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RESEARCH ARTICLE

Histology of gills of *Labeo rohita* infected by crustacean parasite *Argulus* sps.

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Abstract

The present study was conducted on the gills of *L. rohita* of river Tawi parasitised by a crustacean parasite *Argulus* to reveal the histopathological characteristics of the gills of these fresh water carps. Histopathology provides a rapid method to detect effects of pathogens on different tissues and it can be considered as an indicator of the extent of deteoration of fish environment. In Indian major carps argulosis disease is caused by the crustacean parasite.The parasites are leaf like in appearance and were found to attatch with skin, fins and gills of the fishes. The affected sites were haemorrhagic, ulcerated and mucus studded. The histopathological changes in the skin of affected fish exhibited hypertrophy, telangiectasis, aneurism and fusion of lamellae along with the shortening and degeneration of gill lamellae accompanied by edematic or haemorrhagic spots. The histopathological observations due to the presence of pathogens in fish also play a vital role in disease control and health management in aquaculture.

Introduction:-

Parasites are one of the major group of organisms that may or may not cause infection in fishes depending on a number of factors. The occurence of both ecto and endoparasites in fish is very common (Moyle and Cech, 2004). Fish parasites result in huge economic losses as they increase mortality and also increase farm inputs via increased treatment expenses and cause reduction in growth rate due to disease outbreak (Kayis, 2009). Gill parasites are common on cultured and wild fish. Many of these have long been recognized to have the potential to affect the growth; fecundity and survival of hosts (Johnson *et al.*, 1996). Gills are specialized tissues for gaseous exchange, circulation, ionic balance, hormone production and nitrogenous waste excretion (Pelster, 2010). Gill parasites attatch to the gills of fish and feed on the host blood and tissue (Ojha and Hughes, 2001).The organs of attatchment such as suckers and hooks cause extensive tissue damage and inflammation and may render fish susceptible to secondary infection by bacteria, fungi and viruses (Dezfuli *et al*., 2003). Thus the damage to gill tissue can reduce the ability of the fish to maintain normal oxygen uptake by hindering water flow (Ojha and Hughes, 2001). Parasites play an important role in determining the health status of the fishes (Ferguson, 1989). Present study was therefore, undertaken to reveal the effect of different parasites on histology of gills of freshwater carps.

Parasitic infections often give an indication of the quality of water, since parasites generally increase in abundance and diversity in more polluted waters (Poulin 1992, Noga 2010). Parasites are capable of causing damage to the fish through injury to the tissues or organs. Fish parasites result in economic losses or mortality, treatment expenses, growth reduction during and after outbreak of disease .There are no specific pathogonomic clinical signs for parasitic diseases in fish, although a group of clinical signs may be specific for some parasitic infestation (Reavill and Roberts, 2007). Most external parasites however, can be readily identified on direct observation and wet mount preparations while some parasitic infections need specific paraclinical examinations for confirmations.

Argulus species (Family: *Argulidae*), more commonly known as fish lice, are members of a large group of branchiuran parasites infestation that cause disease in fish. The argulids are crustaceans and are related to crabs,

lobsters and shrimp (Mohamed and Kenewy, 2013). Approximately 100 different species of *Argulus* are distributed worldwide depending upon species, can infest freshwater and saltwater fishes. The three most studied species are *Argulus foliaceus*, *A. japonicus*, and *A.coregoni* that are found in freshwater systems. This parasite penetrates the upper layers of the host skin and feeds on blood and body fluids (VanDer *et al*., 2000). The major fishes affected with this disease are fry, fingerlings and adults of Indian major carps (Sheila *et al*., 2002). The affected fishes become restless with erratic swimming movements and attachment sites show signs of ulceration. Adult parasites are oval, flat and leaf like in appearance with transparent to whitish color along with two conspicuous black spots (Sheila *et al*., 2002). Generally, they are found to attach with the skin, fins or gills of Indian major carps but sometime, kidney, liver and spleen are also found to distress with *Argulus* parasites (Hassan, 2005).

Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs such as the gills, muscle, liver and kidney (Dutta, 1996). It however, proves to be a cost effective tool to determine the health of organisms which in turn reflects the health of entire ecosystem. It is a rapid method to detect the effects of irritants and pathogens in different organs (Johnson *et al.*, 1993) and it can be considered as the indicator for abnormal condition for fish environment (Roberts, 2001). It helps to identify the extent of damage in the organs of diseased fish and also the etiological agents harbored in target organs of the fish (Sheila *et al*., 2002). It plays a significant role for understanding the mechanism of disease processor and the course of diseases ranking from acute and chronic stages through fish level reactions in host fish by pathogens.

Materials And Method:-

The present study was conducted in River Tawi, Jammu from April 2015-December 2015.The sampling was done using the cast nets of one inch mesh size and the fishes were brought live to the Fisheries Lab. at P.G. Deptt. Of Zoology,University of Jammu. The total length, standard length and the weights were recorded. The samples were also studied for patho-morphological and anatomical examinations. The patho-morphological examinations comprised of identifying and locating any visible external lesions, haemorrhages, and formations of vista and patches on body surfaces, gills and fins. The patho-anatomical examinations were thus carried out for finding any viable lesion or inflammation in internal organ.

The specimens were dissected and the infected gills were immediately fixed in alcoholic Bouin's fluid for 24 hours. After complete removal of picric acid, the tissue was dehydrated in alcoholic series, cleared in xylene and processed for preparation of paraffin wax blocks. Sections of 4-5μm were taken using a microtome and stained using haematoxylin eosin.Stained histopathological sections were examined under Olympus research microscope O/C 91525. Microphotography was done both at low and high magnification.

Results And Discussion:-

The present observations have been taken on the histopathological changes taking place in response to the infection of *Argulus* in gills of *Labeo rohita.* Many variations were found during the microscopic examination of the specimens. The visual observations showed that parasites are found attached to the skin with head and operculum being the favourite sites of attachment. The affected fishes carrying a heavy load of parasites were highly mucus laden with very slimy surface and pale appearance (Fig. 5). The fins showed the corroded fin filaments which might prove a hinderance in fish movement (Palaq *et al*., 2016). Clinical signs and behaviors observed in infected fish were in accordance with the cases reported by Toksen (2006), Yildiz and Kumantas (2002) and Noaman *et al.* (2010).

Gills of *L. rohita* show the histopathological alterations which included proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary lamellae (Fig 1), severe degenerative and necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucous cells proliferation. Hyperplasia of the filaments was found which was expressed in the expansion of their end sections. Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Marked proliferation of mucous cells, shortening of secondary lamellae, and hemorrhage between gill filaments, dilation and congestion in blood vessels of gill filaments were also observed. Also the tips of the secondary filament were enlarged to form balloon like swellings (telangiectasis) in severe infection (Fig. 3). Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae (Fig 2). Atrophy of secondary lamellae was also observed. The hyperplasia of the epithelial cells caused cloudiness of the skin with excessive mucous secretion. This may lead to

hypoxia of the gills (Darwesh *et al.*, 2014). Similar results have been reported by Taylor *et al.* (2005) which is in agreement with the one obtained from the present study.

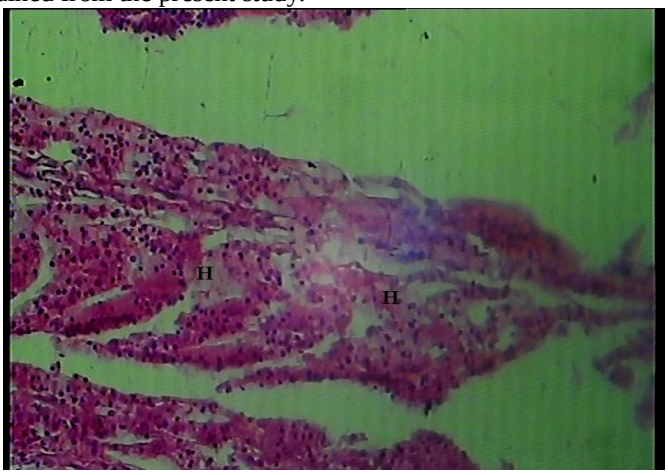

Figure 1:- Hyperplasia (H) causing the fusion of secondary lamellae (100x)

