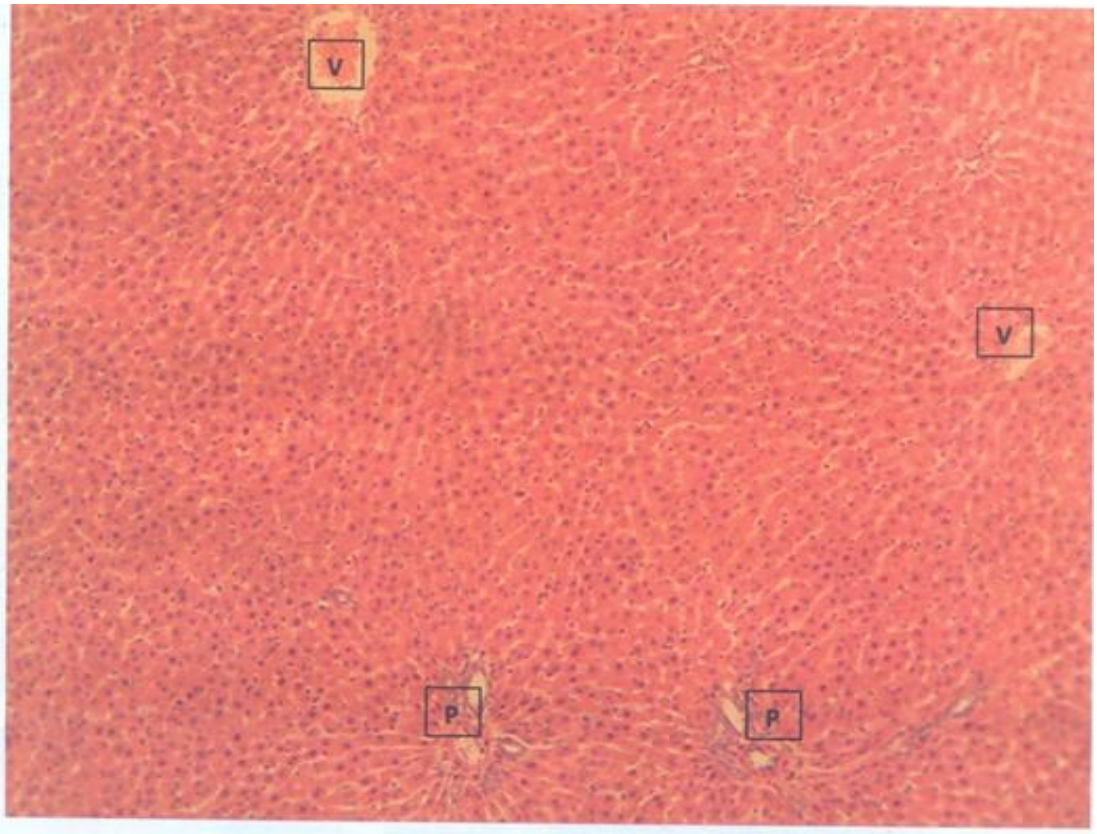

Figure 5: Histo-architecture of the control at x 100 magnification.

