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VARIATIONS IN HAEMATOLOGICAL FACTORS OF GUINEA PIGS INFECTED AND TREATED WITH DIFFERENT ANTI SCHISTOSOMA THERAPY

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Abstract
A study was conducted to examine some variation in haematological standards on Guinea pigs experimentally infected with *Schistosoma haematobium*. Male and female guinea pigs weighing 2.5–6.5 kg were divided into four groups, which were further divided into three replicates with control. The groups were infected with *S. haematobium* and allow for space of 2, 7, 8 and 90 days post infection. Blood sample for White Blood Cell count (WBC), Park Cell Volume (PCV) and Haemoglobin (HBC) determination was collected using orbital technique from the retro-bulbar plexus of the medial canthus of the eye of the guinea pigs, before analysis. The value of white blood cell count on guinea pigs treated with praziquantel two days before and after treatment were not significant 0.0605 ($P > 0.05$), artemether before and after treatment were statistically significant 0.0163 ($P < 0.05$) while artequine pre and post treatment were not significant 0.0539. Actions of praziquantel, artemether artequine and praziquantel/artequine on pack cell volume decreases after treatment (t-Test, $P < 0.01$) two days post treatment. The mean level of PCV seven and twenty eight day's pre and post treatment in the entire drug administered was highly significant (t-Test, $P < 0.01$). The t statistics obtained shows that the mean value of HBC in all the treatment groups as the parasite develops (2-7-28-90 days) were significant ($P < 0.01$; $P < 0.05$).

Keywords: *S. haematobium, Heamatological, Praziquantel, Artequine*

Introduction

Schistosomiasis is a waterborne infection and is one of the most common parasitic diseases in the world, and is of public health global importance (WHO, 2013). Schistosomiasis is prevalent in tropical and subtropical areas, especially in poor communities without access to safe drinking water and sufficient sanitation. This disease has major health and socio-economic impacts, and creates an important public health problem in developing countries as well as a significant hazard for visitors and travellers who visit disease endemic regions (WHO, 2013). It is estimated that 779 million people are at risk of infection, and about 250 million people are currently infected (Colley *et al.,* 2014). The control of schistosomiasis is based on comprehensive treatment of vulnerable population groups. It is estimated that at least 90% of those requiring treatment for schistosomiasis live in Africa (WHO, 2017**).**
Currently, schistosomiasis control strategy is mainly based on the treatment of infected individuals with safe and effective drugs. There has been an intricate intervention procedure on the elimination of *Schistosoma* transmission using combined drug, snail control and behavior change (King, 2017). Implementation of school and community-based Mass Drug Administration (MDA) programs have generated significant benefits in some countries like Uganda, Burundi, Rwanda, Cameroon, Mali, Burkina Faso, Niger, China, Nigeria and other nations. Results have included significant reductions in the prevalence of heavy infections and of many advanced forms of schistosomiasis (King, 2017). Praziquantel is the only drug of choice for treatment of schistosomiasis, however, low cure rate has been observed and development of resistant to the drugs has also been documented (Fallon *et al.,* 1997 and Doenhoff *et al.,* 2008) hence the study of artequine. Aretquine is a free combination of two well-known antimalara, artesunate and mefloquine which offers fast and prolonged schizonticidal

action. Artequine meets todays therapeutic needs, namely fast onset of action and a long lasting therapeutic effects, good tolerability, a short trestment duration (Mapa, 2010)

Many parasitic infections negatively influence the blood qualitatively and quantitatively in the course of their presence in humans. For instance, in schistosomiasis infection during erythrocytic phase; the worm causes destruction of erythrocytes by metabolizing haemoglobin within erythrocyte and also causes blood loss during the movement of their spiny eggs through blood vessels (Agbolade *et al*., 2009). It is generally alleged that iron deficiency due to extracorporeal blood loss is the main cause of schistosomiasis-associated anemia (Leenstra *et al.,* 2006). Infected people having severe anaemia before treatment are the ones most likely to show significant gains in haemoglobin levels following treatment (King and Dangerfield-Cha 2008). This work compared the effect of infection and some anti-malaria drugs treatments on the haematological values of guinea pigs infected with the different developmental stages of *S. heamatobium*

Materials and Methods

Experimental Animals (Guinea Pigs)

Guinea pigs were obtained and kept in the animal house (Animal Breeding and Genetics) in Department of Zoology and Environmental Biology, University of Nigeria Nsukka for a week to acclimatize to laboratory conditions prior to infection. Cercariae were obtained from laboratory infected *Bulinus truncatus* snails which were shed into water, 200 *S. haematobium* cercariae were counted under a dissecting microscope and were used to infect the guinea pigs

