Global Advanced Research Journal of Environmental Science and Toxicology (ISSN: 2315-5140) Vol. 3(4) pp. 049-056, November 2014
Available online http://garj.org/garjest/index.htm

Full Length Research Paper

Effects of *Leurus quinquestriatus* scorpion venom on the histological structure of the liver and kidney

Al–Harbi, H.A.A.[1] and Al-Hasawi, Z.M.[2*]

[1]Poison Control and Medical Forensic Toxicology Center, Jeddah, Saudi Arabia
[*2]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
*Corresponding Author`s E-mail: ahssan555@yahoo.com

Abstract

The present study was planned to examine the effect of the venom of the yellow scorpion Leurus quinquestriatus (Arachnoida) venom on the histological structure of the liver and kidney of albino mice. Ten albino mice with the same age (45 days)and approximately the same the same body weight (25±5 gm) were used . The venom was obtained from L.quinquestrianus which was collected from Al-Khomra area near Jeddah City, by means of an electric stimulation . The mice were subjected to intercellular injections with the scorpion venom with a dose of 0.03 mg/km , and the animals were left 6 hours before being dissected . The animals were anaethetised with diethyl ether ,dissected and small parts of the liver and kidney were removed. Michrotmic sections were made and three materials were used to stain the sections , each specialized in certain characteristics. The venom caused remarkable histological alterations in the liver and kidney tissue structure, like nuclear margination, nuclear pyknosis, karyolysis, cell swelling, cellular necrosis, cellular damage, and inactivation of the antigen marking the endothelial clls (CD34).

Keywords: Yellow scorpion **-** venom - histological structure- liver – kidney- albino mice.

INTRODUCTION

Scorpion venoms are mixtures of salts, proteins peptides, amino acids, enzymes, small molecules, with the proteins forming the main part of the basic compounds (Al-Sadoon and Jarar, 1997). The venom of the scorpion *Leurius quinquestriatus* contains different compounds similar to polypeptide neurotoxin (Brown et al., 1983). Two types of venoms were identified in *L. quinquestriatus* venom (Lqh7) and (lqh6) (Hamon et al., 2002). It contains 5 toxic compounds all of it composed of basic proteins with low molecular weight bound to disulphide bridges (Kopygan et al., 2006). *Leiurus quinquestriatus* caused serious damage to the renal tissues, specially the renal corpuscles, the renal tubules as these cells were suffered damage in the form of cellular degeneration, and cellular necrosis, plus sever cellular infiltration in the pulmonary tissues, and blood congestion in the blood capillaries of the lung, with separation of the cardiac muscle causing empty areas between them (Soheir et al., 2007). Scorpion venom affects many systems resulting in complex defects in the physiological processes, and even complete failure of functions (D´ Suze et al., 2003). Scorpion envenoming is a public health hazard in many

countries specially in the rural areas (D´ Suze et al., 2004; Quiroga et al., 1997). Working on the effect of scorpion venom on male gonad with *Leiurus quinquestriatus* of the family Buthidae Hafeiz et al. (1984) has reported that a single dose of VII from *L. quinquestriatus* given to rats is capable to induce degenerative changes in seminiferous tubules, resulting in necrozoospermia. Scorpion venom encompassing liver and kidney function, inflammatory response, metabolism and circulation plus blood cells (DeSuze et al., 2004). The main gross and striking lesions were observed in the liver and included massive coagulative necrosis, associated with centrotubular haemorrhage, friable consistency, pronounced lobular pattern and areas of haemorrhage (Traverso et al., 2004), with the presence of tubular necrosis. The tissue distribution of venom is complex, and tissue distributes to different tissue sites, and the effects of venom appears after 4 hours, and tissue reaction takes place due to the striking response to the proteins and enzymes (Henriques et al., 2002) . The administration of Tityus n. sp. Scorpion venom to mice at a dose of 3.75 mg/mouse induced alterations in the spermatogenesis, sertoli cell vacuolation, immature germ cells and spermatocyte arrest, also vascular dilation and congestion were detected in the interstitial tissue (Pennavedeau et al., 2000) . Scorpion venom caused histological alterations like, inhibition of edema forming and the cell membrane integrity in cardio-hepatic tissues compared to unenvenomed mice Ali-Bassalem, 2012).

MATERIALS AND METHODS

The animals

A number of 10 adult albino mice were used in this experiment. The mice were in good health , approximately having the same weight (25 ±5 gm) and 45 days in age. They were collected from king Fahad Center for Medical Research in King Abdul Aziz University.

The animals were subjected to intramuscular injection with the scorpion venom with dose 0.03 mg/Kg. according to (Abdoon et al., 2005) and the animals were left for 6 hours before being dissected.