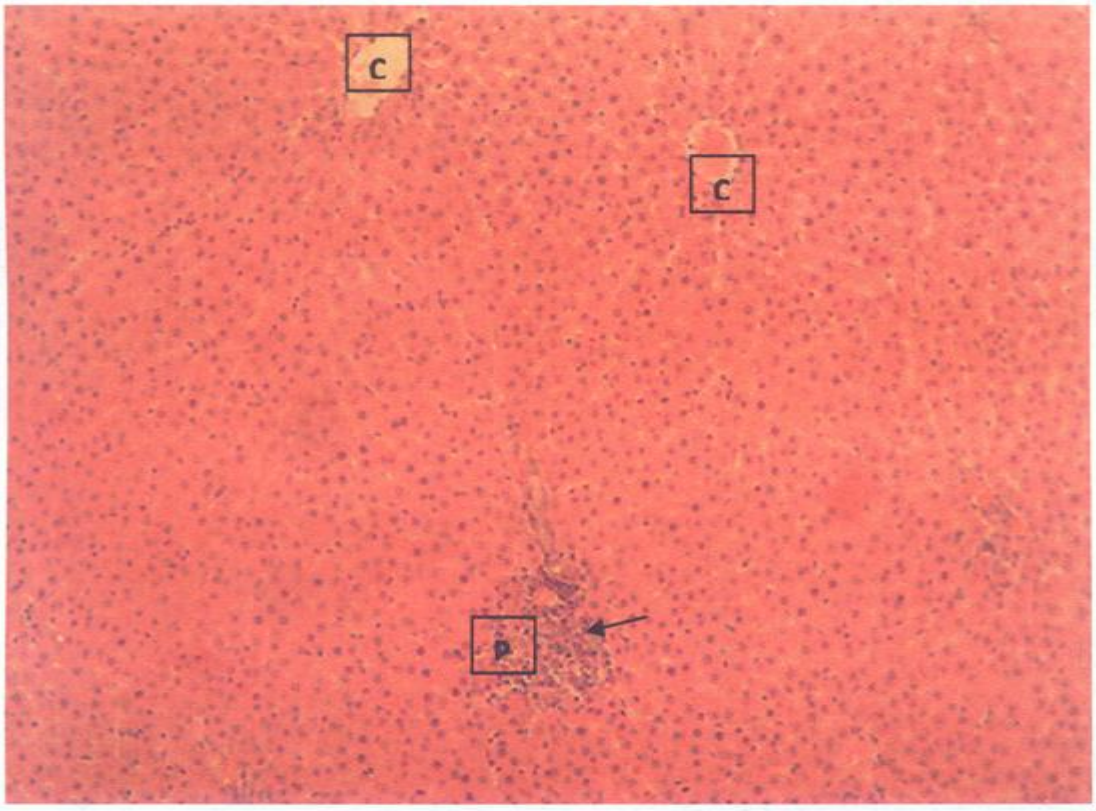

Figure 6: Histo-architecture of the low treatment group (venom 2.5 mg/kg), at x 100 magnification.
Key: (P) – portal area. (C) - Central vein. H & E – Hematoxylin and Eosin stain
X 100 – Is the magnifying power of the microscope used.

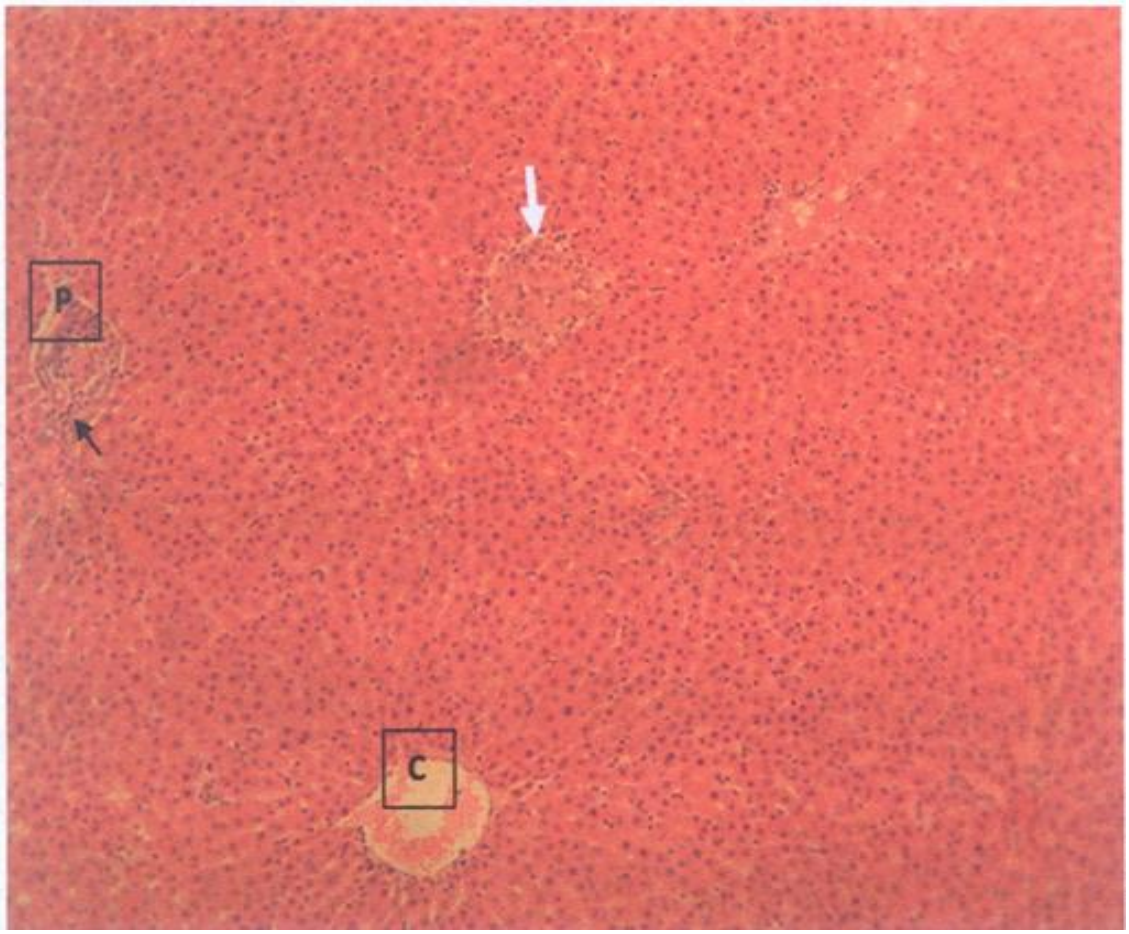

Figure 7: Histo-architecture of the low treatment group, at higher magnification.

