



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2021; 9(3): 16-21

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Received: 12-03-2021

Accepted: 15-04-2021

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Assessment of the biochemical and hematological variations in mice infected with *Schistosoma mansoni* Cercariae

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Abstract

This study investigates the biochemical and haematological variations occurring in mice infected with doses of *Schistosoma mansoni* cercariae using a mouse model. The study was conducted in Jos North Local Government Area of Plateau State Nigeria from March through April 2018. Nine female albino mice weighing averagely 20-45g were subdivided into three (3) groups. Group 1 and 2 were injected intraperitoneally with high dose (2ml) and low dose (1ml) of solution containing *S. mansoni* cercariae while the third group remained uninfected thus served as control. On the 30th day of experimentation blood samples were obtained for evaluation of haematological and biochemical parameters. The study showed a significant ($P < 0.05$) difference in the enzymes and albumin (Alanine Transaminase, Aspartate Transaminase, Albumin) levels of the infected groups as compared to the control group. Lipid levels and electrolytes were also significantly different ($P < 0.05$). Also recorded was a significant increase in hematological parameters (White Blood Count, Red Blood Count, Mean corpuscular volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, Platelets, Lymphocytes, Neutrophils, Monocytes) at $P < 0.05$.

Keywords: Biochemical, Hematological, Variations, Mice, *Schistosoma mansoni* Cercariae

1. Introduction

Schistosomiasis or Bilharziasis is a parasitic disease which is caused by flukes (trematodes) belonging to the genus *Schistosoma*. It is regarded as the third most devastating tropical disease in the world after malaria and intestinal helminthiasis. The disease is considered as a major source of morbidity and mortality for developing countries in Africa, South America, the Caribbean, the Middle East, and Asia ^[1]. Over 207 million people, 85% of them living in Africa, are infected with schistosomiasis ^[1] with an estimated 700 million people at risk of the infection in 76 countries where the disease is considered endemic. This is due to their agricultural work, domestic chores, and recreational activities which expose them to infested water where they can acquire the infection.

Globally, as much as 200,000 deaths are attributed to this infection annually ^[2] with Nigeria being thought of as the most schistosomiasis-endemic African nation ^[3]. It was not until 1908 when the first case of bilharzia was reported in Nigeria and was thought to be more widespread than ever contained in records. According to ^[4], Nigeria's growing population remains vulnerable to schistosomiasis. In view of little or no actively planned control programmes for the disease (schistosomiasis) in Nigeria, it has become a 'come to stay' disease. Schistosomiasis affects virtually all populations, but its effect is worst in children and adults practicing unprotected irrigation farming and fishing. Nigeria as a country is said to have about 24 million people at risk of schistosomiasis with an overall prevalence of 9.5% ^[5]. Several prevalence and intensity reports on schistosomiasis have emerged from around the country; revealing that prevalence and intensity of this infection increase with age and peaks in the 5-14 years age group ^[6] as they are mostly unaware of the risks of transmission of the infection via cercariae-infested water bodies which account for more of the infections occurring in the country ^[7]. The different species of *Schistosoma* have different snail species serving as their intermediate hosts; thus, *Biomphalaria* serves as host for *S. mansoni*, *Oncomelania* for *S. japonicum*, *Tricula (Neotricula aperta)* for *S. mekongi* while *Bulinus* serves as host for *S. haematobium* and *S. intercalatum* respectively ^[8, 9, 10]. When suffering from schistosomiasis, the symptomatic, acute phase of the illness is known as Katayama fever and presents with normal fever, malaise, urticaria and eosinophilia.

Other symptoms can include cough, diarrhoea, weight loss, haematuria, headaches, joint and muscle pain, and enlargement of the liver and spleen ^[11].

Chronic infection with *S. mansoni* and *S. japonicum* causes periportal liver fibrosis and portal hypertension with ascites and oesophageal varices ^[12]. Diagnosis of the disease can be done by microscopy examination of stool or urine preparations for *Schistosoma* ova; by finding ova on rectal biopsy; or with serology to detect antibodies to schistosomal antigens or the antigens themselves ^[13]. This study therefore investigates the biochemical and hematological variations occurring in mice exposed to doses of *Schistosoma mansoni* cercariae.