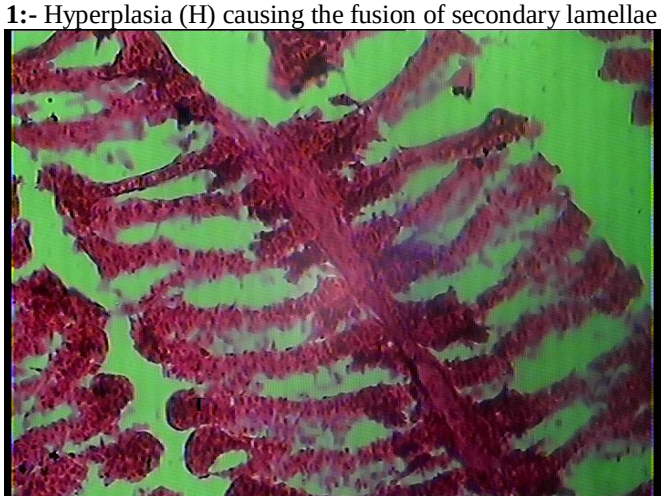

Figure 2:- Fusion of the secondary lamellae with balloon like tips of the filaments (Telangiectatic T) (40x), prominent hyperplasia with edematic spots

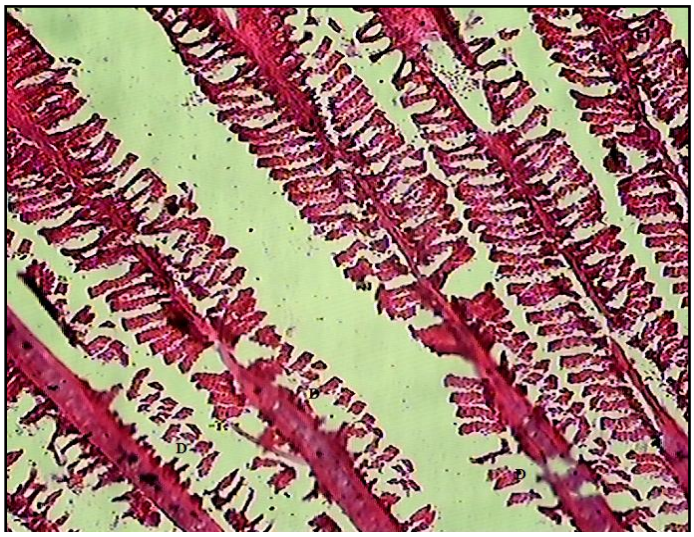

Figure 3:- Showing the disintegration of the cells of gill filaments (10x) and eating up cell stage

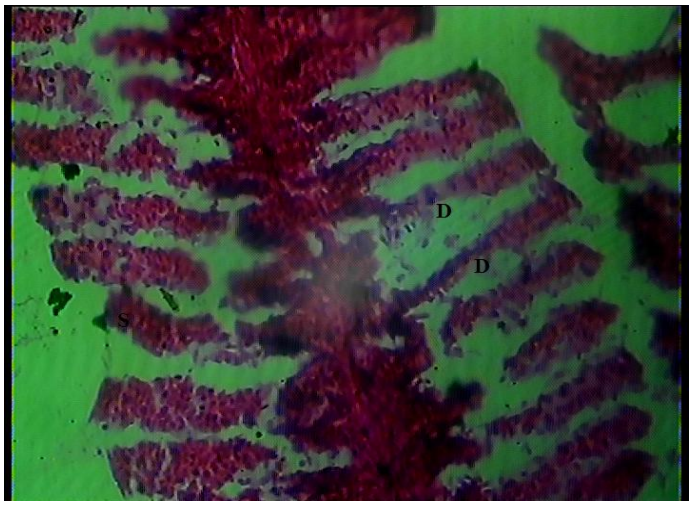

Figure 4:- Showing prominent disintegration of the cells, necrotic changes, shortening of the gill filaments (40x)