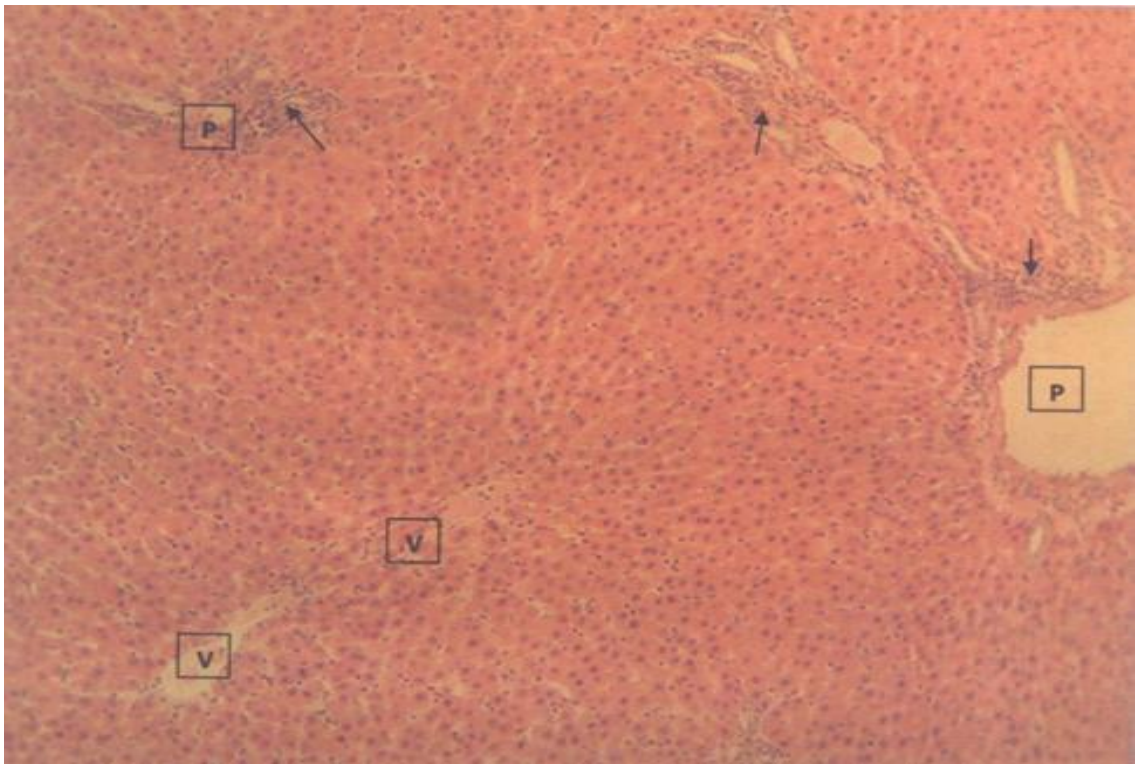

Figure 8: Histo-architecture of the high treatment group (venom 5.0 mg/kg), at x 100 magnification.

action of the venom on the liver. Also, studies by Costal-Oliveira and Co-workers [12,19] showed high toxin concentration in the liver and other vital organs following envenomation supporting increased circulating enzyme levels of ALT and AST.

Studies had also shown that the venom component had direct effect on the morphology of cells including haemolysis, inflammatory changes, edema formation [10,13], and these might lead to cell lyses and damage to tissues. Some histological studies recorded dilation of the organelles which was related to an accumulation of fluid in the sarcoplasmic reticulum [8,10]. This was similar with the gain in the relative weight of the liver after venom administration in this study.

The liver histology revealed cellular damage after venom administration at both doses of the venom. This was in consonance with the inflammatory changes that occur after scorpion envenomation reported by Ghafourian and others [9,13]. Studies had shown that there are increased inflammatory processes on organs such as lungs, kidneys, liver, and heart tissues, characterized by an increased density of mononuclear cells after scorpion envenomation [9,10,13, 21].

CONCLUSION

In conclusion, the scorpion venom caused significant cell damage to the liver tissues, and might lead to liver failure and consequent death, without adequate interventions. The results showed the damage to the tissues was time dependent, with little or no dose dependence. Therefore, the duration of exposure and dose of venom should be targeted in prevention and treatment of scorpion stings and its related morbidities and mortalities.

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