The Bioscan 7(1) : 107-110, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

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

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

Received on :
26.12.2011

Accepted on :
13.02.2012

***Corresponding author**

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 anemia 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.

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.

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 *et 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) = (PCV × 10) / RBC Count, where PCV = Packed Cell Volume/Haematocrit, RBC = Red Blood Cell Count, Units:Cubic microns; Mean Corpuscular Haemoglobin (MCH) = (Hb × 10) / RBC Count, where Hb = Haemoglobin, RBC = Red Blood Cell Count, Units : micro micrograms; Mean Corpuscular Haemoglobin Concentration(MCHC) = (Hb×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 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 normalcy 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

Pararneter change	Batch	Mean ± S. D.	't' valu	%
	Control**	13.3 ± 0.235		
Haemoglobin	Infected	11.3 ± 0.235	0.00794*	-(15.038)
Hb (%)	Treated	14.9 ± 0.200	< 0.0001	+(12.030)
Total erythrocyte	Control	7.64 ± 0.040		
Count(TEC)	Infected	6.80 ± 0.062	< 0.0001	-(10.995)
(million/cu.mm)	Treated	7.91 ± 0.016	< 0.0001	+(3.534)
Haematocrit	Control	39.3 ± 0.180		
(Hct)/Packed	Infected	33.2 ± 0.158	< 0.0001	-(15.522)
Cell volume	Treated	44.4 ± 0.200	< 0.0001	+(12.977)
(PCV) (vol%)				
Platelets	Control	11.24 ± 0.016		
(lakh/cu.mm)	Infected	4.84 ± 0.016	< 0.0001	-(56.939)
	Treated	12.37 ± 0.017	< 0.0001	+(10.053)

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

Parameter	Batch	Mean ± S.D.	't' value	% change
Mean**	Control	51.44 ± 0.449		
Corpuscular	Infected	48.82 ± 0.413	< 0.0001	-(5.093)
Volume(MCV)	Treated	56.13 ± 0.252	< 0.0001	+(9.117)
(cu.microns)				
Mean	Control	17.41 ± 0.254		
Corpuscular	Infected	16.62 ± 0.321	0.0025*	-(4.538)
Haemoglobin	Treated	18.84 ± 0.258	< 0.0001	+(8.214)
(micro microgms)				
Mean	Control	33.84 ± 0.744		
Corpuscular	Infected	34.04 ± 0.558	0.5351(NS)*	+(0.591)
Haemoglobin	Treated	33.55 ± 0.386	0.3654(NS)*	-(0.857)
Concentration				
(MCHC) (%)				

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 helminthiases.

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; Baranski *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.

ACKNOWLEDGEMENT

We are grateful to the Management of Vivek Vardhini College, Hyderabad, for the Laboratory facilities; Dr.G.S.T.Sai, Former Senior Research Executive, Indian Drugs and Pharmaceuticals Limited(IDPL), Dr. J. Surya Kumar, Senior Director, Orchid Healthcare, Chennai, for valuable Pharmacological suggestions, Nizam's Institute of Medical Sciences (NIMS), Hyderabad, for Haematological analysis.

REFERENCES

Anderson, R. M. and Medley, G. F. 1985. Community control of helminth infections of man by mass and selective chemotheraphy. *Parasitol.* **90:** 629-660.

Anosa, V. 1977. Haematological observations of helminthiasis caused by Haemonchus contortus in Nigerian dwarf sheep. *Trop. Anim. Health and Prod.* **443(5):** 438-446**.**

Baranski, M. C., Gomes, N. R., Godoy, O. F., Da Silva and Filho, M. C. 1984. Treatment of Taeniasis and Hymenolepis nana with single oral dose of praziquantel. Study of therapeutic efficacy, tolerance and safety. Materia Medica. *Polona.* **52:** 129.

Becker, B., Mehlhorn, H., Andrews, P. and Eckert, J. 1980. Light and electron microscopic studies on the effect of praziquantel on Schistosoma mansoni, Dicrocoelium dendriticum and Fasciola hepatica (trematoda) in vitro. *Zeits fur. Parasiten.* **63:** 111-128.

Belding, D. L. 1965. Textbook of Parasitology Appleton-Century-Crafts New York.

