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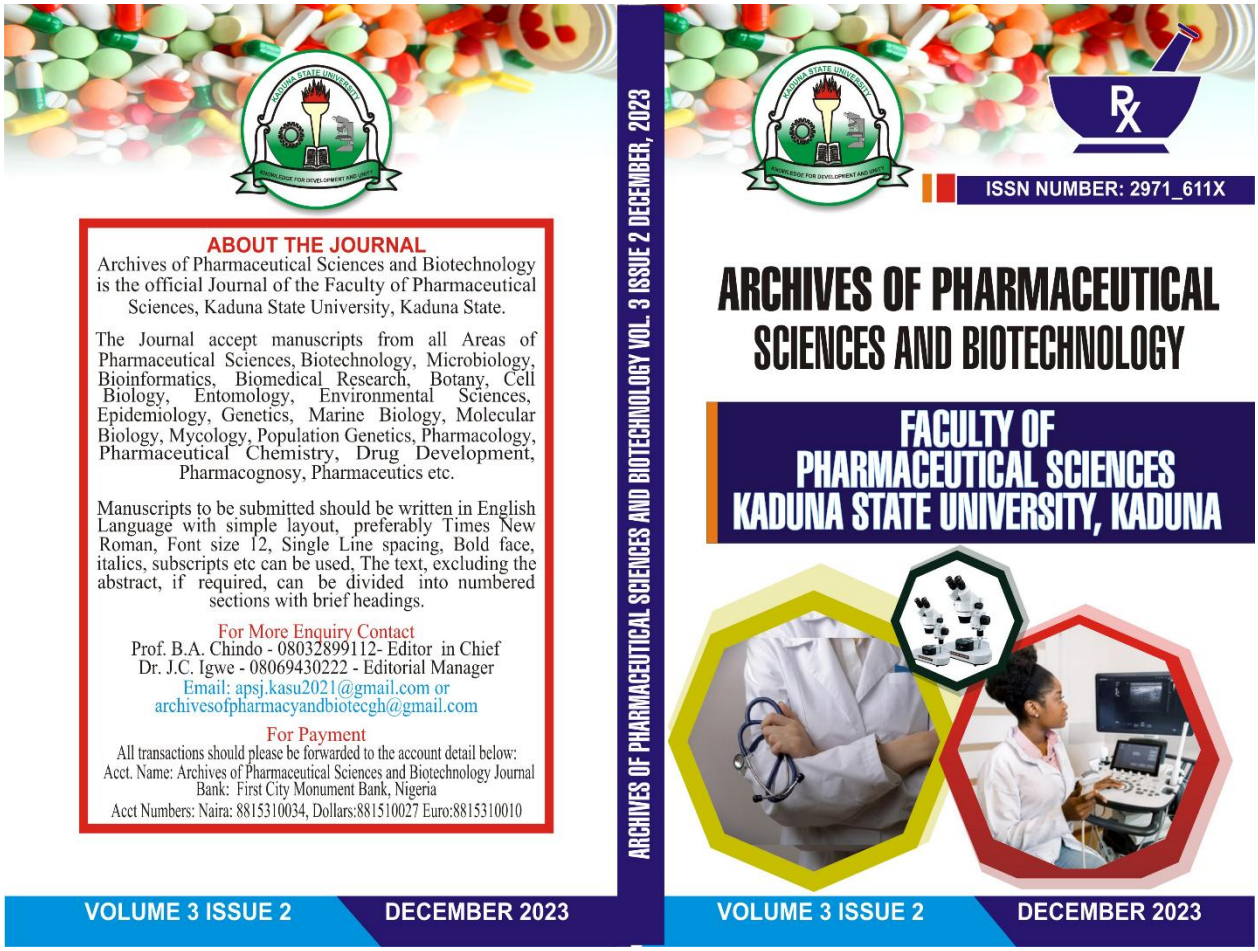

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AMELIORATIVE EFFECT OF ETHANOLIC EXTRACT OF *BAUHINIA MONANDRA* ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF *PLASMODIUM BERGHEI* INFECTED MICE

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ABSTRACT

Introduction: The progression of malaria is characterized by anemia and elevated serum levels of liver enzymes.

Aim: The purpose of the study was to assess the impacts of the ethanol-based extract of *B. monandra* on the hematological and biochemical parameters of *Plasmodium berghei-infected* mice.

