



Global Advanced Research Journal of Environmental Science and Toxicology (ISSN: 2315-5140) Vol. 1(9) pp. 226-234, December, 2012
Available online <http://garj.org/garjest/index.htm>
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Full Length Research Paper

Histological and histochemical studies on the effect of *Rhzya stricta* extract on the mice and the possible protective role against *Leuris quinquestriatus* scorpion Venam

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Accepted 24 December, 2012

Rhzya stricta plant has been considered as medical plant for treating many human and animal diseases. In this study the leaf extract of the plant was orally administered at a dose of 3.75 mg/kg as a pretreatment to albino mice against toxic injuries of the venom of the yellow scorpion *Leuris quinquestriatus*. The durations of the pretreatments were 15, 30, 45 days. The examination was carried out on the histological and anatomical structure of the liver and kidney as it is known that envenomation usually causes many tissue alterations and injuries. The study showed that in spite of the adverse effects and alterations that had occurred on the mice hepatic and renal tissues due to the injected venom, the *R. stricta* leaf extract has caused partial improvement on the histological structure of the liver and kidney depending on the duration of the pretreatment. The improvement increased gradually with increase in the pretreatment period. After 45 days of pretreatment most of the hepatic cells look normal as elongated tapes, and the renal tissue showed normal appearance of the glomerulus, the proximal and distal tubules and the nuclei, and also the thick and thin limbs on Henle loop and the collecting tubules are all normal. The collagen fibers were seen very thick in the liver tissue, and its presence was also observed in the kidney tissue after 45 days pretreatment. Extending the pretreatment period up to 45 days also induced very strong activity of the antigen (CD34) in the kidney tissue similar to that of the control. We suggest more work in this field.

Keywords: Histochemical Studies, *Rhzya stricta*, Scorpion Venam

INTRODUCTION

Medical plants are widely used in curing many diseases, and plant extracts play an important role in treatment of patients subjected to scorpion and snake stings. The

plant extracts are used as traditional medicine to activate the immunity system to resist the action of different types of venoms (Maiorano et al., 2005; Oliveira et al., 2005). Many researchers have used *R. stricta* leaf extract in traditional medicine (Ali et al., 2008, 1998) and this plant is classified as an important medical plant, and it is indigenous to south Asia (Iqbal et al., 2006) and distributes in Saudi Arabia in many places (Hassan et

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al., 2007; Adam et al., 2002).

The previous studied didn't refer to anything about the hepatic and renal tissue and cell structures that are related to the use of *R. stricta* extract in alleviating the adverse effects of envenomation. But the protective activity of the liver against the toxic effect of acetaminophen which causes liver fibrosis was increased when the animals were treated by the extract of the plants *Artemisia scoparia*, *Ambrosia maritima* (Ahmed and Khater, 2001; Gilani and Janbaz, 1993). The leaf extract of the plants of the family *Compositae* is able to mitigate the liver fibrosis (Lee et al., 2007). The leaf extract of the plant *Vernonia amygdalina* was found effective against hepatotoxicity that was caused by administration of carbon tetrachloride by mice (Babalola et al., 2001). The pretreatment of mice and rats with the extract of the plant *Ginkgo Biloba* against *L. quinquestriatus* scorpion venom have significantly extended the life period of the animals (Fatani et al., 2004). Also the pretreatment of mice with the seed extract of *Proanthocyanidin* 10 days before *L. quinquestriatus* venom injection had significantly protected the animals against the scorpion venom effects (Eissa et al., 2004).

The aim of this study is to examine the role of the pretreatment of mice by *Rhazya stricta* leaf extract against the adverse effects that are expected from the envenomation of the yellow scorpion *Leuris quinquestriatus* on the anatomical and histological structures of the liver and kidney.

MATERIALS AND METHODS

Leaf extract

Leaf samples of *R. stricta* dense were collected from Al-Khomrah area south Jeddah along the coastal road during spring season (Jan – April) where the leaves are in fully green color. The leaves were thoroughly washed with water, dried by means of hot air, and then ground to very fine powder with a blender. The powder was then put in distilled water (9g:40ml) and boiled and filtered using bacterial filter. The filtrate was boiled again, cooled to room temperature and again filtered and put in a glass tube inside the fridge at 10°C. The extract was diluted with distilled water according to the weight of each rat. The dose of 2.36 gm /km was demonstrated orally by the use of the stomach feeding tube (Wasfi et al, 1994).

