



Histopathological And Histochemical Changes Of The Intestinal Tissues Of *Channa Punctatus* (Bloch) Infected With A Trematode Parasite, *Genarchopsis Goppo* (Ozaki, 1925)

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Abstract: The trematode parasite *Genarchopsis goppo* infects the intestines of the freshwater fish *Channa punctatus*. In the present study the histopathological and histochemical investigations have been evaluated to understand the extent of damage caused due to the infestation of the parasite and also to assess the histochemical alterations that occurred in the intestine of *Channa punctatus* (Bloch) as a result of *Genarchopsis goppo* infection. It is observed that the histopathological changes included the destruction and shedding of intestinal villi, inflammatory fibrosis characterized by hyperplasia and metaplasia of the submucosal layer. The histochemical tests applied on the intestinal tissues revealed that the total carbohydrates, proteins, and lipids were found elevated in the intestine due to helminth infestation.

keywords - Keywords: *Genarchopsis goppo*, *Channa punctatus*, Histopathology, Histochemistry

Introduction:

Helminth parasites represent a significant threat to fish populations, and the study of trematode parasites in fish remains a largely unexplored area ripe for investigation. Earlier studies were made on this parasite (Madhavi, 1978; Sinha, 1987; Mueleman et al, 1987; Pokora 2001; Urabe, 2001 and). While numerous studies have been conducted on trematodes affecting fish, relatively few have focused specifically on the histophysiological and histopathological aspects, particularly the alterations in the chemical composition of host tissues (Bose and Sinha, 1979; Barbara, 1980; Muzzal, 1980; Chung Yui-Tan, 1981; Christina, 1982; Papaerna and Vanas, 1983; Maqbool and Nizami, 1984; Zarina, 1990; and Bhargavi and Krishna, 1993). The interactions between parasites and their hosts can have detrimental effects on the host (Holmes and Bethel, 1972; Holmes, 1979; Minchella and Scott, 1991). Parasites can negatively impact host physiology in various ways, often inducing stress responses in the infected organism.

The presence of parasites in a host leads to numerous alterations, including changes in the macromolecular composition of the host's tissues, which is supported by histochemical and biochemical studies. Significant contributions to this area of study were made by Lomukhin (1971), Kumar and Singh (1971), and Cheng and Snyder (1972). The impact of parasites on host tissues triggers a cascade of changes that ultimately reduce absorption rates and disrupt other metabolic processes. Biochemical alterations in infected tissues

encompass changes in carbohydrates, proteins, lipids, nucleic acids, and enzyme levels. While some studies have aimed to visualize the biochemical changes in fish due to parasitic infestation (Robinson & Williams, 1971; Gupta and Agarwal, 1984), there is limited information available regarding histochemical studies, which elucidate the changes occurring in host tissues as a result of parasitic infections. Notable contributions in this area have been made by Hoswell (1973), Higgins et al. (1977), Pike and Burt (1983), and Watson et al. (1992). The present study aims to investigate the histopathological and histochemical alterations induced in the intestines of the fish *Channa punctatus* due to the infestation by the trematode parasite, *Genarchopsis goppo*.

Materials and Methods:

In this investigation, fish specimens were obtained from local fish markets as well as directly from fishermen catching them in nearby freshwater bodies. To collect the parasites, the fish were sacrificed, and their alimentary canals were opened for examination. Many parasites inhabit various sections of the alimentary canal hence, the entire tract was isolated and placed in a Petri dish containing normal saline. The intestine was meticulously screened for the presence of parasites, which were then collected onto a slide and fixed for further analysis. The parasites were stained using Alum carmine for identification.

Both the infected and uninfected intestines of the fish were isolated and cut into small segments. These segments were preserved in Bouin's, Susa, Carnoy's, and Zenker's fluids (Gurr, 1962) for subsequent histopathological and histochemical analyses. A range of histochemical tests (Pearse, 1968; Bancroft, 1975) was conducted on microtome-cut sections of both infected and uninfected intestines

Results:

The histopathological examination of the intestine in hosts infected by this parasite revealed significant damage across various intestinal layers, from the mucous membrane to the muscularis layer. The mucous membrane exhibited extensive damage to the villi, including areas where the villi were compromised. This infection resulted in the invasive encroachment of the muscularis layer into the intestinal lumen, with a complete retraction of the submucous membrane. Additional histopathological changes noted in the intestine included fibrosis accompanied by hyperplasia and metaplasia. Other evident alterations included dilation of blood vessels, degeneration of intestinal folds, epithelial necrosis, vacuolation of submucosal cells, and proliferative changes leading to the degradation of various intestinal layers. Inflammation and fibrosis correlated with hyperplastic and metaplastic changes were observed (Fig. 3). The presence of the parasite also induced vascular dilation in the submucosa, resulting in degeneration of intestinal folds, villi reduction, and necrosis of epithelial cells (Fig. 4). Vacuolation and proliferation of submucosal cells contributed to the degeneration observed in several intestinal layers (Fig. 5). Pathological consequences included an increase in mucosal thickness along with damage to the mucosa (Fig. 6). A notable decrease in muscular layer thickness was also common in helminth infections.

