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The Efficacy of a Herbal Drug, Schitozim over Praziquantel in the Management of *Schistosoma mansoni* Infection in BALB/c mice

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Abstract
Schistosomiasis is a major public health problem worldwide. Schistosome eggs are responsible for most pathology. The organs affected are liver and spleen. There has been very little progress on schistosomiasis chemotherapy in Kenya due to the high costs and unavailability of known drugs to the local masses. There has been a resurgence of interest in the study of antischistosomal medicinal plants of local origin. Plants seem to be a cheaper source for drug development. The aim of this study was to compare the efficacy of a herbal drug, Schitozim over Praziquantel in the management of *S. mansoni* infection in BALB/c mice and to provide defined information of the parasitological and immunological responses towards this herbal drug. BALB/c mouse strains, was used. The experimental groups included; normal/naïve control; infected and not treated control group; infected groups administered with three different doses (50 mg/kg, 150 mg/kg, 300 mg/kg) of Schitozim and infected group administered with one standard dose (900 mg/kg) of Praziquantel. Serum were collected at week 6 post infections and week 2 post treatment and assayed to determine total leukocyte counts, differential counts, blood chemistry parameters and the levels of immunoglobulin G using ELISA kit. At week 6, perfusion was done to determine worm load. Data was analysed using SPSS, the data was expressed as mean ± standard error of means. Analysis of Variance (ANOVA) was carried out to compare differences between treatment groups while multiple comparisons between the various treatment groups was done using Dunnet Test and Least Significance Difference Test (LSD). Results show that worm count in the groups treated with Schitozim was not significantly different from the number of worms recovered in the group treated with praziquantel. Secondly, Schitozim was able to maintain a normal level of the three tested transaminases. The levels of bilirubin, albumin were also maintained at a normal range in the infected mice treated with Schitozim and Praziquantel. Eosinophil counts were low in number in both Schitozim and Praziquantel treated groups. Lymphocyes, monocytes and neutrophils counts were high in the both groups. SWAP specific IgG response, 0-3hr release protein specific IgG response and SEA specific IgG responses were not significantly different among the Schitozim and Praziquantel treated groups. Phytochemical screening showed that flavonoids, glycosides, saponins, steroids and tannins were present in schitozim aqueous extract. From the information above on worm recovery, populations of leucocytes in serum, levels of ALT, AST, ALP, bilirubin, total protein, and albumin, Schitozim behaves similar to Praziquentel in the management of *S. mansoni* infection in BALB/c mice. However, further research is needed to determine toxic level of schitozim since all drugs will turn out to be toxic when certain concentration is reached. Other studies which will investigate the levels of total cholesterol, triglycerides, High density cholesterol and low density cholesterol on Schitozim treated- *S. mansoni* infected mice.

Keywords: Schitozim, Praziquantel, Transaminases, Lymphocytes, IgG, Phytochemical screening, BALB/c Mice

1. INTRODUCTION

Human schistosomiasis affects at least 240 million people worldwide, mostly children, in 76 tropical countries with another 700 million people being at risk of contracting the disease (WHO, 2012; Chitsulo *et al*., 2000), and the number of people estimated to be infected or at risk continues to increase as population growth increases. It is also estimated that 85% of the total number of infected people worldwide are in Africa. Schistosomiasis is one of the most widespread of the major parasitic diseases and its negative socio-economic and public health impact in tropical and subtropical regions of the world is second only to malaria (WHO, 2001). Its distributional range has continued to expand due to increased establishment of water resources development projects to boost food and cash crop production and hydroelectric power generation for industrialization (Hunters *et al*., 1993). The disease is transmitted by some fresh water molluscs, notable of the genera, *Biomphalaria, Bulinus* and *Oncomelania.* In Africa, *B. pfeifferi* is probably the most important and the most widely distributed vector (De-Clercq, 2000).

The disease is mostly due to eggs deposited in host tissue when eggs are swept in the blood system from

mesenteries to various organs in the body which induce inflammatory and fibrotic lesions in host organs. The granuloma formed around the schistosome egg is primarily a T-lymphocyte mediated host response: immune complexes formed with antigens from the worms and their products explain some of the symptoms and pathological findings presented by the infected individuals (Jordan *et al*., 1993)

At present, there is no vaccine available, and Praziquantel (PZQ) is the chemotherapeutic agent of choice with good efficacy against the adult worm, however, there are increasing concerns for the emergence of drug resistance in some schistosoma isolates (Mountford, 2005).

Schistosomiasis control can be interrupted at four distinct points: Preventing excreta from reaching fresh water (sanitation), preventing the interaction of free swimming larval stages of schistosomes (miracidia, cercariae) with intermediate host snail (broadly-snail control), preventing exposure to susceptible humans to the infective, free-swimming cercariae (reduction of water contact), Chemotherapeutic attack on the parasite population living within human host (mass or targeted chemotherapy).

Chemotherapy using the drug praziquantel is considered the most cost effective approach for control of human schistosomiasis (WHO, 2001). Other strategies recommended include; snail control, health education, community participation and provision of clean water and improved sanitation. (WHO, 2001).

Schitozim, is a herbal drug sold over the counter in Herbal Shops in Coast Region, Kenya. It has shown a wide activity against *S. hematobium* infection in humans based on subsequent withdrawal of symptoms of *S. hematobium* infection in the individuals using the drug in Coastal Region in Kenya. The aim of this study was to investigate the immunological and parasitological effects of schitozim, and compare its efficacy to that of PZQ in the management of *S. mansoni* infection in BALB/c mice

2. MATERIALS AND METHODS

2.1. Phytochemical screening of Schitozim

Chemical tests were carried out on the aqueous extract for the qualitative determination of phytochemical constituents as described Sofowara, 1993; Trease and Evans, 1989; Siddiqui and Ali, 1997 and Harborne, 1998. The various extracts were tested for terpenoids, steroids, flavonoids, saponins, tannins, glycosides and alkaloids.

