Histopathological and Histochemical Alterations in the Liver of Freshwater Fish, Channa Punctatus Due to Genarchopsis Goppo (Ozaki, 1925)

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Abstract:
The trematode parasite, *Genarchopsis goppo* (Ozaki, 1925) is found infected to the fish, *Channa punctatus*. This parasite brought histopathological and histochemical abnormalities in fish liver. The changes that have witnessed in the liver, include inflammation of liver, change of colour, and development of cysts. The histopathological changes include disarray in the arrangement of hepatochords, enlargement and rupture of hepatocytes. The hepatic sinusoids are filled with blood. Vacuolization and necrosis was also noticed in the infected liver. Histochemical changes were noticed with the enhanced carbohydrate, protein and fat content.

Keywords: Genarchopsis goppo, Channa punctatus, Histopathology, Histochemistry

Introduction:
The impact of parasites on freshwater fish is of significant concern due to their widespread prevalence and their role as an intermediate link in the food chain. Parasites can influence host physiology in various ways, inducing stress in the affected organism. Adult flukes often invade numerous organs, including the gills, digestive system, liver, and kidneys. The liver is a vital gland that performs several critical functions; it is the primary organ for detoxification and plays an essential role in the metabolism of key bodily elements (Pardeshi et al. 2012). Consequently, a histological examination of the liver can provide insights into the health status of fish and help to assess the potential effects of parasites on the functions of different body systems (Kaur et al. 2012). Previous investigations have documented the presence of trematodes in fish (Bose and Sinha 1979; Barbara 1980; Muzzal 1980; Chung Yuitan 1981; Maqbool and Nizami 1984; Bhargavi & Krishna 1993; Christina 1982; Sinha et al. 1988; Gupta and Agarwal 1983).Parasites induce numerous alterations in the composition of host tissues, with significant contributions from researchers such as Lomukhin (1971), Kumar and Singh (1971), Robinson and Williams (1971), Cheng and Snyder (1972), Pandey et al. (1992), Das and Das (1997), Roberts (2001), Mishra et al. (2006), Sinha and Nikhil (1988), and Ginetsinkskay (1960).
India is characterized by abundant freshwater resources, hosting a rich genetic biodiversity of fish (over 2,200 species) and ranking ninth in terms of freshwater mega-diversity (Rubina Mondal and Anuradha Bhat, 2020). In tropical regions, parasites present a significant challenge to freshwater fish populations (Morales-Serna et al., 2019). They are a major limiting factor affecting the growth of farmed fish

(Prangnell, David et al., 2016). Additionally, parasites can devalue nutrients, alter biological processes and behaviors, decrease immunity, reduce growth and reproductive capacity, increase mortality and morbidity, and cause mechanical injuries depending on their number and the sites of infection (Bichi et al., 2020; Nmor et al., 2020). Furthermore, parasites can influence host population dynamics and manipulate community structures (Marcogliese David, 2004). Parasitology remains a continually evolving field of research. The relationship between hosts and parasites is unique as, among the two, it is only the parasite that benefits, while the host endures the adverse effects (Stothard et al., 2018).
The impact of parasites on hosts leads to a series of interactions that ultimately impair nutrient absorption and disrupt various metabolic processes. Changes in levels of carbohydrates, proteins, lipids, nucleic acids, and enzymes are particularly significant. However, there is relatively limited information regarding histochemical studies that specifically elucidate alterations in the chemical composition of various tissues (Higgins et al., 1977; Hoswell, 1973; Pike and Burt, 1983; Watson et al., 1992). Therefore, the present study focuses on the histological and histochemical changes observed in the liver of the freshwater fish Channa punctatus infected with the trematode Genarchopsis goppo.

