FUDMA Journal of Sciences (FJS)
ISSN Online: 2616-1370
ISSN Print: 2645-2944
Vol. 2 No.3, September, 2018, pp 122 - 132

EFFECT OF *SCHISTOSOMA HAEMATOBIUM* INFECTION AND DRUG TREATMENT ON THE IMMUNE SYSTEM OF IMMUNOSUPPRESSED GUINEA PIGS

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ABSTRACT

Guinea pigs were immunosuppressed, before infection with *S. haematobium.* They were treated with different drug combination (Artequine, Artemether and Praziquantel), to ascertain their efficacy. The Guinea pigs were acclimatized to laboratory conditions for a week prior infection with 200 *S. haematobium* cercariae. Male and female guinea pigs weighing 2.5–6.5 kg were divided into four groups, 1- 4 (with 36-42 guinea pigs each). They were further divided into twelve subgroups of A - L with 3-4 guinea pigs each. The grouping 1-4 was done to monitor the effect of the drugs after 2, 7, 28 and 90 days post infection on the haematological parameter. Blood sample for Differential White Blood Cell (WBC) were collected using orbital technique from the retro-bulbar plexus of the medial canthus of the eye of the guinea pigs, before analysis. Infection with *S. haematobium* (Before) decreased the level of lymphocyte when compared with normal control (NC) two days post infection. Except for treatment (After) with 75mg/kg Praziquantel PZQ and 100/200 mg/kg PZQ/ART, all other treatment regimen decreased the level of lymphocyte seven days post infection. Eosinophilia was observed after infection when compared with the NC, hence the mean level of eosinophil decreases after treatment (eosinopenia). Infection with *S. haematobium* increased the level of eosinophil, treatment with 75 and 300 ART and PZQ further increased the level of eosinophil seven days post infection. Infection with *S. haematobium* caused eosinophil in some of the animal and treatment further elevated the value of eosinophil (75 and 300 ART and PZQ) ninety days post infection which was also seen in seven days post infection. Except for 75 and 300 mg/kg ART and PZQ, all other treatment elevated the value of monocyte which were extreme in 75 and 300 mg/kg ARQ, 100/200 mg/kg of PZQ/ARQ and PZQ/ART. Ninety days post infection with *S. haematobium* had increased mean level of monocyte in the white blood cell of guinea pigs

Keywords: *Guinea pigs, Schistosoma haematobium, Differential White Blood Cell, Drug treatments*

INTRODUCTION

Schistosomes also known as bilharziasis are snail-transmitted, water-borne helminthic diseases that are found in fresh water bodies in low and middle-income countries. Current estimates from the Global Burden of Disease Study 2010 suggest that 252 million people are infected with schistosomes, 90% of whom live in sub-Saharan Africa (Hotez *et al*., 2014). Globally two-thirds of Schistosomiasis cases are caused by *Schistosoma haematobium* one-third are *Schistosoma mansoni* and 1% with *Schistosoma japonicum* or *Schistosoma mekongi* (WHO 2017) Human schistosomiasis remains the second most prevalent parasitic disease in the tropics, with a huge impact on the socio-economic development of affected regions (WHO, 1999). Chronic schistosomiasis, can lead to many other pathological conditions including anemia, chronic pain, undernutrition etc especially in children. Three species in particular are considered to be important in human infections, *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum.*

Urogenital Schistosomiasis is a chronic parasitic infection of circulatory system caused by *S. haematobium*, which affects the bladder and subsequently the urinary tract system of man (WHO, 2006). There is apparently no vaccine available for the control of Schistosomiasis (Gryseels *et al.*, 1994), chemotherapy is the available strategy for schistosomiasis control, with praziquantel being the principal drug (WHO, 1999). Low cure rates to praziquantel have been observed from *Schistosoma mansoni* infection in northern Senegal (Gryseels *et al*., 1994, Stelma, 1995; Guissé *et al*., 1997), and studies of an isolate of this parasite suggested praziquantel tolerance (Fallon *et al.*,1997). Hence the requirement for new drugs that are as effective, and non-toxic to patients is important to provide alternative therapy to praziquantel if resistance increases in the future. With an increasing use of praziquantel, there is a possibility of development of resistance to the drug, hence the necessity to explore the activities of other drugs (Bennett *et al*., 1997 and Southgate *et al*., 2005). A series of laboratory studies and chemical trials in Egypt and Senegal have raised

considerable concern about the possible development of tolerance to praziquantel (Geerts and Gryeels, 2000; Liang *et al*., 2000; Utzinger *et al.,* 2000a). The first alarming reports of possibility of praziquantel resistance came from northern Senegal, where the drug had produce very low cure rate (Cioli *et al.*, 1995).

Choice of experimental host has been one of the chief difficulties in experimental chemotherapy of schistosomiasis, hamster is the animal of choice in the study of *S. haematobium* but it's difficult to access here in the tropic. Guinea pigs can be found around here and its manifestation of infection is like that of human beings, hence, it susceptible to *S. haemtobium* (Okeke *et al*., 2011).

WBCs or leukocytes are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system. The number of WBCs in the blood is often an indicator of disease. There are normally between 4×109 and 1.1×1010 white blood cells in a litre of blood, making up approximately 1% of blood in a healthy adult. An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia. The physical properties of leukocytes, such as volume, conductivity, and granularity, may change due to activation, the presence of immature cells, or the presence of malignant leukocytes in leukemia. Therefore, the aim of this work is to find alternative drugs to praziquental and study the effects on the haematological parameters of the model animal used.