The animals

A total number of 80 adult male albino mice were used in this research. 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.

They were housed in groups of 3 animals per cage at a temperature of 18-20°C under a 12h dark–light cycle. They were fed standard pelleted diet (grain soils with plant fibers, vitamin A amino acids, salts of Ca, P, Fe, K) and clean drinking water. The animals were left for one week before start of treatments.

The animals were grouped into four groups each one is ten mice, the first one is control (G1) which was treated orally with distilled water, the second (G2) was treated orally using the stomach tube with *Rhazya stricta* extract at dose of 2.36 gm/kg for 15 days, the third group (G3) was administered the plant extract at the same dose for 30 days, and the fourth group was orally administered the leaf extract of. at the dose of 2.36 gm/Kg for 45 days.

At the end of each treatment the animals were anaesthetized with diethyl ether, fixed on the dissection board and small parts and small parts of the liver and kidney (each sample is 3mm³) were taken for sectioning.

Leuris 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 from Al-Khomrah region in Jeddah city, along the coastal road 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, fluorescent 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 Eosin, 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, 50x DAB 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 formalicinfixation 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%), and 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 30-40 minutes to clear the samples from ethanol.

Paraffin infiltration

The tissues were subjected to paraffin infiltration using melted paraffin wax inside the oven at 60C° for one hour.

Embedding

Molting wax was poured inside embedding moulds, and the tissue samples were transferred 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 24C°. The sections were picked out and put on a

hot plate at 30C° 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 eosin 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 immunohistochemisrty 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

The histological studies on hepatic and renal tissues of the treated mice using Haematoxylin and Eosin stain.

The mice were administered *R. stricta* leaf extract for periods of 15, 30 and 45 days and then dissected after injection with *L. quinquestriatus* venom for 6 hours.

The liver

The hepatocytes of the mice pretreated with *L. stricta* extract for 15 days appear normal in shape with circular nuclei, but most of the cells suffer cytoplasmic vacuolization and cytoplasmic granulation, and nuclear margination can be seen in some cells. The sinusoids separate the hepatocytes and they look normal containing kupffer cells which are circular in shape with darkish stain. The branches of the portal vein and bile duct have normal shapes.

After 30 days pretreatment with the plant extract, the hepatocytes appear normal in shape as elongated taps with homogeneous cytoplasm, and normally circular nuclei separated by sinusoids which are and normal,