2. Materials and Methods

2.1 Study Area

The study was conducted from March through April 2018 in Jos North Local Government Area of Plateau State Nigeria. Jos North Local Government has an area of 291km² and a population of approximately 429,300 people. Plateau State is located in North Central Nigeria. The area was selected for the study as a result of numerous reports from various health facilities about haematuria resulting from Schistosomiasis in the area. The area has a mean annual rainfall of 131.75cm - 146 cm, which draws human activities to water bodies.

2.2 Host-Parasite Model

All samples collected were analyzed at the National Veterinary Research Institute, Vom, Jos Plateau State under the supervision of a Senior Laboratory Technologist while adhering to standard laboratory procedures. The National Veterinary Research Institute, Vom, has a GPS co-ordinate of N 9566.480 and E 85442.880. *S. mansoni* -mouse model was used throughout this study. Nine female mice with average weight of 20-45g were purchased from National Veterinary Research Institute, Jos, Plateau State. They were kept in three groups (containing 3 mice each) and were fed commercial rodent feed and water.

2.3 Collection of fresh water snails

The fresh water snails (*Biomphalaria spp*) were collected by hand picking, from Angwan Rukuba River, Jos North at about 8am to 9am in the morning.

2.4 Infection of mice/inoculation of mice with *S. mansoni* cercariae

100 ± 10 *S. mansoni* cercariae per mouse were inoculated into six (6) mice intraperitoneally using a 2ml syringe. Each of the three mice in the first group was administered 1ml of water solution containing *S. mansoni* cercariae while each of the three mice in the second group was administered 2ml of water solution containing *S. mansoni* cercariae. The cercariae were obtained or harvested from wildy-bred and naturally infected snails (*Biomphalaria glabrata*) by exposing them to artificial light for over 4 hours. The other (third) group of mice was left uninfected. Hence, they served as the control group.

2.5 Haematological examination of mice

2.5.1 Haematocrit (Packed Cell Volume)

The haematocrit or packed cell volume (PCV) determines the percentage of red blood cells (RBCs) in whole blood of infected mice in comparison to the control group at each phase of the experiment.

2.5.2 Micro-Haematocrit Procedure

Two-thirds to three-quarters of heparinized capillary tubes were filled with well-mixed, venous blood. One end of the capillary tube was sealed with wax and placed in a micro-haematocrit centrifuge, with the plugged end away from the centre of the centrifuge. The samples were then spun at a pre-set speed of 10,000 to 12,000 rpm for 5 minutes. If the haematocrit exceeds 50 percent, the sample was centrifuged for an additional 3 minutes. The haematocrit was then read using a microhaematocrit reader.

2.6 Determination of Biochemical Parameters

2.6.1 Determination of Serum Glucose (Randox kit)

Three test tubes labeled T, S and B were set for test, standard and blank respectively. To each of the three tubes, 1000µl of phenol reagent was added. To the test tube labeled 'T' 10µl of serum was added, to the test tube labeled 'S' 10µl of glucose standard was added. While to the blank Test tube 'B', 10µl of distilled water was added. The contents in the test tubes were mixed and incubated at 37 °C for 10 minutes. The absorbance of test and standard were then read against the blank using a spectrophotometer within 60 minutes at 546nm.

Where; A sample = absorbance of test (sample) and A standard = absorbance of standard.

2.6.2 Determination of Total Protein

Protein concentrations of the various samples were determined by means of the Biuret method as described by Gornal *et al.* ^[14] with some modification (potassium iodide was added to the reagent to prevent precipitation of Cu²⁺ ions as cuprous oxide).

2.6.3 Determination of Serum Creatinine

The method of Gornal *et al.* ^[14] was used in the determination of serum creatinine. The method uses the Jaffe reaction which involves the production of coloured complex when creatinine in alkaline solution reacts with picric acid solution.

