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HISTOPATHOLOGY OF FUNGAL DISEASED FRESHWATER FISHES

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ABSTRACT

The histopathological studies on freshwater fungal disease fishes were carried out during the period from feb 2018 to march 2019 in the Lake of Hasanparthy Warangal District. Symptoms of fungal disease freshwater fish species *Channa punctatus* showed variation among the species and its distribution. The fungi were first identified on the body surface and tip of the gills of fishes and attach themselves on the surface by their powerful sucking devices. The histopathological observations were appeared that gills and body surface were the main infested areas and gill filaments were damaged by the *Aspergillus flavus* and *Penicillium sps*. The histochemical studies were shows the disease caused extensive damages to the blood elements by rupturing blood capillaries, causing necrosis, coagulation and haemorrhage.

KEYWORDS: Histopathology, *Channa punctatus*, *Penicillium sps*.

INTRODUCTION

Histopathological studies conducted on ulcerated fish show identical histopathological manifestations. In advanced lesions there is massive necrotizing granulomatous mycosis of the underlying muscle fibers, involving the distinctive branching aseptate, invasive fungal mycelium. The presence of fungal hyphae was demonstrated in the epidermis of some early stages of infected fish from India (Viswanath *et al.*, 1997). Basically fungal granulomata occur in the dermis and hypodermis. *A. invadans* induced epizootics are characterized grossly by the development of deeply penetrating ulcers and microscopically by extensive myonecrosis and granulomatous myositis.

Disease causing organisms are probably widespread and abundant in fishes under culture conditions even in the absence of disease. The diagnosis is tough since ill health fishes cannot be diagnosed easily by observation of external signs or behavior. Advanced Laboratory techniques are most useful but even these are not always successful unless the disease condition is severe (Turnbull, 1993). Tissue level pathological features in the target organ tissues can be known by employing histopathological procedures.

The aim of the current study is to evaluate the occurrence of histopathological alterations in skin, gills of the freshwater fish *Channa punctatus*. *Channa* species are frequently cultured in freshwater systems throughout the lakes, tanks and reservoir regions of Telangana. *Channa punctatus* are common both in large cages (monoculture) and in smaller, familyunit sized ponds (mono and polyculture). It is sold locally on the domestic market or used for family consumption. This is the first description of a commerricially important fishes in India.

MATERIAL AND METHODS

Collection of Samples

The microbial and mycotic infected *Channa punctatus* showing external symptoms were collected from Hasanparthy, lake at Warangal district, Telangana, India.The specimens (fishes) were collected alive by using fishing net and are brought immediately to the laboratory for further clinical examination in plastic containers with oxygen filled water.

The specimens were deeply anaesthetized by immersion into 5 ml/L aqueous solution of ethylene glycolmonophenylether. After dissection of fishes, sample tissues of skin, gills, liver and pancreas were carefully removed and small pieces were preserved in fixatives. Tissues of skin, gills, liver and pancreas were preserved in Bouin's, Zenker's, Susa, Carnoy and Formal Calcium standard fixatives as per protocols. The infected fishes were identified by red spots on their body, excess mucus secretions, damaged and sluggishness.

The Microbial investigation was carried out in different infected parts were separated from fish of *Channa punctatus* namely skin and gills.The fungal microorganisms such as *Aspergillus flavus, Fusarium solani, Rhizophus stolenifer,Aspergillus fumigatus, Penicillium chrysogenum, Aspergillusniger* and *Trichoderma viridae* were determined in above the organs.

Processing for histopathological Investigations

- Samples of skin and gills tissues were taken out of fixative.
- **Hydration:** Washing of these tissues was done with saline water/solutions as per procedures adapted.
- **Dehydration:** The removal of water or dehydration was done by' transferring tissue into the 70% alcohol of sufficient quantity and then graded alcohol upto 90% for one change and two changes ofabsolute alcohol.
- **Clearing:** The clearing of tissues was done by subjecting to a clearing agent *i.e.* xylene.
- **Embedding and Impregnating:** The impregnation agent used wasparaffin wax, as it facilitated easy penetration into the tissuewithout causing structural damage and without much shrinkageor crystal formation.
- **Trimming:** The embedded block containing tissue was furthertrimmed such that only one tissue is subject for cutting intosections.
- **Mounting of the Block:** An iron blocks of 30 mm/was used to holdthe block at the correct angle and in position for cutting.
- **Section cutting:** The block containing tissue was cut at 5µmthickness with the help of a microtome.
- **Floating out and Mounting of sections:** The cutting sections wereallowed to float in a water bath so as to avoid wrinkles. Further it is also seen that the cut sections were adhered to the slides firmly and in a right position. The slide was given an egg albumin coating before affixing the sections over it.
- **Staining the slides with Azan and mounting:** The stained sections were observed under light microscope andmicrophotographs were taken for pathological observations.

