ASSAY OF ANAEMIA DURING INDUCED HYMENOLEPIASIS IN MICE AND RESTORATION WITH PRAZIQUANTEL

J. PARVATHI* AND ARUNA KAREMUNGIKAR
P.G. Department of Zoology, Vivek Vardhini College, Jambagh, Hyderabad - 500 095, A. P., INDIA
E-mail: saip_jayanthi@yahoo.co.in

INTRODUCTION
The host-parasite relationship is so intricate that it almost defies analysis. Moreover, the triumviate interactions of the host-parasite-drug is not complete without the haematological analysis to comprehend a complete picture of parasitism. Hymenolepis nana, a cosmopolitan intestinal cestode helminth, with a unique direct lifecycle, is common in the tropical countries, affecting the general health of adults, mental and physical status of children, especially under malnutrition (Belding, 1965; Busher and Haley, 1972). A very large number of worms may be present, often as a result of internal auto-infection with pronounced intestinal disturbances as well as toxic symptoms (Spencer and Monroe, 1961). The invasion of this intestinal cestode damages the host leading to not only mechanical trauma, but alters the haematology by loss of blood and associated alterations (Sumner, 1988).

The drugs for intestinal helminthiasis should not be absorbed but remain in the gastrointestinal (GI) tract where the organisms are usually located (Katzung, 1987). The broad-spectrum anthelmintic Praziquantel, [2- Cyclohexyl carbonyl - 1, 3, 4, 6, 7, 11b - hexahydro - 2H - pyrazino (2,1-a) isoquinoline – 4-one], showed a hundred percent cure with 25mg/kg body weight dosage in various hosts (Thomas and Gonnert, 1977). The advent and wide use of electronic cell counters, with a great degree of accuracy and reproducibility, has enormously increased the practicability of blood cell counts. Hence in the present work the estimation of haemoglobin, total red cell count, haematocrit and platelets has been done.

ABSTRACT
Helminth infections, the major parasitic diseases, affect the general health and productivity of adults, mental and physical growth of malnourished children, mostly in the tropics. The study of helminth-induced anaemia has not been extensive, although this consequence of parasitism is not uncommon. The present study involves the evaluation of haematological alterations induced in the laboratory mouse, Mus musculus, infested with the intestinal helminth, Hymenolepis nana by the estimation of blood parameters in an automatic coulter counter. Using these parameters, the blood indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated to interpret the extent and type of the resulting anaemia. The healing effects of the broad-spectrum anthelmintic praziquantel in restoring normal haematology in the traumatized host are also envisaged.

KEY WORDS
Hymenolepis nana, Mus musculus
Praziquantel
Haematology anaemia
Coulter counter

MATERIALS AND METHODS
The host, Mus musculus, were grouped into three batches. These were maintained under Good Laboratory Practices (GLP) conditions (Bodil, 1994), weighing about 20-25g and 4-5 weeks old. One-third of the total mice were kept as the uninfected control batch. Two-thirds mice were orally infected with about 100 viable eggs of Hymenolepis nana per mouse, and maintained as the infected batch. On the 16th day, half of the infected mice were given a single oral dose of Praziquantel, a 0.2mL aquatic suspension at 25mg/kg bodyweight of the host mice. This batch was maintained for 3 days as the treated batch. The control, infected and treated batches of mice were sacrificed at appropriate times. Blood was collected by the decapitation method(Dieterich, 1972), anti-coagulated with 1.5mg/ml blood of the dipotasium salt of Ethylene diamine tetra acetic acid (EDTA) and various blood parameters analyzed using an Automatic Blood Analyzer (Stanley, 1976; Lewis al., 1991). The Beckman Coulter HMX Haematology Analyzer with Autoloader was the instrument used in this study, which is a quantitive, automated heamatology analyzer.

The Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance, when a particle (cell) in a conductive liquid passes through a small aperture, based on the fact that blood is a poor conductor of electricity, where as certain diluents are good conductors. The haematological parameters assayed are - Haemoglobin content; Total Red Blood Corpuscles, Haematocrit/Packed cell volume (Hct/PCV); Platelet count. Using these, the Blood indices were calculated as follows — Mean Corpuscular
Volume (MCV) = \( \frac{(PCV \times 10)}{RBC \text{ Count}} \), where PCV = Packed Cell Volume/Haematocrit, RBC = Red Blood Cell Count, Units: Cubic microns; Mean Corpuscular Haemoglobin (MCH) = \( \frac{(Hb \times 10)}{RBC \text{ Count}} \), where Hb = Haemoglobin, RBC = Red Blood Cell Count, Units: micro micrograms; Mean Corpuscular Haemoglobin Concentration (MCHC) = \( \frac{(Hb \times 100)}{PCV} \), where Hb = Haemoglobin, PCV = Packed Cell Volume/Haematocrit.

Units: Percent (%).

The statistical values for each parameter of a batch is the mean of five individual observations, showing Significance (S) at Probability (P) < 0.05, and Non-significant (NS) at p > 0.05. According to the applicability of the values, Parametric ‘t’ test and Non-parametric Mann-Whitney Rank Sum test were applied. All these results are tabulated.

RESULTS

The various blood parameters analyzed in the Coulter counter of the three batches of mice, the uninfected control, infected and treated hosts showed the following variations.

The infected batch of the host blood showed a decrease over the uninfected control host blood in all the blood parameters – haemoglobin (Hg), total Red Blood Cell count (RBC), Haematocrit/Packed cell volume (Hct/PCV), and more pronounced in the Platelet count. Hb showed a 15%, total RBC – 11%, PCV – 15.5% and Platelets 57% decrease over their control counterparts at Probability 0.05. But Praziquantel treated batch of the host blood removed these alterations and showed an increase over the infected batch, closer to the control batch, Hb - 12%, total RBC - 4%, PCV - 13% and Platelets – 10% increase over the control batch, thereby proving its efficacy in curing Hymenolepiasis (Table 1).

The blood indices calculated from Hb, RBC and PCV are analyzed as follows at Probability of 0.05 - Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) showed lower values for the infected host batch over the control batch. But Mean Corpuscular Haemoglobin Concentration (MCHC) showed a marginal increase for the same. The Praziquantel treated batch of the host showed values closer to the control batch for all these blood indices, thus restoring normacy in the helminth infected host (Table 2).

DISCUSSION

Hymenolepiasis is a major cause of morbidity in less developed countries with warmer climates and poor sanitation, especially in children. Due to the presence of intestinal parasite Hymenolepis nana in the present study, the intestinal wall of the host is injured due to mechanical injury caused by the penetration of the parasite into the villi of the host (Parvathi

### Table 1: Dynamics of blood parameters of Mus musculus during Hymenolepis nana infection and treatment with anthelmintic praziquantel

<table>
<thead>
<tr>
<th>Parameter change</th>
<th>Batch</th>
<th>Mean ± S. D</th>
<th>‘t’ value</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (%)</td>
<td>Control**</td>
<td>13.3 ± 0.235</td>
<td>0.00794*</td>
<td>-15.038</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>11.3 ± 0.235</td>
<td>(&lt;0.0001</td>
<td>+12.030</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>14.9 ± 0.200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total erythrocyte</td>
<td>Control</td>
<td>7.64 ± 0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>6.80 ± 0.062</td>
<td>(&lt;0.0001</td>
<td>-10.995</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>7.91 ± 0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count(TEC)</td>
<td>Control</td>
<td>39.3 ± 0.180</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>33.2 ± 0.138</td>
<td>(&lt;0.0001</td>
<td>-15.522</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>44.4 ± 0.200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets (lakh/cu.mm)</td>
<td>Control</td>
<td>11.24 ± 0.016</td>
<td>(&lt;0.0001</td>
<td>-56.939</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>4.84 ± 0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>12.37 ± 0.017</td>
<td>(&lt;0.0001</td>
<td>+10.053</td>
</tr>
</tbody>
</table>

The values are mean of five individual observations.; ± indicates standard deviation figures in parenthesis is percent change of infected and treated batches over control resp. * absolute ‘t’ values.; the values show statistical significance at p < 0.05 ; (ns) – non significant : p > 0.05.; ** non-parametric Mann-Whitney rank sum test applied. for others “t” test applied.

