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The Effects of Co-Administration of Mangiferin and Artemther-Lumefantrine on Hematological Indices of Plasmodium *Berghei Berghei*-Infected Swiss Albino Mice

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ABSTRACT

Malaria, caused by the parasite Plasmodium falciparum, is prevalent in Nigeria, with an average occurrence rate of 75%. However, it is possible to cure this disease. Chemical-based drugs often induce hematological abnormalities in blood tissues when employed as a treatment approach. This study was conducted due to the need to investigate the effect of co-administration of mangiferin and artemether-lumefantrine on hematological indices of plasmodium berghei-infected Swiss albino mice. Forty-two (42) albino Swiss mice were divided into seven groups, with four groups infected with the blood of highly parasitized P. berghei-infected mice, while three were control groups. Artemether-lumefantrine (AL) was administered as the standard drug, and Mangiferin was used for treating the parasite. Group 1 was the normal control and received normal feed and water ad libitum. Group 2 was infected with P. berghei. Groups 3 and 4 were given 8 mg/kg body weight of artemether-lumefantrine and 410.79 mg/kg of mangiferin, respectively. Groups 5 and 6 were infected with P. berghei and treated with 8 mg/kg body weight of artemether-lumefantrine and 410.79 mg/kg of mangiferin, respectively. Group 7 was infected with P. berghei and treated with artemether-lumefantrine and mangiferin at doses of 8 mg/kg and 410.79 mg/kg body weight, respectively. The animals were sacrificed, and blood was obtained for the estimation of hematological parameters. In the study, giving antimalarial drugs and mangiferin together had a big effect on the production of red blood cells, the decrease in infection, and the clumping of platelets in mice that were infected with P. berghei. The study recommends further research to look into the long-term effects and safety issues of giving mangiferin along with antimalarial drugs in cases of uncomplicated malaria.

KEYWORDS: malaria, Mangiferin, Artemether Lumefantrine (Act), Hematological Indices and Parasitemia.

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Introduction

Malaria affects around 280 to 290 million individuals on a yearly basis, and natural antimalarial medications include quinine and artemisinin. Nevertheless, the majority of contemporary antimalarial medications have lost their efficacy against Plasmodium falciparum. Elizabeth et al. identified anemia as the second most prevalent hematological anomaly in cases with malaria infections. The hematological abnormalities associated with malaria infection vary depending on the following factors: Factors that affect the development of immunity to malaria include demographic variables, individual background, the level of malaria incidence in a certain region, and the existence of hemoglobinopathies. The pathophysiological mechanisms behind alterations in blood composition in malaria are complex and not completely comprehended. Nevertheless, the precise diagnosis and comprehension of these alterations enable doctors to respond effectively and avert severe consequences for individuals with malaria. The World Health Organization has indicated for over two decades that hyperparasitemia is a defining feature of severe P. falciparum malaria. Multiple studies have established an association between the quantity of parasites and the severity of malaria illness. Furthermore, an elevated degree of parasitemia causes anemia due to the excessive destruction of red blood cells by the parasites. It is crucial to note that humans have utilized many treatment methods, including chemical-based medications and ethnobotanical treatments, to avoid the blood-related abnormalities induced by malaria infections.

Blood is a connective tissue that flows through enclosed vessels, providing oxygen and nutrients to cells while eliminating metabolic waste products. Red blood cells, also known as erythrocytes, white blood cells, and thrombocytes, are essential for various physiological processes within the human body. Red blood cells make up 6–10% of an organism's total body weight and play a crucial role in the efficient operation of the circulatory system.

Hemoglobin, a pigment responsible for the crimson hue in blood, is a crucial molecule involved in oxygen transportation. It binds with oxygen molecules and aids in the transportation of carbon dioxide waste from various tissues to the pulmonary system. The coloration of red blood cells varies based on the condition of hemoglobin.