Clinical and Physical Signs Of The Diseased Fish

The present study reveals that the following gross observations in the infected fish aredullness, loss of balance, loss of appetite, sluggish movements, swimming near the surface water, lethargic, erratic and spiral swimming. Rough and dark pigmented skin haemorrhage at the base of the fins and sometimes ulcers on the skin, unilateral or bilateral exophthalmia. Theprotrusion of the eyeball, so that the eyelids will not cover it, in consequence of disease, opaqueness of the eyes with haemorrhages and abdominal distention.

Fig. 1: Hasanparthy Lake.

Fig. 2: EUS infected *Channa punctatus.*

RESULTS

Histopathology of Skin

Histological, morbid fish often exhibit epidermal erosion infected with *Aspergillus* species colonization but minimal associated inflammation. In *Channa punctatus* loss of epidermis was wide spread over large portions of the body since then, the infection has repeatedly been diagnosed. Histopathological changes of skin tissue infected with *S. parasitica* had shown loss of epidermis and necrotized hypodermis, Miyazaki and Egusa (1972). Muscle layer shows inflammation with necrotic tissue debris and formation of number of well developed layered granulomas surrounded by hyphae Hatai (1980) Granulomas showed fribrillar structures due to hyphal infection. *A. laevis* injected tissue showed more or less similar symptoms as that with *S. parasitica*. Necrotized epidermis and hypodermis with granulomatous response of musculature. Histological studies of tissue injected with *A. niger* showed no granuloma formation in musculature, no hyphae was seen in deeper layers although epidermis was degenerated and edema was observed in underlying hypodermis and musculature, (Hatai *et al.*,1994 and Hussian *et al.,* 2013). Granulomatous response due to *Aphanomyces* infection was also reported Qureshi *et al.*,(2001); Qureshi (2012). Histopathological studies of tissue infected with *Aspergillus flavus, Fusarium solani, Rhizophus stolenifer,Aspergillus fumigatus A. niger* did not show any granuloma formation, however degenerated musculature was observed with necrotized hypodermis and epidermis, (Rekha Chauhan *et al.,* 2014).

Histopathological investigations of skin had shown epithelial desquamation which displayed erosion finally lead to ulceration in the infected area. Varying degree of destruction had taken

place in the dermis and hypodermis. Epidermal and dermal cells suffered vacuolar degeneration and focal necrosis. Dermis and hypodermis showed necrosis contained fragments from fungal hyphae with focal aggregation of melanomacrophages. Histopathological examination of infected skin and muscles of *Channa maurilus* showed various types of destructions in tissues. Loss of epidermal layer with complete necrotization of dermis and hypodermis. Penetrating fungal hyphae were clearly observedin muscular layer (Rekha Chauhan *et al.,* 2014). Histopathological manifestations due to mycotic; infections were studied (Laxmareddy *et al.,* 2013; Hussian *et al.,* 2013; Chauhan *et al.,* 2014a). Varying degree of destructions has been observed due to *Aspergillus sp.* Infection observed in present study is supported by reportsof above workers. Mostly inflammatory reaction represents a host reaction against the bacteria and fungi. The skin is slightly emerging over the surface. The underlying muscle fibers were characterized by vacuolation, granulomatous inflammation, necrotic tissue debris and presence of inflammatory cells. Fungal hyphae of variable lengths were observed in muscle tissue. The granulomas surrounded hyphae that filled the necrotic spaces of longitudinal and circular muscles. Theskin had shown focal sloughing of the epidermis with hyperplasia of alarm substance and mucous cells. The separated dermis was almost joined by the fibrous connective tissues that were arranged in the parallel position to the epidermal layer. The dead cells formed due to sarcolysis decreased in number. Active macrophages found with the small focal lymphocyte accumulation in the healing area. Congested and hemorrhagic dermis with excessive aggregation of lymphocytes and melanomacrophage cells with hyaline also of epidermal basement membrane.

Morbid fish's exhibits complete mucosal erosion, muscle fibers regeneration was observed. Regenerated muscle bundle almost replaced by the fibrosis area with severe regressive changes in muscles and necrotic changes developed in muscle fibers. The necrotic muscle fibers were increased in their numbers and followed by swollen myoseptum and sarcolysis which is created a bigger area of the lesion. Hemorrhage was observed within the necrosis part, small number of melanin pigment of containing cells began to be encountered under the basement membrane of the epidermis. Damaged portion of the dermis still disconnected. Musculature was clearly observed where the muscle, fibers were hyalinized with prominent destruction of the nuclei. The tissue reaction was less common where the melanophores activation was neglected. (Fig3 & 4).

