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HISTOCHEMICAL LOCALIZATION OF ACID AND ALKALINE PHOSPHATASES IN *MONIEZIA EXPANSA* (RUDOLPHI, 1805)

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ABSTRACT

Cestodes or true tapeworms are important group of the Platyhelminthes, endoparasitic in the alimentary tract and associated ducts of various vertebrates and therefore, of great interest to the medical and veterinary profession.

Moniezia expansa, the tapeworm under study is the common parasite of oldest domesticated animal sheep. It inhabits the small intestine especially the lower portion and absorb the nutrition of host from the intestine and deprive it of many important nutrients and thus deteriorates the quality of meat, wool, skin of host which leads to ecomical losses as well.

Phosphatases are enzymes hydrolysing esters of phosphoric acid and play an important role in many metabolic processes. The histochemical localization of acid and alkaline phosphatase has been studied in Moniezia expansa as an effort to study some of its biological aspects which would help in its control and chemotherapy.

Acid phosphatase was found to be localized in tegument, subtegumental cells, parenchymal muscles, interproglottidal gland tegument, Mehlis glands, testes, uterine wall and eggs and was not observed in parenchyma, interproglottidal gland cells, excretory vessels, ovaries and vitellaria. Alkaline phosphatase activity was observed in tegument, subtegumental cells, parenchymal muscles, parenchyma, interproglottidal glands, excretory vessels, membranes enclosing ovaries, vitellaria, Mehlis gland, testes, uterine wall and eggs.

The presence of acid phosphatase in testes, mehlis' glands, uterine wall and eggs as well as alkaline phosphatase in eggs in Moniezia expansa has been reported for the first time. Both the acid and alkanine phosphatases showed the variations in their activity in the tegument along the strobila with highest in mature region. This gives the idea of high metabolic activity in the mature region of the cestode. These phosphatases play important role in metabolism of the parasite and seems to be involved in transportation, digestion, distribution of nutrients and metabolites and absorption of metabolic end products.

Keywords: Phosphatases, Alkaline phosphatases, Acid Phosphatases, Moniezia Expansa, Cestode, Histochemistry, Enzyme

1. INTRODUCTION

Cestodes or true tapeworms are important group of the Platyhelminthes, endoparasitic in the alimentary tract and associated ducts of various vertebrates and therefore, of great interest to the medical and veterinary profession.

Moniezia expansa, the tapeworm under study is the common parasite of oldest domesticated animal sheep. It inhabits the small intestine especially the lower portion and absorb the nutrition of host from the intestine and deprive it of many

important nutrients and thus deteriorates the quality of meat, wool, skin of host which leads to ecomical losses as well.

Phosphatases are enzymes hydrolysing esters of phosphoric acid and are widely distributed. They play an important role in many metabolic processes especially carbohydrate metabolism and in transphosphorylation transfer mechanisms of a number of helminth parasites. Phosphatases active in acidic pH are acid phosphatases and those in alkaline pH are alkaline phosphatases.

Histochemical distribution of phosphatases has been performed in many cyclophyllidean cestodes. Rogers (1947), Sircar and Sinha (1978), Yamao (1952), Kilejian *et al.* (1961), Erasmus (1957ab), Waitz (1963), Bogitsh (1963), Waitz and Schardein (1964), Lee and Tatchell (1964), Howells and Erasmus (1969), Mayberry and Tibbitts (1972), Moczon (1974), Roy (1979), Khera and Arora (1984), Farooq and Farooqi (1984), Upender and Bhargavi (1985), Varma *et al.* (1985), Suman *et al* (2021).

The presence of acid and alkaline phosphatases in cestode *Moniezia expansa* has been studied earlier by many workers (Rogers (1947), Yamao (1952), Erasmus (1957b), Howells and Erasmus (1969), Khera and Arora (1984) and Varma *et al.* (1985) but most of these studies are basically limited to tegument and interproglottidal glands.

Therefore, in the present work, an attempt has been made to study the histochemical localization of acid and alkaline phosphatases in various tissues and organs of scolex, immature, mature and gravid regions of *Moniezia expansa*.

2. MATERIALS AND METHODS

The parasites were collected from the intestine of sheep obtained from local abbatoir, washed in several times in normal saline till free of debris.

