

**EFFECT OF AQUEOUS LEAF EXTRACT OF *ANDROGRAPHIS PANICULATA* ON  
*SCHISTOSOMA MANSONI* INFECTED MICE**C. Igbeneghu<sup>1\*</sup>, M. J. Olisekodiaka<sup>2</sup> and K. Ariwoola<sup>1</sup><sup>1</sup>Department of Biomedical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.<sup>2</sup>Department of Chemical Pathology, Faculty of Medicine, Nnamdi Azikiwe University, Awka, Nigeria.**\*Corresponding Author: C. Igbeneghu**

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Article Received on 07/06/2018

Article Revised on 28/06/2018

Article Accepted on 19/07/2018

**ABSTRACT**

**Aim:** To investigate the possible effect of aqueous leaf extract of *Andrographis paniculata* (*A. paniculata*) on mice infected with *Schistosoma mansoni* (*S. mansoni*). **Study Design:** Parasitological, haematological and biochemical assessment of treatment activity of the aqueous leaf extract of *A. paniculata* on mice infected with *S. mansoni*. **Place and Duration of Study:** Department of Biomedical Sciences Ladoke Akintola University of Technology, College of Health Sciences, Osogbo, Nigeria between March and May, 2013. **Methodology:** 48 male mice (range 18-22 g) were randomly divided into four groups (A-D) each consisting of 12 mice each and in replicate of two: group A received water (0.5 ml/kg), group B received aqueous *Andrographis paniculata* (10 mg/kg), group C was infected with *S. mansoni* for six weeks and group D was infected with *S. mansoni* for two weeks and then treated with *A. paniculata* (10 mg/kg) for four weeks. Worm burden and egg load, haematocrit, haemoglobin concentration, red blood cell counts, serum total protein and albumin concentration and activities of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined using standard methods. **Results:** Worm burden and egg load were significantly lower ( $p < 0.001$ ) while haematocrit, haemoglobin concentration and erythrocyte count were significantly higher ( $p < 0.001$ ) in infected treated mice than in infected non-treated mice. Mean activities of ALT, AST, ALP and serum total protein and albumin levels were significantly higher ( $p < 0.001$ ) in infected treated mice when compared with respective means of infected non-treated. **Conclusion:** Results from the present study suggest that *A. paniculata* possibly possesses anti-schistosomal properties.

**KEYWORDS:** *Andrographis paniculata*; *Schistosomiasis*; *Schistosoma mansoni*.**INTRODUCTION**

Schistosomiasis is one of the leading parasitic infections in tropical and sub-tropical countries. It is the most widespread chronic debilitating water-borne parasitic disease and remains a public health problem where it occurs.<sup>[1]</sup> Five species of schistosomes infect man but in Africa the most common ones are *Schistosoma haematobium* and *S. mansoni* that cause urinary and intestinal schistosomiasis respectively. Global estimates indicate that 600 million are at risk of schistosome infection and 200 million people from 76 countries are infected with schistosomiasis, out of which 85% are from sub-Saharan Africa.<sup>[2,3]</sup> Schistosomiasis control measures include chemotherapy, health education, good sanitation, avoiding contact with contaminated water and control of the vector snails.<sup>[2]</sup> Currently, the drug of choice for schistosomiasis is praziquantel (PZQ).<sup>[4]</sup> However, the emergence of schistosomes resistant to praziquantel has been reported.<sup>[5,6]</sup> Consequently, there has been increase

in many researchers' interest of developing alternative drugs of plant origin.<sup>[7]</sup>

In Ilie, a rural community in Southwestern Nigeria, *S. mansoni* is endemic and the inhabitants of this community claimed to use *Andrographis paniculata* for its treatment. The chemical composition of *A. paniculata* showed that it is a rich source of diterpenoids and flavonoids including other metabolites.<sup>[8]</sup> The major bioactive component isolated from *A. paniculata* is "Andrographolide" a colourless crystalline appearance with a bitter taste and is responsible for its pharmacological activities.<sup>[9-12]</sup> The underlying molecular mechanisms have also been investigated and attributed to the nuclear transcription factor kappa B (NF- $\kappa$ B) which is the molecular target for the anti-inflammatory activity of *A. paniculata*.<sup>[13,14]</sup> Apart from possessing anti-inflammatory<sup>[13,16]</sup> properties, it has antiviral,<sup>[17]</sup> hepatoprotective and hypertensive activities.<sup>[15,18]</sup> Also, it activates both antigen-specific and non-specific immune responses making it effective against a variety of

infectious and cancer-causing agents.<sup>[19]</sup> The aim of this study was to investigate the curative effect of *A. paniculata* on mice infected with *S. mansoni*.

## 2. MATERIALS AND METHODS

### 2.1 Parasite

A strain of *S. mansoni* cercariae obtained from naturally infected snails from a stream in Ilie, Southwestern Nigeria and maintained in *Biomphalaria pfeifferi* were used in this study.