Leurius quinquestriatus venom

In this study the venom of the yellow *L. quinquestriatus* which belongs to the family Buthidae order scorpions (*Arachnida*) was used. The scorpion was collected for Al-Khomrah region in Jeddah city, along the coastal rood at night through the use of an ultraviolet light from an ultraviolet torch which allows the scorpions to get out and be seen. Special iron picker was used to pick up the scorpions, each one was put inside a glass tube with perforated cover.

Equipments and Tools

The equipments used include light microscope (Nikon Eclipse- Japan), a microtome to cut the sections, disposable micropipette (Gibson) and syringes and a sensitive electronic balance, glass bottles, florescent light, ultraviolet torch, microwave oven, glass slides and covers and paraffin wax.

Reagents

The reagents used for staining the liver and kidney are: 1- Mayer□s Haematoxylin and Eosen, 2- Masson Trichrome Staining, 3- Immunohistochemical kit (Ceu Marque USA). Aniline blue solution, ponceau fusion solution, hydrogen peroxide block, phosphomolybdic acid, streptavidin peroxidase DAB plus substrate, ethyl alcohol, 50xDAB chromogen, and the two antigens hepatocyte specific antigen (OCHIE5) from (CELL MARQUE < USA), and (CD34) (QBEnd/10) antigen from (VENTANA, Germany) which are used to detect the anti-hepatocyte specific antigen and the endothelial cell marker .

Methods

Extraction of the venom

Scorpion venom was extracted by the use of the electric stimulation (15V) method, after fixing the scorpion on a board. The venom dropped was collected on the surface of a glass slide and left for few seconds to dry before being collected in a form of small granules inside small hygienic plastic bottles (Ependorph) at 20 □C. The venom was diluted with 0.85 % NaCl for preparations of the required dilutions .

Antigen retrieval

This step of unmasking of the antigen was carried out using the formalic infixation to break the protein bindings between the antigens to unmask the antigen and make it ready to accept its specific antigen and bind with it.

Preparation of tissues

Tissue samples were fixed in equivalent formaldehyde solution (10%), then washed with tap water for 12 hours. The samples were then dehydrated by being passed through increasing levels of ethanol from 30% up to 100%. Xylene was then applied for 3o-40 minutes to clear the samples from ethanol .

Paraffin infiltration

The tissues were subjected to paraffin infiltration using

melted paraffin wax inside the oven at 60Cº for one hour.

Embedding

Molting wax was poured inside embedding moulds, and the tissue samples were transfered from the oven inside these moulds which were then cooled to room temperature .

Sectioning

The embedding moulds with the samples were fixed on the specimen holder, and rotary microtome (Lieca) was used to provide sample sections 5 µm in thickness The paraffin was removed from the sections by putting in water at 24Cº. The sections were picked out and put on a hot plate at 30Cº to get rid of the extra water. Deparaffinization was carried out by immersing the tissues in xylene for 8 minutes.

The tissue sections were immersed in hematoxylin for one minute then in alcoholic eosen 1%, for the examination of the arrangement of the hepatic and renal cells, their sizes, and the presence or absence of vacuolization or granulation.

The tissue sections were also stained using Masson trichrome staining, for the detection of the structure of Gilson´s capsule, and the collagen fibers. Also the tissue specimens were prepared using the technique immunohistochmisrty which detects certain antigens that are found associated with their antibody, and which are added to the tissue under reasonable conditions. The sites of these antibodies in the tissue are recognized by the use of labeled secondary antibodies which can directly attach to the primary antibodies (AL-Khateeb, 2001).

The (OCHIES) was used for detection of the hepatic specific antigen, and CD34(QBEnd/10) for the kidney sections. Light microscope was used for tissue examinations.

Inactivation of endogenous peroxidase

The slides were inoculated in 3% hydrogen peroxide for 5 minutes for the inactivation of endogenous peroxidase so as to minimize the unwanted peroxides to avoid staining of the tissue background and interfere in the antigens.

RESULTS

Effects of *Leuris quinguestiatus* scorpion venom on the histological and histochemical structure of the liver and kidney of mice.

1- Histological studies of hepatic and renal tissues affected by the scorpion venom using haematoxylin and eosin:

After being injected by *L. quinguestrictus* venom the animals were anaesthetized with diethyl either and dissected for the preparation of the hepatic and renal tissue sections after 6 hours.