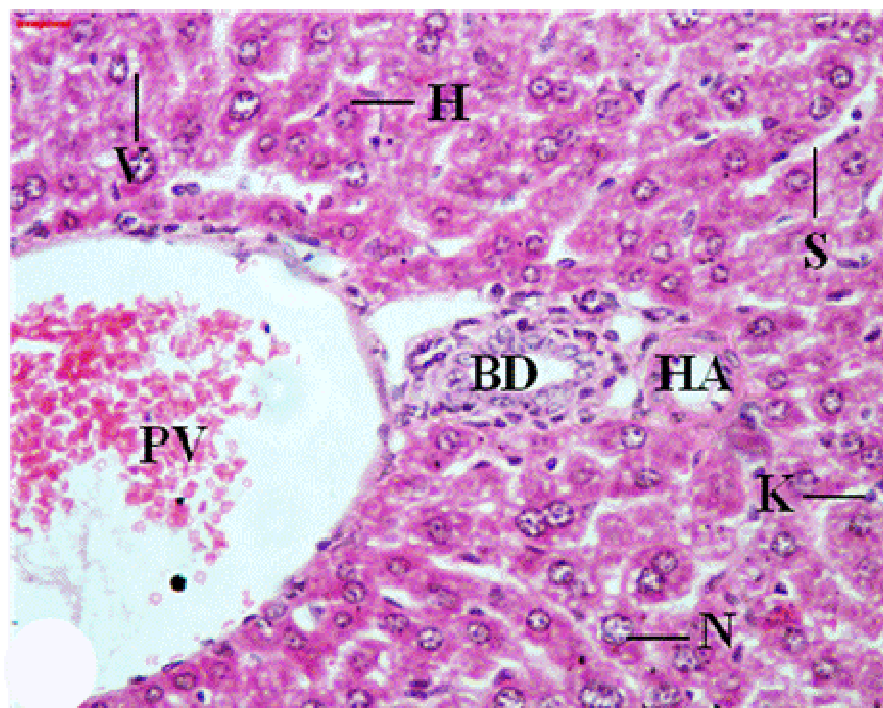


Figure 1. Section of mice liver pretreated with *R. stricta* extract 45days before venomming. (H) hepatocytes separated by sinusoids (S). (N) normal shaped nuclei with some cytoplasmic vacuolization (V) in most cells. (K) Kupffer cells. (PV) branch of the portal vein. (BD) branch of the bile duct.(HA)branch of the hepatic artery.

containing kupffer cells which are active and large.

The hepatocytes after 45 days pretreatment with the plant extract are in shape as elongated taps, but some of it suffers cytoplasmic vacuolization and granulation, with circular nuclei and thick stain. The sinusoids separate the hepatocytes, and contain some kupffer cells. Figure (1).

The kidney

The renal anatomical structure of the mice pretreated with *R.stricta* leaf extract for 15 days against *L. quinquestriatusvenom* show nuclear pyknosis, nuclear margination and an empty looking cytoplasm, with blood cells.

On the other hand the renal tissue structure of the mice administered the plant extract 30 days before vemon injection showed nuclear pyknosis, and an empty looking cytoplasm.

And the pretreatment of mice 45 days before vemon injection resulted in renal structure with normally looking glomerulus, normally looking proximal and distal tubules and nuclei. Also the thick and thin limbs of HenleLoop and the collecting tubules are looking normal. See Figure (2).

Histological studies on hepatic and renal tissues of the treated mice using Masson trichrome staining

Masson trichrome staining was used to detect the structure of the renal connective tissues.

The liver

The anatomical features of the tissues and cells of the liver of the mice subjected to 15 days pretreatment with *R. stricta* leaf extract before injecting the scorpion venom, show disruption of the collagen fibers in the central vein, while appearing very thick around it. The thickness of the collagen fibres is also shown in the portal vein, and collagen fibres are also present in the sinusoidal wall.

Under the extension of the pretreatment with the plant extract to 30 days, the collagen fibers are also observed disrupted in the central vein, and their presence is seen in the central vein, the portal vein and at the sinusoidal wall.

When the pretreatment with the plant leaf extract was extended to 45 days the collagen fibers are seen very thick at the central vein wall and the portal vein wall. Figure (3).

