Chapter 3

HISTOPATHOLOGICAL EFFECTS OF S. ARGUS VENOM ON MICE
Histopathological effects of S. argus venom on mice

3.1. INTRODUCTION

Histology is concerned with the organization of tissues and Pathology is the study of disease. Histopathology is that branch of pathology that deals specifically with tissue abnormalities. The study of cytological and histopathological alterations is an integral part of toxicology. 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.

3.2. REVIEW

Studies involving histopathological effects of fish venoms are very few or limited in number. Studies with spine extracts on gastrocnemius muscle of mice injected with T. nattereri venom have been carried out [Lopes-Ferriera et al., 2001]. Balasubashini et al., [2006] studied the effect of P. volitans venom on the vital organs of mice. Studies have been conducted on the neuropathological alterations in mice brain occurring on administration of verrucotoxin, the purified fraction of fish venom from S. verrucosa [Breton et al., 1999].
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Histopathological studies on the effects of crinotoxins have been carried out. Notable among these is that of Deo [2000] on the epidermal secretions of two marine catfish Arius disumieri and Osteogeneosus militaris and that of Al- Hassaan et al., [1985] on the skin toxin of Arius thalassinus. Histopathological studies on rabbits injected with crude skin toxin of Arabian Gulf catfish A. bilineatus have shown that the toxin causes liver and lung damage [Alnaqeeb et al., 1989].

3.3. MATERIAL AND METHODS

The gross anatomical changes upon autopsy and histopathological changes in various organs of the mice that succumbed to the spine extracts of S. argus were studied.

3.3.1. Gross Anatomical Changes

Autopsy was carried out on mice, which died upon envenomation to observe the gross anatomical changes. The colour changes if any of the brain, pale or dark discoloration as the case may be of the heart, liver, lungs and kidney and also other abnormalities such as internal haemorrhage, retention of fluids were examined.

3.3.2. Histopathology

The metabolic pathway traversed by the toxin often dictates the choice of organs of study. Liver [being the major detoxifying organs, directly receiving materials from intestine], kidney [the major excretory organ involved in xenobiotic excretion], brain [the major organ controlling CNS] and heart [the effect of the toxin on the cardiac system and the cardiotoxic nature of xenobiotic] being the cardinal organs were selected for study.
Brain, heart, liver and kidney were excised from the envenomated mice and fixed in 10% formalin. The tissues were dehydrated in ascending grades [30%, 50%, 70%, 90% and 100%] of alcohol for 1 hour each. The samples were then cleared in xylene for two hours and impregnated in paraffin wax thrice each time for 45 minutes. The samples were embedded in wax and were allowed to solidify and the surplus amount was trimmed off. Sections of 4 μm thickness were made using a hand rotary microtome. The sections were mounted on microslides and dried. The sections were deparaffinised in xylene and hydrated in descending grades of alcohol.

The best sections among them were picked up on a microscopic slide. The excess water was removed using a blotting paper. Dewaxing was done by drying the slides in hot plate for 2-3 hours and by clearing them in xylene. Samples were then hydrated in descending grades of alcohol. Staining was done by using Delafield’s hematoxyline for 7 minutes. These were then passed through descending grades of alcohol [3 minutes each], followed by eosin stain [3 minutes] and absolute alcohol [1 dip] and finally cleared in xylene. These were mounted on DPX. Prepared sections were examined and photographed under a Leica microscope.

3.4. RESULTS

3.4.1. Gross Anatomical changes

Autopsy revealed non-specific heamorrhage inside the body cavity and discoloration of heart and liver in mice injected with crude S. argus venom whereas no discoloration was observed for brain and kidney.
3.4.2. Histopathological Effects

The effect of *S. argus* venom on the vital organs are very well confirmed by the histopathological changes of the vital organs liver, kidney, brain and heart. In the case of liver moderate degenerative changes were noticed and the hepatic cells lost their structural integrity. Extremely vacuolated areas and haemolysis were observed in addition to marked pycnotic nuclei [Fig.3.1.]. Microvesicular types of fatty changes [Microvesicular steatosis] were also seen in hepatocytes. Blood sinusoids were distended, congested, or disrupted with partially haemolysed blood; in most of the cases hepatic cells were disrupted and also cells were occluded by haemolysed blood. Focal areas in the liver revealed hepatic cells undergoing degenerative changes and coagulative necrosis. Occasionally oedematous fluids were also seen at some places.

Noted histopathological changes were observed in the kidney. 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 was observed [Fig.3.2.]. 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 material 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.

In the case of brain focal area lysis [Encephalomalacia] were noticed. Demylinated areas were observed. Coagulative necrosis and pycnotic nuclei were also seen [Fig.3.3.]. Blood sinusoids were highly congested with haemolysed blood and haemorrhage was observed. Glial nodule formation was observed in
Fig. 3.1. Histopathological effects of *S. argus* venom on mice liver. Control and sections showing A. Vacoulation B. Pycnotic nuclei C. Congested blood sinusoids D. Necrosis E. Blood clot.
Fig. 3.2. Histopathological effects of S. argus venom on mice kidney. Control and sections showing A. Thickening of bowmans capsule B. Shrinkening of glomeruli C. Proteinaceous or foreign material present D. Tubular necrosis E. Lysis F.Congested blood sinusoids H. Blood clot.
Fig. 3.3. Histopathological effects of S. argus venom on mice brain. Control and sections showing A. Congested blood sinusoids B. Pycnotic nuclei C. Vacoulation D. Necrosis.
Fig. 3.4. Histopathological effects of S. argus venom on mice heart. Control and sections showing A. Myofibrillar degeneration B. Blood clot C. Pycnotic nuclei D. Vacoulation.
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some areas of the cerebrum. Brain tissue showed spongiosis [oedema] throughout the parenchyma. Prominent histopathological changes of the heart were degeneration of myofibrils, and focal areas of necrosis [Fig.3.4.]. Cloudy areas of swelling were also observed in addition to pycnotic nuclei. Coagulative necrosis was also seen.