Anemia is a medical disorder characterized by a deficiency of hemoglobin in cells, leading to an accelerated loss of red blood cells compared to their replenishment. Polycythemia is a medical disorder characterized by an elevation in the circulating mass of red blood cells beyond the established normal range.

The mean corpuscular hemoglobin concentration (MCHC) is a measure used in hematology to assess the concentration of hemoglobin in red blood cells. Platelets are vital blood components involved in hemostasis and blood clotting. Originating from bone marrow cells, they are smaller than red blood cells and easily disintegrate upon contact with foreign substances. Platelet count ranges from 150,000 to 400,000 per cubic millimeter. White blood cells, also known as leukocytes, protect the immune system against foreign agents. They differentiate from red blood cells due to their nuclei and are categorized into five types: neutrophils, basophils, lymphocytes, and monocytes.

Typically, a high white blood cell (WBC) count signifies an elevated production of white blood cells in response to an infection, a drug-induced reaction that stimulates WBC production, a bone marrow disease that leads to excessive production of white blood cells, or an immune system disorder that enhances white blood cell production (Butura, 2008). Conversely, a low white blood cell (WBC) count is typically attributed to viral infections that temporarily interfere with the functioning of the bone marrow, congenital disorders that result in reduced bone marrow function, cancer or other diseases that harm the bone marrow, autoimmune disorders that eliminate WBCs or bone marrow cells, severe infections that deplete WBCs at a faster rate than they can be produced, medications like antibiotics that destroy WBCs, and sarcoidosis (accumulations of inflammatory cells in the body) (Cowman, 2012).

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The purpose of this study was to validate the efficacy of combining antimalarial drugs with mangiferin in order to prevent hematological abnormalities during the administration of successful therapies.

Objectives of the study

The Objective of this study is to investigate the effects of co-administration of mangiferin and artemether-lumefantrine on the hematological indices of plasmodium *berghei-infected* Swiss albino mice.

Materials and Methods:

Collection and preparation of leaves for *Mangifera indica*

Mangifera indica leaves that had just reached maturity were collected from Nkit Itam in Itam, the local government area of Akwa Ibom State in Nigeria. The University of Uyo's Botany Department handled the authentication procedure. For the Mangifera indica sample, specimen voucher number UUH4361 (Itam) was assigned. The leaves of the *Mangifera indica* plant were gathered, carefully cleaned, and then let air dry for five days at room temperature.

Purification of Mangiferin

Method**: (**Jutiviboonsuk and Sardsaengjun, 2010).

The leaves of Mangifera indica L. var. Alphonso were collected, air-dried, and turned into a powder. The defatted powder was separated using aluminum foil and cotton wool. The defatted leaves were then extracted with 70% methanol for 72 hours, resulting in a semi-solid mass. This mass was divided into five parts using dichloromethane and dissolved in 50% ethanol. The resulting ethanolic phase was hydrolyzed, and the ethyl acetate fraction was divided four times using 100 milliliters of ethanol. The resulting ethyl acetate fraction was dissolved in ethanol and refrigerated overnight. The precipitate was separated using column chromatography. The dried extract was then isolated using three separate columns containing hexane, dichloromethane, ethyl acetate, and ethanol. The isolated compound was then separated and dried into pale yellow, needle-shaped crystals. The isolated compound was further studied using various techniques, including UV/VIS spectroscopy, thin layer chromatography, melting point studies, gas chromatography, and high-performance liquid chromatography. The results were compared with a mangiferin reference standard. The extract was collected and stored in a sterile tube at room temperature.

Procurement of Artemeter-Lumefantrine and Malaria Parasites

The reference antimalarial (Malenter DS) was obtained from Siban Pharmacy, 27 Ikpa Road, Akwa Ibom State, Nigeria, and it was a product of Norvartis Pharmaceuticals UK Ltd.

Plasmodium berghei was acquired from the Department of Microbiology and Parasitology, National Institute for Medical Research, Yaba, Lagos. It was sub-passaged and kept in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.