Material and Methods

For this investigation, fish were obtained from local fish markets as well as directly collected from fishermen who caught them from various freshwater bodies. To isolate the parasites, the fish were humanely sacrificed, and their viscera were examined. The liver was subsequently isolated and placed in a petri dish containing normal saline for thorough screening for the presence of parasites. The collected parasites were then placed on a slide for fixation. Staining was performed using Alum Carmine dye. Both infected and uninfected fish livers were preserved in Bouin's, Susa, Carnoy, and Zenker's fluid for further analysis. Following routine histological preparation, tissue sections of 5 µm thickness were cut using a rotary microtome. To assess the normal histology of the liver, Heidenhain's Azan stain (Gurr, 1962) was utilized. A series of histochemical tests (Pearse, 1968; Bancroft, 1975) were conducted on the microtome-cut sections of both infected and uninfected liver tissues to identify any histochemical changes arising from the infection by Genarchopsis goppo**.**

Results

The normal histology of the liver consists of polygonal hepatic cells, each containing a clear, spherical nucleus. These cells are arranged among sinusoids, forming cord-like structures known as hepatic cell cords. In contrast, the pathological changes observed in the liver of Channa punctatus infected with Genarchopsis goppo encompassed morphological, histopathological, and histochemical alterations.The morphological changes noted in the liver of infected fish included notable shifts in color and signs of inflammation. Specifically, the liver appeared pale red and yellow in infected specimens. Additionally, the presence of cysts and abscesses was observed in the liver tissue. Significant histopathological changes documented in the livers of infected fish (as illustrated in Figures 1, 2, 3, and 4) included enlargement of the hepatocytes. These hepatocytes exhibited a loss of their distinct shape, and vacuolation within the cytoplasm was evident. The arrangement of the hepatic cords was disrupted, with hypertrophy of hepatocytes noticeable. The perilobular regions displayed vacuolation, while the central lobular areas underwent necrosis. The cytoplasm of the affected cells was non-uniform and presented a granulated appearance. The sinusoids were damaged, ruptured, and filled with blood, while the associated blood vessels appeared shrunken and degenerated. The penetration of parasites into the liver led to obstructions in the bile passages, further contributing to the liver dysfunction in the infected fish.

Table – 1: Histochemical Tests For Liver Of *Channa Punctatus*

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'	++	+++
Pyronin-Y	++	+++
Methylgreen/Pyronin – Y	++	+++
Feulgen reaction	+	+++

+++= Strongly positive ++ = Moderately positive
+=positive - = Negative

The histochemical analysis of liver sections from both infected and uninfected fish demonstrated positive reactions to periodic acid-Schiff (PAS) staining, indicating the presence of carbohydrates, particularly glycogen. This presence of glycogen was further confirmed through PAS staining after diastase digestion and by the use of Best carmine's stain, which also yielded a positive result. In the livers of infected fish, the distribution of glycogen was notably higher, as evidenced by strong positivity in the staining results (Figure 5, Table 1).When sections of the liver were stained with Schiff's reagent without prior oxidation by periodic acid, a light pink color was observed, suggesting the presence of aldehyde groups. Further testing showed a negative response to alcian blue at both pH 1.0 and pH 2.5, indicating the absence of mucopolysaccharides, including sialomucins and hyaluronic acid, in both infected and uninfected liver samples (Table 1).The presence of basic proteins was confirmed through staining with the mercuric bromophenol blue method, which exhibited characteristic color changes (Figure 6). Congo red staining indicated that glycoproteins were present in significant amounts within the liver cells. Infected fish livers exhibited a greater protein content, reflected by strong positivity to bromophenol blue (Table 1). Small quantities of tyrosine-containing proteins were detected with the Millon's reaction.The presence of protein-bound amino groups in the liver samples was demonstrated through ninhydrin-Schiff's staining, which showed positivity in both infected and uninfected sections. Moderate levels of sulfhydryl and disulfide compounds were also present in the liver, as indicated by positive responses to the ferric ferrocyanide method (Figure 7) and the AB/KMnO4 test.The presence of nucleic acids (DNA and RNA) was confirmed through positive reactions in the liver sections using Feulgen's reaction and the Pyronin-Y technique. Phospholipids were identified with copper phthalocyanin staining (Figure 8). The abundance of lipids in the liver was demonstrated by a strong reaction of the liver sections to Sudan Black B, indicating higher lipid content.

On the whole the histochemical studies revealed that all metabolites analyzed were present in greater quantities in the infected livers compared to their uninfected counterparts, as suggested by the strong positivity in various staining assays (Table 1). This indicates significant biochemical changes in the livers of fish infected with Genarchopsis goppo.