MATERIALS AND METHODS

Experimental Animals (Guinea Pigs)

Guinea pigs were obtained and were acclimatized to laboratory conditions for a week prior to infection with 200 *S. haematobium* cercariae. They were kept in animal house (Animal Breeding and Genetics) in Zoology Department, University of Nigeria Nsukka. Male and female guinea pigs weighing 2.5–6.5 kg were divided into four groups 1- 4 (with 36-42 guinea pigs each) which were further divided into twelve subgroups of A - L with 3-4 guinea pigs each. The Groups 1-4 were infected and treated 2, 7, 28 and 90 days post infection; they were further divided into 12 subgroups (A – L) comprising of three animals each. The animals were labeled: (A) immunosuppressed animals treated with 75mg/kg of praziquantel 2, 7, 28 and 90 days post infection. (B) immunosuppressed animals treated with 300mg/kg of praziquantel 2, 7, 28 and 90 days post infection. (C) immunosupressed animals treated with 75mg/kg of artemether 2, 7, 28 and 90 days post infection. (D) immunosupressed animals treated with 300mg/kg of artemether 2, 7, 28 and 90 days post infection. (E) immunosupressed animals treated with 75mg/kg of artequine 2, 7, 28 and 90 days post infection. (F) immunosupressed animals treated with 300mg/kg of artequine 2, 7, 28 and 90 days post infection. (G) immunosupressed animals treated with 75mg/kg of praziquantel /150mg/kg of artemether 2, 7, 28 and 90 days post infection. (H) immunosupressed animals treated with 100mg/kg of praziquantel /200mg/kg of artemether 2, 7, 28 and 90 days post infection (I) immunosupressed animals treated with 75mg/kg of praziquantel/150mg/kg of artequine 2, 7, 28 and 90 days post infection. (J) immunosupressed animals treated with 100mg/kg of praziquantel/200mg/kg of artequine 2, 7, 28 and 90 days post infection. (K) Control immunosuppressed, infected without treatment. (L) Control unimmunosupressed, infected without treatment. (M) Normal Control

Immunosuppresant

Mycept is a lavender coloured, oval shaped, film coated tablet. Each film-coated tablet contains Mycophenolate Mofetil 500 mg. 20 mg/kg of Mycept were given to the animals orally every day for five days before infection.

Exposure of Guinea Pigs to Cercariae

Snails harbouring newly patent infections were placed in containers with freshwater (20 snails per 200ml) under strong artificial illumination for a period of four hours, 1ml of water containing cercaraie were poured in petri dish and counted. Guinea pigs were weighed, male and female guinea pigs weighing 200-650g were exposed to 200 *S. haematobium* cercaraie by subcutaneous injection. Animals were fed regularly and monitored. Control animals were included in all sets of experiment.

Drugs and Doses (Artemether, Praziquantel and Artequine)

Artemether injection (Paraline, Shanghai Harvest Pharmaceutical Co., LTD) is a sterile solution in suitable oil for injection, a clear, colourless oily solution. 80 mg/ml in 1-ml ampoule were administered via intramuscular route at a dose of 75, 150, 200, and 300 mg/kg (Xiao *et al.,* 2001 and Utzinger *et al*., 2001). Jerico praziquantel 600mg (manufactured by Sishui Xierkang Pharmaceutical Co., LTD, China) were administerd orally using 1ml syringe at dose of 75, 100 and 300 mg/kg. Artequin 600/750mg (Artesunate and Mefloquine, manufactured by Mepha LTD., Aesch-Basel, Switzerland) were given orally at a dose of 75, 150, 200 and 300 mg/kg. Artequin and artemether were administered singly and in combination with praziquantel. The dose was selected on the basis of previous work done by (WHO, 2006; El-Bassiouni *et al.*, 2007; Van Nassaun *et al*., 2008). The drugs were administered 2days, 7days, 28days, and 12weeks/90days post infection (Xiao *et al*., 2001, Yang *et al.*, 2001, Manneck *et al*., 2010).

Heamatological Studies

Blood sample collection for haematology

Blood sample for haematological determinations was collected using orbital technique from the retro-bulbar plexus of the medial canthus of the eye of the guinea pigs. A nucrocapillary tube was carefully inserted into the medial canthus of the eye to puncture the retro-bulbar plexus and thus enable outflow of 2mls of blood into a sample bottle containing ethylene-diamine-tetra-acetic acid (EDTA). 2ml of blood collected in the sample bottle was shaken gently to mix up the blood with EDTA and prevent clotting.

Differential white blood cell count

The blood sample was shaken gently and a drop of blood was placed on a clean grease-free slide. The drop of blood was carefully smeared on the slide using a cover slip to make a thin smear. The smear was air-dried and thereafter stained by the Leishman technique using Leishman stain. The stained slides were later examined with an immersion objective using a light microscope. 200 cells were counted by the longitudinal counting method and each cell type was identified and scored using the differential cell counter. Results for each type of white blood cell was expressed as a percentage of the total count and converted to the absolute value per microlitre of blood (Schalm *et al*., 1975).

Total white blood cell count (total leukocyte count)

0.02ml of blood was pipetted into a small test tube containing 0.38ml of white blood cell diluting fluid to make a 1:20 dilution of the blood sample. The diluted sample was loaded on to the Neubauer counting chamber, and all cells on the four corner squares were counted using a light microscope at X10 objective. The number of cells counted for each blood sample was multiplied by 50 to obtain the total white blood cell count per microlitre of blood (Schalm *et al.,* 1975).