2.2. Maintenance of the snail host in the malacology laboratory.

The snails were collected from Mwea irrigation scheme in Kirinyaga District, Kenya and screened for schistosomes under strong light (100watts) for 2hrs daily for 5 weeks. Those that were negative of cercariae were housed in temperature controlled (25-27°C) snail room in plastic tanks containing sand and gravel obtained from Mwea. The snails were fed on dried and sterilised lettuce leaves. The tanks were artificially aerated using Daphnea. They were allowed a period of 12hrs of light and 12hrs of darkness. The infected snails were then maintained under the same conditions.

2.3. Infection of snails and shedding of parasites

The snails were infected with free swimming larvae (miracidia) obtained from faecal samples of olive baboons with chronic *S. mansoni* infection. Four weeks post infection; the snails were covered with black clothes to prevent light from stimulating shedding of cercariae.

2.4. Harvesting cercaria and counting

Five weeks post-infection, snails were carefully removed from tanks using forceps and placed in 10 ml beakers containing snail water. They were exposed to 100 watt lamp for 1 h to shed cercaria. The cercariae suspension were then pooled together. Three aliquots of 50µl each were obtained from well mixed cercariae suspension (mixing was done by cross motion to avoid swirling of cercariae, which would bring majority to the centre). Iodine was used to immobilise and give colour to cercariae during counting. An average of the 3 aliquotes was made and a volume containing 250 cercariae was worked out.

2.5. Infection of mice with S. mansoni

Mice of BALB/c strain were used in this study. They were obtained from animal rearing unit and maintained at the animal experimental unit at the Rodent House, Animal Resources Department, IPR. The mice were anaesthesised using a mixture of rompun and ketamine (10mls of ketamine was mixed with 0.5mls of rompum; Agar, Holland) and shaved on the stomach area. Mice were arranged on an infection rack, stomach up. Cotton wool dipped in water was used to moisten the shaven area to allow easy penetration of cercariae. A 1 cm steel ring was placed on the shaven area of each mouse, then, a suspension constituting 250 live cercariae was dispensed in the metal ring using a micropipette and left for 30 minutes for cercariae to penetrate into the mice (Smithers and Terry, 1965).

2.6 Treatment of infected mice

At the 4th week after the start of bioassay, three doses of herbal medicine, Schitozim were determined and administered as 50 mg/kg (Dose 1), 150 mg/kg (Dose 2) and 300 mg/kg body weight of mice (Dose 3). Each treatment group contained 12 randomised block samples of mice. Each mouse in each of the three groups D1, D2 and D3 was given orally 200µl of appropriate dose of Schitozim suspension using a micropipette with truncated

yellow tips. Treatment was also administered to the positive control group using Praziquantel (PZQ ; Biltricide, drug obtained from Bayer AG Germany). A tablet of PZQ that weighs 600 mg was administered at the dose of 900 mg/kg body weight of mice (Njoki *et al.,* 2012). All the 12 mice in the positive control group were each orally given 200 µl of 900 mg/kg standard dose of PZQ. Schtozim and PZQ were ground into powder using separate mortar and pestle. Calculated amount of distilled water was added to the powder and stirred continuously. Two naive treated control group was administered with Schitozim to later monitor the effect of Schitozim where there is no infection. The infected control and the naive not treated control groups were not administered with either PZQ or Schitozim. Picric acid was used to label different parts of mice to mark different experimental groups. Treatment was repeated on alternating days (after every 48 h) for two days PZQ and five days Schitozim.

2.7. Sampling of blood

At week 6 mice were anaesthetized as described above (2.5). A small incision was made at the center of the abdominal skin and the skin torn around the waist of the mouse. The skin was pulled apart to expose the abdominal and thorax wall. A small cut was made on the right side of the mouse just above the diaphragm, through the ribs up to the left side of the thorax, taking care not to puncture the lungs and the heart. Another cut was made on either sides of the sternum taking care not to puncture the interthoracic arteries. The ribcage on the left side was trimmed. The left ventricle was located and 1ml syringe inserted into it with the bevel facing downwards.

Blood was sucked in small jerks in order to create vacuum and prevent the heart from collapsing. When all blood was drained from the left ventricle, the same procedure was repeated to drain blood from right ventricle. The whole volume of blood collected was dispensed into microfuge (eppendorf) tube and left on the bench for 2 h to clot. Thereafter, the clotted blood was processed into serum and stored at -20 °C.

2.8. Schistosome Specific IgG Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA plate (Nunc-Immuno^{TM} plate marxisorp^{TM} surface, Denmark) was coated with prepared 50µl of 5 µg/ml of 0-3hr release protein antigen, Schistosomal egg antigen (SEA) and *S. mansoni* soluble worm antigen (SWAP). It was incubated overnight at 4°C to allow the antigen to bind. There was a comparison of schistosome specific IgG responses for groups; Naive, infected-untreated control, Praziquantel, D1(50mg/kg) schitozim, D2 (150mg/kg) schitozim, D3(300mg/kg) schitozim. The plates were read at 630nm on an ELISA reader, Marxi Kinetic Microplate reader (Molecular Devices, Palo Alto, England). The IgG levels determined by ELISA were presented as absorbance (O.D) at 630nm. Colour developed depending on the strength of binding.

2.9. Hematological Tests

2.9.1 Total leukocytes counts and Differential counts

Thin blood smears were prepared and stained using Giemsa stain and blood film was examined. Counting was done with the help of a tally counter and the number of fields counted to obtain a total of 100 leukocytes was noted. The counting was repeated three times noting the number of fields counted each time. The morphology of different leukocytes, lobulations of neutrophils and presence of cytoplasmic inclusions was also noted.