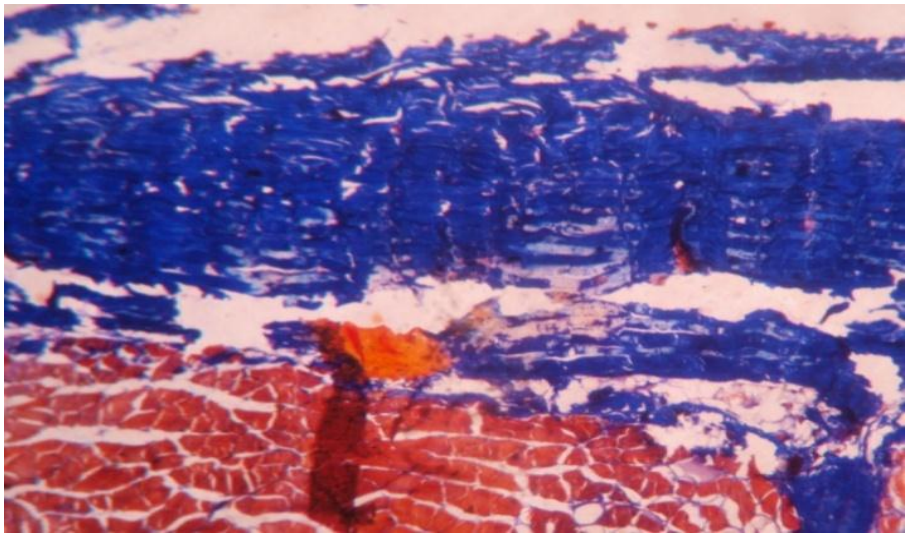

Fig. 3: Section of control Skin of *Channa punctatus* (Azan).

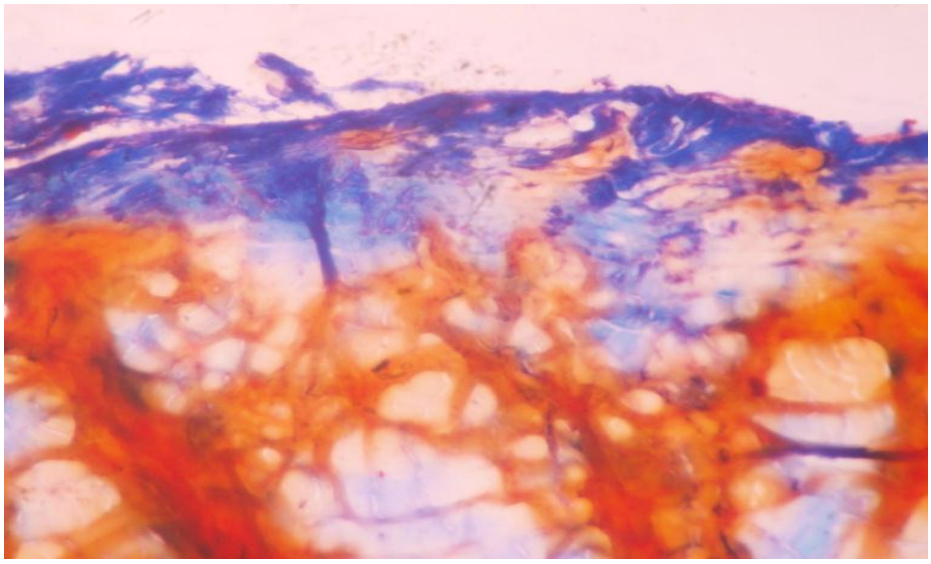

Fig. 4: Section of infected Skin of *Channa punctatus* (Azan).