Methods: A total of 25 mice were injected intraperitoneally with *P. berghei* (resistant ANKA clones), while an additional 5 mice remained uninfected to act as the normal control and were designated as group 6. The negative control (group 5) received distilled water only. The positive control group (group 4) was treated with ACT and served as the positive control. Meanwhile, groups 1, 2, and 3 were orally given *B. monandra* extract at doses of 50, 100, and 150 mg/kg body weight, respectively, for a duration of three days. The effects of the leaf extract of *B. monandra* on liver enzymes (alanine aminotransferase and aspartate aminotransferase) and hematological parameters of *P. berghei*-infected mice were studied using standard methods.

Results: The parasitized control mice showed significantly elevated serum ALT and AST activities compared to all other groups. However, parasitized mice administered the extract and those treated with ACT exhibited similar ($p > 0.05$) serum ALT and AST activities. Furthermore, each of the extract doses utilized demonstrated a stimulating effect on red blood cell production after the treatment was administered.

Conclusion: This plant holds great promise for the amelioration of liver damage and the formation of blood cells.

Keywords: *Bouhinia monandra, Plasmodium berghei,* malaria, hematology, biochemical

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1. INTRODUCTION

Malaria, a parasitic disease transmitted by the anopheles mosquito, has been a worldwide problem for centuries, claiming up to 1.2 million lives yearly, especially in sub-Saharan Africa, Asia, and America [1,2]. This disease remains the greatest contributor to the high morbidity and mortality of sub-Saharan Africa, with over 80 % of cases and 90 % of death tolls[3]. The progression of this disease is marked by the development of hematological complications and alterations in biochemical parameters. Biochemical markers serve as a dependable method to evaluate the liver health of animals during infection. The liver has a vital function in the body's metabolic processes, secretion, and elimination of substances. It is essential for maintaining, executing, and controlling bodily equilibrium and is engaged in numerous biochemical pathways supporting growth, immune defense, nutrient provision, energy generation, and reproduction[4]. In cases of malaria, observed alterations in biochemical parameters involve leakage of the liver enzymes such as ALT and AST into the bloodstream as well as modifications of heme metabolism and cell energy utilization. Variations in blood-related parameters have been linked to occurrences of malaria[5]. Furthermore, the manifestation of malaria results from intense hemolysis of infected red blood cells, which may result in life-threatening conditions such as the development of anemia and the generation of free radicals in the body[6]. Hence, this study evaluated the effect of the ethanolic extract of *B. monandra* on the hematological and biochemical parameters of *Plasmodium berghei*-infected mice.

Bouhinia monandra, a member of the Fabaceae family, is a leguminous tree found widely distributed. In Thailand, it has traditional applications in managing fever, alcohol toxicity, allergies, and cancer[7]. Various studies highlight the potent antioxidant and free radical scavenging abilities of the leaf extract from *B. monandra*. Additionally, investigations into its properties have explored its potential as an anticancer and antimicrobial agent[8].

2.0 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Collection and identification of the plant

The identification of the plant, which was collected from Ndunwamini Amagu, Abakaliki Local Government Area of Ebonyi State, Nigeria, was made by a plant taxonomist at the Applied Biology Herbarium Centre, Ebonyi State University, Abakaliki, Nigeria.

2.1.2 Animal

Thirty healthy, two-month-old male mice were acquired from the Animal House of Veterinary Medicine at the University of Nigeria, Nsukka (UNN), for the experimental study. The animals were kept in metal cages within a controlled environment at the Presco Campus of EBSU, and without any restrictions or limitations, food and water were made available.

2.1.3 Parasites

This study utilized the *P. berghei* strain (chloroquine-resistant), specifically the ANKA strain, which was acquired from the Department of Veterinary Medicine at UNN.

2.1.4 Drug

The standard drug, Lonart DS, manufactured by CIPLA LTD, India, was used for the study.

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2.2 METHOD

2.2.1 Processing and extraction of plant material

After the plant was cleaned, separated, and dried away from direct sunlight, it was finely processed into a powder with an electric mill and sieved. The extraction of the plant extract was conducted using a Soxlet apparatus by adding 200 g of the plant powder to 600 mL of 70 % ethanol using a continuous extraction method involving repeated cycles of solvent boiling and condensation to efficiently extract compounds from the plant material using a rotary evaporator (RE301 USA), and the residue was stored in a freezer (HR 170T, Japan) at 4 ^{o}C.

