Histopathological alteration in Poison gland and hepato pancreas of scorpion species *Hotanta tumulus* from Malakoli region Nanded district (Maharashtra)

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Key words: - Epicuticle, exocuticle , endocuticle , pycnosis, necrosis.

Abstract:-Scorpion follow fossorial mode of life for managing adoptive character. So it is very difficulties in operation during summer. In some invertebrate animal histopathological study throughout to year is difficult due to non-availability of animal. So it is need to study what changes are occurs while summer why death rate is more in summer is try to study by using histological study of more than 200 animal and found conclusion.

Introduction:

Live scorpion specimens were studied with respect to various criteria like morphology, anatomy, histology, etc. Histology is an important branch of biology which deals with study of cells and tissue. It helps determining the action of toxicants or pollutants on the body tissue of organism and their adverse effects. Histopathology deals with the study of pathological changes induced in the microscopic structure of body tissue. Any particular alteration of cell may indicate the presence of disease or the effect of toxic substances. In scorpion it is observed that external organs are affected due to toxic chemicals causing erratic, movement loss of equilibrium increased fungal infection and lesions on head and cephalothorax finally leading to death. Due to fungal infection whitish mass is deposited over body surface. It may disturb bodily functions and cause death. It may be attributed to significant damage caused to the internal organs such as liver, muscle; heart, intestine, book lungs etc.

Thus, histopathological study gives useful data concerning changes in tissue prior to external manifestation. Numerous histopathological changes have been reported in scorpion exposed to a variety of pollutants.

Toxicants impair the metabolic & physiological activities of the organism. Physiological study alone does not give a complete understanding of pathological condition of tissue under toxic stress. Hence, present investigation was undertaken with a view to study the histopathological changes in vital tissue.

Material & Method:

A laboratory acclimated animal was anaesthetized and used for all the primary observations and kept in a dissecting tray having dorsal surface upwards. Freshly collected healthy animals were selected for histopathological study. They were dissected and tissue like Integument, hepatopancreas, book lung, heart, intestine, testis, and poison gland separated from animal immediately.

The tissues were fixed in aqueous Bouin's fluid for 24 hours and then processed through graded series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 6µ thickness were cut and stained with Hematoxylene and Eosin and later on screened and photographed by micro photography.

A] Histopathological procedure before block preparation.

Wash the tissue in bouins fluid in 24 hours and lithium carbonate washing for two minute then washed in different grades of alcohol like 30%,50%,70%,90% and 100% for 24 hours each.

B) Procedure after block preparation (double staining procedure)

Fix the material on slide with fresh egg albumin, wash in tap water for two minute and stain in hematozylene for two minute. Destain the slide in acid water and wash in tap water each for 2 minutes. Dehydration is made in 30, 50, 70 % alcohol for 5-10 minutes each and stain in eosin for two minute. Further dehydration is carried out in 90 and 100 % of alcohol each for 5 – 10 minutes. Clear the slide in xylol or clove oil for 1-2 minutes and mount in DPX immediately.

The slides are dried and washed with xylene and cleared. The clear slides are screened and photographed by microphotography.

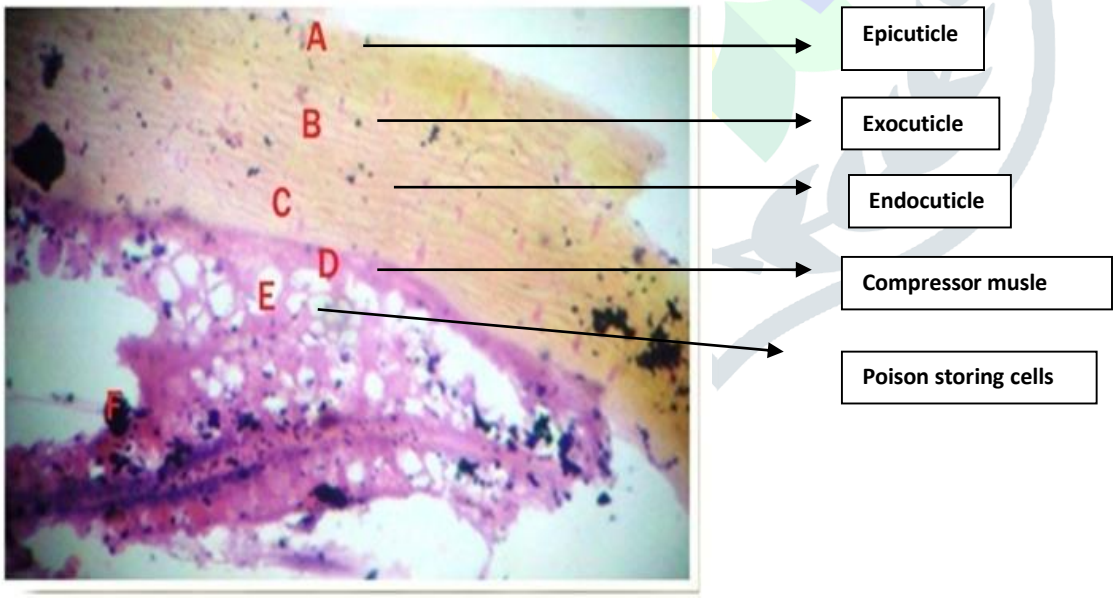

Fig. No. 1 Histology of poison gland a) Epicuticle b) Exocuticle c) Endocuticle d) Compressor Muscle