2.7 Statistical Analysis

Graph pad prism version 7.0 (ANOVA and student T-test) was used to compare the differences in haematological and biochemical parameters between the infected groups and control group.

3. Results

Table 1 shows the enzymes and albumin level of both infected and control groups of mice. There was a rise in ALT levels in both low and high dose groups from 58.000 ± 4.839 (U/L) in the low dose group to 83.533 ± 14.633 (U/L) in the high dose group with a corresponding very low level in the control group 27.600 ± 8.879 (U/L). The ALP levels also increased in the exposed groups from 128.33 ± 15.857 (U/L) in the low dosage group to 213.67 ± 51.096 (U/L) in the high dosage group against the control batch with 110.67 ± 65.730 (U/L). The AST levels significantly increased from 624.67 ± 52.844(U/L) in the low dosage group to 993.33 ± 113.700(U/L) in the high dosage group against the control group with 482.00 ± 120.900 (U/L). Similarly, ALB levels significantly increased from 16.503 ± 1.625 (g/dl) in the low dosage group to 17.970 ± 0.707 (g/dl) in the high dosage group as against the 12.433 ± 0.923 (g/dl) in the control group (*p*<0.05).

Table 1: Enzyme and Albumin Level of Albino Mice

Group	ALT (U/L)	ALP (U/L)	AST (U/L)	ALB g/dl
Control	27.600 ± 8.879	110.67 ± 65.730	482.00 ± 120.900	12.433 ± 0.923
Low Dose	58.000 ± 4.839 ^a	128.33 ± 15.857 ^a	624.67 ± 52.844 ^a	16.503 ± 1.625 ^a
High Dose	83.533 ± 14.633 ^a	213.67 ± 51.096 ^a	993.33 ± 113.700 ^a	17.970 ± 0.707 ^a

Values are expressed as mean ± SEM, n=3.

^aValues are significantly different when compared with control ($p < 0.05$)

Keys

ALT-Alanine Transaminase

ALP-Alkaline phosphatase

AST-Aspartate Transaminase

ALB -Albumin

Table 2 shows the lipid profile of both the infected and the control groups. TP levels increased significantly in both the low and high dosage groups from 30.267 ± 2.469 (g/dl) to 34.400 ± 2.212 (g/dl) respectively against 25.600 ± 1.650 (g/dl) in the controlled group. There was a considerable decrease in the level of TBIL in the infected groups against the control. The low and high dose groups showed lower levels at 29.300 ± 11.753 (µmol/L) and 26.033 ± 5.657 (µmol/L) respectively against the control group with significantly high level at 37.933 ± 8.134 (µmol/L). The UREA levels showed some slight variations as the low dosage group recorded 3.820 ± 0.226 (mmol/L) with the high dosage group recording 5.433 ± 1.783 (mmol/L) while the control group recorded 5.917 ± 1.297 (mmol/L). CREATININE and UREA showed significant variations in their levels in comparison with control and the exposed groups. The exposed group with low dosage recorded CREATININE level of 1.985 ± 0.827 (mg/dl) with a slight increase in the exposed group with high dosage 2.817 ± 1.416 (mg/dl). This was however higher in the control group than in both the exposed groups at low and high dosage, 3.025 ± 1.417 (mg/dl). There was a significant linear increase in UA from 109.800 ± 12.384 (µmol/L) in the low dosage group to 137.500 ± 32.303 (µmol/L) in the high dosage group. The control group recorded a significantly lower value, 79.833 ± 30.084 (µmol/L) ($p < 0.05$).

Table 2: Lipid Profile level of Albino Mice

Group	TP (g/dl)	TBIL (µmol/L)	UREA (mmol/L)	CREATININE (mg/dl)	UA (µmol/L)
Control	25.600 ± 1.650	37.933 ± 8.134	5.917 ± 1.297	3.025 ± 1.417	79.833 ± 30.084
Low Dose	30.267 ± 2.469 ^a	29.300 ± 11.753 ^a	3.820 ± 0.226 ^a	1.985 ± 0.827	109.800 ± 12.384 ^a
High Dose	34.400 ± 2.212 ^a	26.033 ± 5.657 ^a	5.433 ± 1.783 ^a	2.817 ± 1.416 ^a	137.500 ± 32.303 ^a

Values are expressed as mean ± SEM, n=3.