2.9.2 Blood chemistry

Blood biochemical parameters were determined in serums of all mice groups under the study. Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total bilirubin (TB), bilirubin, albumin, Total protein (TP) were done using chemistry analyser (Humalyzer 2000®) and Alkaline phosphatise was determined as a test of the liver function impairment. Determination of the total protein, albumin and bilirubin was done using spectrophotometry in serum.

2.10. Parasitological Assay

The perfusate was collected in glass petridish (20 cm diameter) and transferred in a urine jar to settle. The adult worms were recovered by topping the urine jars with perfusate with phosphate buffered saline (PBS). Haemolizer DL-1 (Erma Inc. Japan) was added to lyse the red blood cells. The worms were poured on a petri dish containing PBS and counted.

The worm maturation and percentage worm reduction of adult worm recovered for each group was calculated using the control group as follows (Yole *et al.,* 1996):

2.11. Data analysis

Data entry management and preliminary summaries was done in Microsoft Excel spreadsheets. Other statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 16, a statistical analysis software. Using the software, one way ANOVA was used to determine whether there are significant differences in the various parameters which were tested in the various treatments such as worm count, IgG responses, Leucocyte counts, Transaminases, albumin, protein and bilirubin levels. Still with the help of SPSS, multiple comparisons were done using Least significance different test (LSD test) and Dunnet test to determine difference between any two groups. The significance level of probability used in the analysis was less or equal to 0.05 i.e $P \leq 0.05$

3. RESULTS

3.1. Phytochemical Screening.

Phytochemical screening of the aqueous extracts of schitozim revealed the presence of Tannins, Steroids, Flavonoids, Glycosides and Saponins (Table 1).

Table 1: Phytochemical Screening Results

Extracts	Alkaloids	Tannins	Saponins	Glycosides	Terpenoids	Steroids	Flavonoids
Aqueous	ND	+	+	+	ND	+	+

KEY: + = Phytochemical detected; ND = Phytochemical Not detected

3.2 Worm maturation

Worm maturation was calculated for infected-untreated control group in order to establish the survival of *S. mansoni* in BALB/c, maintained in snails from Mwea irrigation scheme in Kirinyaga District. Two hundred and fifty (250) cercariae were used during the infection. Worm maturation was calculated as described by Yole *et al*., (1996).

$$\text{Worm maturation} = \frac{\text{Mean Number of worms recovered from infected control}}{\text{Initial number of infecting parasites}} \times 100\%$$

$$\text{Worm maturation} = \frac{61}{250} \times 100\%$$

Worm maturation was 24.4% which is close to a third of the total parasite used in infection.

3.3 Worm Recovery

The number of worms recovered by perfusion from the six groups infected with *S. mansoni* and perfused at week 6 is shown in Table 2 and Figure 1.

Table 2: Mean Number of worms recovered from experimental and control groups

Experimental group	IC	PZQ	D1	D2	D3
Worms	61	22	33	41	24
SE	15.4	3.3	4.8	9.8	2.3

Key: D1= Schitozim (50mg/kg), D2 = Schitozim (150mg/kg), D3 = Schitozim (300 mg/kg),
PZQ = Infected and treated with Praziquantel (positive control), IC = Infected & Untreated (negative control).

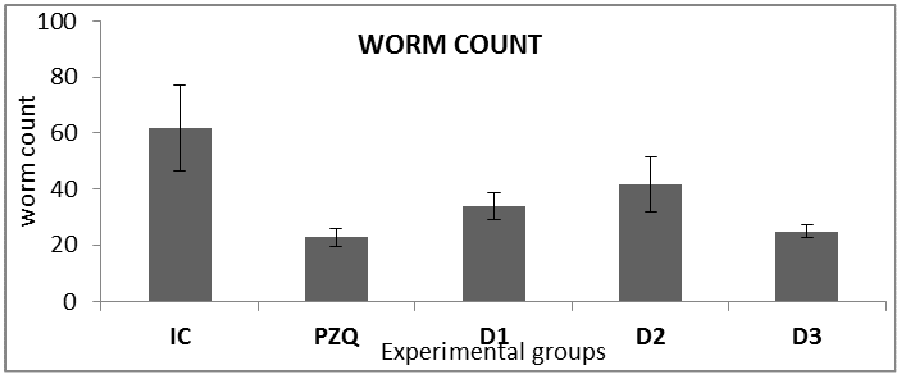

Figure 1: Mean worm count in various treatments

3.4. S. mansoni Specific IgG responses

3.4.1 0-3hr release protein specific IgG responses

The infected-untreated control group (IC) was not significantly different from PZQ, D1, D2 and D3 at $p>0.05$. When PZQ was compared with D1, D2 and D3 the difference was not significant at $p>0.05$. D1, D2 and D3 were not significantly different from each other at $p>0.05$. The naive group was significantly different from all the other groups at $p<0.05$ (Appendix 2).