For histochemical localization of acid and alkaline phosphatases in *Moniezia expansa*, scolex, immature, mature and gravid regions of the tapeworm were fixed in absolute acetone for 24 hours at 4^{o}C, blocks of 3 to 5 proglottids were embedded in paraffin wax. Longitudinal and transverse sections were cut at 6-8 µm.

For cold formalin fixed frozen sections, the fresh tissue sections at 10 µm thickness were cut using the cryocut microtome (Leica CM 1800) and were mounted on a clean slide. These were then fixed in 4% formalin at 4^{o}C for 12 to 24 hrs.

For *in toto* preparations, immature, mature and gravid proglottids were fixed in 4% formalin for 12-24 hrs, washed well to remove the fixative, incubated in substrate media, dehydrated, cleared and mounted.

The histochemical localization of acid phosphatase was done by lead phosphate method by Gomori (1950) as in Pearse (1972).

The histochemical localization of alkaline phosphatase was demonstrated by Calcium cobalt method by Gomori as in Pearse, 1972.

The control sections were immersed in incubating mixture lacking the substrate and also some control sections were treated with boiling water before incubating in the incubating mixture.

The presence of acid phosphatase in the sections was indicated by brownish black colour. The control sections showed no such colour.

Sites of sections possessing alkaline phosphatase activity were stained brownish black in colour and control sections showed negative reaction.

3. RESULTS

Acid phosphatase was found to be present in tegument, subtegumental cells, parenchymal muscles, interproglottidal gland tegument, Mehlis' gland, testis, uterus wall and eggs in *Moniezia expansa*. It was not observed in parenchyma, interproglottidal gland cells, excretory vessels, membranes enclosing lobules of ovaries and vitellaria. Tegument showed the variation in enzyme activity along the strobila. In mature proglottid it showed highest activity whereas in scolex and immature proglottids moderate and in gravid proglottids it showed very low activity.

The enzyme showed no activity in the control sections.

Alkaline phosphatase was present in tegument, subtegumental cells, parenchymal muscles, parenchyma, interproglottidal gland tegument, interproglottidal gland cells, excretory vessels, membranes enclosing ovaries, vitellaria, Mehlis' glands, testis, uterine wall and eggs in *Moniezia expansa*. Alkaline phosphatases showed non-uniform activity in the tegument along the strobila of the parasite. It was found to be highest in mature proglottids as compared to other proglottids. The enzyme was found to be absent in the control sections.

The results are summarized in following table:

Table 1

Table 1 General Distribution and Intensity of Acid and Alkaline Phosphatases Reaction in Various Structures of Moniezia Expansa

Structures		Acid phosphatase		Alkaline Phosphtase	
		Test	**Control**	**Test**	**Control**
Tegument	(Scolex)	++	–	++	–
	(Immature proglottid)	++	–	++	–
	(Mature proglottid)	+++	–	+++	–
	(Gravid proglottid)	+	–	+	–
Subtegumental cells		++	–	++	–
Parenchymal muscles		+++	–	+++	–
Parenchyma		–	–	+	–
Interproglottidal gland tegument		+++	–	+++	–
Interproglottidal gland cells		–	–	++	–
Excretory vessels		–	–	++	–
Membrane enclosing lobules of ovaries		–	–	++	–
Vitellaria		–	–	++	–
Mehlis gland		++	–	+++	–
Testis		++	–	++	–
Uterus wall		+	–	+	–
Egg		++	–	++	–

(+++) Intense reaction, (++) Moderate reaction, (+) Present, (–) Absent.

Figure 1

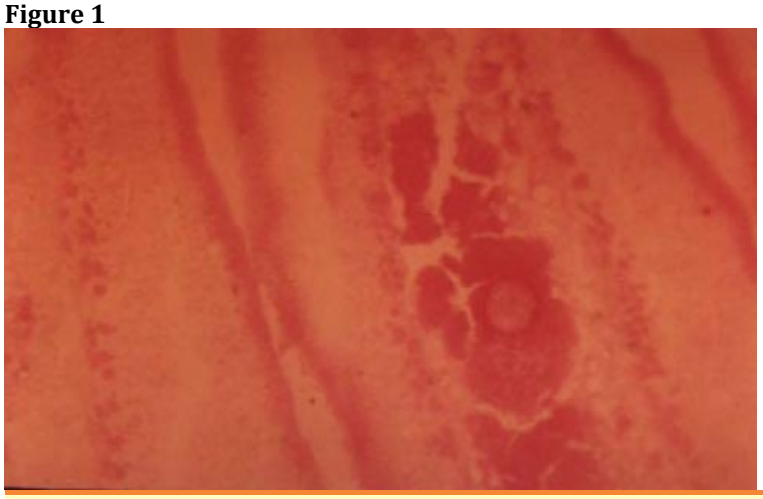

Figure 1 Control Sections of Mature Proglottids of Moniezia Expansa Showing Absence of Alkaline Phosphatase Activity.