The liver:

The histological examination revealed strong swollen hepatocytes that led to change in the shape of cells and disappearance of most of the sinusoids. The hepatocytes suffer from cytoplasmic vacuolization, and appearance of irregular spaces without stain inside the cytoplasm, and the loss of chromatin inside most of the nuclei, in addition to unclear pyknosis. Also karyodegeneration was noticed in most of the hepatocytes together with nuclear margination in other call.

Some cells have mega nuclei and others have nuclear pyknosis. Damaged hepatocytes are also observed, together with cell necrosis of other cells and blood congestion in the branch of the portal vein (Slide 1).

The kidney

The renal histological sections of the mice injected with the venom are showing damaged proximal tubule endothelial cells and an empty looking cytoplasm. And on the other hand swollen cells, nuclear pyknosis and nuclear margination are observed, together with elongated nuclei and cytoplasmic vacuolization) and the damage of renal glomerulus. Also hyaline cast and inflammatory cells are observed, and leaked blood cell can be seen getting out of the blood vessels Slide (2).

2 - Histological studies on connective tissues of hepatic and renal tissue using Masson trichrome stain:
The Masson trichrome stain was used in the sections to detect the structure of the connective tissues in the liver and the kidney .

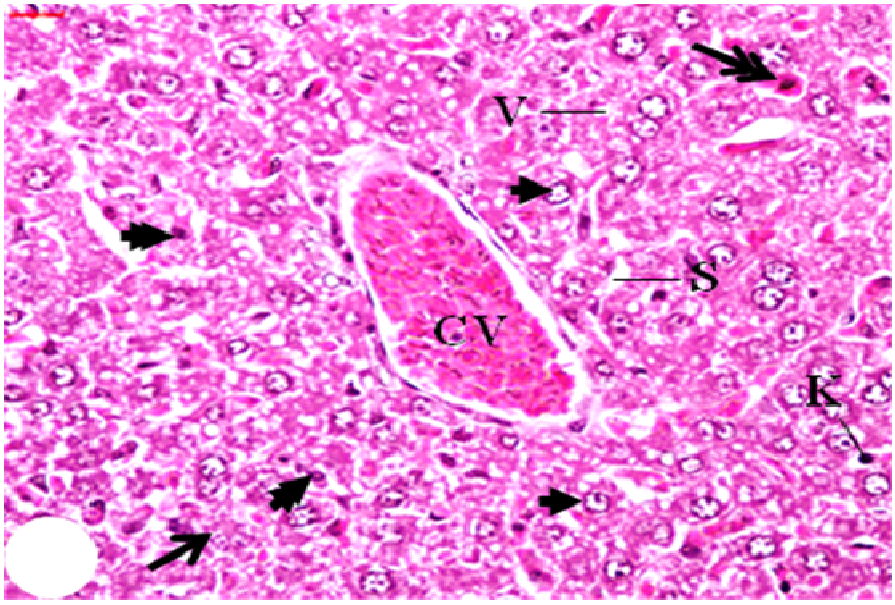
The Liver

The microscopic examinations of the slides showed the disruption of the collagen fibers in the central vein, and in the branch of the portal vein, and in the hepatic sinusoid, and in the hepatocytes Slide (3).

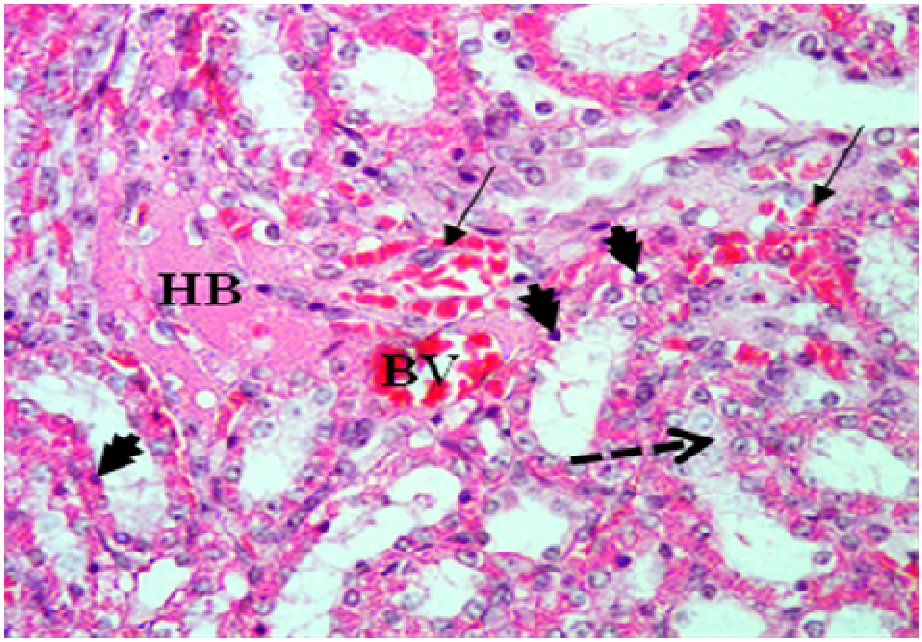
The Kidney

The disruption of the collagen fibers can be observed in the renal glomerulus and in the basement membrane of the glamerulus, and also can be seen in the basement membrane of the renal tubules Slide (4).