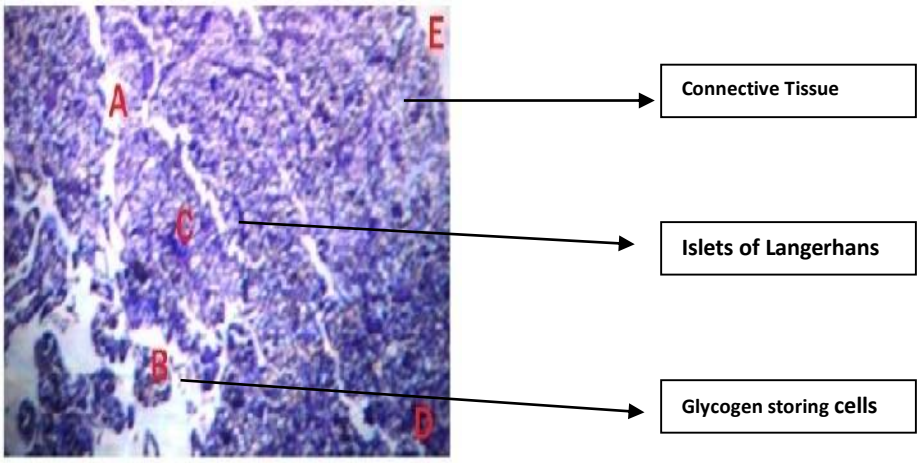

Fig. No. 2 Histology of hepatopancreas a) Hepatic Lobule b) Connective tissue c) Islets of langerhans

Observation and results

Fig. 1 shows the structure of poison gland. The gland has tough outermost chitinous part called cuticle which consist three sub layers: outer epicuticle, middle exocuticle and inner endocuticle which is the thickest. Inner to cuticle, two poison glands are separated by median muscle boundary. Each gland consist a central lumen surrounded by extensively folded secretary epithelium. The poison gland is composed of many folds of columnar cells of secretary epithelium based on layer of connective tissue. These are two types of cells i. e secretary and columnar cells with basal nuclei. In columnar cells cytoplasm is clear. The part below the cuticle of poison gland is covered by strong muscle on dorsal side called compressor muscle which is richly vascular. The contraction of compressor muscle helps in ejection of poison from poison gland. No alteration was observed in histological structure of poison gland in different seasons.

In *Hotantatamulus*, liver and pancreas are fused. They are commonly called hepatopancreas. Its internal structure is discussed separately under two separate heads as follows. Fig. 2.shown the histological structure of Hepatopancreas.

Histological structure of liver is made up of chords of polygonal hepatic cells called hepatocytes that form irregular lobules separated by connective tissue. It is the organ of detoxification of harmful substances. Histopathological changes lead to necrotic effects on hepatocytes, hyperplasia, and rupture of cell membrane resulting in multinucleated regions. Hepatic cells in scorpions exhibit more damage than cells from any other animal exposed to toxicants.

Histological structure of pancreas consist tubules of different sizes. These tubules are surrounded by the intertubular connective tissue with some muscle, collagen fibers and amoebocytes. Epithelium of tubule is packed on a thin basement membrane which consist basal nuclei. Tubular epithelium comprises of two types of cells i. e digestive and secretary cells. The digestive cells are highly vacuolated and elongated, roughly cylindrical with spherical nucleus at the base. In vacuolar cytoplasm granular material is present and second pyramidal cells known as calcium cum secretary cells.

The changes included vacuolation in epithelium, disruption of the basement membrane, shrinkage of tissue, necrosis of epithelial cells and increased calcium deposition in tubule cells. These results are concerned with seasonal changes. During summer, digestive gland shows very irregular vacuolization, basement membrane surrounding the epithelium is broken at a few places. Due to increasing temperature the epithelial cells are seen separated from the basement membrane and cellular identity is lost due to increased calcium deposition. The nucleus of epithelial cells undergoes pycnotic changes. Pancreatic changes lead to congestion, hyperplasia, edema and necrosis.

Discussion:

The venom glands of scorpions were extensively studied by *Werner F.* (1935); *Halse S.S, Prideaux P.L, Cookson A and Zwicky K.T*(1980); *SolegladE and Franckle* (1981); *Sissom W.D* (1990); *Farley R. D*(1999).

Pavlowsky E.N(1925), was among the first authors to survey important anatomical systems of scorpions across many genera and families, with an unusually representative selection of scorpion genera.