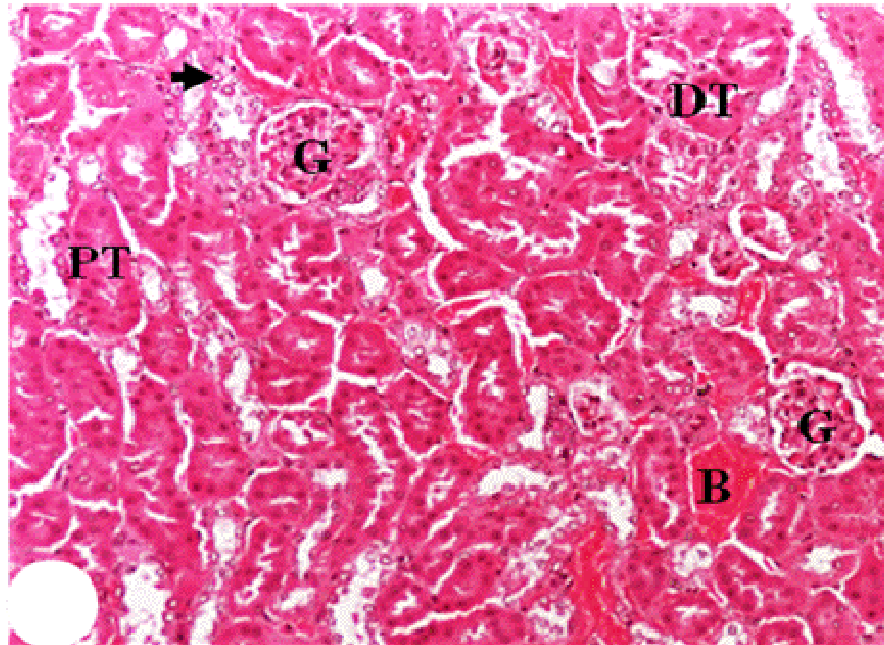


Figure 2. Section of mice kidney pretreated with *R. stricta* leaf extract for 15 days before venom injection. The slide shows the renal glomerulus (G), the proximal tubule (PT), blood cells (BC), and nuclear pyknosis (arrow).

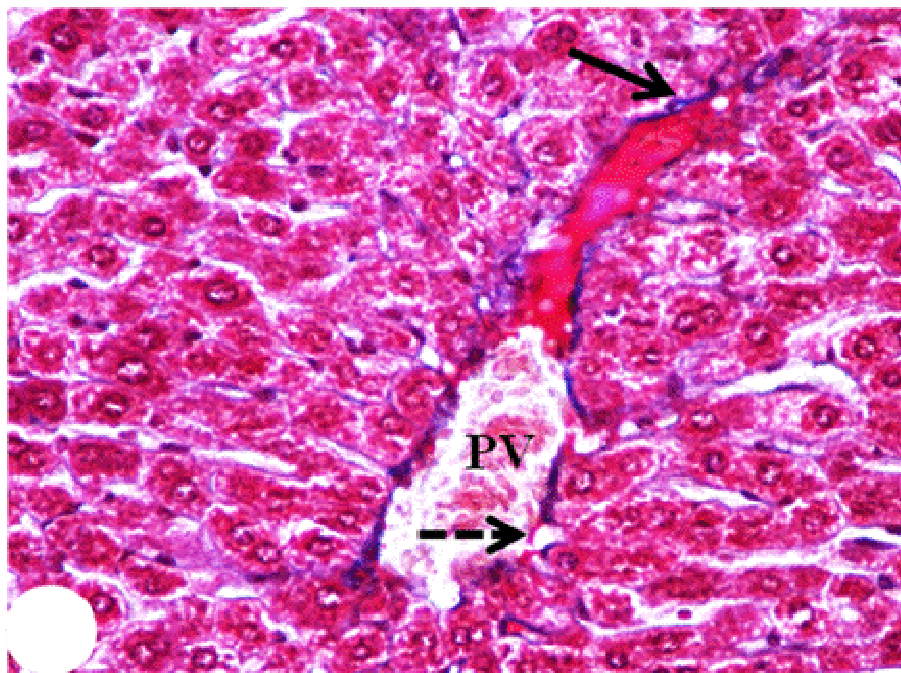


Figure 3. Section of mice liver pretreated with *R. stricta* leaf extract 30 days before venom injection. The section shows the thick collagen fibers (arrow) in the portal vein (PV), and the disruption of the collagen fibers (dotted arrow) in the portal vein (PV).

The kidney

The renal tissues of the mice administered *R. stricta* leaf

extract 15 days before injecting *L. quinquestriatus* scorpion venom show disruption of the collagen fibers in the basement membrane of Bowman's capsule, and in