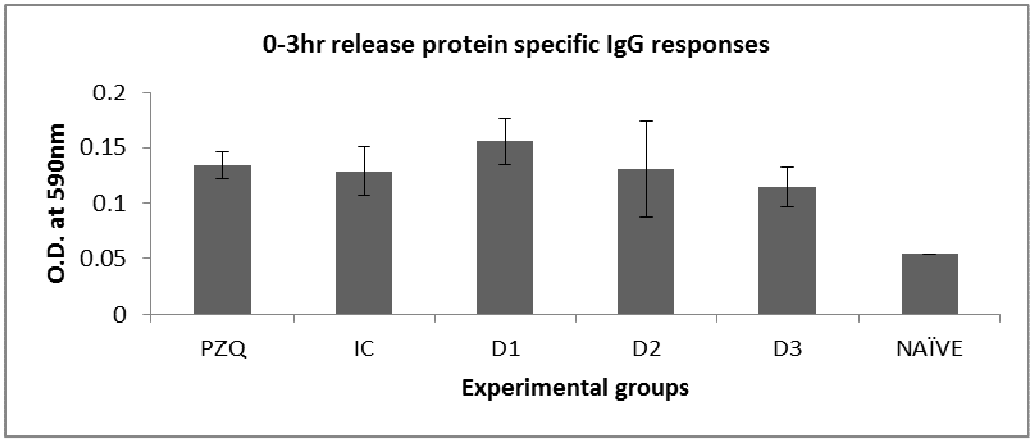

Figure 2: 0-3hr release protein specific IgG responses

3.4.2 SWAP specific IgG responses

When IC was compared with PZQ the difference was not significant at $p>0.05$. IC was not significantly different from D3 ($p>0.05$) but was significantly different from D1 and D2 at $p<0.05$. PZQ was not significantly different from D1, D2 and D3 ($p>0.05$) and the three groups were not significantly different from each other at $p>0.05$. The naive group was significantly different from all the other groups at $p<0.05$ (Analysis in Appendix 2).

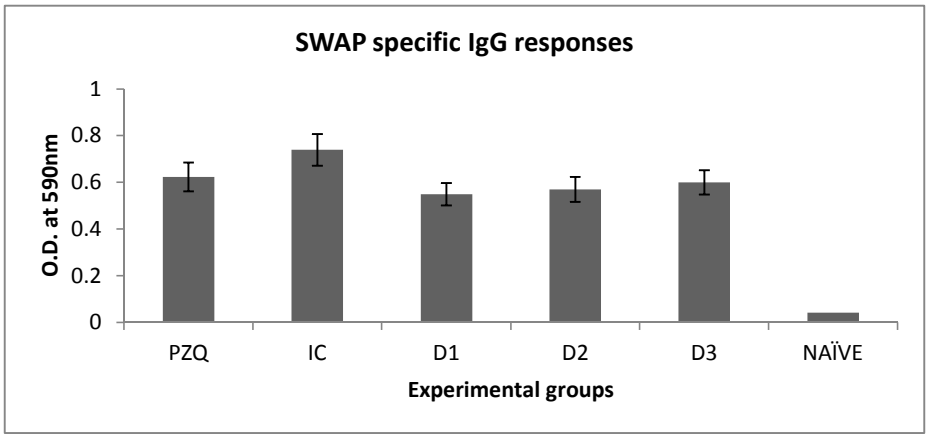

Figure 1: SWAP specific IgG responses

3.4.3 SEA specific IgG responses

When IC was compared with PZQ, the difference was not significant at $p>0.05$. IC was not significantly different from D1, D2 and D3. PZQ was significantly different from D1 at $p<0.05$ but not significantly different D2 and D3 at $p>0.05$. The three doses of Schitozim were not significantly different from one another. Naive group was significantly different from all the other groups at $p<0.05$ (Analysis in Appendix 2).

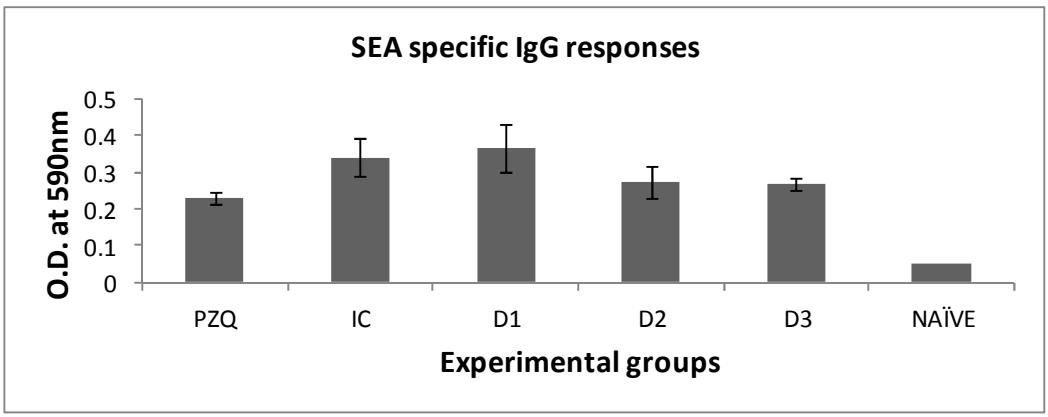

Figure 4: SEA specific IgG responses

Among the three IgG responses in all the treatments, the SWAP specific IgG response was highest (response ranged from 0.7388 to 0.0415) followed by SEA specific IgG response whose response ranged from 0.366 to 0.0485 while the least IgG response was observed in the 0-3hr release protein antigen (response ranged from 0.1556 to 0.0545). In the three immunoglobulin reponses, the least IgG responses were observed in the Naïve group and the highest IgG responses were in the IC group (Figure 2).