(Christensen *et al.* (1984). Male and female guinea pigs weighing 2.5–6.5 kg were divided into four groups (with 36-42 guinea pigs each). The groups were infected and treated 2, 7. 28 and 90. This division was meant to look at different developmental stages of the parasite. The groups were treated with different drug regimen with different concentration as observed in some studies by Xiao *et al*. (2001); WHO (2006); El-Bassiouni *et al.* (2007); Van Nassaun *et al*. (2008)

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. Male and female guinea pigs weighing 200-650g were exposed to 200 *S. haematobium* cercariae by subcutaneous injection. Animals were fed regularly and monitored.

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 combimation with praziquantel. The dose was selected on the basis of previous work done by (Xiao *et al*., 2001; 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.

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).

Determination of haemoglobin concentration

The haemoglobin concentration of the blood samples was determined by the cyanomethaemoglobin method (Kachmar, 1970). 5ml of Drabkin's haemoglobin reagent was added to a clean test tube. Then 0.02ml of the blood sample was added to the reagent and mixed properly. The mixture was allowed to react for 20 minutes, and the absorbance was read at 540nm wavelength against a reagent blank on a spectrophotometer. Standards were also prepared as above and also read at 540nm. The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with a calibration factor derived from the absorbance and concentration of the standard.

Determination of packed cell volume

The packed cell volume (PCV) was determined by the microhaematocrit method (Coles, 1986). A microcapillary tube was nearly filled with the blood sample and sealed at one end. It was centrifuged at 10,000 revolutions per minute for 5 minutes using a micro-haematocrit centrifuge. After centrifugation, the PCV was read using a microhaematocrit reader.

Statisical Analysis

Experimental results were analysed using Genstat and SAS system for windows release 8.01 statistical software for analysis of variance for multiple comparison and student t-Test at probability confidence interval of 95% and 99%.

Results

T-test on the means of pre and post treatment with single and combined drugs on haematological and clinical parameter of guinea pigs infected with *S. heamatobium*

Table 1 shows the different between the means of before and after treatment values of white blood cell on the different developmental stages. Comparing the value of white blood cell on guinea pigs treated with praziquantel two days before and after treatment, statistically it's not significant 0.0605 ($P > 0.05$), artemether before and after treatment is statistically significant 0.0163 ($P < 0.05$) while artequine pre and post treatment were not significant 0.0539. There were no differences between the white blood cell before treatment and after treatment when PZQ was combined with ARQ.

Comparing praziquantel and artemether seven days before and after treatment on white blood cell, there were no significant difference ($P>0.05$) 0.0791, 0.4237 respectively. Artquine is highly significant ($P < 0.01$) 0.0029, the mean value of white blood cell post treatment with artequine is greater than the mean value before treatment. Treatment with PZQ/ARQ was not significant.

Treatments after twenty eight days post infection and treatment were not significant. Ninety days post treatment, administration of PZQ, ART, PZQ/ART were not significant ($P>0.05$) while ARQ and PZQ/ARQ were significant

Table 1: Variation of single and combined drug treatment on the white blood cell before and after treatment on guinea pigs experimentally infected with *S. heamatobium* cercariea

Drug Treatment (mg/kg)	Means in Duration (days) (x10^3/µl)											
	2 (B)	2(A)	t-Test	7 (B)	7(A)	t-Test	28 (B)	28(A)	t-Test	90(B)	90(A)	t-Test
75 ARQ	5.70±0.00	6.50±0.00	0.0539^{NS}	3.25±0.09	6.00±0.58	0.0029*	7.08±0.85	6.30±0.43	0.2440^{NS}	7.70±0.00	11.70±0.00	0.0032*
300ARQ	6.18±1.16	4.03±0.03		4.70±0.23	11.80±0.00		5.15±0.72	6.65±1.18		5.05±0.03	13.10±0.17	
75ART	8.35±0.18	5.50±0.65	0.0163**	8.92±1.96	5.55±0.60	0.4237^{NS}	7.62±1.45	8.10±1.94	0.0807^{NS}	13.40±0.46	11.40±0.58	0.2224^{NS}
300ART	9.37±1.76	8.77±0.45		6.78±0.71	6.50±0.86		7.22±0.54	11.32±1.79		12.55±0.26	9.90±0.06	
75PZQ	8.63±0.10	5.30±0.64	0.0605^{NS}	7.33±0.36	9.33±0.33	0.0791^{NS}	7.55±1.27	7.85±0.38	0.1579^{NS}	9.25±0.72	9.30±0.75	0.4643^{NS}
300PZQ	11.18±2.19	11.08±1.88		7.55±0.36	7.68±1.29		6.25±0.26	7.88±1.49		9.60±0.58	9.20±0.46	
75PZQ/ 150ARQ	5.60±0.00	7.70±0.00	0.1200^{NS}	6.40±0.12	11.45±1.18	0.2626^{NS}	7.23±0.07	8.40±0.23	0.8334^{NS}	7.50±0.00	8.20±0.00	0.0330**
100PZQ/ 200ARQ	4.60±0.00	11.30±0.00		12.70±1.50	8.30±0.23		8.63±0.10	8.60±0.52		6.00±0.00	10.00±0.00	
75PZQ/ 150ART	6.10±0.10	6.80±0.00	0.0265**	6.33±2.18	9.00±0.00	0.0466**	8.73±0.00	9.70±0.43	0.2152^{NS}	6.00±0.00	8.50±0.00	0.2844^{NS}
100PZQ/ 200ART	7.30±1.04	4.55±0.49		4.25±0.49	5.60±0.00		9.23±0.51	9.15±1.15		14.60±0.00	15.10±0.00	