^aValues are significantly different when compared with control ($p < 0.05$)

Keys

TP-Total Protein

TBIL-Total Bilirubin

UREA -Urea

CREATININE-Creatinine

U/A-Urinalysis

Table 3 shows the electrolytes level of the control and the infected mice groups. Assessing the electrolyte balancing of

potassium K⁺ and calcium Ca⁺, the K⁺ level showed some variations between the control group, 5.832 ± 0.712 (mmol/L) and the low dosage, 4.490 ± 0.323 and high dosage group 5.797 ± 0.896. Meanwhile, Ca⁺ increased significantly in the exposed groups from 1.147 ± 0.146 (mmol/L) in the high dose group to 1.217 ± 0.167 (mmol/L) in the low dose group in comparison to the control group 1.117 ± 0.063 (mmol/L) ($p < 0.05$).

Table 3: Electrolytes level of the Albino Mice

Group	K ⁺ (mmol/L)	Ca ⁺ (mmol/L)
Control	5.832 ± 0.712	1.117 ± 0.063
Low Dose	4.490 ± 0.323 ^a	1.147 ± 0.146 ^a
High Dose	5.797 ± 0.896 ^a	1.217 ± 0.167 ^a

Values are expressed as mean ± SEM, n=3.

^aValues are significantly different when compared with control ($p < 0.05$)

Keys

K⁺-Potassium

Ca⁺-Calcium

Table 4a shows the haematological parameters of both the infected and control group. WBC increased linearly in the exposed groups from 4.100 ± 1.150 (×10³/µl), in the low dose group to 7.613 ± 1.371 (×10³/µl) in the high dose group while in the control group it was lower 3.977 ± 1.495 (×10³/µl). The RBC also linearly increased in the exposed groups from 7.737 ± 0.557 (×10⁶/µl) in the low dose group to 8.650 ± 0.577 (×10⁶/µl) in the high dose group respectively while in the control group, it was lower at 4.690 ± 1.076 (×10⁶/µl). The HGB showed some variations in characteristics as the low dose group 10.800 ± 1.419 (g/dl) had a higher value than the control group 10.710 ± 1.355 (g/dl) and the high dose group 10.473 ± 1.205 (g/dl). The HCT was increased exponentially 44.233 ± 3.589 (%) in the low dose group and marginally 37.240 ± 8.616 (%) in the high dose group in comparison to the lower level 29.407 ± 9.860 (%) in the control group. The MCV levels showed linear increase in the exposed groups from 55.933 ± 1.139 (fL) in the low dose group to 57.350 ± 0.794 (fL) in the high dose group respectively while the control group had a lower value 55.460 ± 2.267 (fL). The MCH levels also marginally increased in both exposed groups from 14.137 ± 0.087 (pg) in the low dose group to 14.270 ± 0.173 (pg) in the high dose group respectively in comparison with the control group with a slightly lower level 14.097 ± 0.068 (pg) ($p < 0.05$).

Table 4a: Hematological Parameters

Group	WBC (×10 ³ /µl)	RBC (×10 ⁶ /µl)	HGB (g/dl)	HCT (%)	MCV (fL)	MCH (pg)
Control	3.977 ± 1.495	4.690 ± 1.076	10.710 ± 1.355	29.407 ± 9.860	55.460 ± 2.267	14.097 ± 0.068
Low Dose	4.100 ± 1.150 ^a	7.737 ± 0.557 ^a	10.800 ± 1.419 ^a	44.233 ± 3.589 ^a	55.933 ± 1.139 ^a	14.137 ± 0.087 ^a
High Dose	7.613 ± 1.371 ^a	8.650 ± 0.577 ^a	10.473 ± 1.205 ^a	37.240 ± 8.616 ^a	57.350 ± 0.794 ^a	14.270 ± 0.173 ^a