### Table 2: Dynamics of blood indices of Mus musculus during Hymenolepis nana infection and treatment with anthelmintic praziquantel

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Batch</th>
<th>Mean ± S. D</th>
<th>‘t’ value</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean**</td>
<td>Control</td>
<td>51.44 ± 0.449</td>
<td></td>
<td>-5.093</td>
</tr>
<tr>
<td>Corpuscular (cu.microns)</td>
<td>Infected</td>
<td>48.82 ± 0.413</td>
<td>(&lt;0.0001</td>
<td>+9.117</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>56.13 ± 0.252</td>
<td>(&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>Control</td>
<td>17.41 ± 0.254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpuscular (micro micrograms)</td>
<td>Infected</td>
<td>16.62 ± 0.321</td>
<td>0.0025*</td>
<td>-4.538</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>18.84 ± 0.258</td>
<td>(&lt;0.0001</td>
<td>+8.214</td>
</tr>
<tr>
<td>Mean</td>
<td>Control</td>
<td>33.84 ± 0.744</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpuscular (NS)</td>
<td>Infected</td>
<td>34.04 ± 0.558</td>
<td>0.5351(NS)</td>
<td>+0.591</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>33.55 ± 0.386</td>
<td>0.3654(NS)</td>
<td>-0.857</td>
</tr>
</tbody>
</table>

Parameter batch mean ± S.D.; ‘t’ value % change the values are mean of five individual observations.; ± indicates standard deviation. Fig. in parenthesis is percent change of infected and treated batches over control resp. * absolute ‘t’ values.; the values show statistical significance at p < 0.05 ; (ns) – non significant : p > 0.05.; ** non-parametric Mann-Whitney rank sum test applied. for others “t” test applied.
and Aruna, 2010). This results in excessive blood loss leading to anaemia. There is significant alteration in various other blood parameters depicting the immune status of the traumatized host (Parvathi and Aruna, 2011a). Such results were reported in other helminth infections too (Pike, 1971; Anosa, 1977; Dubashi and Sharma, 1981), their results indicate that the decrease in red blood cell life span results from increased haemolysis, although blood loss from the extrusion of eggs into the intestinal lumen may also be important, and loss of blood by worm attachment leading to local intestinal lesions. This further leads to the alterations in substrate metabolism and the related enzymes (Parvathi and Aruna, 2011b).

The value of Haematocrit/Packed Cell Volume (Hct/PCV) is used along with Haemoglobin, Red Cell Count for the calculation of Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), the three important blood indices used in the diagnosis of various types of anaemias. In the present investigation, the following interpretations are made based on the estimated blood parameters and the calculated blood indices.

The decrease in the MCV of the infected host blood due to the decreased PCV and total RBC count reflects Microcytic Anaemia in the traumatized host due to shortened erythrocyte life-span, as was the case in other helminthiasis.

The decreased MCH due to low Hb and PCV levels in infected host is indicative of Hypochromic Anaemia. The marginal increase in MCHC is suggestive of developing Haemolytic Anaemia. The significant low Platelet count in the infected host is due to the haemorrhage where blood might not clot within its usual time because of the chemicals released by the worm during attachment to host intestinal walls, the chemicals also penetrating other host organs, via blood, damaging the circulating platelets too.

The values of all the parameters in the treated batches of host blood are nearer to the uninfected control host blood values. This very clearly proves the efficacy of the anthelmintic Praziquantel used in the present study in a single oral dose in restoring normal haematological profile in the infected host (Chero et al., 2007; Meltem et al., 2009). Since Hymenolepis nana is common to both mouse and man, these results can be directly extrapolated to humans as well. Hence, there is a need in the tropical countries to develop many more such cost-effective, safe, single, low-dosage regimen, broad-spectrum drugs (Becker et al., 1980; Baransi et al., 1984; Anderson and Medley, 1985), which can be administered orally without specific diet requirements (Bhavana et al., 1996; Ortiz et al., 2002), since multiple infections are common in tropical human populations (Brooker and Alexander, 2006). The research can still further be extended for the immunological aspects (Goodall, 1973; Rickard and Williams, 1982; Pritchard and Behnke, 1985) and development of vaccines too.

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