The cuticle of venom gland of the species is made up of three layers. *Joseph E. Mazurkiewicz and Eldridge M Bertke* (1972), named these as outermost layer epicuticle, a waxy layer. The middle homogeneous layer is exocuticle and innermost thick layer is endocuticle**.** In *Bothriurus vittatus* (Bothriuridae) the transverse section of poison gland shows the chitin of poison vesicle on dorsal concavity is thicker than remaining surface of the ampulla. The hypoderm forms a special organ in the form of many longitudinal folds of the cuboidal epithelium, *Pavlowsky E.N*(1925). The cuboidal epithelial cells are present in between the cuticle and secretary epithelium of venom gland. These cuboidal epithelial cells consist of irregularly shaped centrally placed nucleus. The cytoplasm of these cell is dark granular.

Joseph E. Mazurkiewicz & Eldridge M. Bertke (1972), reported that the muscle fibers are attached to the cuticle by means of tendon cells. The muscle fibers surround glands from all sides and extend in median fissure to separate both the glands. It keeps the glands away from each other. Thick musculature surrounds the venom glands of *Hotanta tamulus* it is densely arranged in dorsal side.*Pavlowsky E.N*(1925), discovered two types of glands: one with simple, smooth epithelium and another with folded epithelium. The venom gland of *Hotanta tamulus* is of type II, because the venom glands enclose a lumen surrounded by folded epithelium.

In *Bothriurus vittatus* (Bothriuridae) the epithelial cells are much longer with rounded nuclei containing fairly granular network of chromatin *Blackwall J*(1952). The venom apparatus of scorpion *Centruroides sculpturatus* (Ewing) consists of paired glands, lined by secretary epithelium with columnar cells. The secretary products are either membrane bound or unbound vesicles released in the lumen of the gland, *Hemprich F.G, Ehrenberg C.G., Eller L. L.* (1991). The venom gland of scorpion *Urodactus novaehollandiae* is complex and contains two types of secretary epithelial cells: goblet and columnar cells. The secretary compound contains proteins, indole compounds, PAS-positive and acidic mucous substances, *Miranda*

F, Kupeyan C and Rochat H(1966). The venom glands enclose a lumen, surrounded by extensive folding of secretary epithelium. The epithelial cells are arranged on basement membrane and connective tissue layers. There are two types of epithelial cells. One type is goblet cells and other columnar epithelial cells. These goblet cells and columnar cells are numerous in *Hotanta tamulus* and are compactly arranged on the both the sides of connective tissue. In *Orobothriurus bicolor* the venom glands consist of very few goblet cells and columnar cells.

In present case, liver glycogen decreases during summer leading to death of animal. In Channa punctatus liver necrosis, inflammation of portal areas, hardening of connective tissue, and shrinkage of nucleus and septa formation around blood vessel is observed. *Shashtri K.V and Gupta P.K* (1978), no fatty infiltration or glycogen depletion was observed various pathological changes were observed. *Dixon D. G*. (2002), studied cyanide effect on liver and pancreas. *Gutierrer M*et.al. (1998), mentioned the hepatic lesion of fatty infiltration nuclear or general hypertrophy of hepatocyte, other degenerative changes in parenchyma loss of hepatic glycogen. *King K.P Kulshekhra R.A*(1996), reported vacuolization in epithelial cells of guppy. Degeneration of epithelial lining and glandular cells is observed by *Bhatnagar S. L. and Shrivastav S*(1975), in between circular and longitudinal muscle fiber, degeneration of connective tissue. Liver cells plays important role in detoxification by breaking down substances of metabolic products as a result of which hepatic cells exhibit more damage than cells of any organ. Effect of many toxicants in liver is reported by many researchers. Necrosis of hepatic cells is reported by *King K.P and Kulshekhra R.A*(1996),who observed splitting, necrosis and vacuolation, formation of multinucleate cells due to effect of temperature. *Warburg M.R* (2001), showed decrease in hepatic mass in heterometrus species and crab during summer. Recently *Zouari N*et.al. (2006)localized lipase activity intra-cellular inside granules located in specific vesicles of the hepatopancreas' digestive cells of *Scorpio maurus*. *Warburg M.R Elias R and Rosenberg M* (2002), studied seasonal changes in the microscopic structure and ultra structure of the hepatopancreticcells in *Scorpio maurus fuscus Hemprich F.G, Ehrenberg C.G., Eller L. L.*(1891). Numerous lipid droplets, pinocytic vesicles, metabolite and heavy metal inclusions can be distinguished in the different cells. *Sorensen E.M* et.al. (1983), mentioned the hypertrophy and hyperplasia of exocrine pancreas in scorpion liver. Their study showed that edema and necrosis also occurred. *Schulz J.W.* (1971), reported the islets cells hyperplasia, sub capsular and acinar cell necrosis, vascular congestion etc. *Jaiswal R.N and Sarojini R* (1990), reported number of morbid changes in hepatopancreas of prawn, however, they noted an increase in number and size of secretary granules.

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