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Assessment of the Antiplasmodial and Haematoprotective Potential of a Polyherbal Extract of *Azadirachta indica, Mangifera indica,* and *Persea americana* Leaves in Mice Infected with *Plasmodium berghei*

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ABSTRACT

The polyherbal extract obtained from the leaves of *Azadirachta indica, Mangifera indica, and Persea americana* is traditionally utilized by the Esan people of Edo State, Nigeria, to treat uncomplicated malaria. This study assessed the antiplasmodial and haematoprotective efficacy of the polyherbal extract via in vivo tests utilizing chloroquine-sensitive mice infected with *Plasmodium berghei*, focusing on parasite count and its effects on haematological markers. The animals were allocated to treatment groups receiving 200, 400, and 800 mg/kg of fresh and heated polyherbal extracts, respectively, with chloroquine as the reference drug. The malaria parasite count was conducted via Field's staining method, while the whole blood count was performed with an auto-hematology analyzer. The results of the study revealed a dose-dependent antiparasitic effect of the polyherbal extract, with the 800 mg/kg dosage reducing red blood cell count. Although the malaria parasite count was not significantly different among the treated groups, those receiving 800 mg/kg daily of both heated and fresh extracts showed parasite levels comparable to the normal and chloroquine-treated control groups. No significant differences were observed in the other red blood cell parameters or white blood cell counts across the groups, including the chloroquine-treated group. The group treated with the heated extract at 800 mg/kg showed the lowest average white blood cell count. This study offers significant insights into the therapeutic potential of the polyherbal extract. Additional research is required to clarify the mechanisms behind the reported effects and to investigate the therapeutic benefits of adequately controlling malaria symptoms.

Keywords: *Azadirachta indica, Mangifera indica, Persea americana,* Antiplasmodial, Polyherbal

Introduction

Malaria is a life-threatening disease caused by parasites transmitted through the bite of infected mosquitoes. It is a considerable global health challenge, especially in sub-Saharan Africa.[1,2] Despite endeavours to manage and treat malaria, the emergence of drug-resistant parasites necessitate an ongoing pursuit of novel and effective therapies. The development of novel antimalarial therapies is essential for tackling this public health issue and mitigating the high morbidity and mortality linked with the disease.[3] Identifying alternative therapies from natural sources such as plant extracts, may provide intriguing opportunities for addressing drug-resistant malaria strains.[1]

Extensive studies have been conducted on the antimalarial properties of herbs, notably about their role in addressing drug resistance in malaria treatment, leading to increased interest in herbal medicines as alternative therapies.[4]

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Artemisia annua, commonly known as sweet wormwood, contains the active compound artemisinin, which is very efficient against drug-resistant strains of malaria parasites. This finding resulted in the formulation of artemisinin-based combination therapies (ACTs). However, resistance of malaria parasites to certain artemisinin-based combination therapy has already been documented in Uganda.[5] Other herbal extracts, including those from *Cryptolepis sanguinolenta* and Cinchona species, have exhibited significant antimalarial activity. These plant extracts comprise chemicals such as cryptolepine and quinine, demonstrating significant antimalarial properties.[6,7] Several herbal formulations exhibit anti-parasitic properties and augment the efficacy of standard antimalarial medications. Extracts from *Curcuma longa* (turmeric) and *Piper guineense* (West African black pepper) have demonstrated this ability, potentially aiding in the mitigation of drug resistance.[8,9]

Herbal remedies utilizing leaves from *Azadirachta indica* (neem), *Persea americana* (avocado pear) and *Mangifera indica* (mango) for the treatment of malaria have been integral to traditional medicine in numerous cultures. Leaves of *Azadirachta indica,* (AI) contain bioactive chemicals with antimalarial activity, whereas leaves of *Mangifera indica* (MI), *and Persea americana* (PA) have different therapeutic effects.[10] These herbs are hypothesized to have synergistic anti-parasitic, antioxidant, anti-inflammatory and immune-enhancing activities, potentially assisting in the management of malaria symptoms.[11]

The justification for investigating the anti-parasitic and haematoprotective properties of the herbal formulation is rooted in its historical application by the Esan people of Edo State, Nigeria, and the necessity to substantiate its effectiveness.[12] The herbal formulation has been utilized as a treatment for uncomplicated malaria in the Esan

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communities, demonstrating its perceived efficacy in addressing various malaria cases, and it has retained its effectiveness despite the evolution of frontline conventional antimalarial medications due to drug resistance.

