ACID PHOSPHATASE ACTIVITY IN CYSTS OF *GIARDIA LAMBLIA*

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Acid phosphatase (E.C.3.1.3.2) activity in cysts of *Giardia lamblia* indicated maximum enzyme activity at pH 5.6 and at a temperature of 60 °C. The Michaelis constant (Km value) was found to be 2.27 mM. The effect of different antibiotics viz. Metronidazole (Aristolyl-F), Erythromycin (Ethimycin), Amoxycilnine (Mox), Doxycycline (Doc-24) and Penicillin (Ampicillin) revealed that Metronidazole had maximum inhibitory effect while Erythromycin had minimum inhibitory effect on the enzyme activity.

Alkaline and acid phosphatases (E.C. 3.1.3.1 and 3.2) are well known for their significant role in metabolic activities of various protozoans. The phosphatases have been estimated biochemically in few protozoans like *Entamoeba histolytica*, *Plasmodium knowlesi*, *Trypanosoma brucei*, *Paramecium spp.*, *P. caudatum* and *P. tetraurelia*, *T. rhodesiense*, *Leishmania donovani* and *Naegleria fowleri*. The present communication deals with the study of the effect of pH, temperature, substrate concentration and various antibiotics viz. Metronidazole (Aristolyl-F), Erythromycin (Ethimycin), Amoxycilnine (Mox), Doxycycline (Doc-24) and Penicillin (Ampicillin) on acid phosphatase activity of the cysts of *Giardia lamblia*, an intestinal protozoan parasite of human beings.

The fresh stool samples were brought to the laboratory and examined for cysts of *G. lamblia* by Formol-concentration method. The cysts were separated by centrifuging the positive faecal samples at 600 g for 10 min. The cysts were washed thrice with chilled distilled water, weighed and then homogenized in Teflon homogeniser. A 10%
homogenate was prepared and centrifuged at 10,000 g for 10 min. The supernatant was used for the determination of phosphatase activity and total protein content as described earlier.

The effect of different concentrations of pH showed that the enzyme activity was maximum at pH 5.6 in *G. lamblia* (Fig.1). While optimum enzyme activity has been observed at pH 3.8 in *T. rhodesiense*; at pH 4.0-4.5 in *P. knowlesi*; at pH 5.0 in *E. histolytica*; in *P. caudatum* and *P. tetraurelia*, in *L. donovani*; at pH 5.2 in *T. brucei* and at pH 5.5 in *L. donovani* and *N. fowleri*.

The effect of different temperatures showed that there was maximum enzyme activity at a temperature of 60°C and after that it started decreasing. The decrease in enzyme activity was because of denaturation of proteins at high temperature (Fig.2). But the maximum enzyme activity at 37°C in *P. knowlesi* and heat inactivation of enzyme activity of *L. donovani* at 100 °C in 5 min, has been observed.

The enzyme activity showed a linear relationship with substrate concentration up to 4 mM and after that it became constant. The Lineweaver-Burk plot of enzyme activity against substrate concentration was drawn (Fig.3). The Michaelis constant (Km value) was found to be 2.27 mM. While Km value for acid phosphatase in *P. caudatum* has been reported to be 1.85 mM at pH 5.0 and in *N. fowleri*, it was 0.60 mM at pH 5.5. The more Km value of enzyme of cysts of *G. lamblia* showed lower affinity of an enzyme towards its substrate concentration in comparison to other protozoans.

The effect of various concentrations of different antibiotics viz. Metronidazole, Erythromycin, Amoxycilnine Doxycycline and Penicillin on phosphatase activity showed that enzyme activity decreased with an increase in concentration (50 μg/ml to 100 μg/ml) of each antibiotic (Fig.4). Considering the phosphatase activity of control as cent percent, it was observed that a concentration of 100 μg/ml of each compound inhibited the activity in the order Metronidazole> Pencillin> Doxycycline> Amoxycilnine> Erythromycin (Table 1). It has been reported that Doxycycline activated and erythromycin inhibited the acid phosphatase in *P. knowlesi*. However, in the present study both Doxycycline and Erythromycin inhibited the enzyme activity of *G. lamblia*. The antibiotic Metronidazole inhibited the maximum and erythromycin inhibited the minimum enzyme activity, thereby
Table 1. Percentage decrease in acid phosphatase activity of cysts of *G. lamblia* at different concentrations of various antibiotics (Mean value of 10 observations in each case).

<table>
<thead>
<tr>
<th>Antibiotic concentration (μg/ml)</th>
<th>Enzyme Activity* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metronidazole Erythromycin Amoxycilne Doxycycline Penicillin</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
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<tr>
<td>60</td>
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<td>90</td>
<td>-56.89</td>
</tr>
<tr>
<td>100</td>
<td>-63.64</td>
</tr>
</tbody>
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Calculated taking activity of control as 100 percent.

showing that Metronidazole is one of the most significant antibiotic affecting the enzyme activity of cysts of *G. lamblia*.

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REFERENCES