2.2.2 Determination of Parasite Density and Inoculum Preparation

The parasitized group of mice received a standardized inoculum containing 10^6 of *P.berghei*, suspended in 0.2 ml of phosphate-buffered saline (Vet Ulay Industries, UK), which was sourced from a donor mouse.

2.2.3 Experimental design

In this research, 30 mice were randomly divided into six groups, each containing five mice. All groups, except the normal control, received an intraperitoneal injection of 10^6 parasitized erythrocytes. Oral treatment began on the third day after the infection and continued for three days.

Groups 1, 2, and 3: Mice in these groups were infected with *P.berghei* and received treatments of 50, 100, and 150 mg/kg body weight of the extract, respectively.

Group 4: Mice in this group were infected with *P. berghei* and treated with a combination of artemether (80 mg) and lumefantrine (480 mg).

Group 5: Mice in this group were infected with *P. berghei* but did not receive any treatment.

Group 6: Animals in this group were neither infected nor subjected to any treatment.

2.2.4 Hematological Assessment

The hematological assessment was carried out using methods described by Cheesbrough[9],

2.2.5 Biochemical Assessment

The assessment of serum ALT and AST levels was carried out using the method of Reitman and Frankel [10].

2.2.6 Statistical analysis

The data were presented as percentage-based averages and analyzed by analysis of variance.

3.0 RESULTS

3.1 Hematological study

There were alterations in the hematological profile of the mice as reflected by a decrease in Hemoglobin (Hb), packed cell volume (PCV), and red blood cells (RBC) three days after infection. However, there was a significantly higher percentage of Hb PCV and RBC content of the extract-treated group compared to the infected untreated group 72 h after treatment, but the extract groups had comparable % Hb, PCV, and RBC with the group treated with ACT as shown in Table 1 and 2. The improvement in these parameters after treatment was in a concentration-dependent manner. However, the increase in PCV did not appear to be in a concentration-dependent manner since mice treated with 100 mg/kg leaf extract recorded higher PCV content (108.8 %) as compared to mice treated with 150 mg/kg of extract (98.68 %).

There was significantly higher white blood cell (WBC) in the parasitized mice

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compared to that of healthy animals 72 h after infection. However, there was a decrease in the WBC content across the groups treated with the ethanol leaf extract of *B. monandry*, which appeared to be concentration dependent, as well as in mice treated with the standard drug, as shown in Table 4. However, the WBC count was still high in the untreated mice.

Table 1: Effect of *B. monandra* on Hemoglobin and Red Blood Cells

S/N	Groups	% Hemoglobin (Hb)		% Red blood cell (RBC)	
		72 hours after infection	After treatment	72 hours after infection	After treatment
1	50 mg/kg	85.32 ± 3.23*	98.56±2.31	76.69±4.29*	81.192±2.26
2	100 mg/kg	88.28± 3.22*	98.45±1.29	77.87±3.62*	95.615±1.62
3	150 mg/kg	86.54 ±4.61*	108.48±2.65	78.33±2.35*	98.973±2.04
4	ACT	85.29±4.12*	102.34±1.99	77.23±1.32*	98.83±2.36
5	Infected and untreated	84.19±1.73*	53.57±1.73*	78.08 ±4.14*	40.544±2.47*
6	Uninfected and untreated	98.80±1.3	99.6±0.89	99.5±0.5	100.3±0.21

* Significant at $p < 0.05$ when compared to uninfected untreated

Table 2: Effect of *B. monandra* on Pack Cell Volume and White Blood Cell

S/N	Groups	% Packed cell volume		% White blood cell	
		72 hours after infection	After treatment	72 hours after infection	After treatment
1	50 mg/kg	85.26 ± 2.67*	98.61 ±1.06	149.047±4.29	114.718±2.26
2	100 mg/kg	88.55± 3.48*	108.85±1.63	135.013±3.62*	110.855±1.62
3	150 mg/kg	86.59 ±3.30*	98.68±1.98	133.863±2.35*	94.113±2.04
4	ACT	80.31±4.62*	102.57±1.21	144.35±1.32*	98.83±2.36
5	Infected and untreated	84.24±2.95*	93.77±2.33*	143.268±4.14*	126.965±2.47*
6	Uninfected and untreated	99.62±1.3	100.02 ±0.09	100.9±0.16	97.3±1.21