Materials and Methods

Collection, Authentication and Preparation of Plant Materials

Fresh leaves of *A. indica* (AI), *M. indica* (MI) and *P. americana* (PA) leaves were harvested in February 2023, from a garden in Ekpoma, Esan West Local Government Area of Edo State, Nigeria. The specimens were identified and authenticated at the Department of Plant Science and Biotechnology, Ambrose Alli University Ekpoma, Edo State. The leaves were air-dried at ambient temperature and subsequently homogenized with an electric blender. The polyherbal mixture was formulated by measuring 200 g of MI, AI, and PA leaf homogenate each (ratio 1:1:1) in 4 L of distilled water (fresh sample). Another portion was prepared, transferred into beakers and heated for 30 minutes in a water bath at 100°C with constant stirring.[13] The mixtures were left to stand for 24 hours and then filtered through muslin cloth. The filtrates were freeze-dried and stored in sealed containers in a freezer until needed.

Grouping of Experimental Animals and Treatment

Twenty-seven (27) chloroquine-sensitive *Plasmodium berghei* ANKA-infected mice (mean weight = 20.5 g) were obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria. The test animals were infected with *Plasmodium berghei* at NIMR and thereafter transported in plastic baskets by an air-conditioned vehicle to the animal house of the Department of Biochemistry at Ambrose Alli University, Ekpoma, within 12 hours post-inoculation. The animals were categorized into 9 groups, each consisting 3 animals and were housed in plastic cages with unrestricted access to pelletized poultry feed and water. Malaria infection was confirmed three days post-inoculation. The animals in Group I designated as the normal control group, were administered distilled water and were not infected with the parasite. Group II, which served as the positive control, was subjected to infection and administered chloroquine phosphate at a dosage of 10 mg/kg body weight daily. Group III, the negative control group was infected with the parasite but administered distilled water. Groups IV, V, and VI served as the experimental groups for the fresh extract. The mice in these groups were parasitized and administered 200, 400, and 800 mg/kg body weight of the fresh polyherbal extract, respectively. Groups VII, VIII, and IX served as the experimental groups for the heated polyherbal extract. The mice in these groups were parasitized and administered 200, 400 and 800 mg/kg of the heated polyherbal extract, respectively. The dosage range was determined based on a preliminary study. The treatment duration was four days, and blood samples were collected via cardiac puncture, in strict compliance with animal use regulations.[14] Chloroquine phosphate (100 mg tablets) was acquired from Joefel Supreme Pharmacy, Ekpoma. A 10 mg/kg body weight solution was made by dissolving a 100 mg tablet in 10 mL of distilled water (10 mg/mL). The mass of the drug (W2) administered was calculated by multiplying the dose by the weight of each mouse (W1), and the volume administered was determined by dividing W2 by the dose.

Haematological Analysis

A complete blood count was performed on blood samples from the groups to determine red blood cell (RBC, 10^8/µL) count, haemoglobin (HGB, g/dL), hematocrit (HCT, %), white blood cell (WBC, 10^3/µL) count, lymphocytes, granulocytes, mean corpuscular volume (MCV, fL), mean corpuscular haemoglobin (MCH, g/dL), red cell distribution width (RDW-CV, %), platelet count (PLT, 10^3/µL), and mean platelet volume (MPV, fL). These parameters were analyzed using the Dymind DH36 3-Part Haematology Analyzer at Eseohe Medical Centre, Ihumudumu, Ekpoma, Edo State, Nigeria. The malaria parasite (MP, /µL) count was determined using Field's staining method.[15,16]

Statistical analysis

Data obtained were analysed using one-way analysis of variance (ANOVA) and the post hoc Tukey's test using GraphPad Prism Version 8.0 (GraphPad Software, San Diego, USA). The results are presented as mean ± standard error of the mean (SEM) and $p < 0.05$ was considered significant.