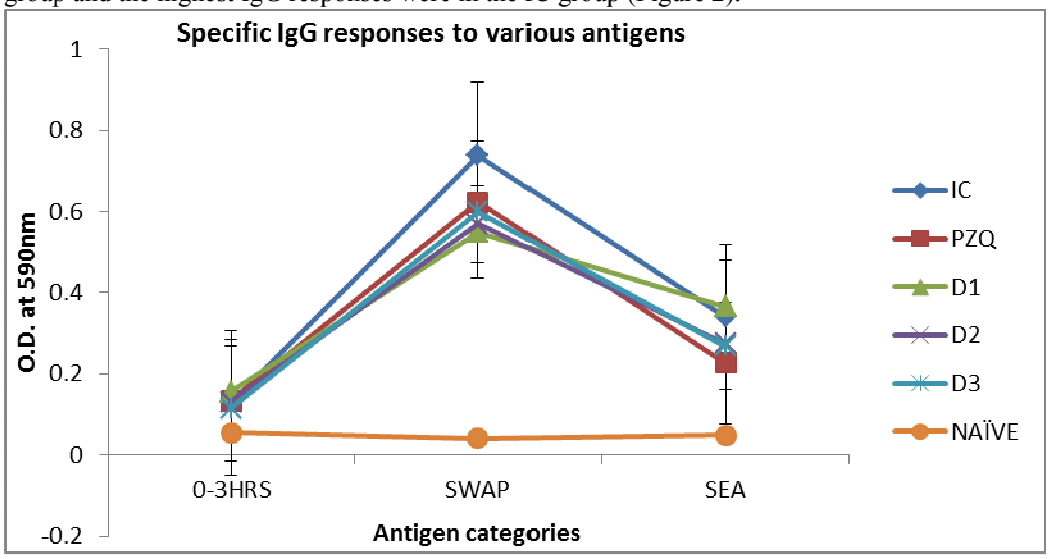

Figure 5: Specific IgG responses to various antigens.

3.5 Blood chemistry

Blood biochemical parameters were determined in serum of all mice groups under the study.

3.5.1 Levels of Transaminases

The trend of ALT levels from the highest to the lowest was IC, D3, D2, D1, PZQ and lowest being the Naïve group. The trend in the levels of ALP from the highest to the lowest was Naïve group, IC, PZQ, D2, D1 and lowest levels being in the D3 group. The trend for the AST levels from the highest to the lowest was D3, IC, D2, D1, PZQ, Naïve. Both ALT and AST were much high in IC and Schitozim groups compared to Naïve and PZQ.

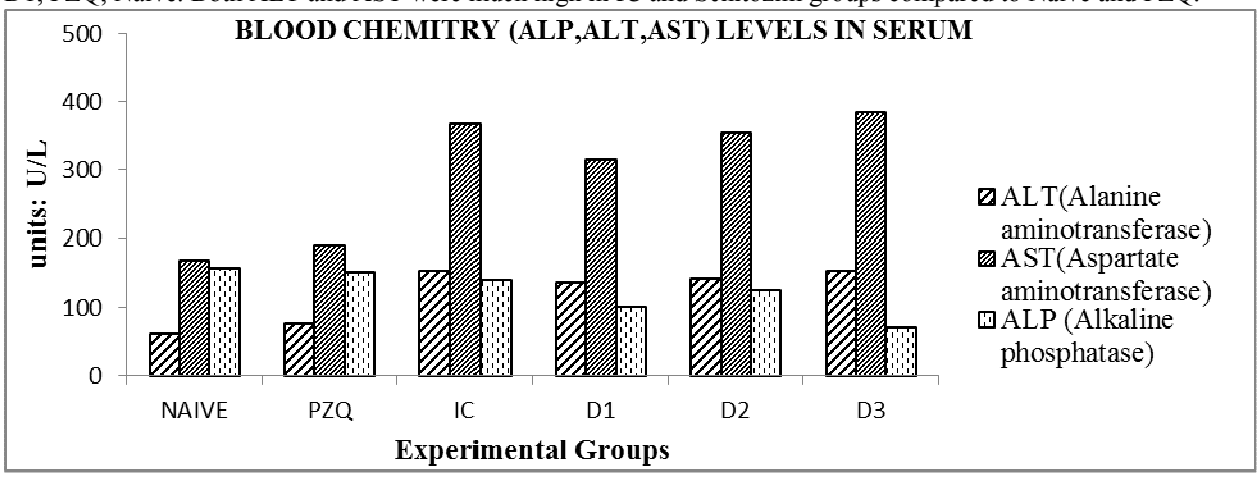

Figure 6: ALP, ALT and AST levels in serum

3.5.2 Total bilirubin, Albumin and Total protein levels in serum

The trend of Total bilirubin from the highest to the lowest was D3, D2, D1/PZQ, IC/Naïve. The trend in the levels of Albumin from the highest to the lowest was PZQ, IC, D2, D1/Naïve, D3. The trend of total protein levels from the highest to the lowest was PZQ, D3, D2, IC, D1/Naïve

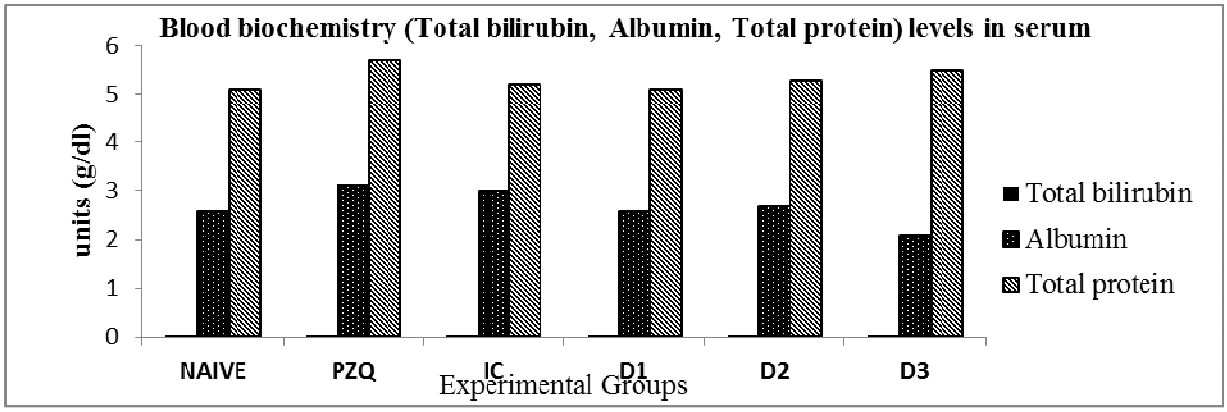

Figure 7: Total bilirubin, albumin, Total protein levels in serum

3.6 Total Leukocyte Counts (TLC) and Differential Counts

Lymphocytes were found in high numbers across all the groups followed by neutrophils then monocytes and eosinophils were generally the least in all the groups.