Experimental Animals

42 Swiss male mice, weighing between 20 and 30 grams, were procured from the animal house of the Pharmacology and Toxicology department, Faculty of Pharmacy, University of Uyo. The animals were made to acclimatize for ten (10) days. Their bedding was changed every two days in well-ventilated cages with a wooden bottom and a wire mesh top, kept at a constant temperature of 29±2°C. The animals received free access to water as well as commercial mouse pellets made by Phizer Livestock Ltd., Aba, but purchased from Uyo Main Market, Uyo.

Inoculum Preparation

Anowi et al. (2015) said that they gave 0.2 milliliters of parasitized red blood cells that were dissolved in normal saline (2 in 10 milliliters) intraperitoneally to mice that had been taken from a group of mice that already had malaria to make them get malaria. The animals that were induced were given therapy after three

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days, during which time blood was extracted from veins near their tails to determine the extent of parasitemia.

Determination of Parasitemia and Calculation

Anowi et al. (2015) conducted a study on parasitemia determination using modified techniques. Infected mice were given blood samples from their tails and placed on a sterile glass slide. The slide was fixed with methanol, stained with Giemsa stain and buffer, and examined with an oil-coated microscope lens. Parasitemia was assessed by counting the infected red blood cells among 200 randomly selected red blood cells. The parasitemia percentage was calculated using the formula: % Parasitemia = (Number of Parasite-Infected RBCs / 200) × 100.

Experimental Design

Exactly 42 male Swiss mice weighing 20–30 g were grouped into 7 groups, each containing six mice, as seen in Table 1.

Table 1. Experimental design showing mice grouping and treatment

Groups	No. of Animals	Treatment
1.	6	Normal feed and water only (NC)
2.	6	Parasitized (normal feed and water only) (PC)
3.	6	Normal + 8 mg/kg A-L only twice daily for 3 days (A-L)
4.	6	Normal + 400 mg/kg M mangeferin only twice daily for 3 days (M)
5.	6	Parasitized + 8 mg/kg A-L twice daily for 3 days (P+AL)
6.	6	Parasitized + 400 mg/kg M mangiferin extract twice daily for 3 days (P+M)
7.	6	Parasitized + 8 mg/kg A-L + 400 mg/kg M twice daily for 3 days (P+AL+M)

NC: Normal Control, PC: Parasite Control, A-L: Arthemeter-Lumefantrine, M: Mangiferin, P+A-L: Parasite and Arthemeter-Lumefantrine; P+M: Parasite and Mangiferin; P+A-L+M: Parasite, Artemeter-Lumefantrine, and Mangiferin

Source: compiled by the researcher (2023).

Extract and Drug Administration

The mice were given both the extract and the drug in a single and combined dosage by carefully inserting an oral canula using the oral method of drug delivery. After three days of doing this twice a day, blood samples were taken for examination.

Termination of the Experiment and Sample Collection for Hematological Analysis

Animals were anesthetized with chloroform, and blood samples were collected from their hearts. Red blood cells (RBC), platelets (PLT), hemoglobin (HGB), packed cell volume (PCV), and mean cell hemoglobin concentration (MCHC) were all found in whole blood samples. An automated hematological analyzer, 2016 model number (SYSMEC XP 300), was used for the analysis of the hematological parameters.

Estimation of blood parameters

The analysis will be carried out using the Sysmex hematological system, which is an automated method for the assay of hematological indices.

Statistical Analysis

The data was analyzed with the Microsoft Excel spreadsheet tool and the Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, Illinois). The data was presented using the mean value together with its corresponding standard deviation. The analysis of variance (ANOVA) was utilized to evaluate the existence of variances among distinct groups. A two-tailed P value that exceeds 0.05 was considered to have statistical significance.