Histopathology of Gill

EUS infection has induced marked pathological changes in fish gills architecture. The changes include epithelial lifting (EL), bulging of tips of primary gill filaments (BTPG), Degenerated secondary lamella (DGSL), Curling of secondary gill filaments (CSG), Atrophy secondary lamella (ASL), Fusion of secondary gill filaments (FSG) The damage was severe in gills of fishes with high level of EUS infection. Shortened and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were well marked. Hyperplasia and hypertrophy of nuclei were also seen. Besides these changes pyknotic nuclei, vacuolization and degeneration of epithelial cells and pillar cells and lifting of the epithelial layer from the secondary lamellae were also observed. In the

present study, hyperplasia and hypertrophy of the epithelial cells, epithelial lifting, lamellar disorganization, lamellar aneurysm, rupture of the lamellar epithelium, rupture of pillar cells and necrosis were observed due to EUS infection. Similar type of pathological changes was observed by many researchers on bacterial and fungal toxicity. De Silva and Samayawardhena (2002) observed irregular appearance of gill lamellae, increased vacuolation in epithelial cell, lamellar fusion and complete destruction of gill lamellae in poecilia reticulate exposed to chlorpyrifos. The damages occurred in the secondary gill lamellae with light precipitation of mucous and exfoliated nuclei, splitting of muscle fibers in the freshwater fishes exposed to acute and chronic EUSInfections. Bacteriaand fungi affect the organ systems specifically during the winter season. This can be speculated that pathological alterations like hyperplasia of epithelial cells, epithelial lifting and lamellar fusion may be increase the space of contact of toxicants with the vascular system of gill, resulting in impairment of respiration as well as fish's health. Severe hyperplasia, results in the fusion of secondary lamellae frequently also results in alterations such as blood congestion, hypertrophy of epithelial cells and lamellar disorganization Marina *et al.*,(2007). Hyperplasia of the epithelial cells and subsequent lamellarfusion, goblet cell proliferation as well as the migration of eosinophilic granular cells (EGCs) to gills of fishes infected with these parasites has been recorded (Mehdi Raissy and Mahsa Ansari, 2011).

According to Rekha Chauhan *et al.*,(2014) gill lamallae with degraded epithelium and fungal hyphae encapsulated by multiple layer of fusion of some secondary lamellae was due to severe infection. In the months of October and November, both the primary and secondary gill lamellae were arranged systematically and no significant pathological symptoms were observed in the structure of gill. However, in December, primary gill lamellae were hypertrophied and few blood cells were accumulated at the base of secondary lamellae. Inflammatory cells and mild haemorrhages were also observed in the primary gill lamellae during this winter period in all farms. However, in the month of January, primary gill lamellae were severely affected followed by marked hypertrophy and hyperplasia and secondary gill lamellae partly missing (Chandra *et al.*, 2012). (Fig. 5 & 6).

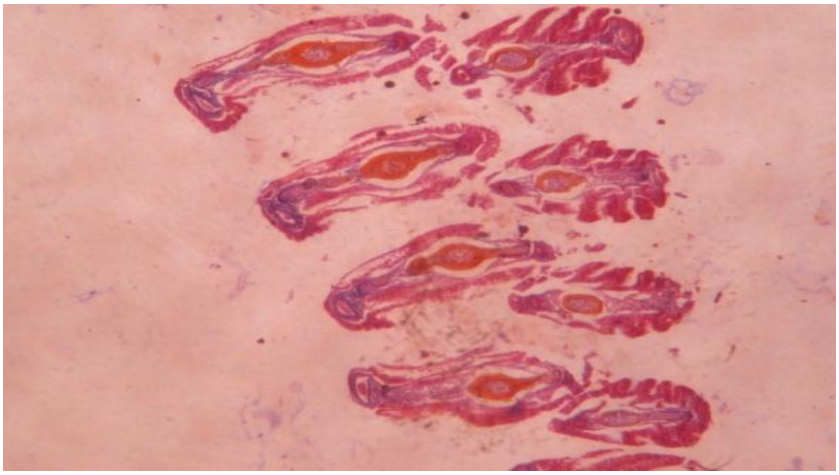

Fig. 5: Section of control Gill of *Channa punctatus* (Azan).

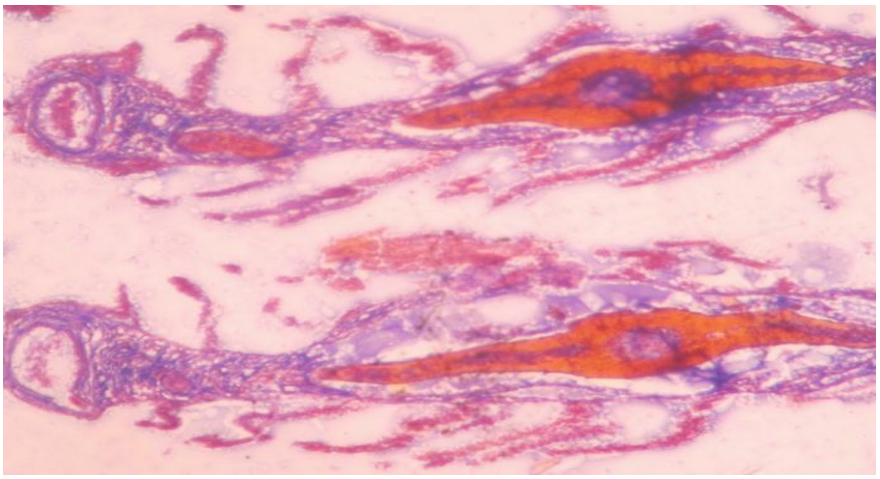

Fig. 6: Section of infected Gill of *Channa punctatus* (Azan).