P < 0.01 (Highly Significant); P < 0.05** (Significant); NS (Not Significant, P > 0.05) PZQ = Praziquantel; ART = Artemether; ARQ = Artequine; A = After; B = Before*

Table 2 summarizes the results of packed cell volume on administration of different drug and drug concentration pre and post 2days (skin phase), 7 days (lung stage), 28 days (schistosomule) and 90 days (adult stage) infection and treatment. Activities of praziquantel, artemether artequine and praziquantel/artequine on pack cell volume decreases after treatment (t-Test, $P < 0.01$) two days post treatment. It was observed also that there were decreased in the mean value of PCV when drugs were administered two days post treatment.

The mean level of PCV seven and twenty eight day's pre and post treatment in the entire drug administered was highly significant (t-Test, $P < 0.01$). Administration of drug after infection seemed to increase the value of PCV seven and twenty eight days post infection.

Except for PZQ, the entire drug treatments were highly significant when compared the pre and post treatment, hence increased in mean value of PCV were seen after drug administration.

The t-Test analysis on the difference between the value of HBC after infection and post treatment were summarised on table 3. The t-Test statistics presented on the Table below illustrates that the mean value of HBC in all the treatment groups as the parasite develops (2-7-28-90 days) were significant ($P < 0.01$; $P < 0.05$). It was viewed that on drug administration after *S. haematobium* infection, the value of HBC decreased.

Table 2: Variation of single and combined drug treatment on pack cell volume before and after treatment on guinea pigs experimentally infected with *S. heamatobium* cercariea

Drug Treatment (mg/kg)	Means in Duration (days) (%)											
	2 (B)	2(A)	t-Test	7 (B)	7(A)	t-Test	28 (B)	28(A)	t-Test	90(B)	90(A)	t-Test
75 ARQ	49.00±0.00	44.50±0.00	0.0021*	48.50±1.44	35.00±0.87	1.3×10^{-10}*	39.25±0.14	30.75±0.43	8.9×10^{-8}*	49.00±0.00	39.50±0.00	2.5×10^{-8}*
300ARQ	44.75±0.43	41.50±0.00		44.50±0.87	32.50±0.00		39.00±1.15	28.25±1.30		44.75±0.43	37.25±0.72	
75ART	41.17±1.42	38.33±1.09	0.0007*	43.33±0.33	36.83±2.40	1.6×10^{-7}*	42.67±0.73	32.67±2.91	5.3×10^{-8}*	43.75±0.14	31.25±0.43	4.6×10^{-11}*
300ART	39.83±2.62	38.58±0.87		43.17±0.33	37.83±2.13		42.67±1.76	32.83±0.33		47.75±0.00	33.50±0.29	
75PZQ	38.00±3.18	37.25±3.03	0.0006*	43.50±1.04	35.33±1.30	1.8×10^{-10}*	39.75±1.59	33.00±0.58	6.5×10^{-6}*	43.50±0.87	53.00±15.01	0.1489^{NS}
300PZQ	42.50±2.02	39.17±0.83		43.00±0.29	33.67±1.01		38.50±0.29	36.75±1.01		35.58±6.74	38.50±0.29	
75PZQ/ 150ARQ	45.50±0.00	43.50±0.00	0.0203**	35.00±4.62	26.25±5.05	1.9×10^{-5}*	40.75±1.01	30.75±1.01	1.7×10^{-8}*	45.50±0.00	35.50±0.00	1.8×10^{-9}*
100PZQ/ 200ARQ	43.00±0.00	46.00±0.00		46.75±0.14	39.25±2.17		41.50±0.29	32.00±0.87		43.00±0.00	33.00±0.00	
75PZQ/ 150ART	43.50±0.00	43.90±0.00	0.0006*	44.00±2.89	40.00±2.89	9×10^{-5}*	40.50±1.15	29.50±2.02	1.8×10^{-6}*	43.50±0.00	35.00±0.00	3.5×10^{-9}*
100PZQ/ 200ART	44.50±0.00	36.50±1.44		43.25±0.43	31.75±4.29		37.50±1.73	34.67±0.67		44.50±0.00	38.00±0.00	

