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Effects of Aqueous and Methanol Extracts of *Zingiber officinale* on the Haematological Profile in *Schistosoma haematobium*-Infected Mice

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Abstract: The aim of the study was to evaluate the immunomodulating effects of aqueous and methanol extracts of *Zingiber officinale* on the haematological profile in *Schistosoma haematobium* - infected mice.The weight and haematological profiles of *Schistosoma haematobium* - infected mice were assessed to evaluate the immunomodulating effects of aqueous and methanolic extracts of *Zingiber officinale* at post-infection and post-treatment. There was a non-significant decrease ($p>0.05$) in the weight of mice in all the groups. However, there was a significant increase ($p<0.05$) in the total white blood cell counts, neutrophils and lymphocytes for group I (*S. haematobium* - infected mice treated with 9.5mg/kg aqueous extract of *Zingiber officinale*). In group II - (*S. haematobium* - infected mice treated with 87mg/kg methanolic extract of *Zingiber officinale*), there was significant increase ($p<0.05$) in the haemoglobin level and white blood cells. Methanolic extract of *Zingiber officinale* has better anti-anaemic effect than the aqueous extract of *Zingiber officinale* . There was no relationship between the level of eosinophils and the treated mice. No significant change in the haematological indices was observed in the group which was administered the standard drug- Praziquantel (PZQ). The immunomodualting effects of aqueous and methanol extracts of *Zingiber officinale* when compared to PZQ are promising in immunosuppressed *Schistosoma haematobium*- infected mice. Fractionation of the crude extract of *Zingiber officinale* may further identify the bioactive components required for treatment of urinary schistosomiasis.

Key words: Haemoglobin, immunomodulation, neutrophils and lymphocytes, *Schistosoma haematobium*, white blood cells, *Zingiber officinale*

INTRODUCTION

Schistosomiasis, also referred to as Bilharziasis is frequently referred to as the second most important parasitic disease after malaria among the most infectious diseases of tropical and Sub-tropical countries (Ojewole, 2004). *Schistosomiasis* is considered one of the Neglected Tropical Diseses (NTDs). It is the third most prevalent parasitic diseases in the world in terms of overall morbidity burden, socio-economic and public heath importance and human impact (Jordan, 2000).

The introduction of relatively safe, effective, broad-spectrum, oral anthelminthic agent, Praziquantel, constituted a significant land mark in the chemotherapeutic control of urinary schistosomiasis. To date, Praziquantel is the drug of choice for infections caused by *Schistosoma* spp. Its only limitation is the cost which restricts its use in many developing countries. However, despite the effectiveness of Praziquantel, there is a high reinfectivity rate in endemic areas even after mass treatment.

There has been an appreciable increase in research on bioactivity of natural products (Aladesanmi, 2007). The isolation and characterization of natural products from African medicinal plants without any biological or pharmacological testing has yielded numerous compounds of novel structure and constitutes the majority of all the recent publications on African medicinal plants (Sofowora, 1993; Sofowora, 2008).

Thus, this study was done to evaluate the immunomodulating effects of *Zingiber officinale* (L) against *S. haematobium* in experimental infection of Swiss Albino mice.

MATERIALS AND METHODS

The study was conducted between September, 2010 and October, 2011

Plant collection and authentication: Ginger (*Zingiber officinale*) was selected on the basis of ethnopharmacological information indicating its medicinal use in schistosomiasis control in some parts of Zaria, Nigeria. Fresh rhizomes of *Zingiber officinale* were obtained from Samaru market, Zaria, Nigeria. The authentication was done in the Herbarium by the

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Table 1: The mean weights (g) of swiss albino mice infected with *Schistosoma haematobium*

	Aqueous *Z. officinale*	Methanol *Z. officinale*	PZQ	NT
Pr-I	22.25	21.75	24.50	21.75
Pi	22.75	22.75	24.75	22.25
Pt	22.50	21.50	24.50-	-

PZQ: Praziquantel; Pr-i: Pre-Infection; Pi: Post-Infection; Pt: Post-Treatment; NT: Not Treated