3.5. DISCUSSION

The histopathological changes revealed the effect of S. argus venom on the vital organs like liver, kidney, brain, and heart. The venom appeared to be injurious to the vascular endothelium and caused congestion of blood vessels and cloudy swellings in liver, kidney, brain, and heart. Liver of S. argus venom treated mice showed congestion, cloudy swelling, microvesicular fatty changes, and infiltration of inflammatory cells around the portal vein. The damage to the hepatocytes in the present study could be attributed to the storage of the toxin in liver for detoxification.

Brain tissue of S. argus venom treated mice showed spongiosis throughout parenchyma. This is in similarity to the effect of P. volitans venom on rat brain [Balasubashini et al., 2006]. Areas of haemorrhage, vascular congestion, and cloudy swelling in renal tubules were observed that revealed the pathological alterations caused in the kidney of S. argus venom treated mice. Heart showed the presence of oedema and degeneration of myofibrils when compared to that of the normal animal.

The effects of S. argus venom on the vital organs like liver, kidney, brain, and heart are in accordance with that of P. volitans venom on rats [Balasubashini et al., 2006]. P. volitans venom caused congestion, microvesicular fatty changes, and infiltration of inflammatory cells around the portal vein. Brain tissue showed
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Spongiosis [oedema] throughout the parenchyma, whereas lungs show areas of haemorrhage, congested blood vessels and inflammatory cells in parenchyma. Areas of haemorrhage, vascular congestion and cloudy swelling in renal tubules were observed in the kidney. Heart of the envenomated mice had presence of oedema and degeneration of myofibrils when compared to the normal animal. [Balasubashini et al., 2006] The oedema of brain and the cloudy swelling in lining cells 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 S. argus 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 T. nattereri [Fonesca and Lopes Ferriera, 2000] Tityus serrulatus [Correa et al., 1997] and Conus lorreossi [Saminathan et al., 2006].

Liver of venom treated mice showed congestion, micro vesicular fatty changes and infiltration of inflammatory cells around the portal vein. Absuma and Venketashvaran [1999] have reported that administration of crude extract of epidermal secretion of Boleophthalmus dentatus causes discoloration of heart, liver and kidney. Alaqaeeb 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. Toxic effect on liver was also observed for the skin toxin of Arius thalassinus [Al-Hassan et al., 1985]. Skin toxin of the giant slender moray eel Thysioidea 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. dissimieri and Osteogeneiosus militaris [Variath and
Venketashvaran, 1999]. Histopathological studies of mice exposed to A. dissimierii mucus extract showed pycnotic nuclei [Deo, 2000]. Studies on the histological evaluation of rat kidney perfused with Thallasophryne nattereri venom showed moderate deposits of proteinaceous material in the renal tubule [Faco et al., 2003].

Kidney cells can release prostaglandins, cytokinins, bradykinin, complement fractions and platelet activating factors [Barraviera et al., 1995]. The histopathological alterations noticed in the kidney cells in the present study could be due to the direct action of the venom in renal glomeruli and tubules or an indirect release of mediators.

Intravital microscopic study with T. nattereri fish venom showed pronounced alterations on microvesicular haemodynamics represented by fibrin depots and thrombus formation followed by complete venular and transient arteriolar stasis [Lopes-Ferriera et al., 2002]. Kristensen et al., [2000] described that intact endothelium is non-thrombogenic. Damage to the endothelial lining of microvessels promotes procoagulant events activating platelets and coagulation cascade resulting in thrombosis.

From the present study it is concluded that the S. argus venom is hepatotoxic, nephrotoxic, cardiotoxic and neurotoxic to the test mice, as it showed pronounced histopathological effects on liver, kidney, heart and brain.

Findings

- Histopathological changes revealed the effect of S. argus venom on the vital organs of mice.
Venom appeared to be injurious to the vascular endothelium of mice and caused congestion of blood vessels.

Tubular necrosis in kidney, coagulative necrosis in liver and myofibrillar degeneration of cells in heart and haemolysis in all organs shows the cytolytic activity of venom.

Microvesicular fatty changes were noted in liver tissue of envenomated mice. Foreign / Proteinaceous material was observed in Bowman's capsule.

Vacoulation and pycnotic nuclei were observed throughout the cells in all organs.

Spongiosis in brain, infiltration of inflammatory cells around the portal vein and the cloudy swellings in the renal tubule all points towards the oedematic activity of the venom.

Congested blood vessels, spongiosis, pycnotic nuclei, glial cell accumulation, focal area necrosis all state the neurotoxicity of S. argus venom.

Thickening of Bowman's capsule, shrunken glomeruli, proteinaceous / foreign material within Bowman's capsule, haemolysis, blood clot, tubular necrosis all revealed the nephrotoxicity of S. argus venom.

Myofibrillar degeneration, pycnotic nuclei, blood clot emphasise the cardiovascular toxicity of S. argus venom.

The hepatotoxicity of the S. argus venom is revealed by the alterations in liver tissue. Micro vesicular fatty changes were noted. In addition pycnotic nuclei and vacoulation were observed. Congested blood vessels, coagulative necrosis and infiltration of inflammatory cells around the portal vein were observed.