Figure 2

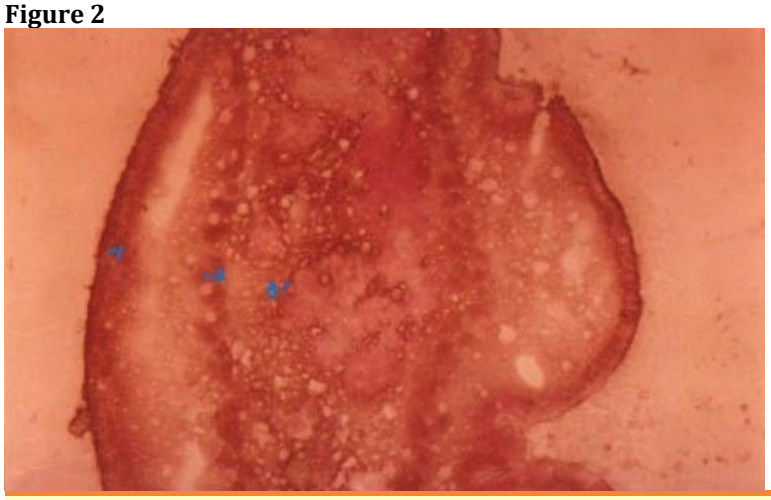

Figure 2 T.S of Mature Proglottid of Moniezia Expansa Showing Alkaline Phosphatase Activity Especially in the Tegument (1), Muscles (2) And Membrane Enclosing Ovaries (3).

Figure 3

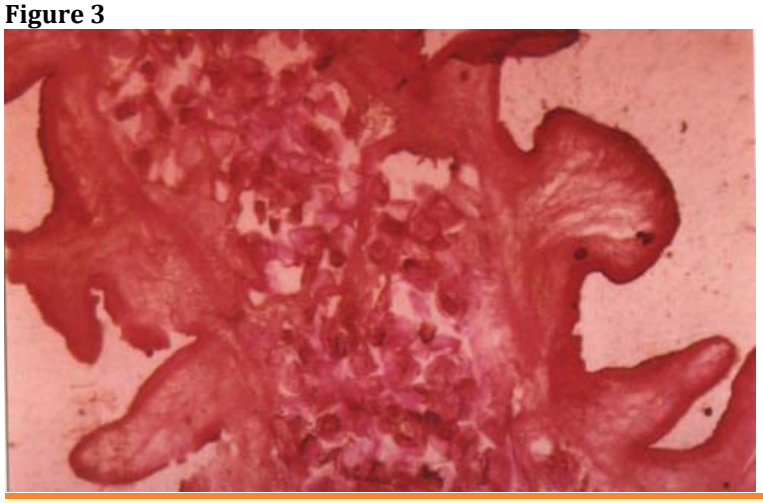

Figure 3 L.S of Gravid Proglottid of Moniezia Expansa Showing Acid Phosphatase Activity.

Figure 4

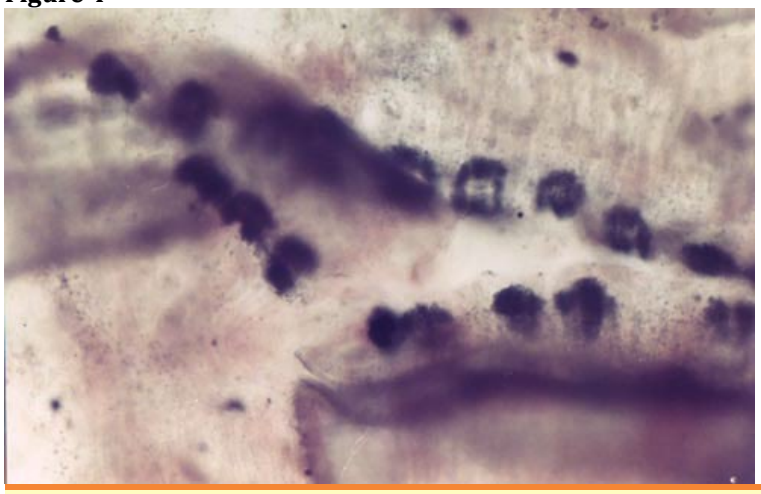

Figure 4 Whole Mount of Mature Proglottid of Moniezia Expansa Showing Acid Phosphatase Activity (Black Colour) in the Interproglottidal Gland Tegument.