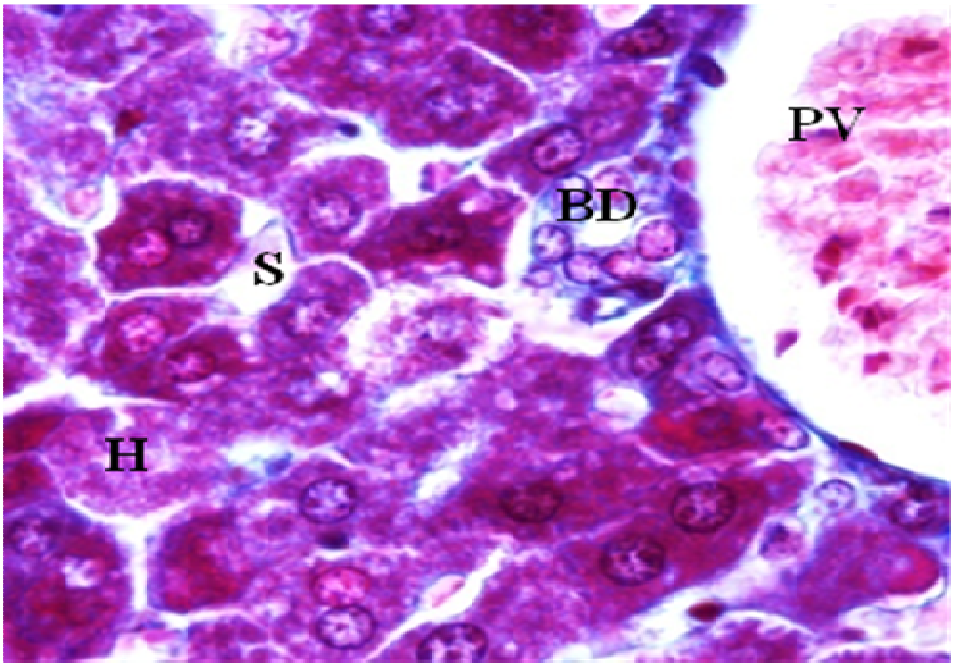
3 - The histological studies of the hepatic and renal tissues affected by *L.quinguestrttus* venom using immunohistochemistry method:



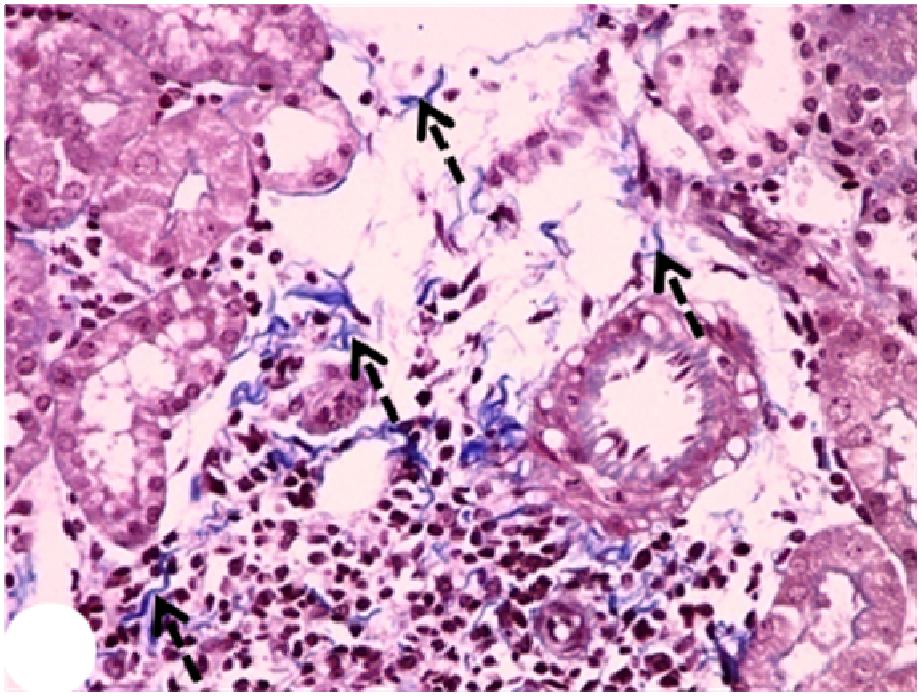
Slide (1) : Section in the liver of mice injected by L.quinquestriatus scorpion venom before 6 hours . It shows very strong hepatocytes swelling led to variation in the cell shape and disappearing of most of the hepatic sinusoids (S). There is cytoplasmic vacuolization (CV), and irregular spaces without staining inside the cytoplasm, and loss of chromatin inside most of the nuclei(arrow head), also nuclear pyknosis(double head arrow), and damaged hepatocytes(arrow) and active kupffer cells (K), and congestion of the central vein(CV) in most of the blood cells.