Statistical Analysis

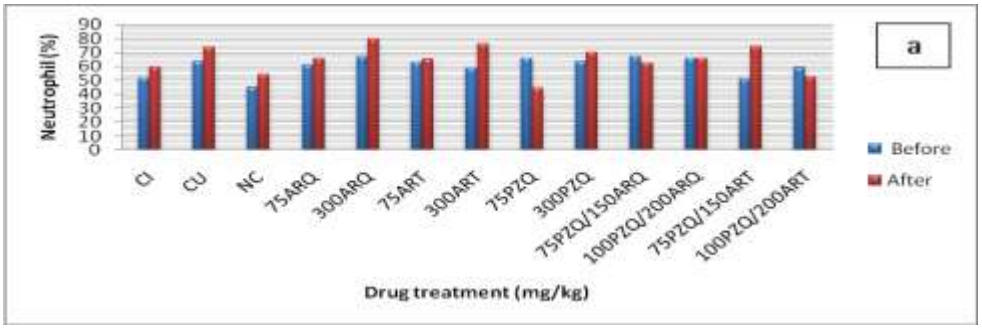
Experimental results were analysed using Genstat and SAS system for windows release 8.01 statistical software

RESULTS

Effect of *S. heamatobium* Infection and Treatment on Differential White Blood Cell of Guinea Pigs

Neutrophil

Figure 1 represents the effect of infection and treatment on neutrophil before and after treatment. Two days post Infection (a) caused an elevation in level of neutrophil (neutrophila) in white blood cell; further elevation was seen even after drug administration when compared with the NC. Distortion by the means of immunosuppression (CI), Infection (CU) and treatment with different drugs except 75 mg/kg PZQ caused an elevation in the level of neutrophia two days post infection. Comparing the value of neutophil before and after treatment, there was observed elevation on the value of neutrophil after treatment except for 75PZQ and 100PZQ/200ART. Seven days post infection (before) and after treatment (after) caused an elevation in the value of neutrophil in most of the animal (b), it was observed that treatment with 300 ARQ and ART and had the highest value. Immunosuppression (CI) had a decreased value in neutophil after infection and as the infection progresses. Immunosuppression, infection and treatment after day twenty post infection (c) increased the value of neutrophil in white blood cell of guinea pigs when compared with the mean value of naïve animals (NC). Infection (Before) decreased the value of neutrophil in almost all when compared with (NC) ninety days post infection (d). Treatment with 75mg/kg ARQ, 75 and 300 mg/kg ART and PZQ, and 75PZQ/150ART decreased the level of neutrophil while the remaining treatment regimen increased the level of neautrophil in the white blood cell of guinea pigs.