Results and Discussion

Malaria, caused by Plasmodium parasites, significantly impacts the blood, especially red blood cells and the entire haematopoietic system leading to profound implications for the health and well-being of individuals affected.[17] During the clinically symptomatic phase of malaria, parasites infiltrate healthy red blood cells (RBCs) and proliferate within them, completing a life cycle of around 48 hours before the cell membrane ruptures, releasing new merozoites into the bloodstream.[18]

This study utilized *Plasmodium berghei* parasite count as a criterion to evaluate the antiplasmodial potential of the polyherbal extracts. The polyherbal extract demonstrated a dose-dependent effect on malaria parasite (MP) count. The groups administered 800 mg/kg of both fresh and heated extracts exhibited significantly lower MP counts in comparison to the untreated negative control group. However, there was no significant difference in MP count between the groups administered 800 mg/kg and the chloroquine-treated group, indicating similar antiparasitic efficacy. The findings indicate a dose-dependent effect with no significant difference among the normal control group, the positive control group and those administered 800 mg/kg of extracts. This outcome corresponds with previous findings about the dose-dependent antimalarial efficacy of polyherbal extracts.[19,20] The lower doses (200 and 400 mg/kg) for both the fresh and the heated extract exhibited elevated MP counts and did not statistically vary from the negative control group. The MP count was lowest in the normal control group (Figure 1).

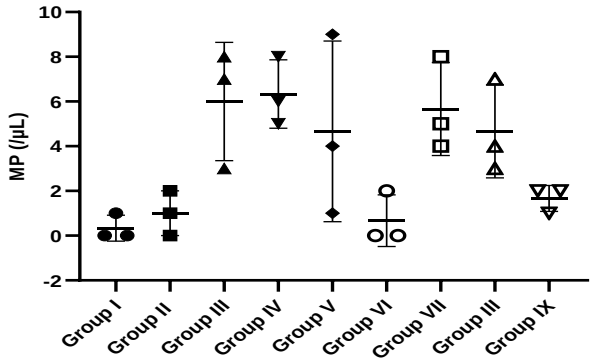

Figure 1: Malaria parasite (MP) count in control and test groups (*Mean* ± *SEM*).

The red blood cell count varied among the treatment groups. Mice administered 800 mg/kg of fresh and heated extracts exhibited reduced RBC counts compared to the normal control and chloroquine-treated groups, although these differences lacked statistical significance. The normal control and positive control groups with lower MP counts had higher RBC counts. This aligns with the previous research reports since malaria parasites destroy red blood cells.[21] The observed reduction in Groups VI and IX which received 800 mg/Kg of the fresh and heated extracts exhibited a negative correlation (-0.223) between the MP count and the RBC count in these groups (Figure 2). This implies that, in addition to its anti-parasitic effects, elevated quantities of the extract may affect the hematopoietic system by either suppressing red blood cell production or directly lysing red blood cells, thus reducing the survival rate of the parasite.[17] Previous reports indicated that reduced RBC counts were noted in populations exhibiting elevated

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parasitaemia.[12,22] The chemo-suppression test utilizing extracts from *Alstonia boonei* and *Carica papaya* in *Plasmodium berghei*-infected mice, exhibiting high parasitaemia demonstrated a reduced mean survival time, weight loss, and diminished haematocrit, all of which were ameliorated in a concentration-dependent manner after treatment with the extracts.[12]

The WBC count was generally lower in groups with lower MP count. Mice administered the heated extract at 800 mg/kg had the lowest mean WBC count among the treated groups. No significant variations in WBC count were noted among the groups. White blood cells are integral to the body's defence against malaria by recognizing and eliminating the malaria parasites that infiltrate the bloodstream. In malaria infection, white blood cell counts are often low to normal, not due to depletion but because the WBCs are sequestered in organs like the spleen and other peripheral pools.[23] Variations in white blood cell count have been recorded, with the developmental stage and species of parasite recognized as two primary factors affecting the severity of infection. The infection and treatment responses are highlighted as potential determinants of this heterogeneity. [23,24] In this study, the groups with lower MP counts also had lower WBC counts. However, the groups administered the heated extract had a lower average WBC count than those receiving the fresh extract, although this difference was not statistically significant (Figure 3). The reason for the reduced average WBC count in the groups administered 800 mg/kg compared to the other treated groups remains unclear. The negative control group with the greatest average MP count did not exhibit the highest WBC count. This observation corresponds with the fact that white blood cells may be removed from peripheral circulation during malaria infection.[23]

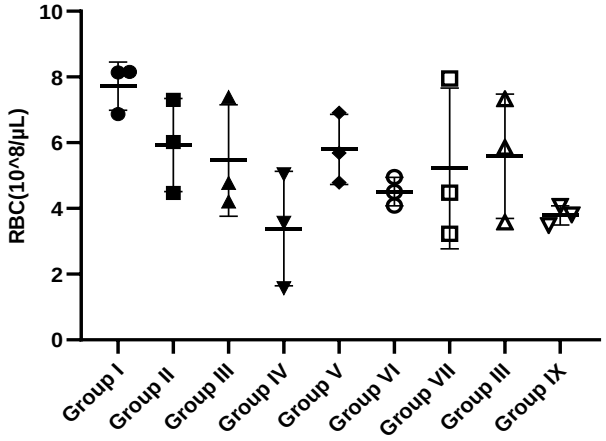

Figure 2: Red blood cell (RBC) count in control and test groups (*Mean* ± *SEM*).