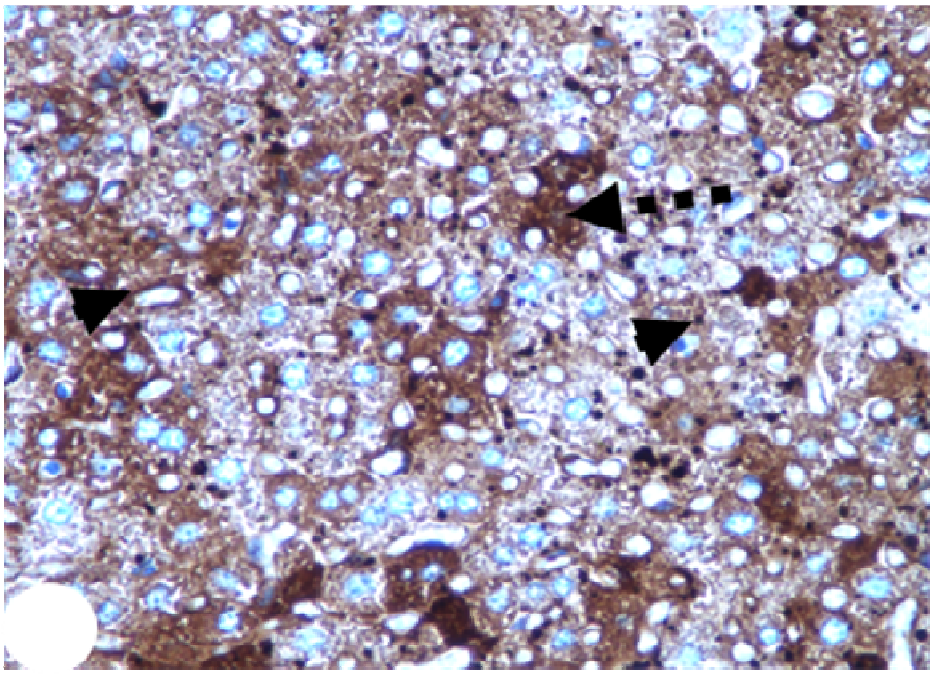
Slide (2): Section in the mice kidney injected with L.quinquesriatus venom for 6 hours. Aggregation of degenerated hepatolitic blood cells (HB) between tubules, and damaged tubular cells(arrow), and nuclear pyknosis(double head arrow) ,and can be seen leaked blood cells from blood capillaries (thin arrow), and a blood vessel(BV).



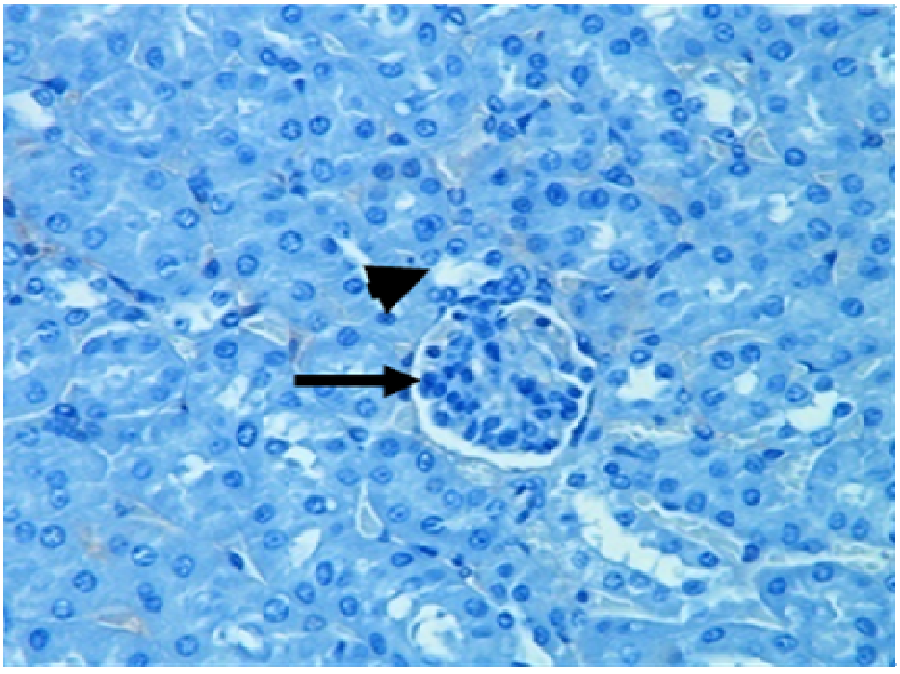
Slide (3): Section in the liver of mice injected by L.quinquestriatus scorpion venom before 6 hours. It shows damage of collagen fibers (dotted arrow) between the hepatic sinusoid (S).



