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ORIGINAL ARTICLE

HISTOPATHOLOGICAL CHANGES IN MICE AFTER INTRAPERITONEAL ADMINISTRATION OF LIONFISH *Pterois russelii* VENOM

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ABSTRACT

The venom of the lionfish *Pterois russelii* was tested for its ability to induce histopathological changes after the intraperitoneal injection of venom at sub-lethal dose of 3.75 µg/kg b.w (10% of the LD_{50} value) on liver, kidney and brain tissues of mice. Histopathological changes observed in vital organs revealed that lionfish venom could be injurious to vital organs. This injury could be considered among the factors that the mice lead to death caused by *P. russelii* envenoming and the effect was due to the site and the mode of action of this poison at tissue levels. This observation may contribute for the discovery of new valuable pharmaceutical products.

Keywords: Lionfish *P. russelii*, Venom, Liver, Kidney, Brain, Haematoxylene, Eosin

1.INTRODUCTION

Histology is concerned with the study of organization of tissues and pathology is the study of disease. Histopathology is a branch of pathology that deals specifically with tissue abnormalities. Histopathological alterations have been used as markers to understand animal health exposed to contaminants in lab (Wester and Canton, 1991; Pawan Kumar *et al*., 2013) and field (Hinton *et al*., 1992; Schwaiger *et al*., 1997; Teh *et al*., 1997). The histopathological markers are necessary to monitor the target organs, including brain, heart, kidney and liver which are responsible for important functions, such as excretion and the deposition and bio-magnifications of toxins in the fish (Gernhofer *et al*., 2001).

The results obtained by using the histopathological techniques are reliable, inexpensive, sensitive, and rapid and have the ability to provide a presumptive diagnosis of the result as well as demonstrating the tissue reaction for the assessment of damage due to xenobiotics. Cell damage is a result of persistent or irreversible biochemical and subcellular dysfunction caused by stress. Though the cell has a great adaptability in responding to changes in internal and external environment by undergoing reversible alterations in both cellular structure and function, often the stressed cells undergo irreversible structural and biochemical changes, which in turn result in alterations in the physiology of the animal. Thus assessment of histopathological manifestation provides insight into the degree of stress, susceptibility and adaptive capability of the stressed organism and is one of the major tools for diagnosis of disease. The objective of this study was to identify and compare the histological changes in liver, kidney and brain tissues of mice after the intraperitoneal injection of lionfish (*P. russelii)* venom at a sublethal dose of 3.75 µg/kg b.w (10 % of the LD_{50} value).

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2.MATERIALS AND METHODS

Venom preparation

Live specimens of the venomous lionfish *P. russelii* were collected from Mandapam coast and brought to the laboratory. Crude venom was extracted as described by Church and Hodgson (2002). The fishes were killed (by cooling) and the venomous spines were removed and stored in 10% glycerol solution at -80^0 C. When required, the spines were thawed and grounded in a chilled mortar and pestle with 10% glycerol solution. The suspension was kept in a magnetic stirrer (overnight) at 2^0 C and centrifuged at 7000g for 15 minutes in a refrigerated centrifuge. The supernatant was removed and the protein was estimated by the method of Lowry *et al*. (1951) and the concentration was

adjusted to 1.0 mg/ml, aliquot and stored at -20^0 C until to use.

Determination of LD_{50}

The LD_{50} value was determined in mice by following the OECD guidelines (up and down method) and the values were calculated by the method of Litchfield and Wilcoxon, (1949).

Experimental animal

Adult male Swiss albino mice *(Mus musculus)* of 10 to 12 weeks old (22 ± 2 g) were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were maintained under controlled conditions of temperature (23 ± 2^{o}C), humidity (50 ± 5%) and light (10 and 14 h of light and dark cycles, respectively) and were fed with commercial standard pellet and provided water *ad libitum*. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Annamalai University (Registration Number: 953/2012/CPCSEA) and the animals were cared in accordance with the "Guide for the care and use of laboratory animals" and "Committee for the purpose of control and supervision on experimental animals."

Experimental design

Twelve matured male swiss albino mice were randomly separated into two groups, as control and lionfish venom treated group.

Control group

The first group was injected intraperitoneally with 100µL physiological saline (0.9% NaCl) solution and marked as control.

Treatment group

The second group was injected intraperitoneally with 3.75µg/kg b.w. (10% of the LD_{50} value) of *P. russelii* crude venom in 100µL saline solution at a single sub-lethal dose.