Table 2: P-values for the comparison between post-infection and post-treatment for groups 1, 2, 3 and 4 using paired sample t-test

	Test		Control	
			[Positive]	[Negative]
Parameters	G1 (Azo)	G2(MZo)	G3(PZQ)	G4(NO TRT)
PCV (%)	0.053	-	0.083	-
Hb (g/dL)	0.192	0.018*	0.124	-
WBC (x 10^9/L)	0.006*	0.010*	0.187	-
Total protein	-	-	-	-
(g/dL)	0.609	0.670	0.134	-
Neutrophils (%)	0.032*	0.089	0.421	-
Lymphocytes (%)		0.019*	0.054	0.397-
Monocytes (%)	-	1.000	0.391	-
Eosinophils (%)	-	-	-	-
Basophils (%)	-	-	-	-
Bands (%)	0.391	0.141	0.391	-

*: $p<0.05$ (Significant difference); -: Test cannot be computed because the standard error of the difference is 0; Azo: Aqueous *Zingiber officinale;* Mzo: Methanolic *Zingiber officinale;* PZQ: Praziquantel; PCV: Packed Cell Volume; Hb: Haemoglobin; G: Group; WBC: White Blood Cell

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Plant extract preparation: The sliced fresh rhizomes of *Zingiber officinale* were dried in the laboratory for four (4) weeks. Extraction was carried out as described by Ojewole (2004) with slight modification. 1kg each of the air- dried plant materials was pulverized into fine particles using a mortar and pestle. It was further extracted in a Soxhlet apparatus for 24 h. The plant material was defatted in about 2.5 L hexane. The resultant marc was further extracted using 2.5 L ethyl acetate and finally with 2.5 L Methanol. The extracts were concentrated *in- vacuo* to dryness using a water bath. The powdered plant was cold macerated with distilled water at room temperature for 24 h. The resultant mixture was then filtered using Whatman's filter paper No. 1 and the filtrate was concentrated to dryness using water bath to obtain aqueous extracts of *Zingiber officinale*. It was scrapped-off and stored at 4ºC. This was done at the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. Aliquot portions of methanol extracts except aqueous extracts were first weighed and homogeneously suspended in TWEEN 80 then with distilled water for use on each day of the experiment.

Phytochemical analysis of *Zingiber officinale:* Phytochemical screening of the methanol and aqueous extracts of both *Z. officinale* for chemical constituents was performed according to standard laboratory procedures by Sofowora (2008) and Evans (2002). The plant extracts were screened for the presence of carbohydrates, glycosides, anthraquinones, cardiac glycosides, saponins, steroids and triterpenes, flavonoids, tannins and alkaloids.

Experimental animals: A total of Twenty-four (24) laboratory bred male and female Swiss Albino mice 18-20 g body weightwere used. The experiment was done according to International guidelines. Mice were housed in polypropylene cages at 25±2ºC with a 12 h light/12 h dark cycle and were given access to water and standard rodent food pellets *ad libitum*. The principles of animal care were also duly followed in this study.

Assessment of the mouse's body weight: The weight of mice at pre-infection, post-infection and post-treatment was assessed.

Acute toxicity test: Lorke's method (1983) was used to estimate the acute toxicity for Lethal Dose (LD_{50}) values. Three dose groups consisting of thirteen (13) mice were administered the aqueous and methanol extracts of *Z. officinale* at 10, 100 and 1000 mg/kg intraperitoneally in the first phase of the investigation. Animals in each group were observed for any immediate signs of toxicity and mortality within 24 h. The second phase of the acute toxicity testing was performed on another different set of animals by administration of different doses of extracts intraperitoneally based on the result obtained from phase one. They were also observed for another 24 h. Their mortalities were recorded and their LD_{50} was also calculated.

Infection of swiss albino mice with *Schistosoma haematobium* cerceriae: Four groups consisting of 4 mice each were infected with *Schistosoma haematobium* at a rate of infection of 130-150 cercariae per mouse (Adamu *et al*., 2006) using body immersion technique (Ismail *et al*., 2007).