DISCUSSION AND CONCLUSION

In the skin of *Channa punctatus*, epidermis, dermis and hypodermis were partly lost and some vacoums were seen in dermis and myotomes were arranged. Dermis and muscles were severely necrotic which created huge vacuums. Analogous observations were also reported. (Ventura Grizzle, 1998) observed histological differences in channel cat fish, *Ictalurus punctatus* (Rafinesque), with naturally occurring bacteria isolated from lesions of skin and superficial muscle and systemic *Aspergillus fumigates* infections *i.e.*, diffuse necrosis into and several internal organs.

In present study, it has been observed that morbid fish exhibited complete mucosal erosion, muscle fibers regeneration. In the regenerated muscle bundle almost replaced by fibrosis area, severe regressive changes in muscles and necrotic changes developed in muscle fibers. Hemorrhages were observed within the necrosis area smallnumber of melanin

pigment of containing cells. These results are closely related with Robertson *et al.*,(1961). Reported that the myocardium had increased collagen fibers and mucopolysaccharides, edema, hemorrhage, vacuolization, loss of fibrils; necrosis and cartilage formation was also found in king salmon *(Oncorhynchus tshawytscha).* In advanced infections the muscle is eventually destroyed by histolytic action on the muscle, Tsut *et al.*,(1988). (Akter *et al.*,2006) observed rough skin, weak body and gray brownish color of the body in *C.punctatus*, *M. tengra* and *H. fossilis.* (Roy, 2006) had observed rough skin, scale loss in *N. nandus.* The presence of melanomacrophage in different layers of the skin and underling muscles of most infected fish may have served as a limiting factor for the disease, since melanomacrophage constitute a part of the fish defense mechanism.

According to Ahmed *et al.*,(2007) the skinand muscle of *M cuchia*, epidermis and dermis were partly lost and some vacoums were seen in dermis and myotomes were arranged normally in November. In December and January dermis and muscles wereseverely necrotic which created huge vacuums. However, marked fungal granuloma, fungal hyphae and melanomacrophage were seen during these periods. He also observed total loss of epidermis and dermis, many fungal granuloma, fungal hyphae and wide empty spaces, with necrotic muscles in Thai *A. testudineus*, collected during December and January from two different farms. In the skin of EUS infected *Channa punctatus*, epidermis, dermis and hypodermis were partly lost and some vacoums were seen in dermis. Dermis and muscles were severely necrotic which created huge vacuums. In present study, it is observed that morbid fish exhibited complete mucosal erosion, muscle fibers regeneration. The regenerated muscle bundle almost replaced by fibrosis area, severe regressive changes in muscles and necrotic changes developed in muscle fibers. Hemorrhages were observed within the necrosis area with small number of melanin pigment containing cells.

The primary gill lamellae are flat leaf like structures with a central rod like supporting axis and a row of secondary gill lamellae on each side of it. They are situated laterally on either side of interbranchial septum. The primary gill lamellae consist of centrally placed rod like supporting axis with blood vessels on either side. Alachlor technical and lasso 50% EC has induced marked pathological changes in the gills which includethe changes include the bulging of tip of primary gill filaments with distortion of the shape of secondary filaments (Butchiram *et al.*,2009). A number of cuts were also observed in secondary gill lamellae. The pillar cell nucleus showed necrosis and developed vacuoles in the secondary gill epithelium.

There is tendency of fusion of disorganised secondary gill filaments. The changes reported in the gill include epithelial proliferation, congestion of blood vessels and hyperplasia. Histopathological changes in the gill of *Labeo rohita* were reported (Vijaya Lakshmi and Tilak 1996). The fish were exposed to organophosphate pesticide monocrotophos. Club shaped lamellae represents progressive degeneration in the gills. (Wannee *et al.,*2002) stated filament cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm in the Nile tilapia. *Aspergillus* and *Mucor sp*. in eyes and *Aspergillus, Rhizopus* on gills and pectoral fins were common. *Aspergillus* was the most common fungus which was isolated from all parts of fish.

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