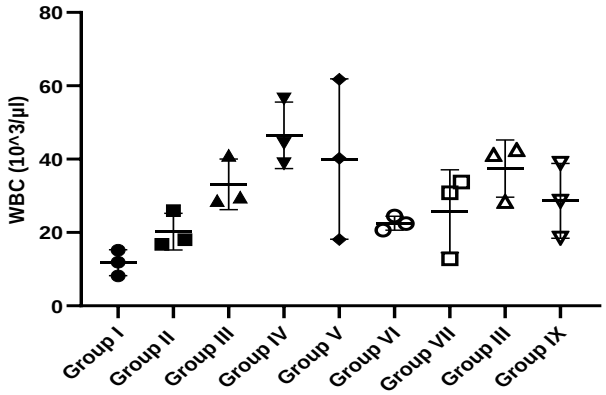

Figure 3: White blood cell (WBC) count in control and test groups (*Mean* ± *SEM*).

Lymphocyte percentages were highest in the groups treated with 800 mg/kg of both fresh and heated extracts. This suggests a potential effect of the extracts on lymphocyte levels during malaria infection. However, the differences observed were not statistically significant when compared to the control and other treated groups (Figure 4).[25]

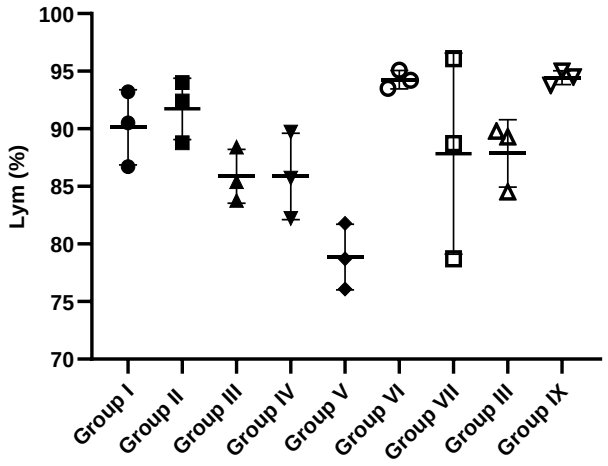

Figure 4: Percentage lymphocytes (Lym) in control and test groups (*Mean* ± *SEM*).

Granulocyte percentages did not vary significantly across groups, though a slight decrease was observed in groups with reduced MP counts. Granulocytes, a subclass of leukocytes characterized by cytoplasmic granules, perform essential roles in the innate immune system through phagocytosis, enzymatic and cytotoxic actions, and the initiation of inflammatory cascades. In malaria, granulocytes are recruited to the site of infection to attack the parasite. The concentration of granulocytes may rise during malaria as a response to the infection, aiding in the fight against the parasite and facilitating the clearance of the infection. The percentage of granulocytes, as shown in Figure 5, exhibited no significant differences across the groups. However, groups with lower MP counts showed a slightly reduced percentage of granulocytes.

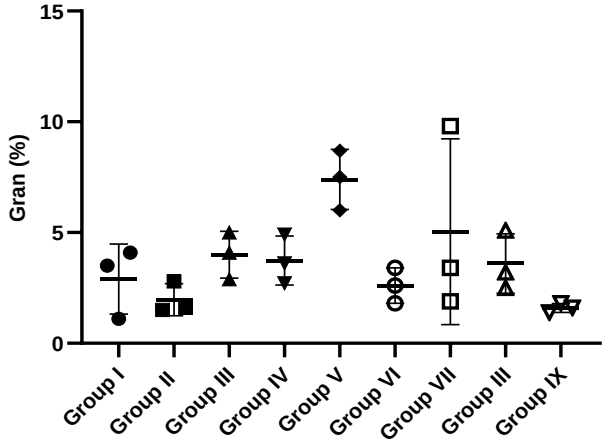

Figure 5: Percentage granulocytes (Gran) in control and test groups (*Mean* ± *SEM*).