### 2.2 Experimental animals

Forty eight (48) male mice weighing 18-22 g were used for this study. The animals were acclimatized for a period of two weeks. Thereafter the mice were randomly divided into four groups (A-D) each group comprised 6 mice in replicate of two (n = 12). Feed (pellets) and clean drinking water were provided to all the animals *ad libitum* during the experiment. In addition, group A: received distilled water (0.5 ml/kg) + normal saline (0.5 ml/kg) orally for six weeks (non-infected, non-treated); group B: received *A. paniculata* (10 mg/kg) via gastric gavage daily using oral cannula for six weeks (non-infected treated group); group C: was infected with *S. mansoni* for six weeks (infected non-treated); group D: was infected with *S. mansoni* for two weeks and then treated with *A. paniculata* (10 mg/kg) via gastric gavage daily using oral cannula for a period of four weeks. All the experimental animals were sacrificed after the last dose by cervical dislocation at the end of the treatment period. The experimental protocol of this study was approved by Institutional Ethics Committee of Ladoke Akintola University of Technology, College of Health Sciences, Osogbo, Nigeria.

### 2.3 Infections of mice

Mice were infected with *S. mansoni* cercariae (100 cercariae per mouse) according to the method described by Oliver and Stirewalt.<sup>[20]</sup>

### 2.4 Preparation of aqueous leaf extract of *A. paniculata*

Harvested *A. paniculata* leaves were authenticated at Department of Botany and Pharmacognosis Obafemi Awolowo University, Ile Ife, Nigeria. The leaves were air dried at a temperature of between 24-27°C for fifteen

(15) days and then milled into powder using electric blender. One Hundred and fifty (150) grams of milled leaves was soaked in 1,500 mL of distilled water and the mixture was filtered and concentrated using a rotary evaporator at low temperature (40-50°C). The extracts were preserved in airtight containers and stored at 4°C during the experiment.

### 2.5 Parasitology and Haematology

The worm burdens were determined by perfusion technique<sup>[21]</sup> while the egg count in the liver and small intestine were carried out using the technique described by Cheevers and Anderson.<sup>[22]</sup> Haematological parameters such as haematocrit (PCV), haemoglobin (Hb) concentration, red blood cell count were estimated using an automated Coulter counter (STKS Model).

### 2.6 Biochemical parameters

The activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were measured using the technique of Reitmans and Frankel.<sup>[23]</sup> Alkaline phosphatase (ALP) was assayed by the spectrophotometric technique described by Babson *et al.*<sup>[24]</sup> and total protein and albumin were measured according to the method described by Doumas *et al.*<sup>[25]</sup>

### 2.7 Statistical analysis

Results were expressed as means  $\pm$  standard deviation. Pairwise comparison of means was done using Student's t-test. A p value of  $< 0.05$  was considered to be significant.

## RESULTS

The results presented in Table 1 show the means of adult worms recovered from infected non-treated mice and infected treated mice. The mean adult worm in infected treated mice was significantly lower than that of infected non-treated mice ( $p < 0.001$ ) with a 79.3% reduction on worm burden. Also, Table 1 shows the means egg load recovered from infected non-treated and infected treated mice. The mean egg load in infected treated mice was significantly lower than that of infected non-treated mice ( $p < 0.001$ ) with an 84.8% reduction on egg load. Therefore, extract of *A. paniculata* caused a highly significant reduction on worm burden and egg load.

**Table 1: Means ( $\pm$ SD) of Adult Worms and Egg Load in infected non-treated and infected treated mice during the experiment.**

| Parameter   | Infected non-treated | Infected treated  | % change | p       |
|-------------|----------------------|-------------------|----------|---------|
| Adult worms | 49.8 $\pm$ 9.2       | 10.3 $\pm$ 4.2    | 79.3     | < 0.001 |
| Egg load    | 5980.0 $\pm$ 980.2   | 908.0 $\pm$ 167.4 | 84.8     | < 0.001 |

Results from Table 2 show the mean values of haematocrit, haemoglobin concentration and red blood cell (RBC) count in the study groups. Infected non-treated group had significantly lower mean values of haematocrit, haemoglobin concentration and erythrocyte count than non-infected non-treated group ( $p < 0.001$ ) or non-infected treated group ( $p < 0.001$ ) or infected treated

group ( $p < 0.001$ ). Also, Table 2 shows the mean values of ALT, AST, ALP, total protein and albumin in the study groups. The mean values of ALT, AST, ALP, total protein and albumin in infected non-treated group were significantly lower than those of non-infected non-treated group ( $p < 0.001$ ) or non-infected treated group ( $p < 0.001$ ) or infected treated group ( $p < 0.001$ ).