Slide (4): Section in the mice kidney injected with L.quinquesriatus venom for 6 hours. It shows disruption of collagen fibers (dotted arrow) in the basement membrane of the renal tubule.



Slide (5): Section in the liver of mice injected b y L. quinquestriatus scorpion venom before 6 hours . It shows strong hepatocyte specific antigen (OCHIE5) inside some hepatocytes (dotted arrow) in addition to granules of high reaction to the antigen inside some other hepatocytes (arrow head) .



Slide (6): Section in the mice kidney injected with L. quinquesriatus venom for 6 hours . Disappearance of the activity of the endothelial cell marker (CD34) of the glomerular capillaries (arrows), and the endothelial cells of the intertubular blood capillaries(arrow head) .

This methods is used to detect certain types of antigens, that are found in the tissues associated with their antibodies (primary antibody) which are added to the tissue under certain conditions. This unknown antigen can be indentified by using as secondary labeled antibody. The antibodies are serum proteins known as immunoglobulin produced by the lymphatic cells in the body and they are five types, (IgG,IGE, IgD,IgA, Agm).

The Liver

Due to the effect of the scorpion venom, there was an inactivation of the antigen marking the endothelial cells (antigen CD34), and the endothelial cells of the glomerular capillaries, and that of the intertubular blood capillaries. While weak activity of the marker antigen (CD34) was observed in the endothelial cells of the interubular blood capillaries Slide (5).

The Kidney

As for the effect of venom of renal tissues there was no sign of activities of the blood vessel endothelial cell marker (CD 34), and the marker of the endothelial cells of the glomeular capillaries and those of the intertubular blood. On the other hand the activity of the marker antigen is clear in the endothelial cells of the interublular blood capillaries, and also weak activity of the marker (CD34) is seen in the endothelial cells of interubular blood capillaries Slide(6).

DISCUSSIONS

Many researchers said that scorpion venom causes kidney faliur, like Mady (2002) on *L.quinquestriatus*, Alves et al. (2005) on *Tityus serrulatus*, Dousset et al. (2005) on *Androctonus astrolabes*. And the present study showed that the alterations on the level of the micro structure and the histology depend on variable periods of time in both of the renal bodies and tubules of the mice subjected to the venom. And the examination of the hepatic and renal tissues under the light microscope showed glomerular aggregation and excess in cell number with swollen cells and cytoplasmic vacuolation and cell necrosis, and the tubular endothelium disruption. Pipelzadeh et al. (2006) also indicated glomerular aggregation and shrinking of Bowman´s space, and disruption of the basement membrane of the glomerulus and Bowman´s space in the renal tissues of the mice injected with the scorpion venom. And the increase in size and number of the glomerular cells many be due to the increase of the epithelial cells in addition to the infiltration by the neutrophilic and monocytes (Burkitt et al., 1996). And (Miner and Sanes, 1996) mentioned in that the safety of the glomerula may change by the micro structural alterations in the glomerular basal membrane.

The cytoplasm degradation in the portal and distal tubules of the kidney may be due to the actions of venom on the renal nephrosis during excretions of venom metabolites, as was also mentioned by Mady (2002). It is known that *L. quinquestriatus* venom works on the sodium and potassium channels of the cell membrane as was said by (Krimm et al., 1999), and this may change the permeability of the cell membrane and thus lead to accumulations of the Na and water inside the cell thus leading to cell swollen and to cytoplasmic vacuolation in the tubular endothelial cells of the mice treated by scorpion venom.