The concentrations of haemoglobin (HGB) exhibited a similar trend to those of red blood cell (RBC) counts. Mice administered 800 mg/kg of the extracts exhibited reduced haemoglobin concentrations relative to the control and chloroquine-treated groups (Figure 6). In malaria infection, a strong link exists between red blood cell count and haemoglobin concentration. The invasion of red blood cells by parasites leads to their rupture, resulting in a reduction of both red blood cell

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count and haemoglobin content. The decrease in functioning red blood cells that transport oxygen and nutrients throughout the body leads to anaemia, marked by diminished red blood cell count and haemoglobin levels.[25,26] The impact of the 800 mg/kg doses on RBC count remains ambiguous; however, the decrease in RBC count also influenced HGB concentration in these groups. This result is consistent with earlier findings by Starck et al. (2021) and Bayisa and Dufera (2022), who noted markedly reduced RBC counts and haemoglobin levels in patients exhibiting severe parasitemia and acute malaria. Their research indicated a reduction in haemoglobin levels in 17,599 children under five years old in Burkina Faso.[26,27]

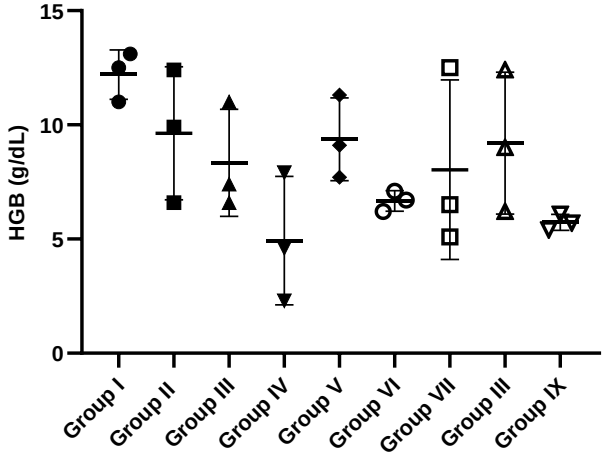

Figure 6: Haemoglobin (HGB) concentration in control and test groups (*Mean* ± *SEM*).

Hematocrit levels were similarly diminished in these groups, concurring with the observed drop in red blood cells and indicating probable erythrocyte injury or impaired erythropoiesis at elevated doses of the extract. Hematocrit is the ratio of red blood cells to the total blood volume. In malaria, hematocrit levels may diminish as a result of the parasitic death of red blood cells. La'lang et al. (2021) reported that in their investigation of the effects of Plasmodium falciparum malaria, 73% of the participants exhibited low hematocrit levels, whereas 27% displayed normal hematocrit levels.[28] The results indicate that hemocrit levels were also diminished in individuals exhibiting significant parasitaemia or administered 800 mg/kg of the extracts (Figure 7).

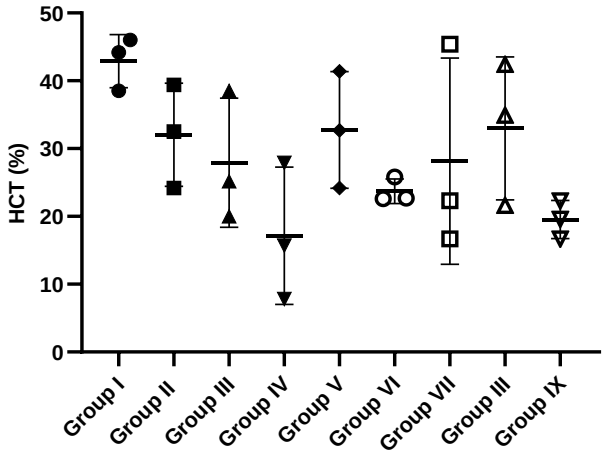

Figure 7: Percentage hematocrit (HCT) in control and test groups (*Mean* ± *SEM*).

During the invasion process, malaria parasites interact with red blood cells (RBCs) through a series of complex molecular interactions. The parasite initially attaches to the RBC membrane, often deforming it, which facilitates the entry of the parasite into the RBC.[18] This distortion affects other factors associated with the RBCs. This investigation found no significant differences in MCV and MCH between the control and treatment groups (Figures 8 and 9). A favourable association with MP count has been previously documented in human research.[29] The platelet count was lowest in the group administered 800 mg/kg of the heated extract; however, this reduction was not statistically significant (Figure 10). Despite no significant differences in platelet count among the groups, the lowest count was observed in the group administered 800 mg/kg of heated extract. Prior reports indicate an absence of association between platelet count and MP count.[29]

Although the sample size limits the ability to draw definitive conclusions, the observed reduction in MP count among the 800 mg/kg treated groups offers valuable insight into the potential antiparasitic effectiveness of the polyherbal extracts.