Figure 5

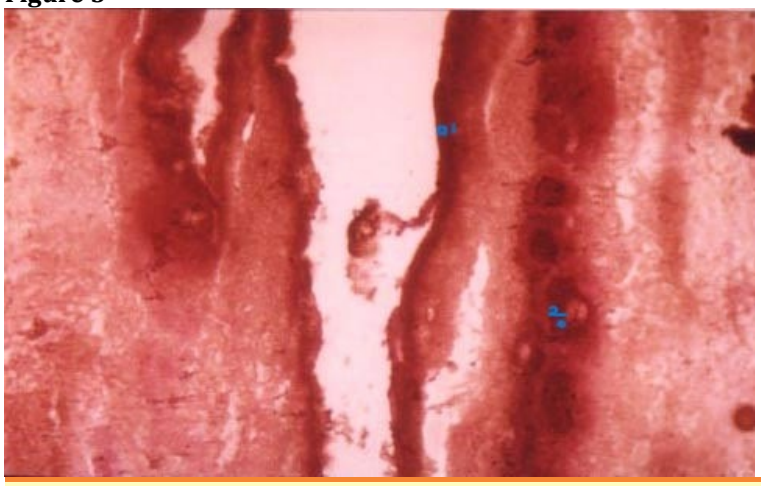

Figure 5 T.S of Mature Proglottids of Moniezia Expansa Showing Acid Phosphatase Activity in The Tegument (1) and Interproglottidal Gland Tegument (2)

4. DISCUSSION

In the present study, both acid and alkaline phosphatases have been observed in the tegument of *Moniezia expansa*. These are in accordance to that of Erasmus (1957b), Howells and Erasmus (1969), Khera and Arora (1984), Varma *et al.* (1985) in *Moniezia expansa* and Erasmus (1957b), Waitz (1963), Bogitsh (1963), Waitz and Schardein (1964), Roy (1979), Farooq and Farooqi (1984), Varma *et al.* (1985), Upender and Bhargavi (1985) and Suman *et. al*. (2021).

The presence of alkaline phosphatase in tegument of *Moniezia expansa* is similar to Rogers (1947) in *Moniezia expansa*, Kilejian *et al.* (1961) in *Echinococcus granulosus*, Mayberry and Tibbitts (1972) in *Hymenolepis diminuta* and Sircar and Sinha (1978) in *Raillietina echinobothrida*.

Non-uniform activity of the phosphatases has been observed along the different (scolex, immature, mature and gravid) regions of strobila with mature region having

highest activity in *M. expansa*. This was in conformity with Rogers (1947) for alkaline phosphatase and Erasmus (1957b), Howells and Erasmus (1969), Khera and Arora (1984) and Varma *et al.* (1985) for both phosphatases in *Moniezia expansa*. This has also been observed in other cestodes by Erasmus (1957a), Mayberry and Tibbitts (1972) and Roy (1979). This non-uniform distribution reflects the metabolic differences between proglottids at different stages.

The activities of phosphatases result in the release of phosphate ions, which may be utilized at cuticular (tegumental) surface for phosphorylated passage of substance through the cuticle or tegument Erasmus (1957). It has been well established that tegument is metabolically active and can take up, as well as digest nutrient through active transport Smyth (1969); Dike and Read (1971), which is accomplished by phosphohydrolases Roy (1979).

The observation of both acid and alkaline phosphatases in the subcuticular or subtegumental cells in *Moniezia expansa* by author is in accordance with that of Waitz (1963), Waitz and Schardein (1964), Roy (1979) and Suman *et.al* (2021). The presence of only alkaline phosphatase was reported by Erasmus (1957ab), Kilejian *et al.* (1961), Bogitsh (1963), Howells and Erasmus (1969), Sircar and Sinha (1978) in the subcuticular cells and this was similar to the present observation. Phosphatases in subtegumental cells may be associated with the secretory functions as these play an important role in formation of the syncytial protoplasmic layer of the tegument.

