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Histochemical studies on *Genarchopsis goppo* from fresh water Murrel, *Channa striatus*

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ABSTRACT

Genarchopsis goppo, a trematode parasite very commonly infects the fresh water murrel, *Channa striatus*. This parasite brought about histopathological and histochemical abnormalities in fish intestine. The histopathological changes include severe damage to the villi and other layers of the intestine. In the infected fish, carbohydrates, glycogen, protein and lipid contents are increased significantly. The present study deals with the histochemical nature in the intestine of infected and un infected fish.

Key words : *Genarchopsis goppo*, *Channa striatus*, *Histochemistry*, Histopathology

The helminth parasites not only alter the morphology of the infected organs, but also cause interruption for nutrition and metabolism. It disturbs secretory functions of glands and other organs. It is therefore assumed that all these factors adversely influence on the host and may lead to the disease status and at lost the death of the host. However, the degree of pathogenecity and the damage depend up on the intensity of the infection, number of parasites found in the host tissues and the secretions of the parasite. Adult flukes invade different organs such as gills, digestive tract, liver and kidney. Earlier studies have been made on the trematodes found in fish (Srivastava and Mukherjee, 1976; Bose and Sinha, 1979; Barbara, 1980; Lester, 1980; Muzzal, 1980; Chung Yuitan, 1981; Gupta and Agarwal, 1984; Maqbool and Nizami, 1984; Zaman, 1990; Lakshma Reddy *et al.*, 2006; Lakhsma Reddy and Benarjee, 2006; Benarjee and Lakshma Reddy, 2006; Benarjee *et al.*, 2006; Benarjee and Laxma Reddy, 2008; Paradeshi and Hiware, 2010). In the present study histochemical changes have been observed in the intestine of fresh water fish, *Channa striatus* due to parasite, *Genarchopsis goppo*.

MATERIALS AND METHODS

For the present investigation, fishes were procured from the local fish market and also collected directly from the fishermen who directly caught fish from the local fresh water bodies. To collect the parasites, the fishes were sacrificed and screened after opening the alimentary canal. Since most of the parasites infect various organs of the alimentary canal, the entire alimentary tract was isolated from the fish and kept in Petridish containing normal saline. Intestine was thoroughly screened for the presence of the parasites. The parasites then collected on a slide and tied for fixation. The parasites were stained with Alum Carmine. The intestine of the infected and uninfected fish were isolated and preserved in Bouins, Susa, Carnoy and Zenker's fluids for the histopathological and histochemical studies (Pearse, 1968; Bancroft, 1975). A battery of histochemical tests was applied on the microtome cut sections of intestine both infected and uninfected to realize the histochemical changes that occur in the tissues if any due to infection of *Genarchopsis goppo*.

RESULTS AND DISCUSSION

The battery of histochemical tests conducted on the infected and uninfected intestine of *Channa striatus* revealed some interesting features of these tissues in various stages of physiological activity. The trematode infections interfere with the digestion and absorption of food material causing metabolic disturbances. The excretory products and the metabolic end products excreted by the parasite into the intestine produce toxicity. These abnormal conditions in the infected tissue alter the quantity and the secretory nature of various chemical

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substances.

Periodic acid / Schiff's (PAS) technique yielded an intense positivity in all the layers of intestine in uninfected fish. This reaction has suggested the occurrence of vicinyl hydroxyl groups in these tissues. Further, this technique indicated the simultaneous occurrence of muco-polysaccharides. The presence of neutral muco-polysaccharides was indicated by PAS / Saliva technique. Similarly the presence of free aldehydes was demonstrated with Schiff's reagent and without oxidizing in periodic acid.

The changes in the distribution of carbohydrates therefore, showed a relationship between the host and the parasite. The distribution of carbohydrates in the infected intestine with the trematode, revealed a change. Even the glycogen content showed a difference from the uninfected intestine, which was evidenced when the sections were subjected to PAS after diastase digestion. In the infected intestine the carbohydrate and the glycogen content increased significantly.