* Significant at $p < 0.05$ when compared to uninfected untreated

3.2 Biochemical Study

ALT and AST activities 72 h after infection were significantly lower ($P<0.05$) in the uninfected group compared to all other groups (Table 3). However, the enzyme activities of parasitized mice administered 50, 100, and 150 mg/kg of *B. monandra* were statistically comparable ($P>0.05$) to those of the uninfected untreated mice 72 h after treatment. The liver enzyme activities were significantly increased ($P<0.05$) in the serum of the infected-untreated mice as compared with all other groups 72 h after treatment.

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Table 3: Effect of *B. monandra* on Aspartate aminotransferase and Alanine aminotransferase

Groups	% Aspartate aminotransferase		% Alanine aminotransferase	
	72 hours after infection	After treatment	72 hours after infection	After treatment
50 mg/kg	119.41± 3.22*	100.05±1.18	118.17±3.36*	98.02 ±4.09
100 mg/kg	120.82± 4.61*	96.55±3.84	119.79±2.92*	95.31±3.93
150 mg/kg	119.43±3.82*	94.48±2.64	118.42±3.67*	92.24±3.45
ACT	117.18±4.47*	101.8±2.33	119.89±2.41*	96.09±2.22
Infected and untreated	119.45±6.21*	124.84±3.37*	117.15±3.39*	123.12±5.65*
Uninfected and untreated	100.09±0.4	98.972±1.06	99.2±0.16	98.3±2.01

* Significant at $p < 0.05$ when compared to uninfected untreated

4.0 DISCUSSION

Malaria is linked to alterations in hematological markers. This was evidenced by the decrease in percentage RBC, Hb, and PCV of the parasite-infected groups three days after infection when compared to that of healthy animals. The observed decrease may be due to invasion by the malaria parasite, which brings about extensive changes in the host blood cells[11]. It has been established that during the first phase of *Plasmodium* infection, the malaria parasites proliferate in the liver of an infected individual prior to attacking red blood cells in the circulation. Parasites thrive and multiply within the red blood cells of an individual, causing the infection to spread[12]. As a result, the red blood cells eventually burst, leading to flu-like symptoms[13]. The rupture of the RBCs is the primary cause of decrease in percentage RBC, Hb and PCV counts. The breakdown of red blood cells could stem from two primary causes. First, it might result from the direct breakdown of red cells containing parasites when their numbers are high. Alternatively, it could be triggered by the immune system attacking both infected and uninfected red cells. This immune response occurs because the invasion of parasites alters the structure of red blood cell antigens, prompting the body to generate antibodies targeting these altered red blood cells. Furthermore, the buildup of RBC, Hb and PCV observed 72 h after treatment in the treated groups suggests that the extract was effective in malaria treatment. The cells begin slowly proliferating once the parasites are removed from the bloodstream, replenishing, and reestablishing the blood supply.

Meanwhile, the significant increase in white blood cell count in all the parasite-infected groups could be because it accounts for the body's immune response to disease, and the increase in WBC confirms the event of infection by the *Plasmodium* parasite. However, after treatment, a decrease in WBC was noted in groups 1, 2 and 3 owing to the clearance of the parasite by the extracts.

Liver enzyme makers refer to specific enzymes employed to distinguish or identify diseases[14]. Specifically, ALT and AST are in use as marker enzymes for liver function. The significant increase in serum ALT and AST values observed 72 h after infection of the mice when compared to the uninfected group corroborates the results of other studies[14-17]. The rise in AST and ALT levels could be due to liver damage and changes in liver cell structure caused by the

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Plasmodium infection. This leads to the enzymes being released into the bloodstream.

The observed decrease in the ALT and AST levels of the groups treated with the extract suggest the ameliorative effect of the extract on liver damage.

5.0 CONCLUSIONS

In conclusion, this work illuminates the possible medicinal properties of this plant. The findings suggest a promising role for the extract in negative effects of malaria infection on the hematological and biochemical profiles.

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