Rogers (1947), Sircar and Sinha (1978), Yamao (1952), Kilejian *et al.* (1961), Erasmus (1957ab), Waitz (1963), Bogitsh (1963), Waitz and Schardein (1964), Lee and Tatchell (1964), Howells and Erasmus (1969), Mayberry and Tibbitts (1972), Moczon (1974), Roy (1979), Khera and Arora (1984), Farooq and Farooqi (1984), Upender and Bhargavi (1985), Varma *et al.* (1985), Suman *et al* (2021)

Both acid and alkaline phosphatases activities were observed by author in the parenchymal muscles in *Moniezia expansa*. This was similar to Roy (1979), Farooq and Farooqi (1984), Upender and Bhargavi (1985) and Suman *et.al* (2021) who reported both acid and alkaline phosphates in parenchymal tissues. The presence of only alkaline phosphatases was reported by Erasmus (1957ab). Keljian *et al.* (1961) Bogitsh (1963), Waitz (1963), Waitz and Schardein (1964), Howells and Erasmus (1969) and Sircar and Sinha (1978). The present observations are not in accordance with Erasmus (1957), Bogitsh (1963), Waitz (1963), Waitz and Schardein (1964), Howells and Erasmus (1969) who failed to observe acid phosphatase activity in muscles in studied cestode models.

Parenchyma in the present study was found to have very low activity for alkaline phosphatase and acid phosphatase showed no activity. This has been in accordance to Waitz and Schardein (1964). Different workers reported the presence of acid phosphatases in parenchyma *viz*. Roy (1979) and Farooq and Farooqi (1984).

The presence of alkaline phosphatases has been observed in excretory canals of *Moniezia expansa*. This is in accordance to Erasmus (1957), Bogitsh (1963), Lee and Tatchell (1964), Mayberry and Tibbitts (1972), Sircar and Sinha (1978), Roy (1979) and Farooq and Farooqi (1984) in different cestodes. But the absence of acid phosphatase in excretory canal in *M.expansa* in present study by author was different from that of Roy (1979) and Farooq and Farooqi (1984) who reported acid phosphatase in the excretory canals of *Raillietina (R.) johri* and *Avitellina lahorea* respectively. The presence of phosphatases along excretory canal suggests its role in absorption of metabolic end products and distribution of nutrients to *different* parts of *Moniezia expansa* Howells (1969).

Acid and alkaline phosphatases showed intense reaction in the interproglottidal glands tegument. It was observed that acid phosphatase has been absent and alkaline phosphatase being present in the gland cells of interproglottidal gland in *M. expansa.* These findings were similar to the findings of other authors.

Observation of phosphatases in reproductive organs are significant and in accordance to those reported by various workers in different cestodes Erasmus (1957ab), Moczon (1974), Sircar and Sinha (1978), Roy (1979), Farooq and Farooqi (1984), Upender and Bhargavi (1985). Acid phosphatases were observed for the first time in testes, Mehlis' glands, uterine wall and eggs in *Moniezia expansa*. Alkaline phosphatase activity has been observed in membranes enclosing lobules of ovaries, testes, vitellaria, uterine wall and eggs. Both acid and alkaline phosphatases in eggs are reported for the first time. However, the present observations are different from those of Kilejian *et al.* (1961), Bogitsh (1963), Waitz (1963), Waitz and Schardein (1964), who did not observe the acid or alkaline phosphatases in the reproductive organs of respective cestodes under their studies.

Phosphatases in reproductive organs might be involved in transport of metabolites in addition to bring important components of cells enforced in rapid synthesis. Moczon (1974) suggested that the presence of acid phosphatase in spermatozoa of *Hymenolepis diminuta* might facilitate the formation of lysosome. This may be the same for *Moniezia expansa* also. In mature spermatozoa this enzyme may help in the breakdown of non-specific phosphate ester in order to maintain a phosphate pool for the production of high energy-yielding compounds Anderson et al. (1968).

Thus, the presence of phosphatases in tegument, reproductive organs, interproglottidal glands and excretory canals seem to play an important role in metabolism of cestode *Moniezia expansa.*

CONFLICT OF INTERESTS

None.

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