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Results

Parasite count before and after treatment

Table 2 shows that, as compared to the host, Plasmodium berghei significantly (P<0.05) raised the plasmodium count in the parasite control group. The group of parasitized mice treated with ACT showed a substantial (P<0.05) drop in parasite count compared to the parasite control group. The number of parasites in parasitized mice treated with Mangiferin was significantly higher (P<0.05) than in normal mice treated with artemether lumefantrine. On the other hand, the number of parasites was significantly lower (P<0.05) in both parasite control mice and parasitized mice treated with ACT. Parasitized mice treated with ACT and Mangiferin showed a significant (P<0.05) decrease when placed side by side with parasite control and parasitized mice treated with Mangiferin.

Effect of Co-administration of Mangiferin and Artemether Lumefantrine on Hematological Indices of Plasmodium-Infected Swiss Albino Mice

As shown in Table 3 and represented in Figures 1–3, this study found that the parasite control group significantly increased in the WBC group compared to the other groups, including NC. But, worthy of note, there was also a substantial decrease in the single treatment groups and combined treatment groups compared to the parasite control group. High WBC counts indicate various conditions, such as an increased need to fight an infection, side effects from medication, bone marrow diseases, immune system disorders, and medications like antibiotics.

In comparison to the NC group, the PC, A-L, and P+M groups showed a significant drop in lymphocyte count, suggesting that medications are effective in treating malaria. The redistribution of lymphocytes due to splenic sequestration may explain the decrease in lymphocyte numbers linked to malaria.

P+A-L significantly decreased in comparison to NC, A-L, and M, while HGB decreased in P+M relative to NC. P+A-L+M significantly increased in comparison to P+M, suggesting parasitemia and even anemia. Packed cell volume (PCV) is a useful tool for screening for anemia when it is not possible to measure hemoglobin.

A-L and M had a substantial increase in platelet concentration, suggesting parasitemia. The parasite's impact on blood affects platelets, which are crucial for maintaining healthy homeostasis. Red blood cells (RBCs) are essential for cardiovascular health and are involved in various biological processes related to cardiovascular health.

Table 2: Parasite Count Before and After Treatment

Post infection	Day 1	Day 2
Host	3.00 ± 0.00	3.00 ±0.00
Normal mice treated with artemether-lumefantrine	0.00 ±0.00	0.00 ± 0.00
Parasite control	8.17 ± 1.90	32.40 ±.17*
Parasite and Artemether Lumefantrine	10.17 ±3.64	0.00 ± 0.00[b]
Parasite + Mangiferin Lumefantrine and Mangiferin	14.33 ± 4.92[a]	10.50 ±1.44[a,b,c]
Parasite and Artemether Lumefantrine and Mangiferin	8.90 ± 3.25	0.00 ± 0.00[b,d]

Values are expressed as mean ±SEM.

* = p<0.05 vs. host

a = p<0.05 vs. normal mice treated with artemether-lumefantrine

b = p<0.05 vs. parasite control

c = p<0.05 vs. parasite + ACT

d = p<0.05 vs. parasite + Mangiferin

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Table 3: The Effect of Co-administration of Mangiferin and Artemether Lumefantrine on Hematological Indices of Plasmodium-Infected Swiss Albino Mice

GROUPS	TREATMENT	WBC(10^3µl)	LYM (%)	RBC(10^6 µl)	HGB(g/dl)	PCV(%)	PLT(10^3 µl)
1	Normal Control	9.34±0.80	93.0±1.99	8.72 ± 0.26	14.43±0.60	48.10±2.65	252.07±171.01
2	Parasite Control	27.42±5.56*	81.40±6.35*	6.80 ± 0.51	10.93±0.44	35.97±1.67	234.00±2.31
3	A-L	8.92±0.35[a]	81.33±3.14*	8.42 ± 0.38	13.60±0.53	47.73 ± 3.57	724.00±51.19*[a]
4	Mangiferin	11.69±3.46	92.17±1.68	7.91 ± 0.07	12.60±0.06	42.40±0.46	756.33±41.56*[a]
5	P+AL	10.26±3.64[a]	84.93±5.08	5.19 ±1.94*[b]	8.30±3.16*[b,c]	27.10±10.33*[b,c]	366.3±104.27[b]
6	P+M	12.98±0.10[a]	81.34±0.77*	6.34 ± 0.03	10.32±0.05*	32.89±0.35*[b]	108.21±0.70 [b,c]
7	P+A-L+M	10.95±0.43[a]	92.30±3.18	8.07 ± 0.33[d]	12.53±0.38[d]	44.47±1.67[d]	360.00±121.82 [b,c]