Values are expressed as mean ± SEM, n=3

^aValues are significantly different when compared with control ($p < 0.05$)

Keys

WBC -White Blood Count

RBC -Red Blood Count

HGB -Haemoglobin

HCT -Haematocrit

MCV -Mean corpuscular volume

MCH -Mean corpuscular haemoglobin

Table 4b shows the hematological parameters of both the infected and control group of mice. The MCHC values increased marginally in the exposed groups from 24.130 ± 0.187 (g/dl) in the low dose group to 25.337 ± 0.117 (g/dl) in the high dose group in comparison to the lower values in the control group at 24.103 ± 1.083 (g/dl). The platelets count (PLT) also increased significantly in the exposed groups from 490.67 ± 16.826 ($\times 10^3/\mu\text{l}$) in the low dose group to 826.67 ± 167.74 ($\times 10^3/\mu\text{l}$) in the high dose group as against the lower value recorded in the control group at 419.67 ± 41.874 ($\times 10^3/\mu\text{l}$). In assessing the differential cell counts of lymphocytes (LYM), there was a significant increase in the percentage of lymphocytes in the exposed groups compared to the control group at 63.137 ± 3.015 (%) in the low dose group and 76.373 ± 7.200 (%) in the high dose group while the control was lower at 61.413 ± 11.989 (%) respectively. The neutrophils also significantly and exponentially decreased in percentage in relation to the dosage of exposure in the different groups. The low dose group recorded 30.000 ± 3.512 (%) while the high dose group recorded 18.667 ± 6.045 (%) and the control group had a neutrophil count of 61.413 ± 11.989 (%). The monocytes (MONO) exhibited the same irregular characteristics with an increase in percentage in the low dose group, 6.297 ± 0.889 (%) and a drop in percentage in the high dose group, 4.960 ± 1.190 (%) which was lower than in the control group, 5.927 ± 0.515 (%).

Table 4b: Hematological Parameters

Group	MCHC (g/dl)	PLT ($\times 10^3/\mu\text{l}$)	LYMP (%)	NEUT (%)	MONO (%)
Control	24.103 ± 1.083	419.67 ± 41.874	61.413 ± 11.989	61.413 ± 11.989	5.927 ± 0.515
Low Dose	24.130 ± 0.187^a	490.67 ± 16.826^a	63.137 ± 3.015^a	30.000 ± 3.512^a	6.297 ± 0.889^a
High Dose	25.337 ± 0.117^a	826.67 ± 167.74^a	76.373 ± 7.200^a	18.667 ± 6.045^a	4.960 ± 1.190^a

Values are expressed as mean \pm SEM, n=3.

^aValues are significantly different when compared with control ($p < 0.05$)

Keys

MCHC -Mean corpuscular haemoglobin concentration.

PLT -Platelets

LYMP -Lymphocytes

NEUT -Neutrophils

MONO-Monocytes

4. Discussion

This study finds that the enzyme and albumin (ALT, ALP, AST and ALB) levels in plasma of infected mice was elevated significantly ($P < 0.05$) in comparison to a significant decrease of albumin as observed in the control group. ALT in both low and high dose groups was elevated when compared to the control group who had lower level. Also, ALP levels increased in the exposed groups as against the control batch which had a lower level. Similarly, AST levels were also observed to be significantly higher in the infected groups than in the control group with the ALB levels also being significantly increased in both low and high dosage groups compared to the control group which had lower levels. These results agree with that obtained by Nahla *et al.* [15], Naglaa *et al.* [16], Mahmoud *et al.* [17] and Marwa *et al.* [18]. They observed that infection of mice with *S. mansoni* results in

significant increase in serum levels of ALT, ALP, AST and ALB. These enzymes have been shown to be commonly employed as biological markers for hepatic cell damage and impaired cell membrane permeability which is due to heavy *Schistosoma* egg deposition [19]. The increase in enzyme and the decrease in plasma albumin may have been due to liver dysfunction. The increased levels of enzyme, associated with the intensity of infection, could also have been due to immunological reactions and the production of immunoglobulins.