Intraperitoneal administration of *Zingiber officinale* extracts: The animals were divided into six subgroups of 4 mice each, 8 weeks post- infection. Extracts of *Zingiber officinale* (ZO) was administered intraperitoneally (i:p.) for five (5) consecutive days as outlined below:

Group 1: Mice were administered 9.5 mg/kg aqueous extract of *Zingiber officinale* (ZO)
Group 2: Mice were administered 87 mg/kg methanol extract of *Zingiber officinale* (i.p.)
Group 3: Positive control (Praziquantel) 200 mg/kg i.p.
Group 4: Negative control (infected but not treated).

Table 3: Post Hoc comparison for post-infection and post-treatment with aqueous andmethanolic extracts of *Zingiber officinale* using Tukey-HSD

Parameters	G1 (Azo)(Pi) to(Pt)	G2 (Mzo) (Pi) to (Pt)	G3 (PZQ)(Pi) to (Pt)	G4 (NO TRT)(Pr-i) to (Pi)
PCV (%)	47.00-40.75	45.50-43.40	48.00-42.00	43.00-40.50
Hb (g/dL)	15.65-14.15	15.13-14.35	15.98-15.18	14.40-13.93
WBC ($\times 10^9$/L)	5.45-11.80	5.43-13.03	8.40-12.70	7.05-11.33
Total protein (g/dL)	6.35-5.63	7.00-7.25	6.15-5.75	6.90-6.78
Neutrophils (%)	21.50-43.25	23.75-39.50	28.75-34.50	24.50-41.25
Lymphocytes (%)	78.50-56.50	75.50-60.00	71.00-67.00	79.50-70.75
Monocytes (%)	0.00-0.00	0.25-0.25	0.00-0.25	0.00-0.00
Eosinophils (%)	0.00-0.00	0.00-0.00	0.25-0.25	0.00-0.00
Basophils (%)	-	-	-	-
Bands (%)	0.00-0.00	0.50-0.00	0.00-0.00	0.00-0.00

-: The test cannot be computed because the standard error of the difference is 0; G: Group; Azo: Aqueous *Zingiber officinale;* Mzo: Methanolic *Zingiber officinale*, Pr: i-Pre- Infection, Pi: Post- infection; Pt: Post treatment; TRT: Treatment; PZQ: Praziquantel; PCV: Packed Cell Volume; Hb: Haemoglobin; WBC: White Blood Cell

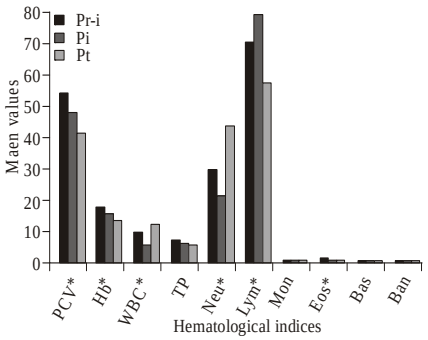

Fig. 1: Haematological profile of mice treated with Aqueous *Zingiber officinale* (AZo) at pre- infection (pr-i), postinfection (pi) and post treatment (pt), Parameters with asterisk(*) vary significantly ($p<0.05$). Others did not vary significantly

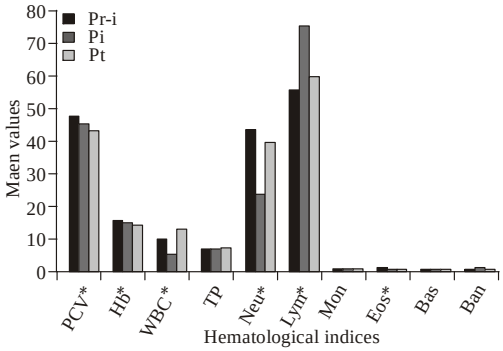

Fig. 2: Haematological indices of mice treated with methanolic *Zingiber officinale* (MZo) extract at pre-infection (pr-i), post-infection (pi) and post treatment (pt).Parameters with asterisk (*) vary significantly ($p<0.05$). Others did not vary significantly