**Table 2: Means ( $\pm$ SD) of the Haematological and Biochemical Parameters of mice Examined in the study groups during the experiment.**

| Parameter                | Group A          | Group B          | Group C  | Group D          |
|--------------------------|------------------|------------------|--|------------------|
| <b>Haematological</b>    |                  |                  |  |                  |
| Haematocrit (%)          | 41.35 $\pm$ 2.10 | 41.68 $\pm$ 2.54 | 29.42 $\pm$ 1.74 <sup><math>\alpha\beta\gamma</math></sup> | 40.80 $\pm$ 1.12 |
| Hb conc.(g/dL)           | 13.42 $\pm$ 0.78 | 13.54 $\pm$ 0.43 | 8.13 $\pm$ 0.61 <sup><math>\alpha\beta\gamma</math></sup>  | 12.82 $\pm$ 0.67 |
| RBC ( $\times 10^9$ /mm) | 7.30 $\pm$ 0.55  | 7.41 $\pm$ 0.49  | 4.53 $\pm$ 0.34 <sup><math>\alpha\beta\gamma</math></sup>  | 6.96 $\pm$ 0.68  |
| <b>Biochemical</b>       |                  |                  |  |                  |
| ALT (IU/L)               | 50.20 $\pm$ 8.62 | 49.30 $\pm$ 7.44 | 98.70 $\pm$ 6.02 <sup><math>\alpha\beta\gamma</math></sup> | 54.40 $\pm$ 8.10 |
| AST (IU/L)               | 52.81 $\pm$ 7.40 | 51.72 $\pm$ 7.20 | 64.53 $\pm$ 6.31 <sup><math>\alpha\beta\gamma</math></sup> | 54.10 $\pm$ 6.80 |
| ALP (IU/L)               | 60.41 $\pm$ 9.28 | 61.61 $\pm$ 8.30 | 90.52 $\pm$ 5.10 <sup><math>\alpha\beta\gamma</math></sup> | 65.70 $\pm$ 4.61 |
| T. Protein(g/dL)         | 7.56 $\pm$ 0.65  | 7.63 $\pm$ 0.46  | 5.12 $\pm$ 0.51 <sup><math>\alpha\beta\gamma</math></sup>  | 6.93 $\pm$ 0.36  |
| Albumin(g/dL)            | 3.12 $\pm$ 0.45  | 3.04 $\pm$ 0.34  | 2.01 $\pm$ 0.36 <sup><math>\alpha\beta\gamma</math></sup>  | 2.94 $\pm$ 0.42  |

Significantly different at  $p < 0.05$ ;  $\alpha$ : group C (Infected non-treated) compared to group A (Non-infected non-treated);  $\beta$ : group C (Infected non-treated) compared to group B (Non-infected non-treated);  $\gamma$ : group C (Infected non-treated) compared to group D (Infected treated).

## DISCUSSION

Results from the present study showed that aqueous extract of *A. paniculata* exhibited significant antischistosomal activity in infected mice. Results showed that *A. paniculata* caused a highly significant reduction in egg load and worm burden. This reduction might be due to the effect of the extract on worm fecundity which could arise from cessation of egg laying or hindering of oviposition.<sup>[4]</sup> Also, it might be attributed to the anti-oxidant properties of *A. paniculata*<sup>[26]</sup> which eliminated the product of oxidative reaction and assisted in immune-mediated destruction of worms and eggs. The eggs of schistosomes are very crucial in the pathology of schistosomiasis mostly due to the toxins released by the eggs. These toxins cause the host to react in a number of ways. Previous reports<sup>[27]</sup> showed that the reaction of the host to the eggs could vary from granulomas to fibrosis and then possibly cirrhosis.

Our results showed that *A. paniculata* caused a highly significant reduction on egg load and worm burden. Our result of erythrocytic parameters showed that mean values decreased significantly in infected, non-treated mice. This is in line with the reports of previous studies.<sup>[28-30]</sup> However, treatment of *S. mansoni* infection with *A. paniculata* resulted in restoration of the mean values of these parameters.

In this study, infection of *S. mansoni* resulted in significant increase in serum ALT, AST and ALP levels. Similar observations were made by some researchers.<sup>[30,31]</sup> Membrane damage had been implicated for the significant increase in serum enzymes AST, ALT and ALP.<sup>[32]</sup> However, infected treated mice had significant reduction in serum ALT, AST and ALP to levels comparable to levels obtained in the control group. This showed that *A. paniculata* restored the hepatic activities of ALT, AST and ALP lowered as a result of *S. mansoni* infection.

Our results showed a significant decrease in serum levels of total protein and albumin which is in line with the reports of previous studies.<sup>[30,33]</sup> However, the administration of *A. paniculata* resulted in restoration of total protein and albumin levels comparable to those of non-infected, non-treated group.

From the above discussion, we report that aqueous extract of *A. paniculata* significantly improved the alterations induced by *S. mansoni* infection. The anti-inflammatory and hepatoprotective properties of *A. paniculata* could be responsible for its schistosomicidal effect. We could not carry out histological study on the liver to demonstrate and compare granulomas in the infected non-treated and infected-treated mice. Further studies including histological examinations are suggested to confirm the anti-schistosomiasis properties of *A. paniculata*.

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