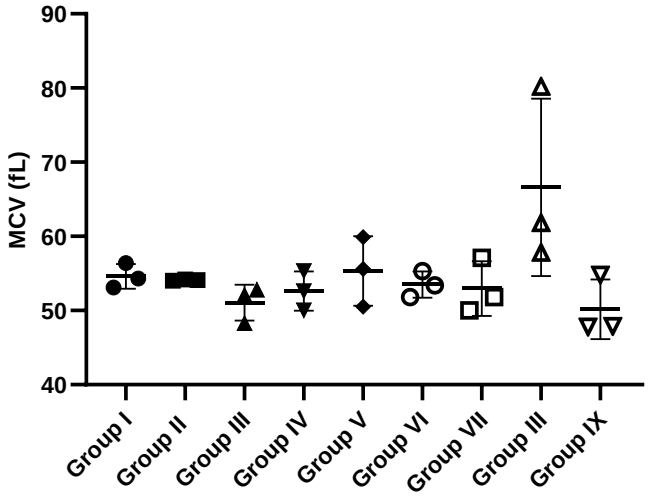

Figure 8: Mean corpuscular volume (MCV) in control and test groups (*Mean* ± *SEM*).

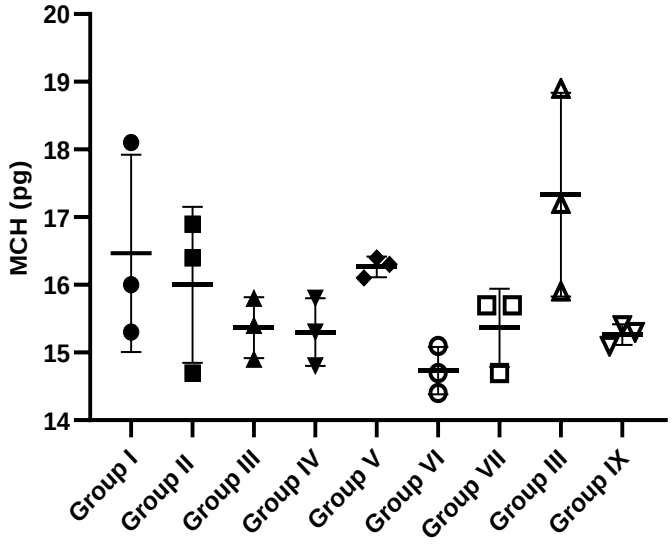

Figure 9: Mean corpuscular haemoglobin (MCH) in control and test groups (*Mean* ± *SEM*).

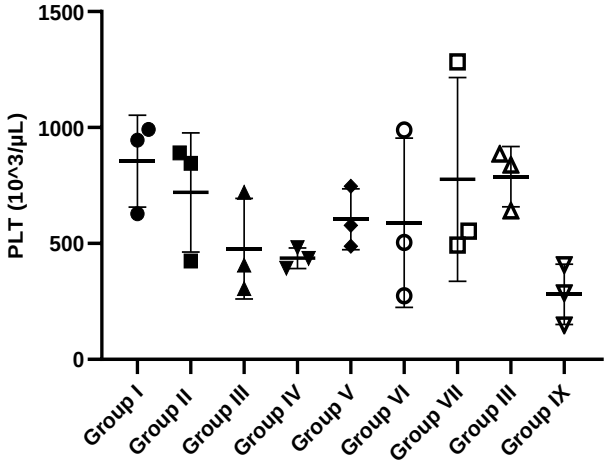

Figure 10: Platelet count (PLT) count in control and test groups (*Mean* ± *SEM*).

Conclusion

The study revealed that the crude combined polyherbal extract exhibits anti-parasitic activity in a dose-dependent manner. The 800 mg/kg body weight doses of the fresh and heated extracts reduced malaria parasite count and red blood cell count. The results suggest that the extract may impact the haematopoietic system, an important aspect of the progression of malaria. This understanding is crucial for exploring the therapeutic potential and applications of the extract.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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