

Original Research Article

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Dietary *Ficus glumosa* Leaf Consumption Alters Parasite Dynamics and Total Protein Levels in *Plasmodium berghei*-Infected Mice

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ABSTRACT

Malaria remains a significant public health challenge, particularly in Africa, where it leads to hundreds of thousands of deaths annually. This study investigated how dietary consumption of *Ficus glumosa* leaves (mountain fig) influences parasite load and protein levels in *Plasmodium berghei* infected mice. Fresh leaves of *Ficus glumosa* were harvested from the University of Cross River State (UNICROSS), Calabar, Nigeria. The leaves were blended and incorporated into diets at concentrations of 5g, 10g, and 20g per 100g of normal rat chow. A total of 36 mice, weighing between 23g to 35g, were acclimatized and thereafter 30 of these mice were inoculated intraperitoneally with 0.2ml blood suspension containing 1×10^7 parasitized erythrocytes of chloroquine-sensitive *Plasmodium berghei* (NK-65) obtained from the Nigerian Institute of Medical Research (NIMR), Lagos. The mice were divided into six groups: groups one, two, and three received diets incorporated with 5%, 10%, and 20% of powdered *Ficus glumosa* leaves per 100g of rat chow respectively; group four received 5mg/kg BW of Artesunate; group five served as the negative control (parasitized but untreated); and group six served as the normal control (non-parasitized, normal diet). Results showed significant variability in mean parasite counts among the groups ($p < 0.05$), with Group five having the highest mean parasite count (279,137.92 P/ μ L) and Group three the lowest (110,034.92 P/ μ L). Chemo-suppression rates were highest in Group three (61%), followed by Groups two and four (58%), and lowest in Group one (54%). Serum total protein levels varied, with Group four showing the highest mean level (5.8348 g/dL) and Group one the lowest (5.7635 g/dL). This indicates that *Ficus glumosa* may have enhanced the host's immune system, providing a dual mechanism of action; directly targeting the parasite and boosting the host's natural defenses. The modulation of protein levels suggests that *Ficus glumosa* consumption impacts both clearance and the regulation of the inflammatory response, which is critical in the pathology of malaria. However, the exact mechanisms behind these effects remain unclear and require further biochemical analyses.

Keywords

Anopheles mosquitoes, global health, antimalarial treatment, geography

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Introduction

Malaria remains one of the most prevalent tropical and infectious diseases in the world, with estimated clinical

cases of 200 million every year (WHO, 2018). It is caused by *Plasmodium berghei* in experimental models and *Plasmodium falciparum* in humans. Previous research has highlighted a potential link between malaria

infection and alterations in serum total protein levels (Alqasoumi *et al.*, 2014). Malaria often leads to systemic inflammation and immune responses in the host (WHO, 2018). Studies have shown that during malaria infection, there is a significant decrease in serum total protein levels, attributed to increased protein catabolism, liver dysfunction, and the release of acute-phase proteins (Talakpo *et al.*, 2019). Additionally, malaria-induced hemolysis and tissue damage contribute to the release of protein breakdown products into the bloodstream, further affecting serum protein levels. Targeting serum total protein levels in malaria patients could offer insights into disease severity, prognosis, and treatment response (Tuteja, 2007). Therefore, understanding the dynamics of serum total protein alterations during malaria infection is essential for developing effective diagnostic and therapeutic strategies to combat this debilitating disease (Talakpo *et al.*, 2019). Additionally, medicinal plants have given humans access to a wide range of powerful medications that can treat or completely remove illnesses and infections (Nana *et al.*, 2012). Globally, the usage of plant-based medications is growing, which has created a pressing need to produce better medications for the treatment of gastrointestinal problems, diabetes, liver diseases, and inflammatory disorders that are safe for both humans and the environment (Olaokun *et al.*, 2013). Through recent researches on herbal plants and medicines, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine (Schulz *et al.*, 2001). Consequently, plants can be described as a major source of medicines, not only as isolated active principles to be dispensed in standardized dosage form but also as crude drugs for the population (Agbodeka *et al.*, 2016). Modern medicines and herbal medicines are complementarily being used for health care program in several developing countries such as countries in Africa, Asia and some part of Europe (Angell & Kassirer, 2010).

Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are often prescribed, even if their chemical constituents are not always completely known (Indu and Crozier, 2010). The therapeutic potency of a medicinal plant is due to the presence of some bioactive components. These bioactive components are ascertained using phytochemical screening such as phytochemical tests and thin layer chromatography (Agbodeka *et al.*, 2016). Moreso, one of such medicinal plants which have demonstrated effective therapeutic behavior is *Ficus glumosa*.

Ficus glumosa is a medicinal plant used in East Africa, Nigeria, Cameroon and Senegal for the treatment of skin diseases and diabetes (Madubunyi *et al.*, 2012). The plant's leaves and bark are used in food in Northern Cameroon and Southern Chad to stimulate the production of milk in both women and animals (Di-Gennaro *et al.*, 2020). Additionally, they are used to treat hemorrhoids, edema, and cardiovascular conditions like hypertension (Ntchapda *et al.*, 2014). Recent research by Abu *et al.*, (2020) showed how gastrointestinal motility is impacted by the methanol extract of *F. glumosa* leaves. Awolola *et al.*, (2019) suggested that *Ficus glumosa* is reputed for the treatment and management of various health conditions, including diarrhoea, dysentery, oedema, headache, stomach ailments and ulcers.

In addition, Madubunyi *et al.*, (2012) demonstrated the antidiabetic and antioxidant properties of the *F. glumosa* Del. (Moraceae) stem bark methanol extract in alloxan-induced diabetic mice. Methanolic extract of *F. glumosa* stem bark had subacute antidiabetic and in vivo antioxidant properties on alloxan-induced hyperglycemic rats, as reported by Onoja *et al.*, (2014). Based on the findings of previous research, this current study is focused on accessing the how powdered *Ficus glumosa* leaf influences parasite load and total protein levels in *Plasmodium berghei* infected mice.

Materials and Methods

Plant sample collection, identification and preparation

Fresh leaves of *Ficus glumosa* were harvested from the campus of University of Cross River State (UNICROSS) Calabar campus, Calabar South Local Government Area of Cross River State, Nigeria.

The Voucher Specimen of the plant was deposited in the herbarium of the Department of Biology of same University. Thereafter, the leaves were thoroughly washed, shade dried at 25⁰C for two weeks and subsequently blended into fine powder using an electric blender.

Feed formulation

The diets were prepared by incorporating 5g, 10g, and 20g of blended *Ficus glumosa* leaves with 95 g, 90 g, and 80 g normal rat chow respectively.

Handling of animals

A total of 36 mice weighing between 23g to 35g were purchased from a disease-free stock in the Faculty of Basic Medical Sciences animal house, University of Cross River State, and housed in metallic cages under standard conditions (12 hr light/12 hr dark, 28°C±3°C and 40-55% humidity) for one week of acclimatization before commencement of the experiment.

The mice were fed normal rat chow and tap water *ad libitum* throughout the durations of the experiment. The animals had free access to food and tap water and were treated according to the international guidelines for the care and use of laboratory animals.

Parasite inoculation

Chloroquine sensitive *Plasmodium berghei* (NK-65) obtained from the Nigerian Institute of Medical Research (NIMR), Lagos State, Nigeria, and maintained alive in the mice. One milliliter of parasitized erythrocyte was obtained from a donor-infected mouse by cardiac puncture and made up to 5 ml with normal saline.

Mice were inoculated intraperitoneally with 0.2ml blood suspension containing 1×10^7 parasitized erythrocytes on day zero. The mice were observed to produce clinical signs such as salivation, reduced activity, body weakness, convulsion, etc., before the commencement of treatment. Mice in groups one to five were inoculated. Mice in groups one, two and three were treated with dietary incorporation of *Ficus glumosa* leaves while mice in group four were treated with Artesunate, mice in group five were not given any treatment.