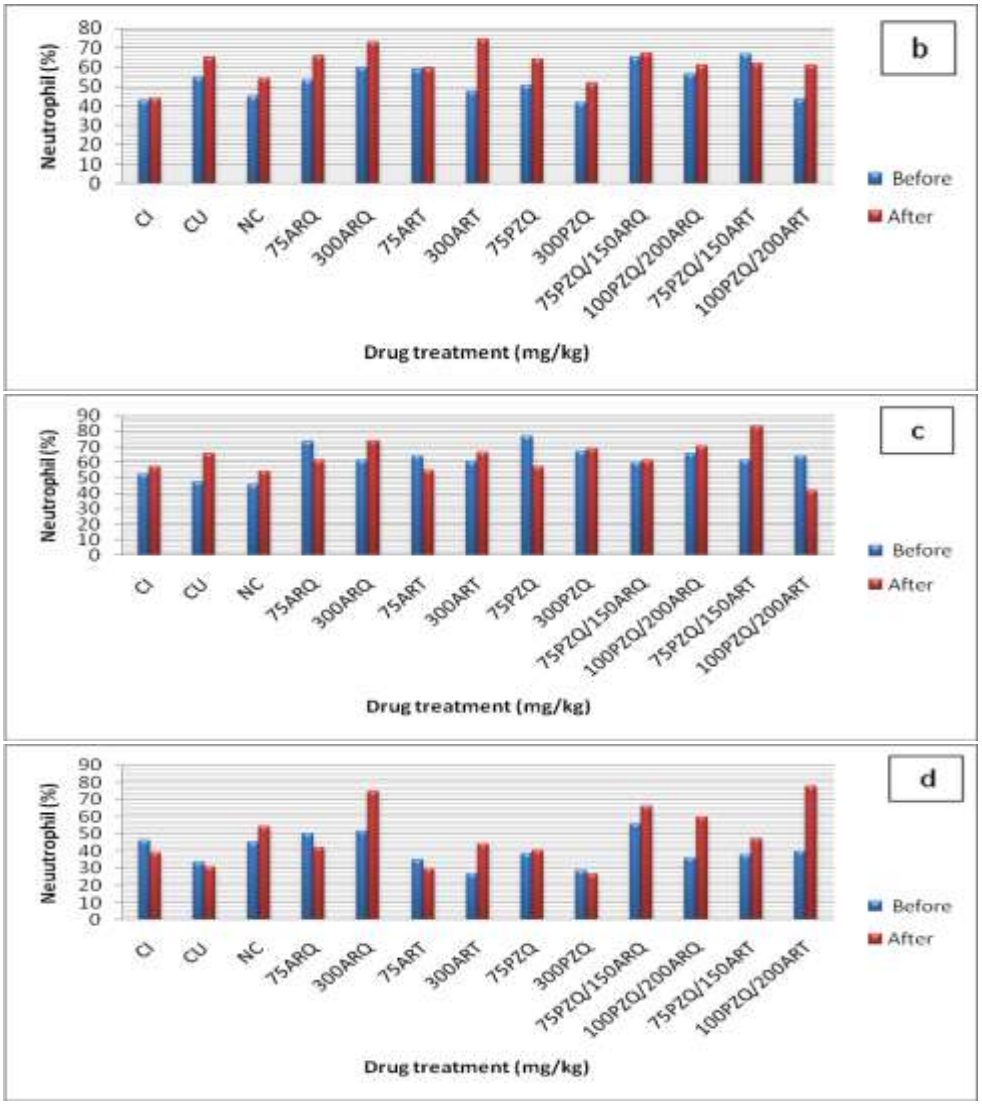
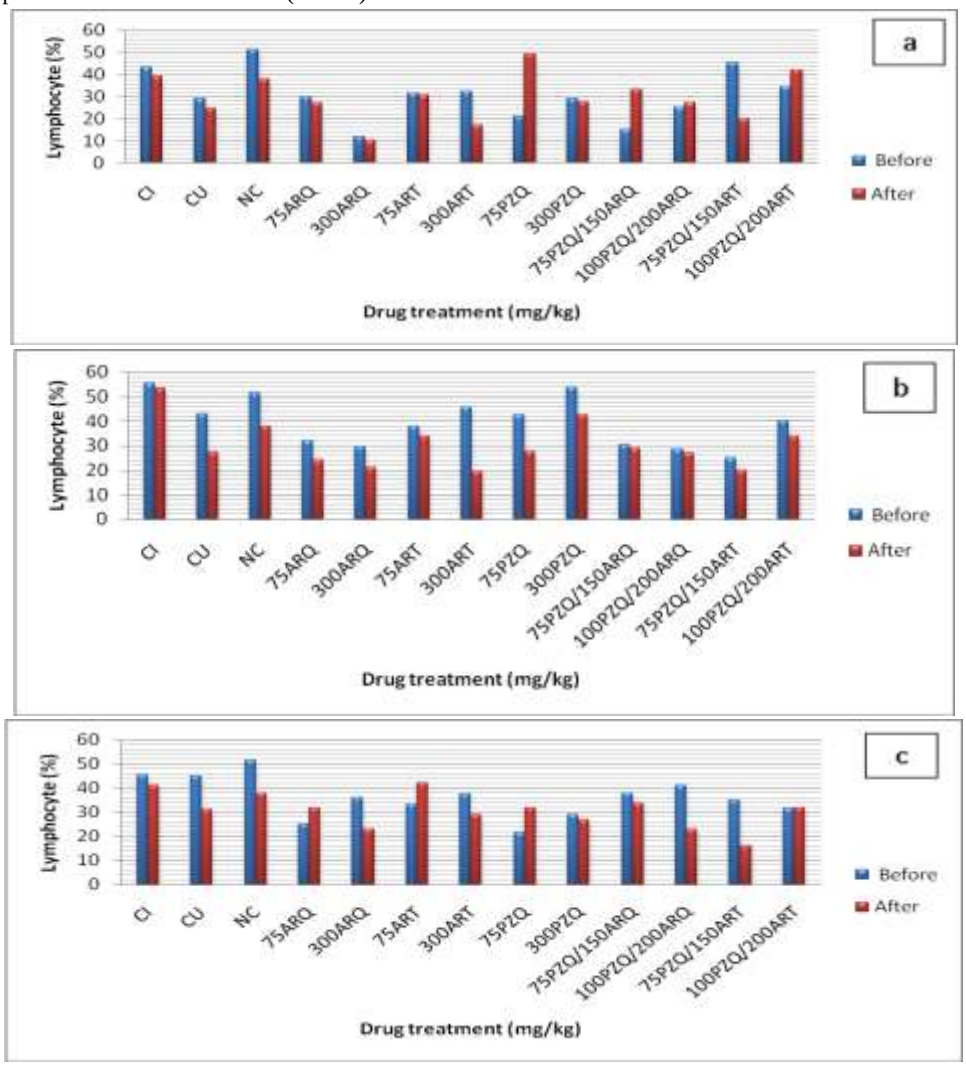


Fig. 1: Effect of different drug treatments on neutrophil administered at different developmental stages on guinea pigs infected with *S. haematobium.* The data are presented as (a) 2days, (b) 7 days (c) 28 and (d) 90 days post infection and treatment. Art (Artemether), Pzq (praziquantel), Arq (Artequine), CI (control immunosupressed, CU (Control unimmunosuppressed), NC (Normal control).

Lymphocyte

Figure 2 illustrates the behaviour of lymphocyte in the white blood cell of guinea pigs experimentally infected with *S. haematobium* after seven days post infection and treatment with different drugs. Infection with *S. haematobium* (Before) decreased the level of lymphocyte when compared with normal control (NC) two days post infection (a). Except for treatment (After) with 75mg/kg PZQ and 100/200 mg/kg PZQ/ART, all other treatment regimen decreased the level of lymphocyte seven days post infection. Infection (Before) with *S. haematobium* and treatment with different drug regimen (After) decreased the level of lymphocyte when compared with normal control (NC) seven days post treatment (b). It was observed also that the mean level of lymphocyte decreased when different drugs were administered. Infection (Before) with *S. haematobium* and treatment with different drug regimen (After) had a decreased level in the of lymphocyte in almost all the drugs treatment when compared with normal control (NC) twenty eight days post treatment (c).



