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Aqueous extracts of Zingiber officinale rhizomes and Hibiscus sabdariffa leaves enhanced Plasmodium berghei-infected mice's hematological parameters.

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Authors declare no conflict of interest

Data Availability

The author confirms that the data supporting the findings in this study are available within the manuscript. Raw data that support the findings of this study is available from the corresponding author, upon reasonable request.

Author Contributions

FA; Data curation; Formal analysis; Methodology; Supervision; Project administration; Resources; Writing-original draft; review and editing; RA; Project administration; Validation; Visualization; AA; Funding acquisition; Investigation.

REVIEW

Aqueous Extracts of *Zingiber officinale* Rhizomes and *Hibiscus sabdariffa* Leaves Enhanced *Plasmodium berghei*-infected Mice's Hematological Parameters

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Abstract

Malaria remains a global health challenge, leading to severe hematological complications. Natural alternatives such as *Zingiber officinale* and *Hibiscus sabdariffa*, known for their antioxidant and bioactive properties, are being investigated for their therapeutic potential. This study examines the hematological benefits of Z. officinale and H. sabdariffa in mice infected with the common malaria research model, NK65 chloroquine-sensitive Plasmodium berghei. Forty-two albino mice, averaging 24.5 ± 1.23 g, were randomly divided into six groups. Mice in group A were uninfected which served as the normal control group. Mice in groups B, C, D, E, and F were inoculated intraperitoneally with *P. berghei*. Group B were not treated while Groups C, D, E, and F received oral treatments for four days with 5 mg/kg body weight of chloroquine, 100 mg/kg body weight of *Z. officinale*, 100 mg/kg body weight of *H. sabdariffa*, and extract mixture at 150 mg/kg body weight (100 mg/kg bw of *H. sabdariffa*: 50 mg/kg bw of *Z. officinale*), respectively. Hematological markers that were measured and compared with the control group were hemoglobin, packed cell volume (PCV), platelet counts white blood cells (WBC), and red blood cells (RBC). Treatments with Z. officinale and H. sabdariffa extracts significantly ($p < 0.05$) improved the platelet counts, RBC, WBC, Hb, and PCV, in comparison with untreated P. berghei-infected mice. The extracts combinations significantly improved hematological health in malaria-infected mice, suggesting potential interactions between the biologically active substances in both plants and highlighting their therapeutic potential. The mechanisms behind these synergistic effects and the efficiency of this herbal combination in clinical settings require more investigation.

Keywords: *Zingiber officinale*, *Hibiscus sabdariffa*, Hematological complications, Therapeutic potential, *Plasmodium berghei*

1. Introduction

The high rates of morbidity and mortality associated with malaria, which is caused by Plasmodium parasites, make it a major global health concern, especially in tropical and subtropical regions.

The most common and harmful implications of malaria is anemia [2]. This anemia lowers hemoglobin levels and hinders oxygen delivery in infected patients due to red blood cell death and bone marrow suppression [3]. Malaria treatment has mostly depended on the usage of antimalarial medications; however, drug resistance and the adverse effects of many synthetic medications have brought attention to the need for safe, accessible, and effective alternative remedies [1].

The possible therapeutic benefits of medicinal herbs have long been studied for the purpose of treating malaria and reduce its hematological effects [4]. Among these, *Z. officinale* and *H. sabdariffa*, are two promising plants that are extensively grown and prized for their therapeutic qualities. H. sabdariffa leaves are abundant in vitamins, minerals and antioxidants as well as are known to improve hematological parameters and lower oxidative stress [5].

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However, likely bioactive compounds in Z. officinale rhizomes contain gingerols and shogaols that have anti-inflammatory, antioxidant, and immune-stimulating properties [6].

Extractions obtained from these plants were reported to reduce malaria-induced anemia by improving hemoglobin levels, RBC, and other hematological indices affected by Plasmodium infection [7–10]. The hematological alteration in the infected mice can be studied using the rodent malaria parasite, which replicates many of the hematological abnormalities observed in human.

