HISTOCHEMICAL ANALYSIS OF GASTROINTESTINAL MUCOSUBSTANCES OF FRESH WATER FISH Mastacembelus armatus INFECTED BY HELMINTH PARASITE Circumonco bothrium sp.

Laxmikant B. Dama¹,² and Amjadkhan V. Pathan²,³

¹Department of Zoology, D.B.F. Dayanand College of Arts and Science, Solapur, 413002 (M.S.), India
²Department of Zoology, Azaad College, Ausa-413520, (M. S.), India
³Email: southraj@gmail.com; amjadkhanp391@gmail.com; ORCID: 0000-0003-3157-8457

Supporting Information

ABSTRACT: Present study was conducted to investigate the histochemical changes induced by Circumonco bothrium sp. in the intestine of freshwater fish Mastacembelus armatus. During present investigations the infection of Circumonco bothrium sp. in Mastacembelus armatus with various histochemical reactions showed localization of carbohydrate, protein, lipid and glycogen. During histochemical study intestine infected by cestodes, the numbers of mucous cells those containing acidic or mixed glycoconjugates were significantly higher than those seen on sections from uninfected fish, which is a protective interaction of the host against parasitic infection. In the current study, a highly significant increase in the number of mucous cells was seen within the infected intestines of Mastacembelus armatus when compared to uninfected counterparts.

Keywords: Circumonco bothrium sp., Histochemical, Intestine, Mastacembelus armatus

INTRODUCTION

The gastrointestinal system is primarily involved in breaking down food for absorption into the body. It is essentially a muscular tube lined by a mucous membrane which exhibits regional variations reflecting the changing functions of the system from mouth to anus. The Alimentary canal is an organ which is involved in various important physiological functions. It is the primary site of food digestion (absorption) and nutrient uptake.

According to Srivastava (1975) and Chandra et al. (2011), most of the species of helminths in adult stage live in the alimentary canal these parasites have detrimental effects upon fish in more ways than one. Different parts of the cell are biochemically different, they take up specific stains to varying degrees. Histochemical tests are used in an attempt to identify cell and tissue components by virtue of their specific chemical reactions. The alteration in the state of cell constituent can be studied by using histochemical techniques, these techniques helps to analyze not only the localization of carbohydrate, protein, lipid and glycogen etc. but also molecular changes at cellular level. The noteworthy contributions towards the expansion and development of histochemistry are those of Lillie (1954), McManus (1948), Pearse (1968) and Bancroft and Stevens (1992), Sonune (2014). In 2012, Ghosh and Chakrabarti observed the histochemistry of the olfactory rosette of Cyprinus carpio.

The present study includes the Histochemical analysis of gastrointestinal mucosubstances of fresh water fish Mastacembelus armatus infected by helminth parasite Circumonco bothrium sp.

MATERIALS AND METHODS

Preparation of slides for histochemical studies:

For histochemical analysis, small fragments from the anterior, middle and posterior parts of infected intestine were used. The infected intestine and normal were cut into small pieces and were fixed in Bouin’s fluid. After 48 hours, washed several times with water, dehydrated in graded series of alcohols, cleared in Cedar wood oil and xylene, blocks were made in cavity blocks by usual method. Thick sections were cut with a rotary microtome at 4-5 micron thick. After removing the wax by xylene, hydration was carried out, dehydrated, cleared in clove oil and xylene and mounted permanently in Canada balsam. Sections were stained with various histochemical staining methods. Best slides or sections were selected and observed under the microscope for histochemical study. Photographs were taken with digital camera Nikon Coolpix L24.

Methods used for histochemical tests were:

1. Periodic Acid- Schiff (PAS) (McManus, 1948)
2. Alpha-amilase-PAS (McManus, 1948)
3. Alcian blue pH 2.5 (Martoja and Martoja-Pierson, 1970)
4. Alcian blue pH 0.4 (Martoja and Martoja-Pierson, 1970)
5. Congo red (Pearse, 1968)
6. Sudan black B (McManus, 1948)
7. Ferric ferricyonide (Pearse, 1968)
8. Free aldehydes (Sawhney and Randhir Singh, 2014)

All the data of results were tabulated according to color intensity into different grades ranging from + to ++++