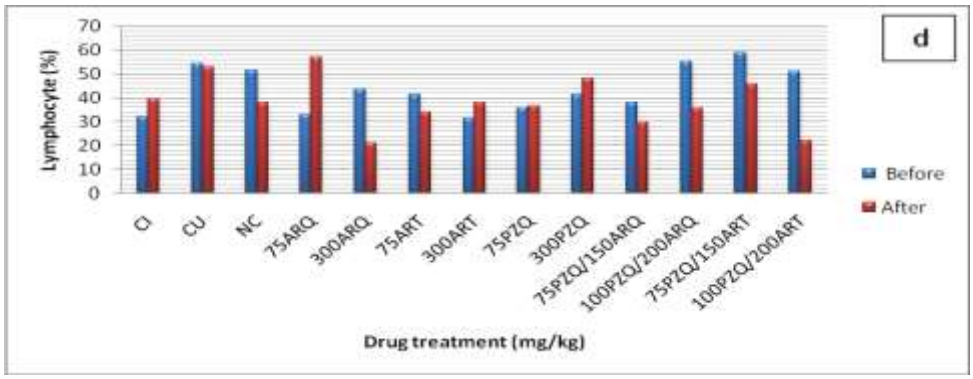
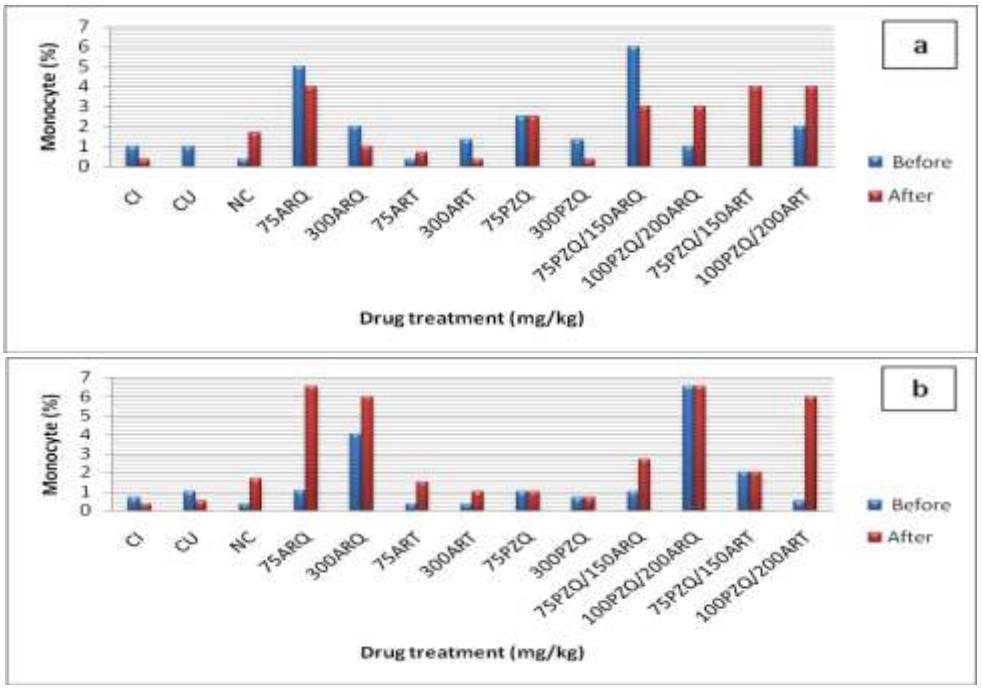


Fig. 2 Effect of different drug treatments on lymphocyte administered at different developmental stages on guinea pigs infected with *S. haematobium.* The data are presented as (a) 2days, (b) 7 days (c) 28 and (d) 90 days post infection and treatment. Art (Artemether), Pzq (praziquantel), Arq (Artequine), CI (control immunosupressed, CU (Control unimmunosuppressed), NC (Normal control).

Monocyte

Figure 3 illustrates the activity of monocyte in the white blood cell in guinea pigs infected and treated with different drug regimen. Infection increased the value of monocyte when compared with NC. Treatment with 75 mg/kg ARQ and PZQ, and all combined drugs increased the level of monocyte two days post infection (a). Infection with *S. heamatobium* increased the mean level of monocyte (Before). Except for 75 and 300 mg/kg ART and PZQ, all other treatment elevated the value of monocyte which were extreme in 75 and 300 mg/kg ARQ, 100/200 mg/kg of PZQ/ARQ and PZQ/ART (b). Ninety days post infection with *S. haematobium* had increased mean level of monocyte in the white blood cell of guinea pigs (d).