The battery of histochemical tests performed on the intestinal sections of *Channa punctatus* illuminated significant changes in the histochemical properties of the intestine due to the parasitic infection. The histochemical profiles of the infected fish diverged markedly from those of the uninfected ones. The application of the Periodic Acid/Schiff (PAS) technique revealed a strong positive reaction across all intestinal layers of the uninfected fish, indicating the presence of vinyl hydroxyl groups in the tissues. This reaction further suggested the existence of mucopolysaccharides and related carbohydrate groups. In contrast, the distribution of carbohydrates in the intestines of fish infected with the trematode *Genarchopsis*

goppo illustrated a significant shift in their chemical composition, reflecting the host-parasite relationship. Additionally, the glycogen content in the intestines of infected fish differed from that of their uninfected counterparts, as evidenced by sections subjected to PAS staining after diastase digestion. Infected fish exhibited a marked increase in carbohydrate and glycogen content, potentially representing a protective response by the fish to mitigate the effects of the infection.

Uninfected fish sections demonstrated a positive response to Alcian Blue at both pH 1.0 and 2.5, while infected fish showed intensified reactions for Alcian Blue at both pH levels, indicating a higher quantity of mucopolysaccharides. A strong positive reaction for Bromophenol Blue confirmed the presence of proteins, with intensity notably greater in infected fish compared to uninfected specimens. This increase in protein levels in the infected fish could be attributed to a higher nutrient supply to the affected organs. Infected fish exhibited an increase in various lipid groups, including total lipids and phospholipids. The total lipids were assessed using the Sudan Black B method, while phospholipids were evaluated using copper phthalocyanine. The results from the histochemical tests on the intestinal sections are summarized in Table 1.

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	+	++
Copperphthalocyanin	+	++
Sudan Black 'B'	+	+++
Orcein/Van Gieson	+	++
Orcein	+	++

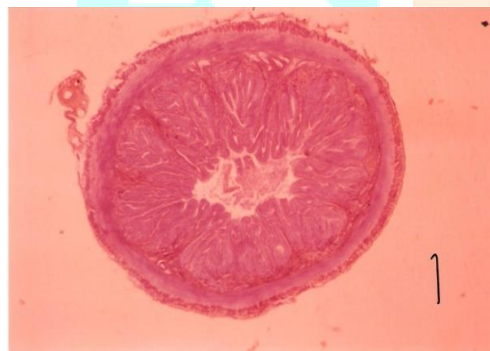
+++ = **Strongly positive**++ = **Moderately positive**+ = **Positive**— = **Negative**