<table>
<thead>
<tr>
<th>No.</th>
<th>Stain/Method</th>
<th>Intensity Normal intestine</th>
<th>Intensity Infected intestine</th>
<th>Inference about mucosubstances Normal intestine</th>
<th>Inference about mucosubstances Infected intestine</th>
<th>Fig. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PAS</td>
<td>++</td>
<td>++</td>
<td>Neutral mucin present</td>
<td>Neutral mucin present</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Alpha-amilase-PAS</td>
<td>+</td>
<td>+</td>
<td>Mucin present</td>
<td>Increase mucin</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Alcian – blue pH 2.5</td>
<td>++</td>
<td>+++</td>
<td>Acidic mucins present</td>
<td>Acidic mucins Increase</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Alcian – blue pH 0.4</td>
<td>++</td>
<td>+</td>
<td>Sulphated mucins predominant</td>
<td>Few sulphated mucins</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Congo red</td>
<td>+++</td>
<td>++</td>
<td>Amyloid elastic fibres few</td>
<td>Increase Amyloid</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Sudan black B</td>
<td>++</td>
<td>+++</td>
<td>Less lipid</td>
<td>Increase lipid</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Ferric ferricyonide</td>
<td>+</td>
<td>++</td>
<td>Less iron deposits</td>
<td>More iron deposits</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>Free aldehydes</td>
<td>+</td>
<td>++</td>
<td>No PPT</td>
<td>Dark PPT</td>
<td>8</td>
</tr>
</tbody>
</table>

DISCUSSION

A heavy mucus production has also been described from several other fish-helminth systems including those detailed by Chambers et al. (2001). The attachment organ of helminth parasites often provokes an inflammatory response within the hosts gastrointestinal tract (Dezfuli et al., 2011). Inflammation is a protective reaction in response to parasitic invasion which results stimulation of specific chemical alterations to the cellular community and tissues at the site of infection. Hur et al. (2013) although the factors that govern mucus discharge are partially defined for mammals, they are not well studied in fish. The present findings are more or less similar to the observations made by Kaur (2014). Who reported the pathological changes mainly enhanced mucus secretion in...
*Channa punctatus* and *Channa striatus* infected by cestode, *Senga* sp. A heavy mucus production has also been described from several other fish-helminth systems including those detailed by Benarjee and Reddy (2006).

Observation of the selected infected slides reveals that average amount of amyloid is present, which stained brownish black in colour when stained with Congo red whereas in normal intestine show the relatively low amount of amyloid. The gastrointestinal tract is typically covered by mucus, the properties of which change in different regions of the alimentary canal (Shephard, 1994). The mucus, which can be considered an aggregate secretion, is produced by mucous cells. Whereas, mucin is glycoproteins within this secretion (Theodoropoulos et al., 2001). There is a high trace of glycogen particularly in the muscularis mucosa, but in infected intestine moderate quantity of glycogen is seen (Sonune, 2014).

There is no complete agreement on the role of excessive mucus secretion which, in the intestines of fish infected with helminths, appears as protective blanket of gel or mucus. Although it has been suggested that increased mucus production in mammals may facilitate the expulsion of intestinal nematodes (Ishikawa et al., 1993), this is not the case in the current *Circumonco bothrium* sp. and *Mastacembelus armatus* situation. In the present study *Circumonco bothrium* sp ensures a secure attachment to the intestinal wall of its host even they crossed the intestine and enters the coelom. Thus it will be suggested that, the main role of mucus is to protect the underlying intestinal mucosa as a physical barrier against the mechanical and biochemical damage induced by parasites (Schroers et al., 2009).
CONCLUSION

The gastrointestinal tract is typically covered by mucus, the properties of which change in different regions of the alimentary canal. The mucus, which can be considered an aggregate secretion, is produced by mucous cells; mucus, however, differs from mucin which refers to specific glycoproteins within this secretion. Intestinal mucus, which is continuously secreted to renew the coating, is a dynamic system which is coupled to the immune system. An accelerated secretion is characterized by a rapid, massive mucous cell discharge in response to physiologic or pathologic stimuli by the parasite. Moreover, the histochemical investigations provide an insight into the nature of various physiological and pathological processes in the gastrointestinal tract occurred due to parasites. It has been observed that the different constituents are stimulated by particular parasite and particular loss in different organs of the digestive system of the fish studied. Histochemical study may provide a valuable with low cost effective tool for the diagnosis of diseases in histopathology, parasitic investigation and for the researchers in histopathology.

DECLARATIONS

Corresponding author

Laxmikant B. Dama is Associate Professor at Dayanand Institutions, Solapur (M.S.), India. In 1998, he completed his Ph.D. in Zoology. His expertise is applied parasitology, fishery science, and his current interests include the isolation of bioactive compounds and to study their effects on animals. Additionally, he is actively involved in writing, editing, and
reviewing research articles for different journals. His publication list is available on Google Scholar and ORCID. E-mail: amjadkhanp391@gmail.com; ORCID: 0000-0003-3157-8457

Acknowledgement
We thank to Principal, Dayanand College of Arts and Science, Solapur for providing laboratory facilities.

Conflict of Interest
The authors declare they have no competing interests.

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