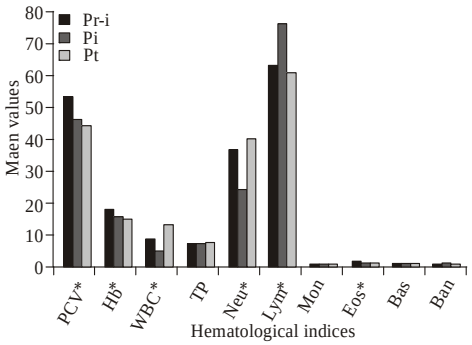

Fig. 3: Haematological responses of mice treated with praziquantel (PZQ) at pre-infection (pr-i), post-infection (pi) and post treatment (pt), Parameters with asterisk(*) vary significantly ($p<0.05$). Others did not vary significantly

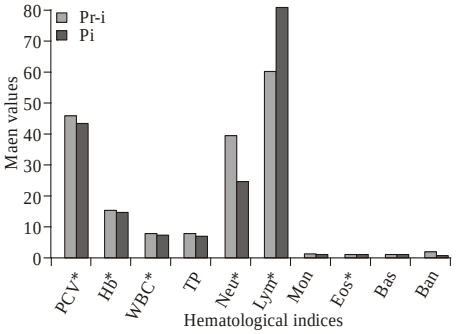

Fig. 4: Haematological indices of mice not treated (NT) at pre-infection, post-infection and post- treatment, Test statistic - Parameters with * vary significantly ($p<0.05$). Others did not vary significantly

Monitoring of haematological indices in cercariae-infected swiss albino mice: The immunomodulating effects of *Zingiber officinale* (ZO) extracts were assessed by monitoring some haematological indices at post-infection and post-treatment viz: Packed Cell Volume (PCV), White Blood Cell (WBC), Total plasma protein, Differential Leucocyte Counts (DLC) using Standard methods.

Statistical analysis: Data analysis was performed using Statistical Package for Social Science (SPSS) version

16.0. Statistical methods employed included paired sample t-test, Post Hoc comparison using Tukey-HSD and Analysis of Variance. p-values less than 0.05 ($p<0.05$) were considered significant.

RESULTS

Phytochemical analysis: Phytochemical analysis of the aqueous extract of *Zingiber officinale* (ZO) showed the presence of carbohydrates, glycosides, cardiac glycosides, saponins, flavonoids, steroids and triterpenes and tannins. However, methanol extract of *Zingiber officinale* (ZO) contained the same compounds as the aqueous extract though tannins were absent.

Table 1 shows the non-significant decrease ($p>0.05$) in the mean weight of mice infected with *Schistosoma haematobium* for all groups at pre-infection, post-infection and post-treatment.

Acute toxicity studies: The intraperitoneal median lethal dose (LD_{50}) values of the plant's extracts were: Aqueous *Zingiber officinale* (31.6 mg/kg body weight) and Methanol *Zingiber officinale* (288.5 mg/kg body weight).

Comparison between post-infection and post-treatment in all groups: Table 2 shows the comparison between Post-infection and Post- treatment for groups 1, 2, 3 and 4.

There was a significant increase ($p<0.05$) in White Blood Cell counts (WBC), neutrophils and lymphocytes in group 1 (mice infected and treated with 9.5mg/kg aqueous extract of *Zingiber officinale* -AZo).This means that the aqueous extract had immunomodulating effect on these haematological indices. While in group 2 (MZo), the level of haemoglobin and the white blood cell count improved significantly ($p<0.05$) in mice treated with the methanolic extract of *Zingiber officinale* in S. haematobium infected mice. This means that the methanol extracts (MZo) of *Zingiber officinale* had better anti-anaemic effect than the aqueous extract (AZo).

There was also no relationship between the level of eosinophilia and the intensity of the infection in all groups. PCV, monocytes, basophils and bands were not significant ($p>0.05$) for all groups. There were no significant levels of serum total protein for all groups including group 3 (PZQ) as shown in Table 2.