Histological study

The histological changes were observed in mice by following the standard histological method of Luna, 1968. After 24 hour of envenomation, the animals were anesthetized using chloroform and sacrificed by cervical dislocation. Liver, kidney and brain were removed carefully and washed thoroughly with 0.9% normal saline to remove any trace of blood. The dissected tissue was treated with Bouin's fluid (fixative) for 16-18 hour and subsequently washed under running tap water for one hour until complete removal of most of the Bouin's fluid from the tissues. Followed by washing, dehydration of the tissues was conducted by immersing in a series of gradually increasing concentrations of alcohol (50%, 70%, 80%, 95% and absolute alcohol) and embedded into paraffin wax for making blocks. Sectioning of the tissue was performed by using a microtome machine. The microtome was pre-set to cut 6µm thickness of the tissue. Small ribbons of tissue sections were placed on microscopic slide with the help of warm distil water containing a few drops of Mayer's albumen and deparaffinized with xylene solution. Haematoxylene and eosin solutions were used to stain the tissue section for preparing permanent slides. Histopathological changes were observed under light microscope.

3.RESULTS

Histopathological effects

The effects of *P. russelii* venom on the vital organs are very well confirmed by the histopathological changes in the treated mice organs like liver, kidney and brain.

Effect on hepatic tissue

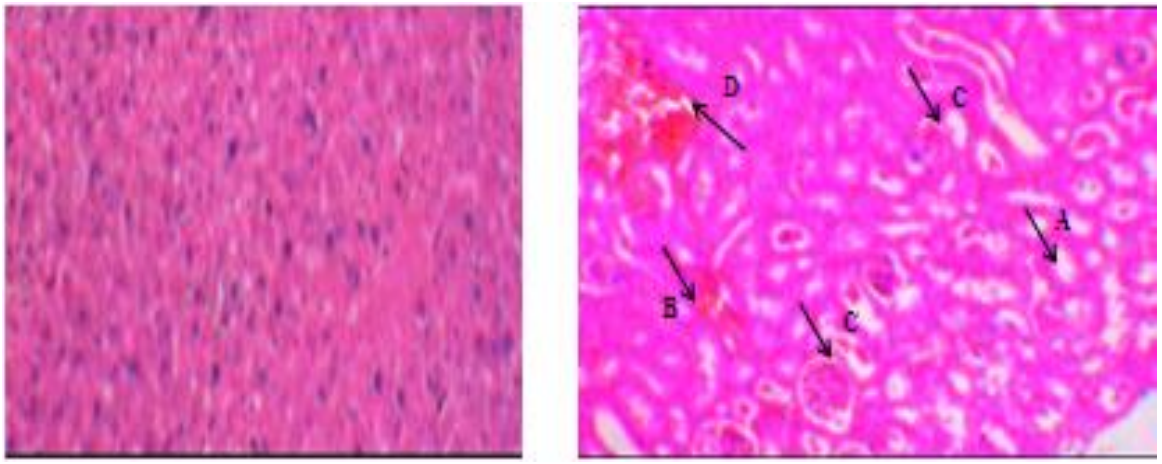
The histological sections of the liver tissue of the control mice showed a regular and compact configuration with well hepatic cell, central vain, and sinusoid and associated with other accessory vain and capillaries. In the envenomated mice liver cells some severe alterations were noticed and the hepaticcells lost their structural integrity. Extremely vacuolated areas and haemolysis were observed in addition to marked pycnotic nuclei. Micro vesicular types of fatty acid changes (Microvesicular steatosis) were also seen in hepatocytes. Blood sinusoids were distended, congested, or disrupted with partially haemolysed blood. The hepatic cells were undergoing degenerative changes and coagulative necrosis. Occasionally oedematous fluids were also seen at some places (Fig-1).

Effect on renal tissue

Renal tissues of control mice showed the normal organization of the renal corpuscles with intact Bowman's capsules each enclosing by a tuft of glomerular capillaries. In the envenomated mice kidney some severe histopathological changes were presented. Histopathological analysis indicated that the venom and its fraction acted on the renal tubules and glomeruli. Blood vessels were highly congested with haemolysed blood and haemorrhage. Cloudy swellings in the lining of renal tubules were noted. In addition to tubular necrosis, pycnotic nuclei were also seen. The parietal epithelium of Bowman's capsule was found to be prominent. Proteinaceous /foreign materials were found accumulated with the glomerulus and often shrinkage of the glomerular tuft could be seen. Moderate degenerative changes were noticed and the cells had lost their normal structure (Fig-2).