The PCV, hemoglobin concentration, RBC, and WBC are measured to investigate the hematological advantages of H. sabdariffa leaves; Z. officinale rhizomes aqueous extracts in infected P. berghei mice. The study's objective is to assess these factors to see if these plant extracts might be employed as supportive treatments for malaria to lower anemia and improve overall hematological health. The results might aid in the creation of supplementary treatments to combat the spread of malaria, which might provide a safe, easy way to promote blood health and healing in infected individuals.

2. Materials and method

2.1. Plant material and authenticity

Hibiscus sabdariffa leaves and *Zingiber officinale* rhizomes were obtained from Oja Tuntun, Ilorin, Kwara State, Nigeria, and identified at the Department of Plant Biology, University of Ilorin, Nigeria. The herbarium received specimens with voucher numbers UIH 830 and UIH 1083, respectively.

2.2. Plant preparation

The plant parts were cleaned, air dried to a constant weight, and then ground into powder using a blender (Marlex excella, Model no: L 31454). Each 100 g powder sample was dissolved in 2 L distilled water in a polypropylene container and stirred occasionally over a duration of 48 h. The mixture was then freeze-dried after filtration with Whatman No. 1.

2.3. Parasites

Plasmodium berghei chloroquine-sensitive NK65 strain was provided by the Institute of Advanced Training and Research on malaria (IMRAT), Medical College at the University of Ibadan. Mice were injected with parasitized blood to keep the parasite surviving in the laboratory.

2.4. Animals for experiments and ethical approval

For the experiment, mature albino mice weighing 21.5 ± 1.4 g were acquired from the Department of Biochemistry Animal House Unit, University of Ilorin. Standard pellets were supplied to the mice, and they had unrestricted access to water. Prior to the start of the experiment, they were acclimated for two weeks. The study received approval from the Ethical Review Committee (UERC) of the University of Ilorin, and the number UERC/ASN/2018/1416 was given.

2.5. Experimental design

Forty-two (42) mice were randomly divided into six groups of seven each. Group A served as a control, was not infected, whereas groups B-E were inoculated intraperitoneal using 0.2 ml containing 1×10^7 erythrocytes with the parasite and permitted parasitaemia three days to develop. Group A received 0.2 ml of distilled water, group B received 0.2 ml of distilled water, without treatment. Group C was treated with chloroquine at dosage of 5 mg/kg body weight. Groups D and E were treated with aqueous extracts of Z. officinale rhizomes and H. sabdariffa leaves at dosage of 100 mg/kg body weight respectively. Group F was treated with the combination of the extract at dosage of 150 mg/kg body weight in ratio 1: 2 (50 mg/kg Z. officinale and 100 mg/kg H. sabdariffa).

2.6. Blood sample collection and hematological assay

Twenty-four hours after the last dose of therapy, animals were stunned, and blood sample were collected into EDTA vials from a punctured jugular vein, Using the methods outlined by Ref. [11]. Hemoglobin (Hb) levels, platelet counts, PCV, WBC, and RBC were all determined using an automated hematologic analyzer, Sysmex KX-21 (Japan).

2.7. Analytical statistics

Data were presented using the standard error of the mean, which is the average of seven determinations. Duncan's multiple range analysis and one-way Analysis of Variance (ANOVA) were utilized to determine the group differences. The post-hoc evaluation test employed. A p-value of less than 0.05 was considered significant [12]. For the assessment, Graph Pad prism 8 was deployed.

3. Results

Hematological indicator was assessed in treated P. berghei-infected mice with chloroquine, Z. officinale rhizomes and H. sabdariffa leaves aqueous extracts following the four-day treatment period. The results show the untreated P. berghei-infected mice had considerably decreased RBC, hemoglobin, and PCV as represented in Figs. 1–3 respectively. However, mice treated with H. sabdariffa extract had significantly (P > 0.05) higher level of hemoglobin, RBC, and PVC, than mice treated with chloroquine and other extract respectively.