Table 3 shows Post hoc comparison for post-infection and post-treatment with aqueous and methanolic extracts of *Zingiber officinale* using Tukey-HSD. There was an increase in the number of White Blood Cells (WBC) at post-infection (p.i.) and post-treatment (p.t.) for the groups 1 and 2.

Figure 1 and 2 shows the haematological profile of mice treated with aqueous and methanol extracts of *Zingiber officinale* at pre- infection, post-infection and post treatment respectively.

DISCUSSION

A critical study on the haematological profile of the mice shows some degree of immunological processes occurring at post-infection and post-treatment as can be seen in (Fig. 1 to 4).

Effects of aqueous and methanolic *zingiber officinale* in cercaria-infected mice: The methanol extract of *Zingiber officinale* (MZo) improved the Haemoglobin level in *Schistosoma haematobium* infected mice than the aqueous extract (AZo). This means the former had better anti-anaemic effect.

AZo and MZo treated mice also showed significant levels of white blood cells, neutrophils and lymphocytes. This indicated a positive immunostimulatory effect of the plant extract in response to the circulating schistosomula. This is unlike the immune response in the positive and negative control groups which showed no level of significance ($p>0.05$).

These findings are in consonance with those of Arinola and Salimonu (1999) who found that neutrophils destroy circulating schistosomula by both direct physical means using pseudopodia and enzyme action of the vacuoles. They extrude enzymes such as betaglucoronidase, lactic dehydrogenase, myeloper oxidase and certain vasoactive amines along the free border of their cells. After which the adherent neutrophils extend their pseudopodia processes into the tegument resulting in the death of schistosomula. Therefore, the aqueous and methanol extracts of *Zingiber officinale* exhibited immunomodulating effects by stimulating the production of neutrophils (Immunostimulation).

ANOVA test of p-values for comparisons between groups showed that at post-infection, monocytes were highly significant ($p<0.05$). Monocytes are less mature circulating forms of macrophages. When matured, they engulf schistosomula in the host's blood. This explains the significant level of the monocytes for all the groups at post-infection while at post-treatment, lymphocytes were highly significant ($p<0.05$) when compared with other haematological indices for all groups. The p-values for all haematological indices can be seen with significant lymphocytes in host's blood circulation at post-treatment.

This is probably due to the fact that, there was proliferation of lymphocytes in circulation. However, the significant reduction observed in the circulating lymphocytes after treatment indicates that this immunological response was due to the downregulation of T-cell mediated immune response hence indicating the potent activity of all extracts when compared with praziquantel.The post hoc comparisons using Tukey-HSD shows an increase in the number of White Blood Cells (WBC) at post-infection (p.i.) and post-treatment (p.t.) for

individual groups as represented in Fig. 1-4. A significant rise in the level of total WBC counts after treatment could be attributed to a significant rise in lymphocytes and neutrophils. This indicated a general immunological response. Immunomodulation is an essential mechanism in directing the clinical and pathologic outcome of schistosomiasis. Lymphocytes responded to the schistosomulae in circulation and proliferated leading to quite a high number above normal in the host's immune system. Studies conducted in Brazil confirmed a previous finding that, lymphocytes from patients with early *S. mansoni* infection are hyper-reactive to worm and egg antigens in proliferation assays (Gazzinelli *et al*., 1983).

No significant relationship was observed between the degree of eosinophilia and the intensity of infection for all groups post-infection and post-treatment as documented by other workers (Elagba *et al*., 2006; McPherson and Pincus, 2007). There was a decrease in eosinophil numbers in the absence of treatment, suggesting active downregulation of the eosinophilia. This is in agreement with the findings of Klion and Nutman (2004).

The increased levels of serum total plasma protein indicating liver function or dysfunction is evidence of hepatocellular injury. This agrees with the work and Elagba *et al*. (2006) where low levels of serum total proteins and albumin in *S. haematobium* patients.

The immunomodulating effects of aqueous and methanol extracts of *Jatropha curcas* when compared to Praziquantel are promising in immunosuppressed *Schistosoma haematobium*- infected mice.

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