The TP levels increased significantly in both the low and high dosage groups against the controlled group. Conversely, there was a considerable decrease in TBIL level in the exposed groups against the control group. The low and high dose groups showed lower levels against the control group with significantly high level. The UREA levels showed some slight variations as the low dosage group had the least level, followed by the high dosage group with the control group showing the highest level. The CREATININE and UREA showed significant variations in its levels in the control and the exposed groups. The exposed group infected with low dosage recorded low levels, with a slight increase in the exposed group with high dosage. The CREATININE in the control group was higher than in either of the exposed groups. There was a significant linear increased in UA in both exposed groups with the control group recording a significantly lower level.

Phospholipids are believed to act as important mediators in immunological modification and signal transduction [20]. However, the irregular characteristics of phospholipids in this study might suggest that Schistosomes are incapable of synthesizing fatty acids *de novo*, thus they must absorb and utilize exogenous lipids from the host's blood and incorporate them into their membrane structures in order to evade the host immune response [21]. The decreased level of phospholipids such as phosphatidylcholine in plasma from the *S. mansoni*-infected mice could be due to absorption of phosphatidylcholine by the parasite. Alternatively, the parasite could provide free choline by its breakdown to diglycerides synthesized within schistosomes, which may also explain the lower levels of glycerophosphorylcholine in the plasma of infected mice. These findings agree with the work done separately by Rumjanek [22] and Li [23], in which they both reported similar findings on lipid utilization and incorporation by *S. mansoni* worm *in vitro*.

Assessing the electrolyte balancing of potassium K^+ and calcium Ca^+ revealed that there were some variations in the K^+ and Ca^+ levels in the control group in comparison to the exposed groups. Several of the metabolic changes observed in the *S. mansoni*-infected mice such as increased albumin level suggest an alteration in energy metabolism. Taken together with the decreased plasma K^+ level, this suggest that activities of glycolysis-involved pyruvate kinase and phosphofructokinase in the liver were greater in *S. mansoni*-infected mice as was also described Rumjanek *et al.* [22]. This also suggests an infection-induced stimulation of glycolysis. The decrease in K^+ and increase in Ca^+ also suggests a disturbance in amino acid metabolism, and is likely to result into liver dysfunction as observed by Rumjanek *et al.* [22].

Mice infected with the *S. mansoni* cercariae showed an increase in blood values (WBC, RBC's, HCT, MCV, MCH, MCHC, PLT, LYM) through the two interval times. The HGB showed some variations in characteristics as the level was higher in the low dose group than in the control group and a

corresponding significant decrease in the in the high dose group as compared to the control group. The characteristic observed was similar to NEU and MONO cells in the different groups of experimental animals. All the pathophysiological changes that accompanied the course of the infection indicated that *S. mansoni* is a pathogenic parasite and all the infected mice showed a severe level of anemia. These findings are in agreement with those of Abdel-Ghaffar *et al.* [24], Szeranfin *et al.* [25] and Omran *et al.* [26]. The above findings of anemia might be likely due to due to blood loss, increased hemolysis and iron deficiency.

The direct loss of erythrocytes may arise from the extrusion of schistosomal ova or because of the consumption of blood by schistosomes as recorded by Mahmoud *et al.* [27] and Mahmoud *et al.* [28]. Infection with *S. mansoni* was found to cause an elevation in WBC's counts and differential WBC's, which has been indicated by the increase in Lymphocytes and a decrease in Neutrophils and monocytes in response to *S. mansoni* infections. However, this study contrasted the works of Marwa *et al.* [18], Szeranfin *et al.* [25] and Abbas *et al.* [29] as they all suggested linear increase in differential cell counts of infected mice with *S. mansoni*. The current study however observed a downward trend in the percentage of neutrophils and monocytes.

5. Conclusion

These findings have shown the basic haematological, and biochemical changes that could occur due to infection by *S. mansoni* cercariae and has provided a baseline information for proper planning and implementation of Schistosomiasis control activities in the rural area where this disease is endemic. Proper health measures should be taken to treat already infected people and limit the rate of infection of non-infected people.

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