P < 0.01 (Highly Significant); P < 0.05** (Significant); NS (Not Significant, P > 0.05) PZQ = Praziquantel; ART = Artemether; ARQ = Artequine; A = After; B = Before*

Table 3: Variation of single and combined drug treatment on the haemoglobin concentration before and after treatment on guinea pigs experimentally infected with *S. heamatobium* cercariea

Drug Treatment (mg/kg)	Means in Duration (days) (g/dL)											
	2 (B)	2(A)	t-Test	7 (B)	7(A)	t-Test	28 (B)	28(A)	t-Test	90(B)	90(A)	t-Test
75 ARQ	15.19±0.00	12.78±0.00	0.0003*	14.80±0.34	12.44±0.86	5.3×10^{-7}*	12.14±0.12	11.01±0.14	1.6×10^{-5}*	15.17±0.01	18.25±0.00	0.2233^{NS}
300ARQ	14.11±0.06	13.21±0.00		13.52±0.51	9.93±0.35		12.14±0.12	9.79±0.56		14.11±0.06	12.44±0.86	
75ART	14.16±0.41	12.56±0.43	0.0016*	14.05±0.21	12.76±1.32	0.0042*	13.61±0.52	11.50±0.50	7.7×10^{-6}*	12.64±0.06	10.44±0.12	1.4×10^{-6}*
300ART	13.48±0.86	13.02±0.43		13.55±0.23	24.60±0.12		13.28±0.35	10.74±0.68		12.84±0.06	10.03±0.37	
75PZQ	12.73±1.08	12.03±0.57	0.0189**	13.54±0.65	13.94±0.48	0.0002*	13.42±0.46	11.22±0.48	6.5×10^{-5}*	14.40±0.85	11.53±0.00	0.0002*
300PZQ	14.35±0.90	14.21±0.23		14.01±0.30	11.70±0.26		12.04±0.11	11.53±0.18		11.56±0.79	10.67±0.25	
75PZQ/ 150ARQ	14.21±0.00	10.49±0.00	0.0165**	11.84±0.46	7.16±1.32	8.2×10^{-7}*	11.93±0.00	10.77±0.56	4.9×10^{-5}*	14.21±0.00	12.32±0.00	1×10^{-4}*
100PZQ/ 200ARQ	13.42±0.00	15.51±0.00		14.51±0.17	9.58±0.79		12.94±0.35	11.25±0.57		13.42±0.00	6.84±0.00	
75PZQ/ 150ART	13.42±0.00	13.68±0.00	0.0048*	13.42±0.91	10.08±0.64	2.3×10^{-8}*	12.55±0.59	10.28±0.56	0.01143**	13.34±0.00	7.76±0.00	0.0017*
100PZQ/ 200ART	13.61±0.00	12.32±0.79		12.63±0.11	9.35±0.00		9.67±2.02	9.40±2.09		30.61±0.00	10.04±0.00	

P < 0.01 (Highly Significant) ; P < 0.05**(Significant) ; NS (Not Significant, P > 0.05) PZQ = Praziquantel ; ART = Artemether; ARQ = Artequine; A = After; B = Before*