Values presented as Mean ± Standard Error of Mean (SEM), n = 3,

* = significantly different from the normal control at $p < 0.05$.

a = significantly different from parasite control at $p < 0.05$.

b = significantly different from A-L, at $p < 0.05$.

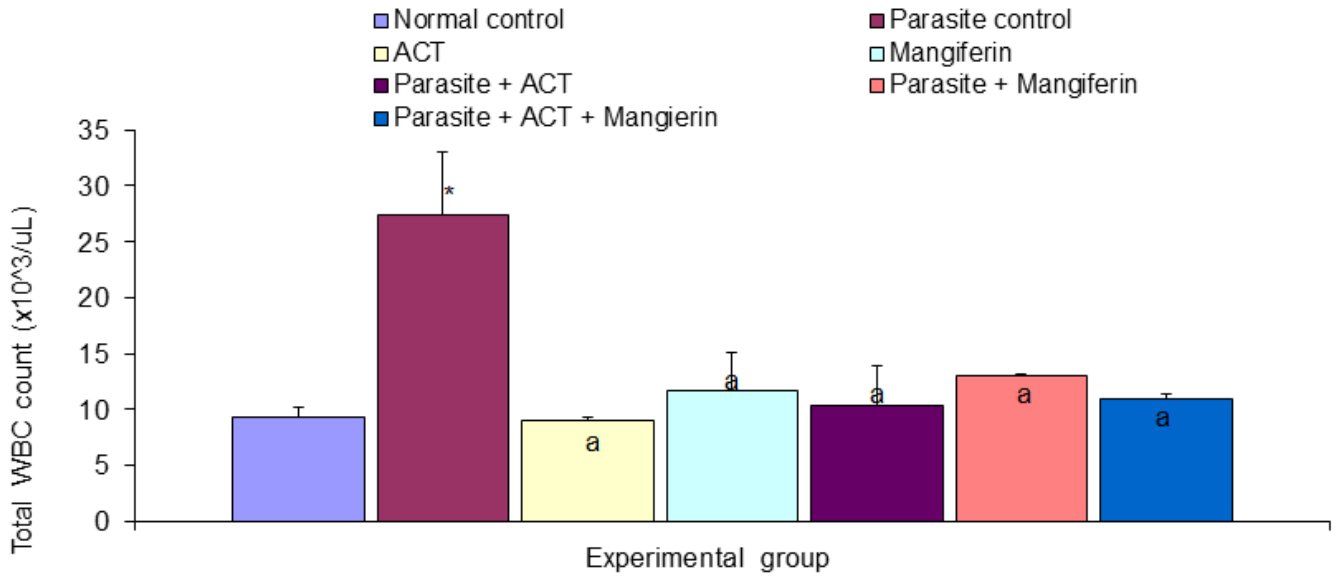
c = significantly different from Mangiferin at $p < 0.05$,

d = significantly different from parasite + A-L at $p < 0.05$

A-L = artemether-lumefantrine

M = Mangiferin P = Parasite

Fig. 1: Bar Chart Showing Total White Blood Cell Count in Different Experimental Groups



Values are expressed as mean +SEM, n = 3.
* = significantly different from normal control at p<0.05
a = significantly different from parasite control at p<0.05

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Fig. 2: Bar Chart Showing Total Red Blood Cell Count in Different Experimental Groups