Fig.1. T.S. of uninfected intestine shows several layers

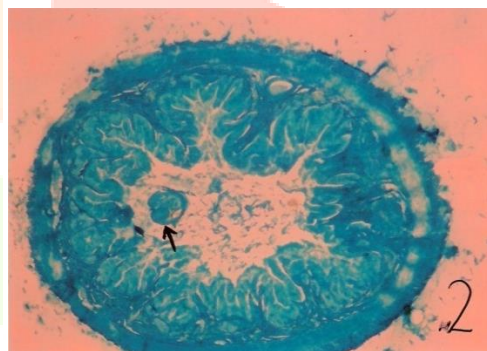


Fig. 2. T.S. of infected intestine shows initial parasitic infection

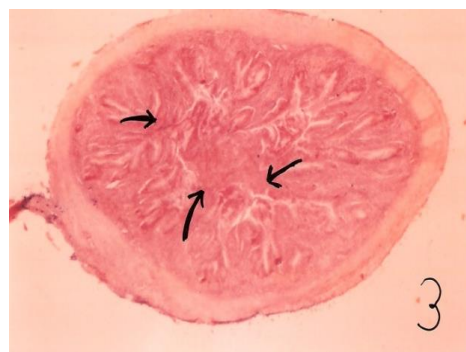


Fig.3.T.S. of infected intestine shows and hypertrophy of

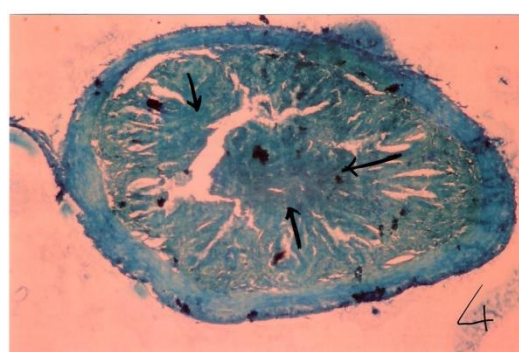


Fig.4.T.S. of infected intestine shows inflammation necrosis of villi

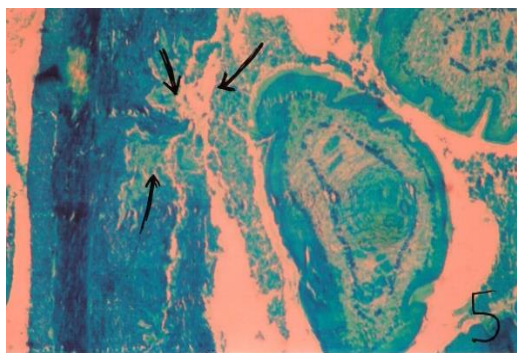


Fig.5. T.S. of infected intestine shows



Fig. 6. T.S. of infected intestine degeneration shows empty lumen with parasite.

Discussion:

The pathological impacts of parasitic infections on fish are well-documented, with parasitic fauna significantly affecting fish health, contributing to mortality rates, and consequently reducing fish populations. Various species of helminth parasites pose a substantial threat to fish fauna, with the extent of damage and pathogenicity largely depending on the number of parasites present and their specific localization within the fish. It is widely recognized that helminth parasites induce pathological changes in various organs of the infected host. These alterations can result from mechanical damage and the release of toxins by the parasites. The helminth infestation causes considerable histological damage in the intestines, which appears to be among the most severely affected organs in the fish's digestive tract. In this study, it was observed that the intestine was significantly effected by helminth invasion, as evidenced by the complete erosion of villi and also the mucous membrane. This disruption in the structural organization of the intestine could have profound impact on the fish's digestive processes and nutrient's absorption. The studies made by Bose and Sinha (1979) reported the presence of the helminth *Genarchopsis goppo* in the stomach of *Channa gachua*, noting extensive damage to the mucous and submucous layers, including a flattening of the surface epithelium, complete destruction of the lamina propria, and edema in the submucosal layer. The histopathological findings in the present study align with these observations, indicating that the digestive tracts of infected fish experience similar effects. The muscularis layer suffered severe damage due to the parasite, leading to degeneration, necrosis, and the development of secondary folds within that layer. Barbara (1980) investigated the pathological changes caused by *Bucephalus polymorphus* and *Rhipidocotyle illensis* in cyprinid fry, while Muzzal (1980) documented damage to a white sucker fish caused by the trematode, *Triganodistomum* attenuation. The studies made by Meakins (1973), Lester (1980), Skinner (1982), Gupta and Agarwal (1984), and Singhal et al. (1984) have also described about the pathological conditions caused by trematodes in various fish species. In addition to the pathological effects on host tissues, it is crucial to consider the physiological state of the affected host. The host's pathological condition can significantly alter the levels of various metabolites, leading to changes in its metabolic and physiological functions. Such metabolic changes can be evaluated through biochemical analyses of the infected tissues. In this study, histochemical tests were performed on the intestinal tissues of infected fish to assess their physiological condition.

Parasites not only alter the morphology of the host organs but also disrupt the physiology, metabolism, and secretory efficacy of the associated intestinal glands, negatively impacting the host. The interaction between the parasites and the host leads to a series of cascading effects that ultimately diminish the efficiency of nutrient absorption and other metabolic processes. Significant alterations in the levels of carbohydrates, proteins, lipids, and related enzymes were observed. The increased protein content in the infected fish could result from enhanced nutrient supply to the affected organs, as suggested by Maqbool and Nizami (1984),

who focused on the histochemical and histoenzymological characteristics of the metacercariae of *Clinostomum complanatum*. Additionally, the increases in other metabolites within the intestine may reflect the fish's protective responses to parasitic infestation, along with the accumulation of food material arising from the impaired absorption rates in the intestine.

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