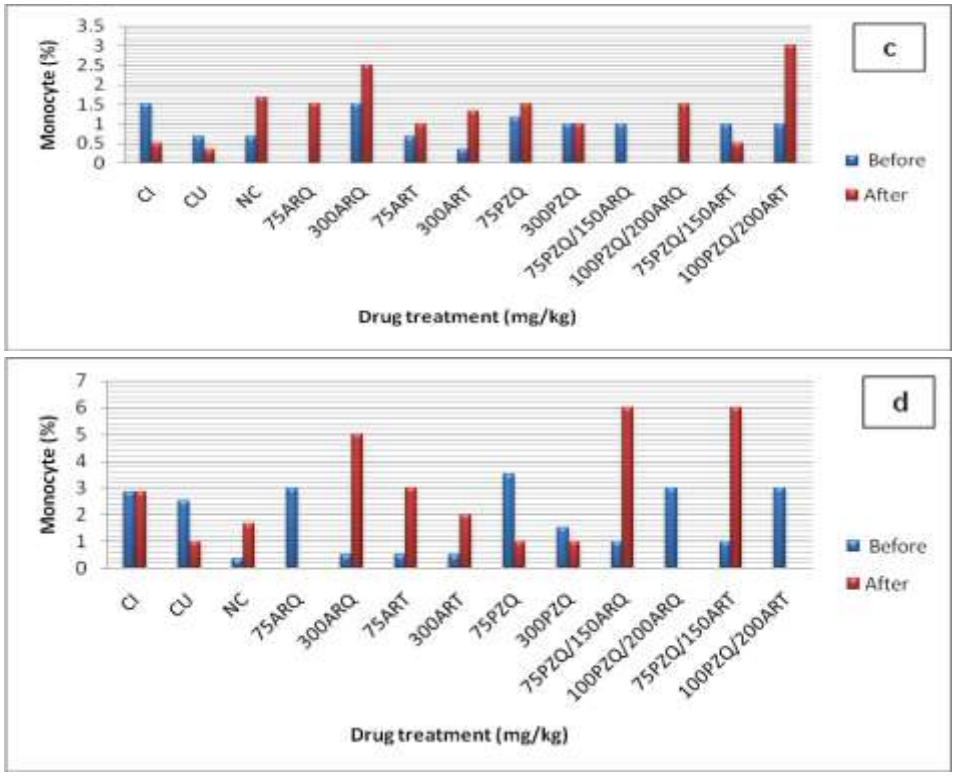
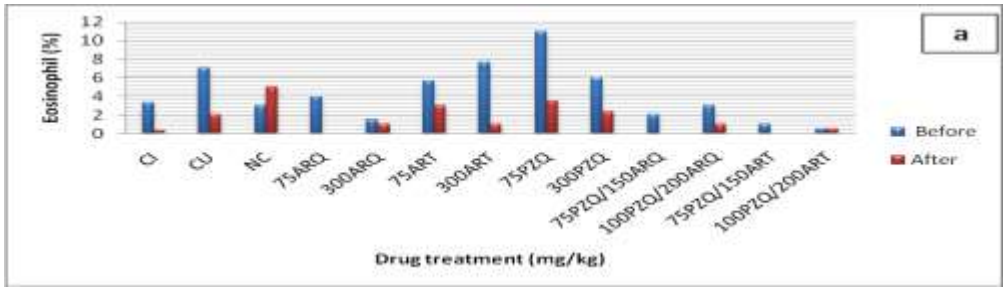


Fig. 3: Effect of different drug treatments on monocyte administered at different developmental stages on guinea pigs infected with *S. haematobium.* The data are presented as (a) 2days, (b) 7 days (c) 28 and (d) 90 days post infection and treatment. Art (Artemether), Pzq (praziquantel), Arq (Artequine), CI (control immunosupressed, CU (Control unimmunosuppressed), NC (Normal control)

Eosinophil

The result of the mean level of eosinophil following drug administration of single and combined treatment regimen on guinea pigs infected with *S. haematobium* at different developmental stages is presented on the Figure below. Eosinophilia were observed (elevation of eosinophil) after infection when compared with the NC, hence the mean level of eosinophil decreases after treatment (eosinopenia) (a). Infection with *S. haematobium* increased the level of eosinophil, treatment with 75 and 300 ART and PZQ further increased the level of eosinophil seven days post infection (b). Infection with *S. haematobium* caused eosinophil in some of the animal and treatment further elevated the value of eosinophil (75 and 300 ART and PZQ) ninety days post infection (d) which was also seen in seven days post infection (Figure 4).