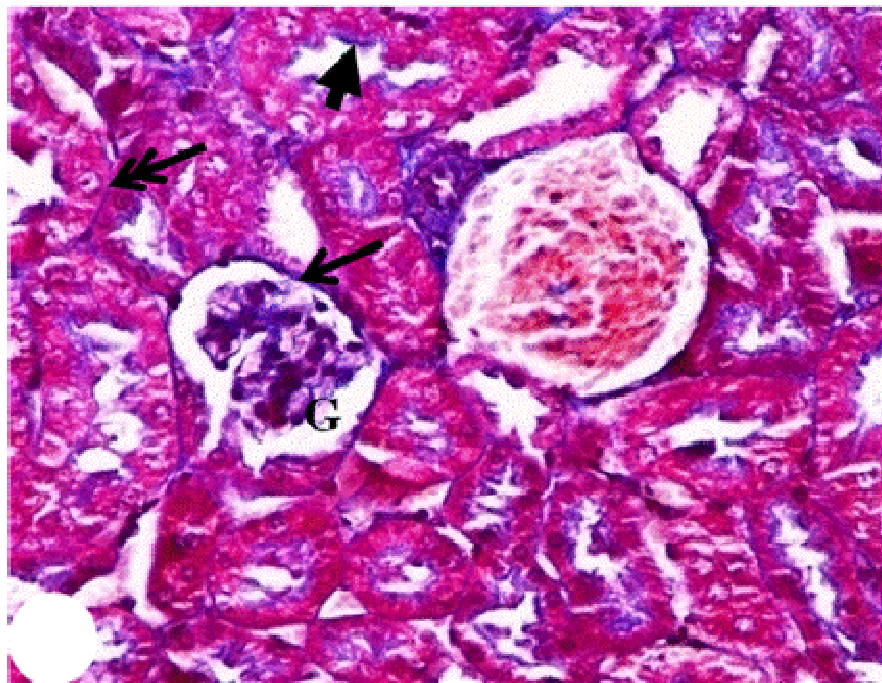


Figure 4. Section of mice kidney pretreated with *R. stricta* leaf extract 45 days before venom injection. The section shows little presence of collagen fibers in the basement membrane of Bowman's capsule (arrow), it also appears in the basement membrane of the renal tubules (double arrow) and in the brush border (arrow head).

the basement membrane of the renal tubules. Some disruption of the collagen fibers is seen in the basement membrane of the glomerulus, and in some of the renal tubules and also in the basement membrane of the renal tubules. Consequently the return of the collagen fibers is detected in the glomerulus, and in the basement membrane of Bowman's capsule and the collagen fibers are seen very thick in the brush borders. The return of the collagen fibers is also observed in the basement membrane of the proximal tubules, accompanied by only little return of these fibers in the thin and thick limb of Henle Loop and in the collecting tubules.

At 30 days pretreatment of the animals with the plant leaf extract, the collagen fibers are seen returning in a thick manner in the glomerulus, and in the basement membrane of Bowman's capsule and in the basement membrane of the renal tubules. The return of the collagen fibers can also be seen in the proximal and distal tubules, in the basement membrane of the renal tubules and in the brush borders as can be seen very thick.

When the plant leaf extract pretreatment was extended to 45 days, the presence of the collagen fibers is observed only in small manner in the basement membrane of Bowman's capsule and in the renal tubules and the brush borders. It is also observed in small manner in the proximal tubule and in the thin limb of Henle Loop, and seen in thick manner in the brush border. Figure (4).

Histological studies on hepatic and renal tissues of the treated mice using Immunohistochemistry technique

This technique is used to detect certain antigens that are found attached to their antibodies under certain conditions. The site of this antigen in the tissue can be identified by labeling the antigen through a labelled secondary antibody. And the antibodies are serum proteins known as immunoglobulin produced by the lymphatic cells in the body and there are five types (IgG , IgE , IgD , IgA , IgM).

The liver

After pretreatment of the mice for 15 days before *L. quinquestriatus* venom injection, large number of hepatocytes is seen around the central vein showing strong activity of the hepatocyte specific antigen (OCHIE5). Some cells can be seen having many granules that have strong positive action of the antigen, and this strong reaction of the antigen is observed in many hepatocytes, and also in the sinusoidal endothelial cells .On the the other hand only little reaction of the antigen can be seen inside the central vein endothelial cells, with the presence of some hepatocytes with no antigen activity.