Reifel and Travil (1977), Reifel (1978, 1979), reported the complex morphology and carbohydrate histochemistry in oesophagus, stomach and intestine of certain teleost fishes. They demonstrated the presence of Sulfomucins, Sialomucins and neutral mucosubstances. Others who have also contributed to the knowledge of histochemical nature of alimentary canal regions are Rogick (1931), Chakrabarty *et al.* (1983), Hirji (1983), Woodword and Bergeron (1984), Maqbool and Nizami (1984), Chakravarthy and Sinha (1987), Chakravarthy and Tandon (1989), Ramagouda *et al.* (1989) and Ramesh Babu *et al.* (1989).

The increase in the carbohydrates may be a resistance to pathological conditions and increase in the glycogen content is due to toxicity of the parasite. Similarly, when the sections of intestine gave a positive response to alcian blue at 1.0 pH and 2.5 pH for muco-polysaccharides, their increase in the infected intestine suggested the increase due to the internal secretions of the intestine and the parasite. The enhanced mucous helps in the resistance of pathological conditions. The results of the band of histochemical tests of intestine are presented in Table 1.

The parasites live in the intestine of fishes which may also interfere with the protein metabolism of the host. During this course, the host tissue may show a decrease in the level of protein content, due to the absorption of the same by the parasite. So, the level of protein content depends upon the metabolic activity of the normal fish and defense mechanism in the infected fish. The proteins play a major role in the metabolic processes of the body

Table 1: Histochemical tests for intestine

Histochemical tests applied	Results	
	Un-infected	Infected
Periodic Acid/Schiff (PAS)	++	+++
PAS/Saliva	++	++
Schiff's without oxidation	+	+
Acetylation / PAS	-	-
Deacetylation/PAS	++	++
Alcian blue 1.0 pH	++	+++
Alcian blue 2.5 pH	++	+++
Alcian blue 1.0 pH/PAS	++	+++
Alcian blue 2.5 pH/PAS	++	+++
Alcian blue / safranin	++	++
Alcian blue / Aldehyde fuchsin	++	++
Mercuric bromophenol blue	++	++
Ninhydrin / Schiff	++	++
Ferric ferricyanide	++	++
Congored	++	++
p-DMAB nitrite	+	+
$KMnO_4$/Alcian blue	++	++
Millon's reaction	+	+
Copperpthalocyanin	++	++
Sudan Black 'B'	++	+++
Orcein / Van Gieson	++	++
Orcein	+	+

+++ = Strongly positive; ++ = Moderately positive;
+ = positive; – = Negative

and also in the cellular integrity. The general test for basic proteins, Bromophenol blue (BPB) gave a strong positivity indicating the highly proteinaceous nature of the intestine. The intestine of infected fish showed more positively than the uninfected fish. The increase in the protein content in infected intestine may be partly due to the contribution by higher rate of supply of nutrients from the host tissues. However, the quantum of increase depends upon the degree of infection, the degree of pathogenicity and the degree of resistance.

Other tests have also been conducted to determine the specific type of proteins. Millon's reaction and P-DMAB nitric method have revealed the presence of little quantities of tyrosine and tryptophan. The presence of protein bound NH_2 groups, Sulfhydryls, disulphides and glycoproteins were revealed with Ninhydrin / Schiff, ferric ferricyanide (FFC), $KMnO_4$ / AB and Congo red, respectively.

The lipid also occurs widely, distributed in all the tissues of the body and its content depends upon a number of factors. The toxicity and pathogenecity are some of important factors. The increase or decrease of lipid content, therefore, depends upon the diet, metabolic

processes, toxic diseases, starvation etc. The lipids constitute relatively an important source of energy supply and are widely distributed in the intestine of fishes. But, the pathological conditions due to parasitic infections cause changes in the physiological functioning of the effected organ and affects the rate of absorption of intracellular digestion. The distribution of lipids and phospholipids was studied using Sudan Black 'B' and copper pathalocyanin, respectively. The strong positively with these two stains suggested the involvement of trematode parasite in the elaboration of lipid contents.

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