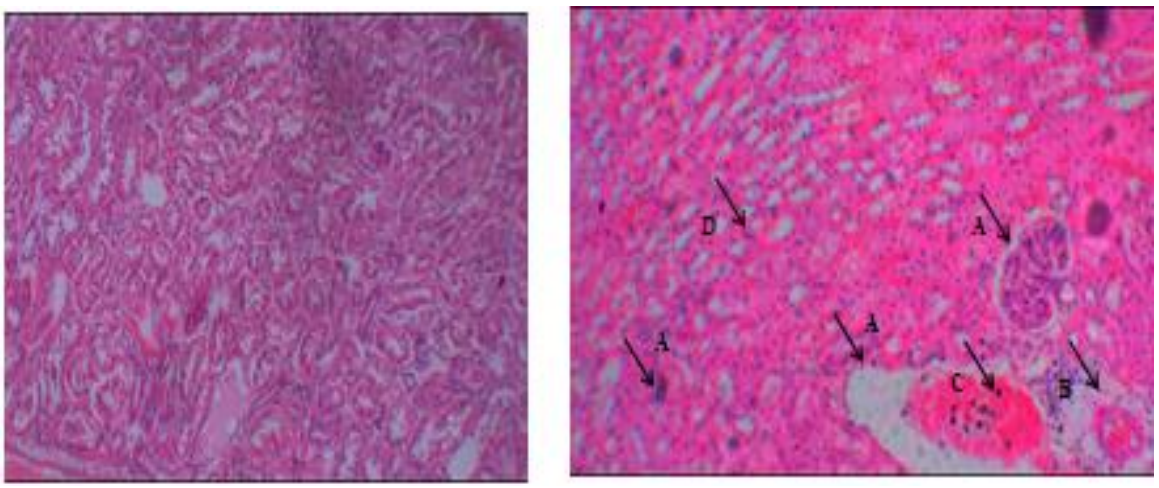
Effect on brain tissue

The histological section of the control mice brain tissue showed a regular and compact configuration with blood sinusoids. But in the treated mice they were highly congested with haemolysed blood and haemorrhage. Glial nodule formation was observed in some areas of the cerebrum. The mild congestion of capillaries and pycnotic nuclei, a condition formed by the condensation of chromatin in the nucleus of a cell undergoing necrosis were found in the cerebellum (Fig-3).



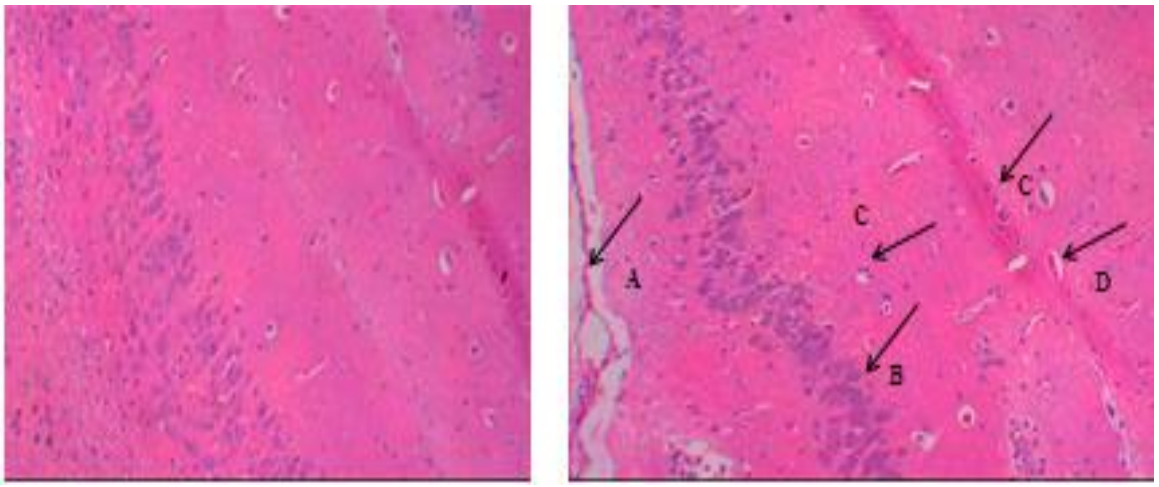
Normal liver **venom treated liver**

Fig.1: Histological effect of *P. russelii* venom on mice liver. A. Vacoulation, B.Pycnotic nuclei,C.Congested blood sinusoids, D.Necrosis.



Normal kidney **Venom treated kidney**

Fig.2: Histological effect of *P. russelii* venom on mice kidney. A.Thickening of bowmans capsule, B.Shrinkening of glomeruli,C.Proteinaceous or foreign material present, D. Tubular necrosis



Normal brain **Venom treated brain**

Fig- 3: Histopathological effects of *P. russelii* venom on mice brain. A.Congested blood sinusoids B.Pycnotic nuclei C.Vacoulation D.Necrosis.

4.DISCUSSION

The in vivo effects of *H. Lepturus* venom have previously been investigated using rats and rabbits as animal models (Ahmadizadeh *et al*., 2006; Zare Mirakabbadi *et al*., 2007; Khamechian *et al*., 2009; Lowe, 2010 and Zayerzadeh *et al*., 2011). However, in the present study mice were chosen as the preferred as alternative animal model. The present study was conducted to know the histopathological changes due to *P. russelii* venom on the vital organs like liver, kidney and brain tissues.