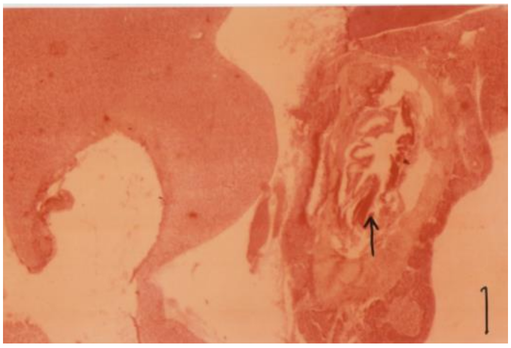

Fig.1. T.S. of infected liver *shows* encysted parasite

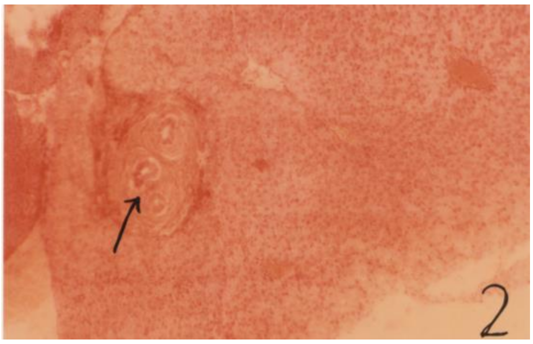

Fig. 2. T.S. of infected liver shows vacuolation.

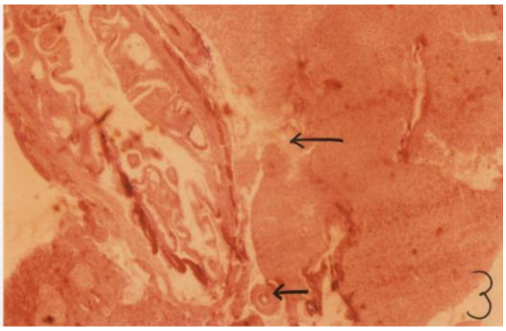

Fig.3 T.S. of infected liver shows hypertrophy and vacuolization

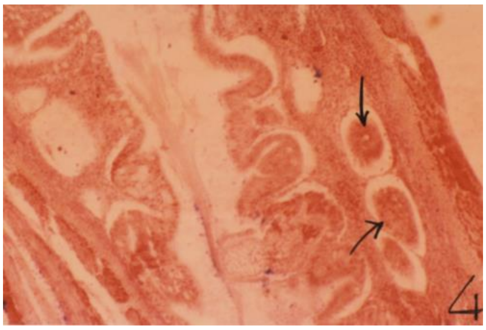

Fig.4. T.S. of infected liver shows necrosis

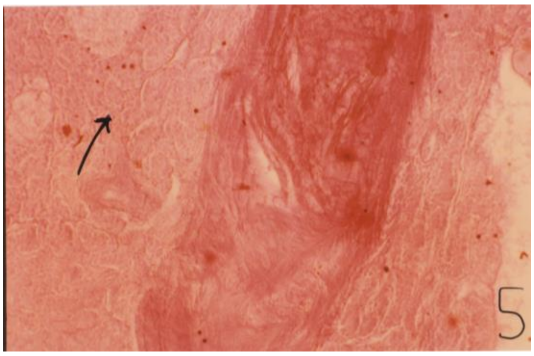

Fig.5. T.S. of infected liver shows positivity to PAS

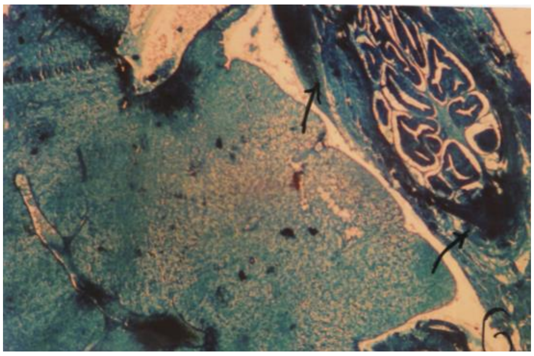

Fig. 6. T.S. of infected liver shows positivity to bromophenol blue.