The trend in the TLC from highest to the lowest was IC, PZQ, D2, D1, D3 and Naïve. The trend of eosinophil counts from the highest to the lowest was PZQ, D2, Naïve, D3, D1 and IC. The trend of lymphocytes from highest to the lowest was IC, PZQ, D2, D3, D1 and Naïve. The trend of neutrophils from highest to lowest was IC, D1, Naïve, D3, D2 and PZQ. The trend of monocytes counts from highest to lowest was D1, IC, D2 and D3 were equal, PZQ and Naïve.

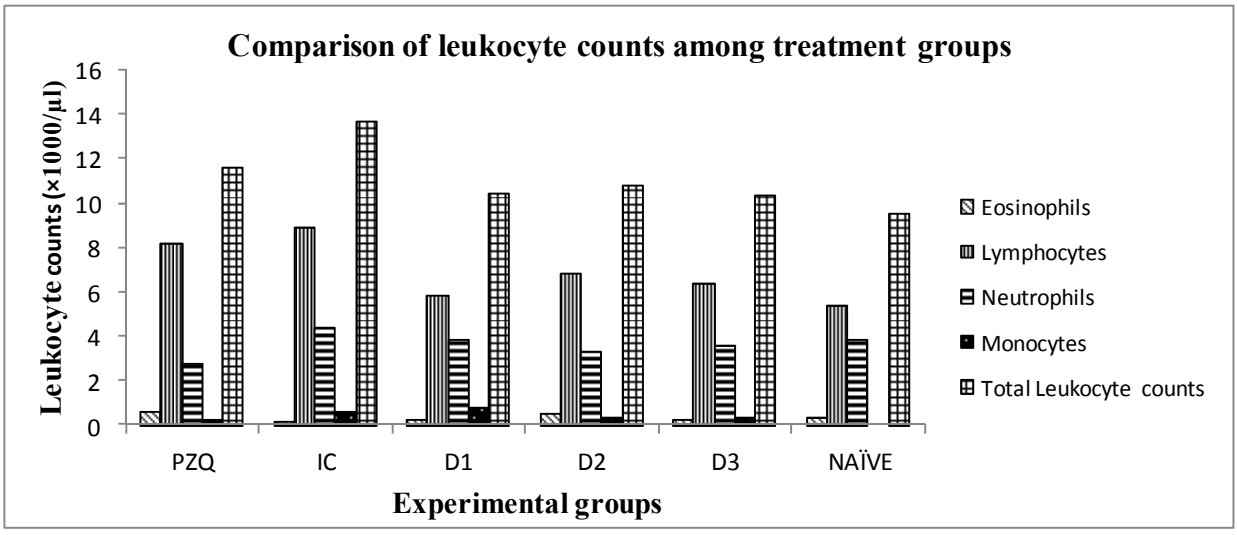

Figure 8: Comparison of leucocytes among the different treatment groups.

4. DISCUSSION

Morbidity due to *S. mansoni* is mainly as a result of the host's responses to schistosome egg antigens to form granulomas mainly in the intestines and the liver where the eggs are trapped (Bindeseli *et al.,* 2004).

4.1 Phytochemicals

There are appreciable number of compounds that were present in Schitozim aqueous extract. They include; Flavonoids, glycosides, saponins, steroids and tannins. The presence of these secondary metabolites has a great importance in the understanding of the basis of the use of Schitozim in traditional medical practice. Tannins were shown as one of the chemical constituent. Tannins have been shown to produce anti-helminthic activities (Niezen *et al.*, 1995). Chemically tannins are polyphenolic compounds (Bate-Smith, 1962). Some synthetic phenolic anti-helminthics such as niclosamide, oxyclozanide and bithionol have been shown to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation (Martin, 1997) causing death of the schistosomes. Flavonoids, sesquitepenes, saponins, tannins, anthraquinones, steroids, glyocises and

generally phenolics have been reported to be potent plant secondary metabolites with broad spectrum of bioactivities. Not only their presence, but also the quantity of these phytochemical constituents in a given extract determines the extent of extracts' bioactivity. In addition, presence of more than one class of secondary metabolites in a given plant extract also determines the nature and extent of extract's biological activity. (Wang *et al*.,2010).Praziquantel and the three doses of Schitozim have been shown to have anti-schistosomal activity. Biological activity is attributed to the presence of various secondary metabolites. The qualitative differences in the constituent compounds of these extracts may partly explain the different dimension of action of different dosages of Schitozim on the worm recovery and maturation, immunological assays and blood chemistry parameters. As a result these secondary metabolites detected in the Schitozim aqueous extract could have been the active constituents with anti- schistosomal activity.

4.2 Worm maturation

The *S. mansoni* isolate originally from Kibwezi and maintained in *B. pfeiferi* from Mwea irrigation scheme used in this study had worm maturation of 24.4% in BALB/c mice. There was an *S. haematobium* worm maturation of 12% obtained in Golden hamsters-*Mesocricetus auratus* Kijiwe isolate (Njoroge *et al*., 2007). Comparison of these two isolates of different species showed that the Kibwezi isolate worm maturation in this study is higher than the Kijiwe isolate.