However, Fig. 4 shows that untreated P. berghei-infected mice had significantly (P > 0.05) higher WBC as compared with the chloroquine or extract treated mice. Furthermore, the significant (P > 0.05) increased noticed in Z. officinale rhizomes extract treated was compared appreciably with the observed increased noticed in the control group.

Additionally, untreated infected *P. berghei* mice exhibited significantly (p < 0.05) lower platelet counts when compared with the extracts or chloroquine treated groups as shown in Fig. 5. Moreover, the elevated platelet count noticed in *Z. officinale*-treated mice was not different significantly with the observed values in the control mice.

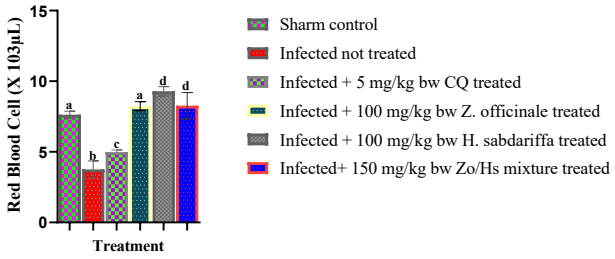

Fig. 1. Red Blood Cells in Plasmodium berghei-*infected mice receiving aqueous extracts of* Hibiscus sabdariffa, Zingiber officinale, *and their combination. Key: CQ = chloroquine, Zo/Hs =* Z. officinale *and* H. sabdariffa combinations, *bw = body weight. Bars with various letters are statistically significant at p value less than 0.05.*

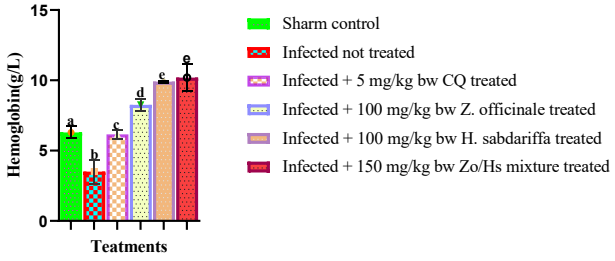

Fig. 2. Hemoglobin levels in Plasmodium berghei-*infected mice treated with* Zingiber officinale, Hibiscus sabdariffa *aqueous extracts, or their combination. Key: Key: CQ = chloroquine, Zo/Hs =* Z. officinale *and* H. sabdariffa combination, *bw = body weight. Bars with various letters are statistically significant at p value less than 0.05.*

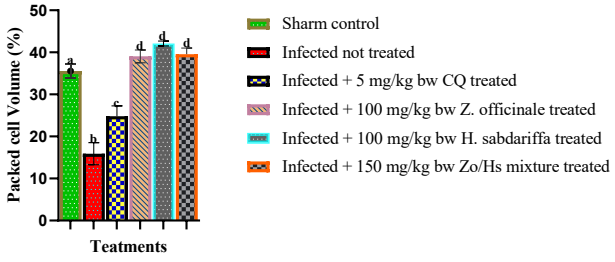

Fig. 3. Packed Cell Volume in Plasmodium berghei-*infected mice receiving aqueous extracts* Hibiscus sabdariffa, Zingiber officinale, *and their combination. Key: CQ = chloroquine, Zo/Hs =* Z. officinale *and* H. sabdariffa *combination, bw = body weight. Bars with various letters are statistically significant at p value less than 0.05.*

4. Discussion

According to Ref. [13], haematological indicators show higher predictive values in the relationship between blood cells and malaria infection. Even at the earliest stages of infection, they are reliable and appropriate methods to assess the severity of malaria [14]. By invading the host cells and breaking down the hemoglobin in red blood cells,