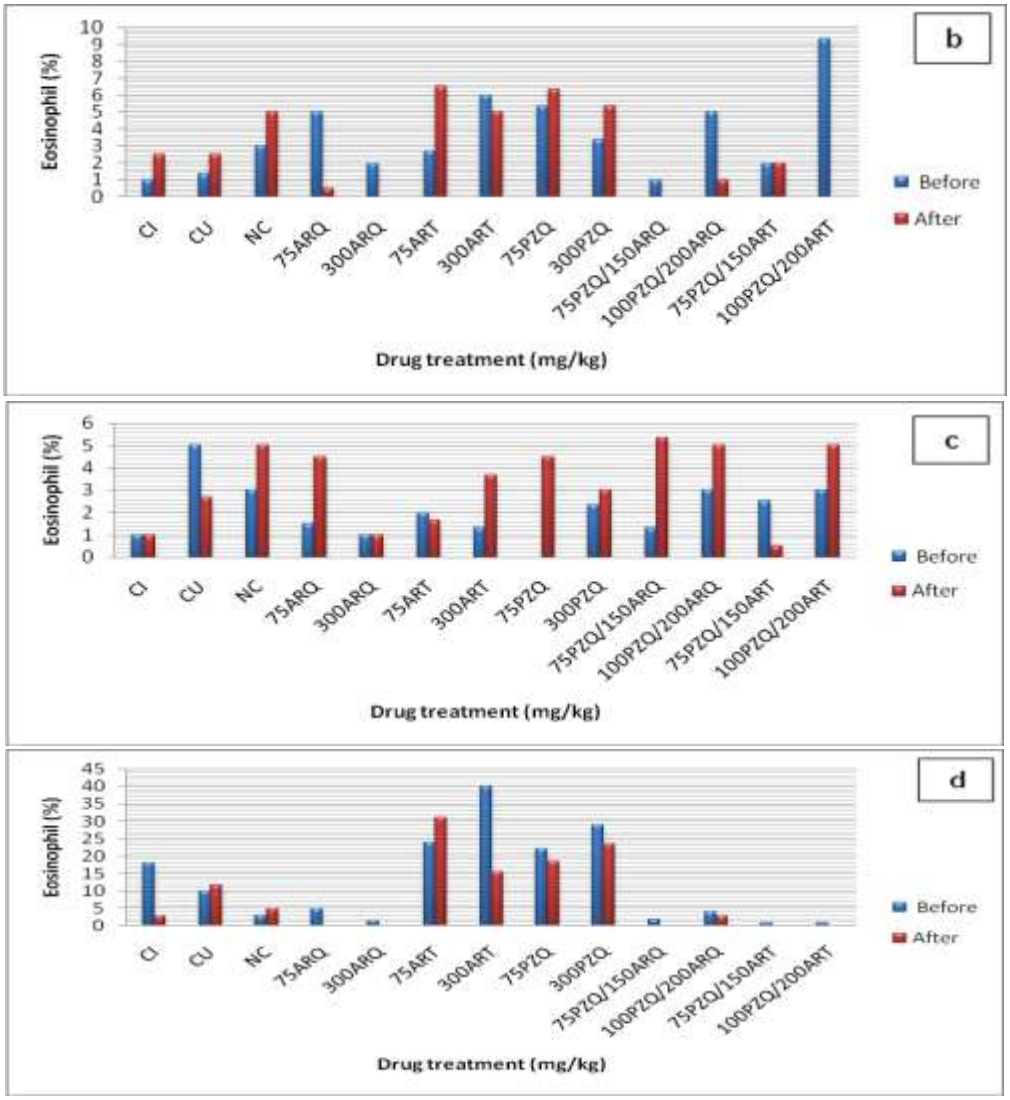
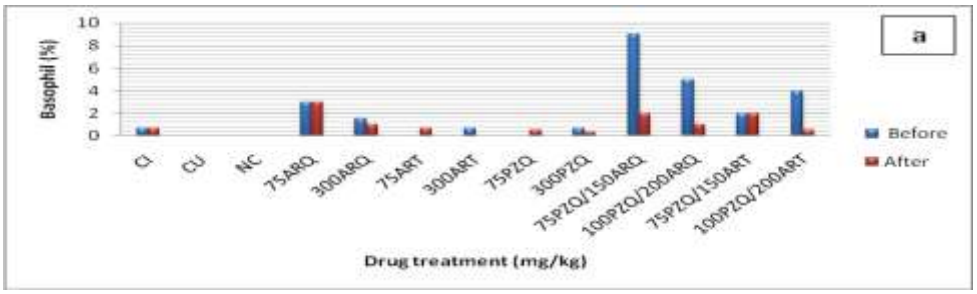


Fig. 4: Effect of different drug treatments on eosinophil administered at different developmental stages on guinea pigs infected with *S. haematobium.* The data are presented as (a) 2days, (b) 7 days (c) 28 and (d) 90 days post infection and treatment. Art (Artemether), Pzq (praziquantel), Arq (Artequine), CI (control immunosupressed, CU (Control unimmunosuppressed), NC (Normal control).

Basophil

Figure 5 represents the activity of basophil in white blood cell of guinea pigs infected with *S. haematobium*. Considering the value of naïve guinea pigs (NC) infection increased the value of basophil and treatment had little or no effect on the level of basophil in all the different developmental stages. (a, b, c and d).