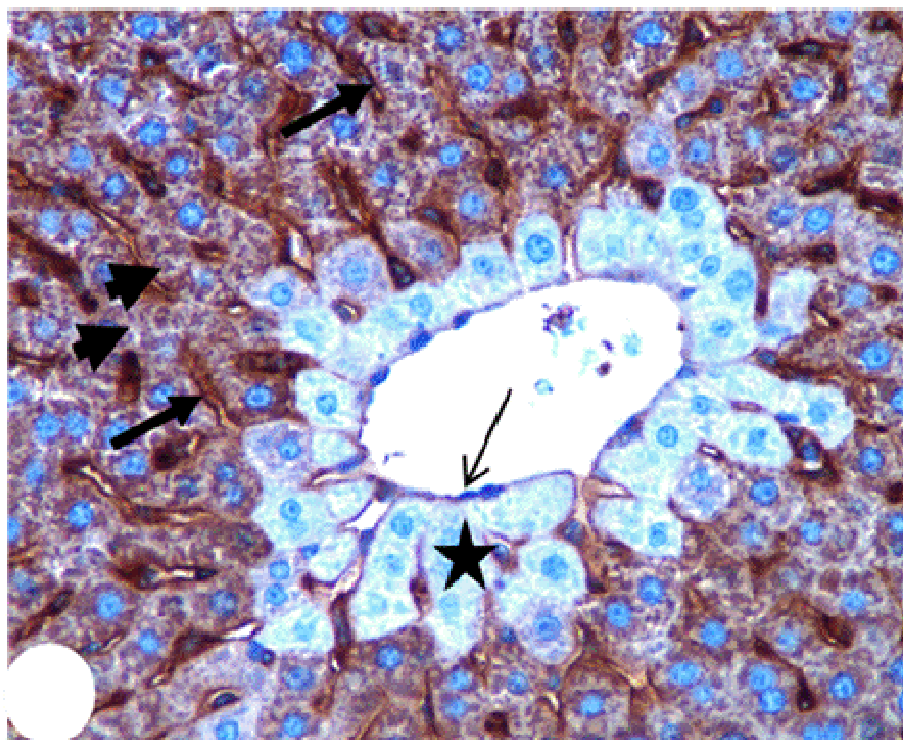


Figure 5. Section of mice liver pretreated with *R. stricta* leaf extract 45 days before venom injection. The section shows little activity of the antigen (OCHIE5) inside the hepatic endothelial sinusoids (arrows), while most hepatocytes contain many granules with strong positive reaction of the antigen (arrow head).

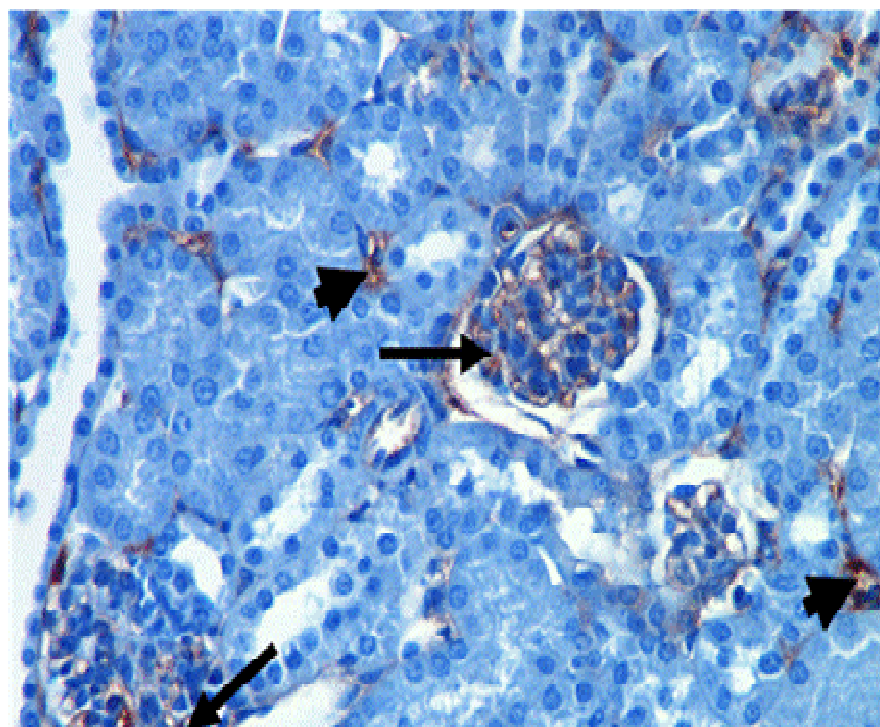


Figure 6. Section in the cortex region of mice kidney pretreated with *R. stricta* leaf extract 45 days before venom injection. The section indicates very strong activity of the antigen marker (CD34) in the endothelial cells of the blood capillaries (arrow), the glomerular capillaries (arrow) and the renal intertubular blood capillaries (arrow head).