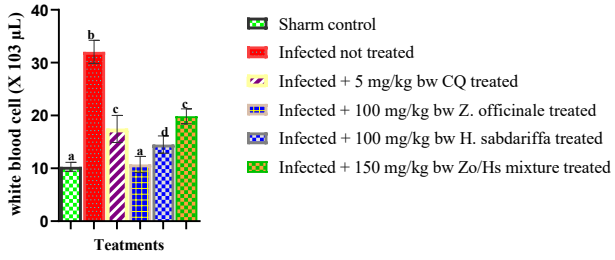

Fig. 4. White Blood Cell in Plasmodium berghei-*infected mice receiving aqueous extracts* Hibiscus sabdariffa, Zingiber officinale, *and their combinations. Key: CQ = chloroquine, Zo/Hs =* Z. officinale *and* H. sabdariffa combinations *bw = body weight. Bars with various letters are statistically significant at p value less than 0.05.*

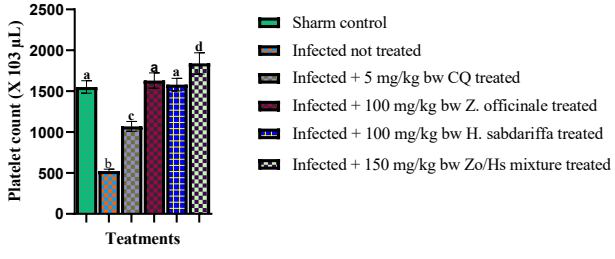

Fig. 5. Platelet counts in Plasmodium berghei-*infected mice receiving aqueous extracts* Hibiscus sabdariffa, Zingiber officinale, *and their combination. Key: CQ = chloroquine, Zo/Hs =* Z. officinale *and* H. sabdariffa *combinations, bw = body weight. Bars with various letters are statistically significant at p value less than 0.05.*

Plasmodium reduces the lifespan of the parasite [7]. Anaemia, leukocytosis, and thrombocytopenia are the most common haematological problems linked to malaria, and the whole blood count ascertains changes in erythrocyte, leukocyte, and platelet counts during diagnosis [15].

A decrease in red blood cell (RBC), hemoglobin (Hb) concentration, and packed cell volume (PCV) are some common indicators of anemia in malaria infection, which are caused by haematological changes [14]. In this study, the reduction in RBC, Hb, and PCV after P. berghei infection may be a sign of hemolysis, which can lead to anemia. The direct destruction of infected red blood cells (RBC), RBC rupture, and hypersplenism are the mechanisms by which Plasmodium infection results in anemia [16]. However, the fact that the RBC count, Hb, and PCV increased following treatment with the extract and the extract mixture at doses of 100 mg/kg bw and 150 mg/kg bw, respectively, suggests that the anemia caused by the parasite was able to be reversed.

White blood cells, or WBCs, are essential for fighting illness in the body. As a malaria infection progresses, the body's WBC numbers can change. Acute malaria frequently results in leucopenia, or a decrease in white blood cells, while severe malaria may cause leukocytosis, or an increase in white blood cells [17].

Additionally, malaria changes leukocytes [18], and following infection, WBC levels typically fall [19]. The leukocyte count in this study may have increased because of the malaria infection, which may indicate an inflammatory response. This is anticipated as the body system produces and mobilizes more WBCs as a result of an infection-related immunological response. Although the treatments did not restore the WBC countdown to control levels, they did demonstrate a downward trend when compared to the infected mice that were left untreated. In treated mice, a lower WBC count may indicate less inflammation and immune system activation.

Platelet counts may drop as the number of malaria parasites increases, and thrombocytopenia may be used to assess the presence and severity of malaria [10,20,21]. The elevated platelet counts in the treated P. berghei mice in this study may suggest that the parasite density was drastically reduced, leading to an increase in platelet counts.

5. Conclusion

In conclusion the *Zingiber officinale* and *Hibiscus sabdariffa* extracts improved hematological parameters in *Plasmodium berghei*-infected mice, with the combined extracts showing greater effect, highlighting the therapeutic potential of natural alternatives.

Ethics Information

The study received approval from the Ethical Review Committee (UERC) of the University of Ilorin, and the number UERC/ASN/2018/1416 was given.

Source of Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Authors declare no conflict of interest.

Data Availability

The author confirms that the data supporting the findings in this study are available within the manuscript. Raw data that support the findings of this study is available from the corresponding author, upon reasonable request.

Author Contributions

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