Figure 5:- Infected fish showing corroded fins and pale mucous laden body

Pathological effects also revealed the proliferation of bronchial tips, thinning, deshaping, shortening and fusion of secondary gill lamellar epithelium. Extensive disintegration of the gill lamellae was also reported (Fig 4). The maximum histopathological damage were induced by scraping and sucking activities of the parasites on host tissues Derwa (1995), Endrawes (2001), Hanna (2001) and Osman (2001). The sections of gills revealed that the functional respiratory components of the gills namely the gill lamellae were also affected badly by the parasitic infection. Swelling of the host tissue was observed particularly at the attatchment site of the parasites. There were no cellular changes in the adjacent uninfected tissues. Large sized argulids hamper the normal respiratory function of the gills by replacing the lamellar spaces with the argulids, thereby reducing the surface of air exchange and thus leading to stress and anoxic conditions. Also changes were found in the primary and secondary lamellae due to the attatchment of parasites. The parasites were found attatched to the tips of gill filaments. Jalali (1997) revealed that lesions caused due to attatchment and feeding of parasites may be secondarily infected with fungi and bacteria whereas Fadaei *et al*. (2001), Barzegar and Jalali (2004), Shamsi *et al*. (2009), Raissy and Ansari (2011), Raissy *et al*. (2011), Campos *et al.* (2001) have recorded hyperplasia of the epithelial cells and subsequent lamellar fusion to be the characteristic feature of the infection.

The parasites pierce the host tissue with the pre-oral stylet, inject a cytolytic toxin and feed on the blood released by the resultant wound. The surface of the host at the point of stylet entry can become erythemic and hemorrhagic. A hemorrhagic factor is produced by some species. Several parasites feeding in close proximity may cause edema and

localized swelling of tissues. However, repeated piercing of the skin by stylet may inject a toxic enzyme causing irritation, in addition to the mechanical damage caused by hooks and spines. This may result in the development of inflammatory lesions characterized by increased mucous secretion, hemorrhages and necrosis of the injured areas (Toksen, 2006 ., Noaman *et al*. 2010., Noga, 2010., Purivirojkul, 2012., Sharma *et al.* 2012., Vasilean *et al.* 2012 and Mayer *et al.* 2013). *Argulus* parasites released some toxic substances from the proboscis glands and certainly have adverse effects on the fish and localized reddening or haemorrhages and also swelling of tissues at the site of infection (Dulin, 1979). Hyperplasia of epithelial cells and fusion of some secondary lamella are examples of defense mechanism of gills. The most significant lesions of *Labeo rohita* were secondary lamellar hyperplasia, haemorrhages in the tips of primary lamellae and fusion of both primary and secondary gill lamellae. *Argulus* parasites released some toxic substances from the proboscis glands and certainly have adverse effects on the fish and localized reddening or haemorrhages and also swelling of tissues at the site of infection (Dulin, 1979). Severe infection by *Argulus* can cause significant mortality in Indian Major Carps (Nandp and Das, 1991).

Histopathological observations as a whole, affect the health background of the host fish resulting in the depletion of growth, susceptibility for other diseases etc. The damage to the gills affects the respiration of the fish, resulting in hypoxia or anoxia and thereby affecting the health status of the host. The findings accomplished that disease argulosis are serious threats in Indian major carps and can cause extensive damage to the yield of fishes. The infection may be visualized with naked eyes and need to manage promptly to secure the health of Indian major carps.

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