After 30 days pretreatment of the mice strong activity of the hepatocyte specific antigen (OCHIES) is observed inside the hepatic sinusoidal endothelial cells. Also most of the hepatocytes are observed containing many granules with strong positive reaction of the antigen, and at the same time there are hepatocytes with no antigen reaction.

When the pretreatment period of the mice was extended to 45 days before the scorpion venom injection, no strong activity of the hepatocyte specific antigen was observed inside the hepatic sinusoidal endothelial cells, while most of the hepatocytes are containing many granules with positive reaction of the antigen. Figure (5).

The kidney

Administering *R. stricta* extract 15 days to mice before venom injection caused no activity of the antigen of the endothelial cell marker (CD34) of the blood and glomerulus capillaries, but there is a weak activity of the antigen (CD34) in the intercellular blood capillaries endothelial cells. But there is an intermediate antigen (CD34) activity in the endothelial cells of the glomerular capillaries and in the intertubular blood capillaries in the cortex region. And also there is an intermediate activity of the antigen in the endothelial cells of the intertubular blood capillaries in the medullary region.

After 30 days pretreatment of the plant leaf extract, the intermediate activity of the antigen (CD34) in the renal tissues is observed in the blood capillaries endothelial cells and in the intertubular blood capillaries endothelial cells in the cortex and the medullary regions. But some cells indicate strong activity of the antigen. When the pretreatment period was extended up to 45 days before venom injection to the animals, a very strong activity of the antigen (CD34) similar to that of the control group can be seen in the endothelial cells of the glomerular capillaries and in the intertubular blood capillaries in the cortex and medullary regions. Figure (6).

DISCUSSION

In spite of the adverse effects of the scorpion *L. quinquestrianus* venom on the hepatic and renal tissues of mice, the results of the pretreatment of the animals by *R. stricta* leaf extract 6 hours before venom injection, had caused partial improvement depending on the plant extract treatment. The improvement included both the tubular endothelial cells of the renal tubules and the glomerulus. And generally only few researchers have worked on the treatment of renal anatomical changes that result from scorpion venom injuries (Sugimoto et al., 1996; Rahmy and Hemmaid, 2001; Soares et al., 2005).

The disruption of the hepatic and renal cells of the mice pretreated by *R. stricta* extract 15 days before

venom injection, may indicate that this period is not sufficient for good protection against the adverse effects of scorpion venom. So these cellular changes were reduced when the plant extract pretreatment period was extended to 30 and 45 days. Ali (2002) suggested that the dose of 0.25 mg/kg of *R. stricta* extract is not effective in treating kidneys subjected to venom injuries, and the higher dose gave significant increase in the activity of SOD and the concentration of GSH, and reduction of the lipid peroxides in the renal cortex. These results may indicate that *R. stricta* leaf extract may contain compounds that can improve kidney of mice subjected to scorpion venom injuries, like the phenolics, flavonoids which have been found in this extract (Hanif et al., 2011).

Many workers have mentioned the role of the antioxidants in protecting the hepatic and renal tissues against adverse effects of venom (Abdel Gawad, 2001; El Ridi and Rahmy, 2000). Many active antioxidant materials were found in about seven plant families including Apocynaceae, which is *R. stricta* family (Nino et al., 2011), and proved its ability to protect hepatic and renal tissues against venom effects (Wong et al., 2001).

The reduction in the number of the inflammatory cells in the treated animals is due to the uninflammatory action of the plant extract (Rasheed et al., 1997). And many compounds that treat inflammatory tissues have been isolated from plant resources like terpenoids in the leaves of *Aspitia Africana* (Okoli et al., 2009), sesquiterpene lactones from medical plants of the family *Asteraceae* (Mino et al., 2004).

The action of *R. stricta* leaf extract in this study in mitigating liver and kidney venom injuries may be due to its antioxidant activity as was recorded by (Iqbal et al., 2006).

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