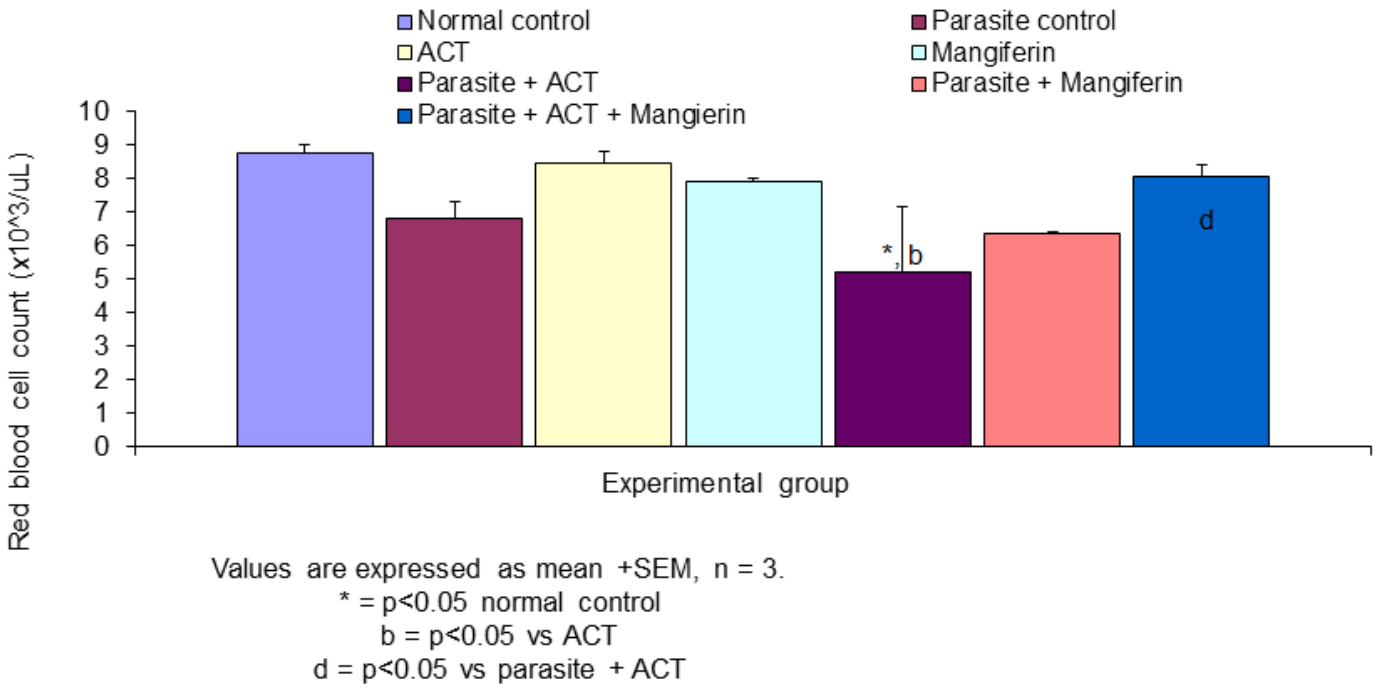
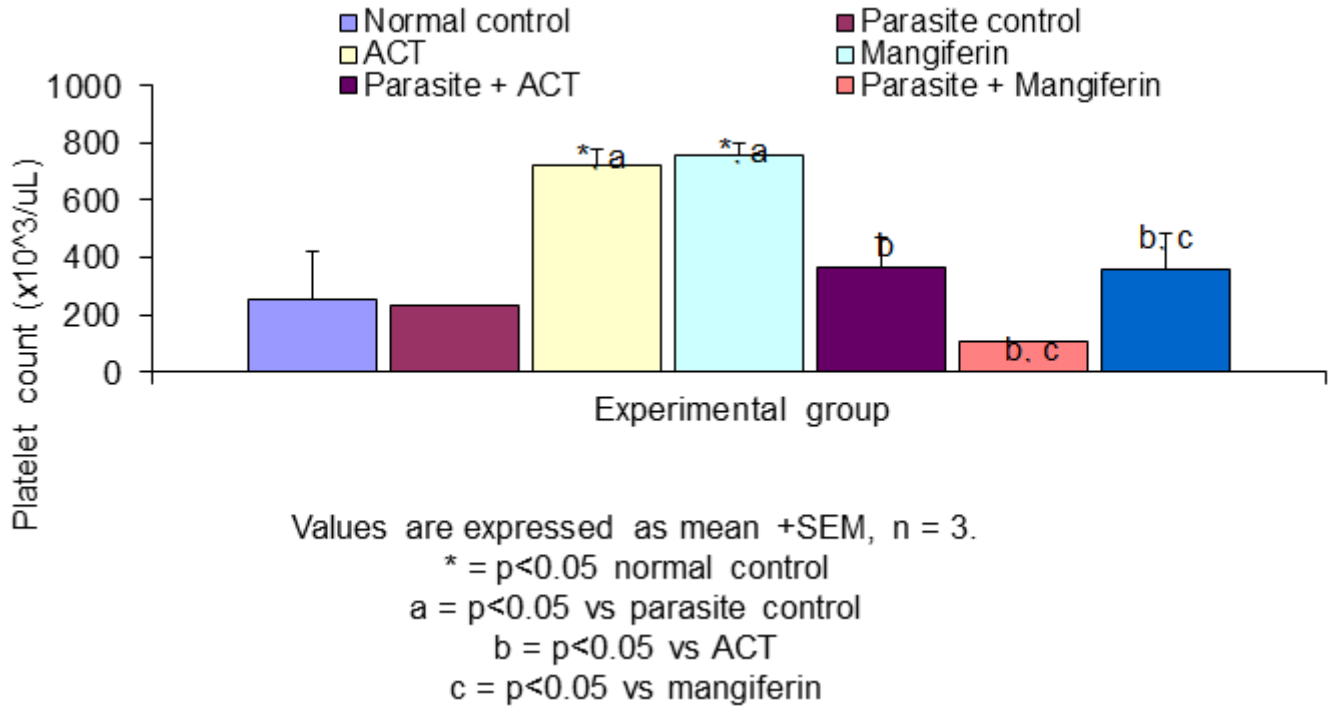