The great numbers of vacuolation inside the tubular cytoplasm and the swelling of the portal tubule endothelial cells of the venom treated mice was observed by Abd El-Gawad (2002) who said that this may be due to the vacuolation of the endothelial cell endoplasmic reticulum. On the other hand Burkitt et al. (1996) added that the swelling in the cell is resulted from the passage of the excess cell water to inside the cell and with presence of granulated cytoplasm result in cell swelling. This swelling of the cells may be due to the disruption observed in the cells and led to scuttering of the cell contents in the tubular lumen in the form of excess granulated materials .

The presence of inflammation cells in the intraspace of the tubular tissue may refer to the presence of inflammation in the tubular tissue, which represent a response or defensive action of the renal tissues when subjected to venom (Eriksson et al., 1996; D'Suze et al., 2003). The renal changes that follow scorpion sting was due to direct effect of the venom on the renal function (Alves et al., 2005).

The necrosis of the renal tubular endothelium due to scorpion venom observed in this study was also recorded by Omran (1992) who injected the mice with L. quinquestriatus venom. The same cell necrosis due to venom was also detected by (El Nasr et al.,1992; Aznaurian and Amiryan, 2006). The small aggregates seen spreading inside the tubule may be due to stripping of the tubular endothelial cells which are subjected to necrosis and which have fallen from its underline basal membrane with remains of damaged cells inside the tubular lumen, and these small aggregates were also mentioned by Pipelzadeh et al. (2006), who worked on *Hemiscorpious lepturus*.

The presence of the small granulated parts in the mice kidney injected by venom in this study may be due to accumulation of the remnant cells or due to tubular endothelial cells came from its underline basement membrane accumulated inside the lumen of the tubule (Eriksson et al.,1996).

It is clear from this study that the scorpion venom has large effect on the collagen, and it can be said that the stripping off of the basement membrane of Bowman´s capsule and the renal tubules is due to the collagen activity (Mortia et al.,1998).

REFERENCES

Adi-Bassam S (2012). A pharmacological investigation of the scorpion venom effect on the cardio-hepatic tissue . Faculty of Biological Science, Dept. of Cellular and Molecular Biolog., Laboratory of Research of venom , Algeria.

Al-Hazmy MA, Fahem B, Salih A (2001). Effect of R. stricta leaf extract on physiol histological tissue structure of mice. M.Sc. , Faculty of Science, King Abdul Aziz University .

Al-Saggaf AI (2004). Rhazya stricta seed water extract on the physiology, biochemical, and histology of the tissue of the liver and kidney of mice. Ph.D. Thesis, Faculty of Science, King AbdulAziz University.

Yahia TA (2004). Medicinal plants. Arabic House for Science, Lebanon, Bairot

AL-Sadoon M, Jarrar B (1997). Study of the frequency and incidence of scorpion stings and snakebites in Riyadh city. *J. King Saudi Univ. Sci.* 6: 217-266.

Alves RD, Nascimento NR, Barbosa PS, Kerntopf MR, Lessa LM, de Sousa CM, Martins RD, Sousa DF, de Queiroz MG, Toyama MH (2005). Renal effects and vascular reactivity induced by *Tityus serrulatus* venom. Toxicon, 46(3): 271-276.

Abd El-Gawad AM (2001). Histopahological and ultra-structural studies on the antidotal effect of vitamin E against flagentyl (Secnidazole) toxicity in albino mice. *J. Egypt. Ger. Soc. Zool*., 34(C): 141-153.

Aznaurian VA, Amiryan VS (2006). Histopathological changes induced by the venom of the snake *Vipera raddei* (Armenian adder). *Toxicon*, 47(2): 141-143.

Brown G, Johnston J, Tolbert L (1983). The effect of toxin from *Leiurus quinquestriatus* scorpion venom on the polymerization and stability of microtubules. *Toxicon*, 21(5): 699-708.

Burkitt HG, Stevens AS, Lowe JS, Yong B (1996). "Wheater's Basic Histology" 3rd ed., Churchill Livingstone, New York, pp. 3-34.

de Sousa L, Bonoli S, Quiroga M, Parrilla P (1996). Scorpion sting epidemiology in Montes Municipality of the State of Sucre; Venezuela: Geographic distribution. *Rev. Inst. Med. Trop. São Paulo*, 38*,* 147-52. [Links]

de Sousa L, Parrilla P, Tillero L, Valdiviezo A, Ledezma E, Jorquera A, Quiroga M (1997). Scorpion poisoning in the Acosta and Caripe Counties of Monagas State, Venezuela. Part 1: characterization of some epidemiological aspects. *Cad. Saúde Pública,* , 13*,* 45-51 [Links]

Dousset E, Carrega L, Steinberg JG, Clot-Faybesse O, Jouirou BB, Sauze N, Devaux C, Autier Y, Jammes YY, Martin-Eauclaire MF (2005). Evidence that free radical generation occurs during scorpion envenomation. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*., 140(2): 221-226.