4.3 Worm recovery

Based on this study, the highest worm count was observed in the infected control group, while the lowest worm count was observed in the PZQ group. There was a significant difference, ($p<0.05$) between PZQ and infected control. This supports reports that PZQ has good efficacy against the adult *S. mansoni* worm (Utzinger and Keisser, 2004). Owing to PZQ high efficacy against all five human schistosome species, good tolerability and ease of administration as a single oral dose, it has become the drug of choice for the treatment and morbidity control of schistosomiasis the world over (WHO, 2002; Cioli and Pica-Mottoccia, 2003; Utzinger and Keisser, 2004). The worm count in the three doses of Schitozim were not significantly different from the worm count in the PZQ ($p>0.05$). This implies that the three doses of Schitozim and Praziquantel had similar efficacy in reduction of the number of *Schistosoma mansoni* in the BALB/c mice. All the treatments showed antischistosomal activities, however, Schitozim D3 at 300m/kg was the most similar in efficacy to PZQ at 900mg/kg.

4.4 Immunoglobulin G responses

In this study, serum antibody analysis was done using the 0-3hr release protein, soluble adult worm antigen preparation (SWAP), and schistosomal egg antigens (SEA).The immune responses to patent *S. mansoni* infection is generally directed into a systemic Th2 type response at the onset of egg production, with elevated production of IL-4, IL-5 and IL-10 in response to soluble egg antigen (SEA) as well as non-parasite antigens (Grzych *et al*., 1991).The elevated levels of IgG responses in infected-untreated control can be associated with a high worm burden leading to a high level of circulating parasite antigens inducing lymphocytes to produce more IgG. This high IgG level did not confer protective immunity; this can be concluded basing on the number of worms recovered in infected-untreated control. High absorbance is correlated to increased antigen-antibody binding which denotes high schistosome infection. (Nutman *et al*., 1985); (Ji *et al*., 2008).Comparison of IgG responses in Naïve with treatment groups indicates that the immunological activities of lymphocytes were going down, and so was the protective immunity, but this implies that the treatments had continued to stimulate an increased lymphocytes activity to provide a higher protective immunity. Using mathematical models, Woolhouse (1995) and Woolhouse and Hagan, (1999) have argued that protection in non-treated humans develops slowly since it requires antigens released at the end of the life span of the parasite, which can be several years. Therefore this similarity of IgG response in all treatment groups could be due to a short time the parasite had lived in the host. PZQ and Schitozim groups had high responses similar to IC although the number of worms were significantly reduced when compared to IC. This is due to the fact that PZQ is known to destroy worms directly (Ali, 2006) and also via the immune system (Jordan *et al.,* 2006). This same mechanisim of action of destroying *S. mansoni* seem to be happening with the three doses of Schitozim. D1 Schitozim had a higher 0-3hr release protein IgG responses and SEA IgG responses than Praziquantel (Fig.2 and Fig.4), but similar to the infected control group and yet D1 had a lower worm count compared to infected control (Fig.1). This may imply that D1 has a higher protective immunity than PZQ. A similar trend was shown in all three antigens used (Fig.6). Naïve had the lowest responses and this was because there was no schistosome infection hence no schistosome specific antigens. However, the background reaction could have been as a result of antigens in nature. Almost in all cases, IC had the highest responses, this was due to no treatment hence more worms releasing more antigens in circulation therefore highest responses expected. Generally, in this study, IgG responses to SWAP in all treatments were higher, compared to responses in 0-3hr release protein and SEA. The low IgG responses to 0-3hr release protein and SEA antigens could be due to reduced schistosome antigens. Therefore at these antigen stages, the specific IgG responses were due to shared antigens. However, IgG responses to SWAP antigens were

high in the treatments and this could be associated to the consistency in the antigens exposed on the surface due to maturity of the worm (adult worm). There might have been also a consistency in the egg secretions produced which were directly stimulating the humoral and cellular immune responses. This variation in the level of IgG to schistosome specific antigens shows that there is greater protective immunity to adult worm than to immature ones as high IgG level indicates higher immune protection (Kanyugo *et al*., 2009).

5.5 Blood Chemistry

Transaminases such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) increase as a result of metabolic changes in the liver such as administration of toxin, liver diseases such as schistosomiasis, cirrhosis, hepatitis, liver cancer and jaundice. In humans, normal levels for AST, ALP and ALT in serum are usually less than 1000µ/l and the levels of these transaminases are increased to some extent in almost all liver diseases (Thapa and Walia 2007). Due to that, moderate, mild and severe elevations of the levels of ALT, AST and ALP in the liver can be used to determine the extent of liver damage. Conversely, moderate to low levels of ALT, AST, and ALP in blood denote a healthy liver or a liver which is recovering from a given disease in most cases as argued by Jeong *et al*., (2010). AST is a protein made by liver cells. At times when the liver is infected, liver cells are damaged and AST leaks into blood stream and the levels increase in serum (Thapa and Walia 2007). AST is also found in heart, kidneys, muscles and brains (Krier and Ahmed 2009). Although enzyme levels may reflect the extent of hepatocellular necrosis, in other cases, elevated ALT levels can be attributed to some side effects of a drug (Krier and Ahmed 2009). ALT and AST are enzymes which help all chemical activities within cells to take place. Injury to cells releases the enzymes into blood thus increased levels of the enzymes in serum. ALP enzyme is found primarily in bones and liver. Damage to bone or liver results in release of ALP in blood and just like AST and ALT, increased levels of ALP also can be as a result of a side effect of a drug and may not correlate with eventual outcome, Thapa and Walia (2007). This study shows that the levels of the three transaminases were least in the naive group and highest in the infected control group. This is due to the deposition of *S. mansoni* eggs in liver causing liver damage hence high levels in infected untreated group and low levels in Naïve group where there was no infection. PZQ and the three doses of Schitozim had higher levels when compared to Naïve but lower than IC. However, the three transaminases were still in the normal range required for a healthy animal. Hence, Schitozim had a hepatoprotective activity similar to that of Praziquantel because it was able to keep the level of the three tested transaminase within the normal range.