Discussion

Studies have been done on *S. haematobium* infections and treatments; these studies have treated together with schistosomiasis and other helminth infections making it difficult to determine the exact benefit of treatment for schistosomiasis (Stephenson *et al.,*1989, Befidi-Mengueet *et al*., 1983 and Olsen *et al*., 1998**)** . However, most of those studies also used antischistosomal drugs other than praziquantel. Mohammed *et al*., (2006) reported that treatment with praziquantel has proven to be effective in normalizing haematological parameters. In this present study the post treatment showed no effect on white blood cell on administration of PZQ as the infection progresses; although, the values of white blood cell post treatment were higher than before treatment. The infection and treatment with ART were found to be significant on the mean value of WBC as the diseases develops. Treatment with ART lowers the value of WBC at the early stage of infection (skin and lung stage) as seen in this result, it might be due to initial protective attack by the blood cell to fight against the infection and as a result the WBC were used up. Treatment with ARQ after 90 days of infection had cumulative higher value of WBC; it can be said that the used up WBC after infection were found to increase after treatment with ARQ when compared with the post treatments. Similarly, comparing the events that follow post infection and treatment using Tukey-HSD, there was an increase in the white blood cells; the authors further suggested that the significant rise in WBC counts could be attributed to a rise in lymphocytes and neutrophils (Labe and Inabo, 2012). A group of researchers in their study also reported a higher but insignificant total WBC and neutrophils (Afrifa *et al.,* 2017). A critical study on the haematological profile of the guinea pigs shows some degree of immunological processes occurring at post-infection and post-treatment.

Effect of *S. heamatobium* infection on the level of PCV as the infection progresses were not stasitically significant; this result is in agreement with that obtained by Bugarski *et al*. (2006) who mentioned insignificant changes in the PCV levels in subjects infected with schistosomiasis. On the contrary, our result is in variance with the results obtained by Mahmoud and Elbessoumy (2014), Al-hroob (2010), Abd EL-Mottleb *et al*. (2008), and El Shenawy *et al*. (2008), who documented a significant decrease in the PCV with schistosoma infection. The discrepancies in the results may be ascribed to differences in parasite species and the dose of parasitic infestation. However, the value of PCV generally increased after drug (PZQ, ART and ARQ) were administration.

The mean values of HBC after different drug administration were significant, which probably means that the treatment used in this work reduces the value of HBC. Haemoglobin is a protein found in the red blood cells that carry oxygen in the body, iron in haemoglobin is also very important because it helps in production of haemoglobin. Furthermore, haemoglobin is a molecule that normally becomes reversibly oxygenated rather than irreversibly oxidized. Erythrocyte is designed to maintain haemoglobin from oxidation so that it can carry oxygen and is not damaged in structure. Decreased levels of haemoglobin, can cause anaemia. More so, the significant reduction observed in the circulating hemoglobin after treatment indicates that this immunological response was insensitive to T-cell mediated immune response. Schistosomiasis infection is related to cognitive impairment, which might be mediated by iron deficiency (Nokes *et al*., 1999 and Ezeamama *et al.*, 2005). A conclusion was reached by Stephenson *et al* (1989) who conducted a study in a *S. haematobium* endemic area of Kenya; in that trial, they reported that treatment with praziquantel had no significant effect on haemoglobin. Ayoya *et al.,* (2009) also reported that treatment with praziquantel had no significant effect on hemoglobin. Likewise, A quasi-randomized controlled trial conducted in Cameroon compared four groups of subjects infected with *S. haemetobium* which were treated with either placebo or praziquantel. Six months after therapy, there were no significant differences in the change in hemoglobin between the four groups (Befidi-Mengue *et al.,* 1993).

On the contrary, a combined drug study conducted in Tanzania examined the effect of albendazole and praziquantel on hemoglobin.15 months after therapy using a quasi-randomized controlled trial design. Children receiving either albendazole alone or both drugs experienced haemoglobin increases. They further stated that there was no significant improvement in haemoglobin in children treated only with praziquantel compared with the control groups (Bhargava *et al*., 2003). Another combination therapy (albendazole and praziquantel) conducted in Tanzanian schoolchildren reported significantly greater increase in haemoglobin in the treatment groups (Beasley *et al*. 1999). Likewise, a randomized controlled trial of the impact of praziquantel on *S. japonicum* morbidity was conducted by McGarvey *et al.*(1996) in Leyte; the study demonstrated a significantly higher haemoglobin concentration in subject six months after therapy. Furthermore, Friedman *et al*., (2005) recorded a rise in haemoglobin 16 weeks after therapy for men treated for *S. haematobium*, similar rise was also recorded in men who were not infected and not treated for *S. haematobium*. The discrepancies in most of the result especially in the result that recorded elevation in haemoglobin are probably due to time interval or the animal models used in the experiments. Most of the elevation was recorded several months after treatment. Hence, size and duration of treatment should be taken into consideration which may vary across different settings and population.

In conclusion, the use of the antimalarial drug artequine possesses interesting antischistosomal properties and also had an increased in level of some of the heamtological parameters, especially after treatment as observed in this work. It is recommended that these antimalarial drug artequine which possesses antischistosomal properties which can be a substitute for praziquental, hence prevent mono drug treatment which can enhance drug resistance.

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