Bhavana Vora, Khopade, A. J., Jain, V. V. D. and Shelly Jain, N. K. 1996. Targeted Oral Drug Delivery. Review Article. *Ind Drugs.* **33(8):** 365-373.

Bodil, L. 1994. Good Laboratory Practice:Handbook of Laboratory Animal Science: Selection and Handling of animals in Biomedical Research. Vol I. CRC Press. London, New York. p. 37-40.

Brooker, S. and Alexander, N. 2006. Contrasting patterns in the small-scale heterogeneity of human helminth infections in urban and rural environments in Brazil. *Int J. Parasitol.* **36(10-11):** 1143.

Busher, H. N. and Haley, A. J. 1972. Epidemiology of Hymenolepis nana infection of Punjabi villagers on West Pakistan. *Amer. J. Trop. Med. Hyg.* **21:** 42.

Chero, J. C., Saito, M., Bustos, J. A., Blanco, E. M., Gonzalvez, G. and Garcia, H. H. 2007. Hymenolepis nana infection: symptoms and response to nitazoxanide in field conditions. *Trans. R. Soc. Trop. Med Hyg.* ***101(2):*** *203–205.*

Dieterich, R. A. 1972. Haematologic values for five northen microtines. *Lab Anim Sci.* **22:** 390-92.

Dubashi, N. G. and Sharma, N. G. K. 1981. Anaemia and hypoalbuminaemia in ancylostomiasis. Clinician. **45(15):** 182-187.

Goodall, R. I. 1973. Hymenolepis diminuta in the mouse: effects of heterologous antigens on worm rejection- a possible biological model for antigenic competition. *Parasitol.* **67:** xviii.

Katzung, B. G. 1987. Basic and Clinical Pharmacology. 3rd ed. Lange Med. Book. pp.733-743.

Lewis, S. M., England, J. M. and Rowan, R. M. 1991. Current concerns in haematology. III. Blood count calibration. *J. Clin Path.* **144:** 881-884.

Meltem, U. E., Aynur, G. and Handan, A. 2009. *Efficacy of praziquantel (injection formula) in the treatment of Hymenolepis diminuta infection in laboratory rats by oral application. Trop Med and Health, Adv Publ. Online. pp.1349-4147.*

Ortiz, J. J., Favennec, L., Chegne, N. L. and Gargala, G. 2002. *Comparative clinical studies of nitazoxanide, albendazole and praziquantel in the treatment of ascariasis, trichuriasis and hymenolepiasis in children from Peru. Trans R Soc Trop med Hyg.* ***96:****193–196.*

Parvathi, J. and Aruna, K. 2010. Histopathological assay of induced hymenolepiasis in Mus musulus and restoration of normalcy with

praziquantel. *The Bioscan.* **5(4):** 661-664.

Parvathi, J. and Aruna, K. 2011a. Leucocyte Variation, an Insight of Host Defenses During Hymenolepiasis and Restoration with Praziquantel. *Ind. J. Pharma. Sci.* **73(1):** 76-79.

Parvathi, J. and Aruna, K. 2011b. Correlation of hyperglycemia and succinate dehydrogenase activity during hymenolepiasis in mice and treatment with praziquantel. *IOSR J. Pharmacy.* **1(1):** 23-30.

Pike, E. H. 1971. Erythrocyte life span and haemoglobin levels in mouse trichuriasis. *J. Parasitol.* **57:** 311-315.

Pritchard, D. I. and Behnke, J. M. 1985. The suppression of homologous immunity by soluble adult antigens of Nematosporoides dubius. *J. Helminthol.* **59(3):** 251-256.

Rickard, M. D. and Williams, J. F. 1982. Hydatidosis / Cysticercosis : Immune mechanisms and immunization against infection. *Advances in Parsitol.* **21:** 229-296.

Spencer, F. M. and Monroe, L. S. 1961. The Colour Atlas of Intestinal Parasites Charles C Thomas. p.119.

Stanley, R. S. 1976. Lynch's Medical Laboratory Technology. 3rd Ed. W.B.Saunders Co. Philadelphia. p. 782-783.

Sumner, B. E. H. 1988. Basic Histochemistry. John Wiley & Sons.

Thomas, H. and Gonnert, R. 1977. The efficacy of praziquantel against cestodes in animals. *Zeit Fur Paras Parasitol Res.* **52(2):** 117-127.