Bilirubin is the yellow pigment created by breakdown of haemoglobin which occurs in the liver, (Thapa and Walia 2007). Liver removes this pigment from the blood and low levels of bilirubin in serum can denote an impaired liver function due to deposition of schistosome eggs in the liver affecting the breakdown of bilirubin and its subsequent clearance from the liver and removal from blood. Elevated serum bilirubin levels generally reflect an imbalance between production and conjugation followed by excretion. An elevation in direct bilirubin is highly specific for biliary tract obstruction by schistosomes and their eggs (Krier and Ahmed 2009).

In this study, the lowest levels of bilirubin was observed in the Naïve group. The levels in the infected control group, PZQ and D1(50mg/kg) Schitozim were similar to Naïve therefore indicating that the ability for liver to clear bilirubin was not impaired. However higher levels of bilirubin were observed in D2 (150m/kg) and D3 (300mg/kg) Schitozim indicating that the ability of liver to clear bilirubin was impaired.Albumin is a protein made by liver. It prevents fluid from leaking out of blood vessels into tissues (Krier and Ahmed 2009). Hence a damaged liver will synthesize less of albumin compared to a normal liver. However serum albumin level is not a reliable indicator of hepatic protein synthesis in acute liver disease (Thapa and Walia 2007). Albumin synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure. Liver is the only site of synthesis of albumin. (Rosalki and McCintyre 1999). In this study, Albumin levels in the Naïve group were similar to D1, D2, PZQ and IC indicating normal liver functioning in the synthesis of albumin. A damaged liver will synthesize less of albumin compared to a normal liver (Thapa and Walia 2007), and hence elevated levels is an indication of impaired liver functioning. Levels in D3 Schitozim were much lower than Naïve showing probably that there was increased activity of liver in albumin synthesis as compared to all the other groups. Generally total protein levels, albumin and bilirubin levels in blood can be used to determine the extent of hepatic necrosis. Liver is one of the most important organs in body and is responsible for many physiological functions suitable for survival of any organism. Schistosomiasis leads to deposition of eggs in livers and in other parts of the body. High deposition of schistosome eggs in the liver impairs its function. As a result, deamination of excess amino acids, breakdown of haemoglobin in the liver and metabolism of albumin in the liver are reduced. (Pearce and MacDonald 2002).

Leucocytes increase in numbers in response to introduction of foreign particles in the body. Schistosomes and their eggs are very antigenic and once they are produced, they induce antibody-mediated immunity leading to proliferation of various classes of leucocytes around them forming granulomatous tissue. Leucocytes are expected to increase in blood at times of infection and hence their numbers were estimated in the various

treatment groups. The leucocytes determined include: eosinophils, lymphocytes, basophils, monocytes and neutrophils. In all treatments, Naïve had lowest counts of lymphocytes, eosinophils and monocytes. Lymhocytes and neutrophils were highest in infected control group while eosinophils were highest in the PZQ group. Generally, the eosinophils counts were few in all the treatment groups while the number of lymphocytes and neutrophils were high in all the treatment groups. Studies have shown that eosinophils are produced in large number during parasite infection and they have been argued to be the only cells responsible for antibody mediated damage of schistomula and reduced levels of eosinophils may denote a reduced immunity (Butterworth *et al*., 1997). High number of eosinophils and low number of neutrophils are needed for effective mediated antibody-dependent damage of schistosomula. Lymphoctes, moncytes and neutrophils do not enhance eosinophil mediated cytotoxity, Butterworth *et al*., (1997). Low numbers of eosinophils in the Schitozim and PZQ group may imply that there was reduced eosinophil mediated cytotoxicity possibly due to reduction of the number of schistosomes in mice when the drugs acted.The high total leucocytes counts in the infected group when compared to low counts in Naïve could imply that pathology was high in the IC group compared to the other groups. High number of neutrophils and lymphocytes can be attributed to high number of granulomas in various tissues of mice which cause proliferation of these cells since even with the reduction of the number of worms, they can still produce more eggs.

5. CONCLUSION AND RECOMMENDATION

The results obtained from this study show that there was no significant difference between Schitozim and praziquantel. In terms of worm recovery, the number of *Schistosoma mansoni* adult worms recovered in the mice groups treated with Schitozim was not significantly different from the number of worms recovered in the group treated with praziquantel. Secondly, Schitozim was able to maintain a normal level of the three tested transaminases which include: ALT, AST and ALP in mice infected with schistomes just like the praziquantel. Similarly, the levels of bilirubin, albumin were maintained at a normal range in the infected mice treated with Schitozim and Praziquantel. Eosinophils have been shown to be low in number in both schitozim and praziquantel treated groups. Lymphocytes and neutrophils were found to be high in numbers in the schitozim and praziquantel groups. 0-3hr protein release specific IgG responses, SWAP specific IgG responses and SEA specific IgG responses shows that the three responses were not significantly different among the Schitozim and Praziquantel treated groups. Lastly, phytochemical screening showed that flavonoids, glycoside, saponins, steroids and tannins were present in schitozim aqueous extract. These phytoconstituents were the active constituents in Schitozim which had anti-schistosomal activity. The study shows that there is a similarity in the efficacy of Schitozim compared to Praziquantel in the management of *S. mansoni* infection in BALB/c mice. Further research is needed to determine toxic level and effective dose of schitozim. Other studies which will investigate the levels of total cholesterol, triglycerides, High density cholesterol and low density cholesterol on Schitozim treated- *S. mansoni* infected mice are also needed as the metabolism of these compounds is directly linked to the liver.

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