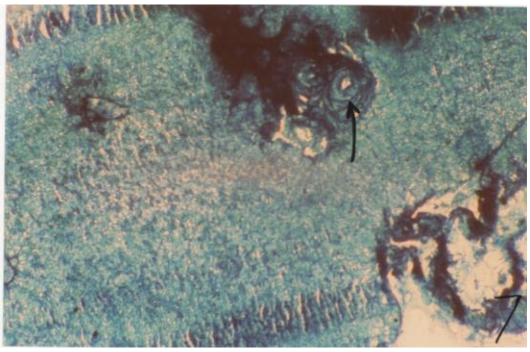

Fig. 7. T.S. of infected liver shows positivity toFerric ferry cyanide.

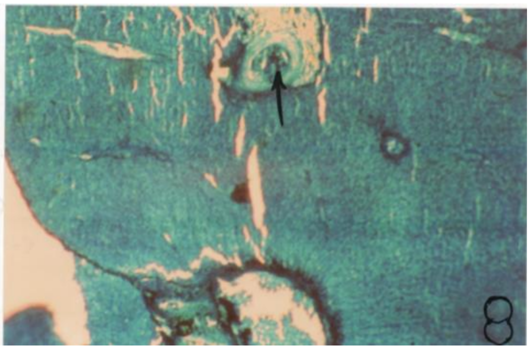

Fig. 8. T.S. of infected liver shows positivity to copperthalocyanin

Discussion:

The pathological changes observed in the livers of fish infected with parasites can largely be attributed to mechanical damage inflicted by the parasites as well as the potential release of various toxins. For instance, significant pathological alterations have been documented in Cyprinid fry infected with trematodes such as Bucephalus polymorphus and Rhipidocotyle illensis (Barbara, 1980; Muzzal, 1980). Additionally, earlier studies conducted by Sinha and Nikhil (1988) highlighted that the metacercariae of E. heterostomum were primarily localized within the liver, which exhibited the highest levels of infection. Infected livers were characterized by atrophy and a pale coloration, contrasting sharply with the reddish hue of healthy fish livers.

Gupta and Agarwal (1983) investigated encysted metacercariae of Euclinostomum heterostomum in the liver tissue of Channa punctatus ; Sinha and Nikhil (1988) also observed considerable histopathological changes in the infected liver, which included compression and distortion of hepatic cells. The bile duct epithelium in these fish was inflamed, leading to bile congestion. Additionally, signs of vaso dilation and congestion of blood vessels were evident in the livers of infected fish. The findings of the present study align well with these previous reports, underscoring the consistent pathological impacts that parasitic infections can have on liver structure and function.The liver serves as a critical digestive gland responsible for numerous vital processes within the body. It is primarily involved in detoxification, metabolism of carbohydrates, proteins, and lipids, glycogen storage, desaturation of fatty acids, and amino acid synthesis, among other essential functions. When a parasitic infection occurs, it disrupts the regulation of sugar levels in circulation, thereby affecting various metabolic pathways. The pathological state induced by such infections can significantly alter the levels of important energy molecules, leading to changes in the metabolism and physiological activities of the host organism. These metabolic alterations are revealed through chemical analyses of the infected tissues compared to those from uninfected fish. In the current study, various histochemical tests were employed to quantify these changes. It is important to note that parasites not only alter the morphology of the host but also interfere with nutritional uptake, metabolic processes, and the functioning of the alimentary canal and its associated glands. Consequently, the influence of parasites on their hosts is predominantly adverse.Particularly interesting findings from the study indicate that the distribution of glycogen within the liver cells was increased in infected fish. As the liver is a principal site for glycogen storage, any parasitic infection in this organ can severely disrupt its crucial physiological functions. These disturbances have direct implications for the chemical composition of the infected tissue, either by depleting or augmenting the levels of critical molecular constituents.The observed increase in glycogen content in the livers of infected fish suggests that the parasites depend on the host's fluids to sustain themselves. Furthermore, since proteins play a vital role in the body's metabolic processes, the examination of protein content in helminth-infected fish has gained significant interest. Understanding the dynamics of protein levels helps elucidate the host-parasite relationship and the extent of parasitism experienced by the fish.Different species of helminth parasites thus present a serious threat to fish populations. The severity of damage or the pathogenic conditions that ensue depend on the quantity of invading parasites and their localization within the host's body.

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