D'Suze G, Moncada S, Gonzalez C, Sevicik C, Aguilar V, Alagon A (2003). Relationship between plasmatic levels of various cytokines, tumour necrosis factor, enzymes, glucose and venom concentration following *Tityus* scorpion sting. *Toxicon*, 41: 367-375.

D'Suze G, Salazar V, Díaz P, Sevcik C, Azpurua H, Bracho N (2004). Histopathological changes and inflammatory response induced by *Tityus discrepans* scorpion venom in rams. Toxicon, 44(8): 851-860.

El Nasr MS, Abdel Rahman M, Shoukry NA, Ahmed M, Eid SM, Fawzi AF, Sarwat MA (1992). The effect of scorpion envenomation on the different organs of albino mice. *J. Egypt Soc. Parasitol*., 22(3): 833-838.

Eriksson P, Denneberg T, Enestrom S, Johansson, Lindstrom F, Shogh T (1996). Urolithiasis and distal renal tubular acidosis preceding primary sjogrens sydrome: A retrospective study 5-53 years after presentation of urolithiasis. *J. Intrn. Med*., 239(6): 483-488.

Hafeiz A, El-Asmar M, Mahmouf K, Halawa F, El-Safori L, Kainel E, Marie N (1984). Effect of scorpion venom (fraction VII) on the rat testis (*in vivo* study). *Egyp. J. Dermat. Venereol*., 4, 61-8. [Links]

Hamon A, Gilles N, Sautiere P, Kopeyan C, Ulens C, Tytgat J, Lancelin J, Gordon D (2002). Characterization of Scorpion a-like toxin group using tow new toxins from the Scorpion *Leiurus quinquestriatus hebraeus. Eur. J. Biochem*., 269(16): 3920-3933.

Henriques MC, Gazzinelli G, Diniz CR, Gomez MV (2002). Effect of the venom of the scorpion Tityus serrulatus on adrenal glands. *Toxicon*, 5(3): 175-179.

Krimm I, Gilles N, Sautiere P, Stankiewicz M, Pelhate M, Gordon D, Lancelin JM (1999). MR structures and activity of a novel alpha-like toxin from the scorpion *Leiurus quinquestriatus hebraeus*. *J. Mol. Biol*., 285(4): 1749-1763.

Kopeyan C, Martinez G, Rochat H (2006). Primary structure of toxin IV of *Leiurus quinquestriatus quinquestriatus*. *INSERM. U.CNRS.UA*. 181(2): 553-559.

Mady AA (2002). Effect of different fractions of the scorpion and snake venoms on lipid peroxidant and some metabolic and histological changes. Ph.D. Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt.

Miner JH, Sanes JR (1996). Molecular and functional defects in kidneys of mice lacking collagen alpha 3 (IV)-implications for alport syndrome. *J. Cell Bio*., 135 (5): 1403-1413.

Mortia T, Yamamoto T, Churg J (1998). Mesangiolysis: an update. *Am. J. Kidney Dis*., 31: 559-573.

Muhammad MA (2011). The acute effects of scorpion (*Leiurus quinquestriatus*) venom on some clinicalpathological parameters in Guinea pigs. *Am. J. Sci*. 7(9): 794-801.

Penna-Videau S, Quiroga-Notti J (2000). Histopathological changes in albino mice testis induced by the administration of Tityus n. sp. Venom. J. of Venomous Animals and Toxins , vol.6(2):391-413 .

Pipelzadeh MH, Dezfulian AR, Jalali MT, Mansouri AK (2006). *In vitro* and *in vivo* studies on some toxic effects of the venom from *Hemiscorpious lepturus* scorpion. *Toxicon*, 48(1): 93-103.

Quiroga M, Marval MJ, Parrilla-Alvarez P, Desousa L (1998). *Tityus caripitensis* n. sp. scorpion venom gland histology. *Toxicon*, 36, 1269. (Abstracts from the 12th World Congress on Animal, Plant and Microbial Toxins*,* 12, Cuernavaca, 1997). [Links]

Traverso A, Sandra D (2004). Experimental poisoning by *Trema Micrantha* (*Ulmaceae*) in cattle. *Pesq. Vet. Bras. J*. 24(4): 211-216.

Williamson JA, Fenner PJ, Burnett JW, Rifkin JF (1996). Multi-tentacled box jellyfish (chirodropid) marine envenomation worldwide: rationale of clinical management. *Toxicon*. 34(2): 155-156.