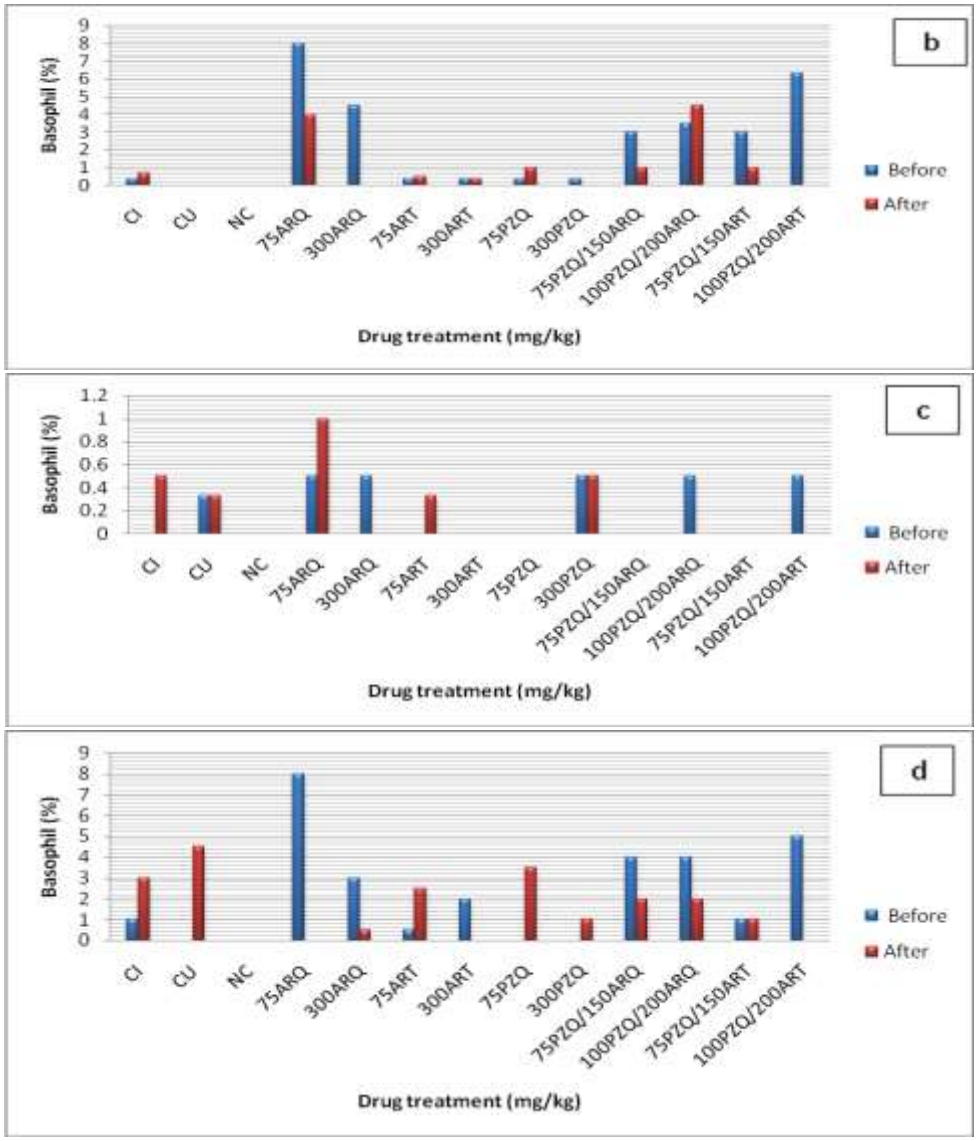


Fig. 5: Effect of different drug treatments on basophil administered at different developmental stages on guinea pigs infected with *S. haematobium.* The data are presented as (a) 2days, (b) 7 days (c) 28 and (d) 90 days post infection and treatment. Art (Artemether), Pzq (praziquantel), Arq (Artequine), CI (control immunosupressed, CU (Control unimmunosuppressed), NC (Normal control)

DISCUSSION

It is widely accepted that most animals, have a common leucocyte pattern consisting of monocytes and lymphocytes. Knowledge about the reference values of haematological of (lymphocytes (39-72) monocyte (2-6) and eosinophil (0-5) in guinea pigs) mammals provides useful information for researchers in their studies and for veterinary and health situations of animals in clinical practice (Archetti *et al.*, 2008). Haematological parameters generally provide information on inflammation, necrosis, various infections of visceral organs and the presence of stress factors (Betancourt-Alonso *et al*., 2011).

There was an observed increase in lymphocytes 2 days post infection, the observed increased might probably be the immune reaction toward the foreign body, the animal tying to fight the infection. Lymphocytosis is a common response to antigenic stimulation and both monocytosis and lymphocytosis may develop in diseases (Archetti *et al.*, 2008). It was also observed that Absolute monocytosis and lymphocytosis did not developed to levels of significance in the animal regardless of continual development of the parasite in the guinea pig system but decrease in lymphocyte was observed in after infection in almost all the developmental stages of the parasite (2, 7, 28 and 90 days) There were eosinophilia (elevation of eosinopil) after 2 days of infection and with animal treated with PQZ, they were also marked increase in eosinophil after 90 day of infection especially with animal treated with PZQ and ART. The observed rise after two days post infection could probably be as a result of

activity of antibodies trying to fight the infection at initial contact which were also seen to reduce after seven and twenty eight days and raised after ninety days when the parasite has started laying eggs and multiplying (powerful defense reaction and allergic manifestation against the schistosomes and/or their egg). Eosinophilia is frequently observed after treatment of patients with infections due to parasitic helminths. Infections due to parasitic helminths share with atopic diseases, the distinction of being the condition most frequently associated with eosinophilia in the peripherial blood and tissue. The magnitude of this eosinophil is generally thought to relate in some way to the degree of infection while observation in animal experimentally immunized or infected suggest the existence of such a quantitative relationship. Others have reported that increased eosinophil can develop after treatment of infections due to the parasitic helminths. They demonstrated that magnitude of this increase in eosiniphila was directly related to number of microfilariae circulating in the blood before therapy (Eric *et al.,* 1979). They further demonstrated that after therapy for eight days with niridazole, both groups developed significant increases in their levels of eosinophilia, which peaked in two to four weeks maximal levels averaged two and one-half to three times the levels before treatment. This probably could be the reason for increased observed after treatment with PZQ and ART after 90 days. Furthermore, Tissue damage contributes to the increase in eosinophil, if treatment reduces the number of eosinophil, the symtoms decreaes or disappears (Rothenberg and Hogan, 2006), hence total reduction were seen in animal treated with all the drugs after 90 days and with ARQ after 2 and 7 days post treatment.

A rise in some level of total leukocyte counts could be attributed to the rise in eosinophils, but likewise a significant rise was encountered in monocytes, lymphocytes and neutrophils especially after infection with *S. haematobium*. This indicated a general immunological response. A number of systems employing antibodies and/or eosinophils, neutrophils, monocytes were produced to kill schistosomes. A significant relationship was observed between the degree of eosinophilia and the intensity of infection (Davis et al 1981). However, the significant reduction observed in the circulating leukocytes and eosinophils after treatment indicates that this immunological cell response was due to *S. haematobium* infection, as the level remained high in the patients still passing eggs after treatment, unlike patients with other parasitic infections.

In conclusion, immunological parameters and its knowledge can be used to assess the health as well as the physiological status of animals under infection.

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