Fig. 3: Platelet Count in the Different Experimental Groups



Conclusion

Overall, when Swiss albino mice were given therapeutic doses of both mangiferin and the standard regimen of antimalarial drugs, the hematological indices showed that WBC levels, RBC levels, and platelet levels all

got better. In mice infected with P. berghei, the synergistic effect had a big effect on the production of red blood cells, the decrease in infection, and the clumping of platelets.

Recommendation

1. **Further Research and Clinical Trials:**

a. Perform more research and clinical studies to verify and reproduce these findings in a wider and more varied sample size.

b. It is important to carefully look into the long-term effects and safety issues of giving mangiferin along with antimalarial drugs to humans.

2. **Optimization of Combination Therapy:**

a. Investigate the most effective dose schedules for mangiferin and antimalarial medications to optimize therapeutic advantages while limiting potential adverse reactions.

b. Assess the effectiveness of this combination at various phases of malaria infection.

3. **Synergistic Mechanism:**

a. Investigate the underlying mechanisms of the observed synergistic effects, including potential interactions at the molecular and cellular levels.

b. Explore whether the combination therapy has a broader application in combating other infectious diseases.

c. Encourage the integration of this combination therapy into malaria treatment protocols, if proven effective and safe in human studies.

4. **Regulatory Approval:**

a. Collaborate with regulatory authorities to initiate the process for approval and inclusion of the combined therapy in national and international malaria treatment guidelines.

5. **Production and Distribution:**

a. If proven effective and safe in human trials, promote the production and distribution of pharmaceutical formulations containing mangiferin and antimalarial drugs as a combined therapy.

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