Absuma and Venketashvaran (1999) have reported that administration of crude extract of epidermal secretion of *Boleophthal musdentatus* causes discoloration of heart, liver and kidney. Skin toxin of the giant slender moray eel *Thyrsoidea macrura* showed extensive necrosis and haemorrhage in kidney and liver of mice (Raju and Venketashvaran, 1999). Similar effects were also observed for skin toxins from the three arid catfish *Arius caelatis, A. dissumieri* and *Osfeogeneiosus militaris* (Variath and Venketashvaran, 1999). Histopathological studies of mice exposed to *A. dissumieri* mucus extract showed pycnotic nuclei (Deo, 2000). Marta *et al*, (2011) observed that, the skin toxin from the venom of *P. falkneri* is responsible for the development of an early necrosis with mild inflammatory reaction, probably due to the reaction of the venom. Dehghani *et al*. (2012) reported that the *H. lepturus* venom which can affect both local and systemic on the mice including skin tissue damages and internal organs respectively. The *H. lepturus* venom with neurotoxic, cytotoxic and haemolytic characters has toxic effects on the mice and human. The similar results were observed in *Bungarus caeruleus* envenoming rat and the effect was considered to be due to the site and the mode of action of this poison at tissue level and this finding may contribute for the discovery of antivenom related valuable pharmaceutical products (AL-Mamun *et al*., 2015).

Liver of lionfish venom treated mice showed congestion, micro vesicular fatty changes and infiltration of inflammatory cells around the portal vein. Toxic effect on liver was observed for the skin toxin of *Arius thalassinus* (Al-Hassan *et al*., 1985). Alnaqeeb *et al*. (1989) observed extensive haemorrhage in liver tissue which was attributed to tissue destruction, due to blocking of blood flow in turn leading to necrosis. Taha Shawi Morad *et al*. (2014) were also reported that, the liver of mice after treated with single dose of LD_{50} *Naja naja* snake venom induces intrahepatic hemorrhage, liver necrosis and hepatotoxicity in mice.

Haemorrhage, vascular congestion and cloudy swelling in renal tubules were observed in the kidney of *P. russelii* venom treated mice. The venom also caused congestion, microvesicular fatty acid changes and infiltration of inflammatory cells around the portal vein. Kidney cells can release prostaglandins, cytokinins, bradykinin, complement fractions and platelet activating factors (Barraviera *et al*., 1995). Studies on the histologicalevaluation of rat kidney perfused with *Thallasophry nenattereri* venom showed moderate deposits of proteinaceous material in the renal tubule (Faco *et al*., 2003). Lougin *et al*. (2010) have reported that, the histological and immunohistochemical studies on the nephrotoxic effects of *Naja nigricollis* venom showed intense dose and time dependent abnormalities including signs of glomerulolysis, tubular necrosis and damage, formation of hyaline and granular tubular casts as well as signs of intertubular medullary hemorrhage at early stages of envenoming. The histopathological alterations noticed in the kidney cells during the present study could be due to the direct action of the venom in renal glomeruli and tubules or an indirect release of mediators.

Brain tissue of *P. russelii* venom treated mice showed spongiosis throughout parenchyma. This change was similar to the effect of *P. volitans* venom on rat brain (Balasubashini *et al*., 2006). Brain tissue showed spongiosis (oedema) throughout the parenchyma. The oedema of brain and the cloudy swelling in liningcells of renal tubule suggest that the venom might contain oedema-causing factors that could have crossed over the blood-brain barrier (BBB) and damaged the brain (Saminathan *et al*., 2006). Cloudy swellings were observed in all the vital organs under study. The effects of *P. russelii* venom on the vital organs are very well confirmed by the histopathological changes of the venom treated mice. Similar results were observed during administration of venom from *Tityus serrulatus* (Correa *et al*., 1997), *T. nattereri* (Fonesca and Lopes Ferriera, 2000), *Conus lorreossi* (Saminathan *et al*., 2006) and *Scatophagus argus* (Sivan, 2007). The present research revealed that the venom of *P. russelii* was appeared to be injurious to the vascular endothelium and caused congestion of blood vessels and cloudy swellings in liver, kidney and brain tissues and lead to death.

5.CONCLUSION

The present study was conducted to investigate the histopathological changes in vital organs of the *P. russelii* venom treated mice. This is one of the possible of important characterizations of proteins in *P. russelii* venom and also for understanding their action in the process of development of specific antivenom. Therefore, further studies need to be carried out for the isolation and purification of lionfish venom and applying these valuable natural raw materials in sophisticated research for discovery of antivenom related drug and other pharmaceutical valuable product.

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