Animal treatment

Animals were grouped for experimental studies and were fed with the dietary incorporation of *Ficus glumosa* leaves, following the inoculation of *Plasmodium berghei* parasite. The mice were weighed at the start of the experiment and randomly assigned into six study groups of six mice in each group. Group six (Normal control) received normal diet and tap water *ad libitum*, group five (negative control) was parasitized and received normal diet and tap water only, group four (Artesunate DS®-treated) received 5mg/kg BW of Artesunate, groups three, two, and one were fed with diets incorporated with 5g, 10g, and 20g of *Ficus glumosa* leaves respectively.

Sample collection and determination of parasitemia

After an overnight fast, all mice were sedated with ketamine and dissected. The samples were collected through cardiac puncture using sterile syringes and needles. Blood smears were made, stored with Giemsa for microscopic examination. Percentage parasitemia was obtained by counting the number of parasitized erythrocytes out of 20 erythrocytes in random field of the microscope. Percentage parasitemia is calculated using the formula.

$$\% \text{ Parasitemia} = \frac{\text{Number of infected RBCs} \times 100}{\text{Total number of RBCs (1000)}}$$

Sample collection and Serum total protein

After an overnight fast, all mice were sedated with ketamine hydrochloride and dissected. Blood obtained by cardiac puncture using plain bottles, sterile syringes and needles. Serum total protein was measured using spectrophotometer (Agilent Technologies, Inc. 5301 Stevens Creek Blvd Santa Clara, CA 95051 USA) at the biochemistry department of University of Calabar, Cross River State, Nigeria, following the methods described in RANDOX kits.

Statistical analysis

All data collected were summarized as mean ± SD. Post hoc comparisons to evaluate pairwise differences among group means were conducted with the use of Turkey HSD test on SPSS (version 26). The differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Parasitemia profiles of mice infected with *Plasmodium berghei* and treated with dietary incorporation of *Ficus glumosa* leaves and Artesunate

The result for parasitemia profiles of mice infected with *Plasmodium berghei* is presented in table 4.1. The table presents the mean parasite count (P/μL) of *Plasmodium berghei*-infected mice across five groups, showing significant variability in both mean values and standard

deviations. Group five had the highest mean parasite count at 279,137.92 P/μL with a substantial standard deviation of 220,527.50, indicating significant within-group variability. Conversely, Group three exhibited the lowest mean parasite count at 110,034.92 P/μL with a much lower standard deviation of 8,207.72, indicating more consistent and effective parasite suppression within this group. Group one had a mean parasite count of 128,950.73 P/μL and a high standard deviation of 75,453.80, reflecting considerable variability. Group two showed a mean parasite count of 116,765.52 P/μL and a standard deviation of 47,848.73, while Group four had a mean parasite count of 117,474.17 P/μL and a standard deviation of 8,563.82, both indicating moderate to high variability. The overall mean parasite count for all groups combined was 150,472.65 P/μL with a standard deviation of 112,169.01.

Furthermore, table 4.2 provides chemo-suppression percentages across four groups of *Plasmodium berghei* infected mice, highlighting the effectiveness of different treatments in reducing parasite counts. Group three achieved the highest chemo-suppression rate at 61%, suggesting it may be the most effective treatment among the groups. Both Group two and Group four exhibited a chemo-suppression rate of 58%, indicating similar levels of efficacy. Group one had the lowest chemo-suppression rate at 54%, this represents a substantial reduction in parasite count.

Effect of the treatment on serum total protein of *Plasmodium berghei* infected mice

In table 4.3, it presents serum total protein levels in *Plasmodium berghei* infected mice across six groups, showing variability in both mean values and standard deviations. Figure 2, presents the graphical illustration of the variability between different groups. Malaria remains a leading cause of morbidity and mortality worldwide. Despite the conventional pharmaceutical interventions to control the disease, challenges such as drug resistance and vector control still persist, necessitating the exploration of alternative therapeutic approaches. The development of plant-derived medicines in modern medicine was prompted by the use of plant materials as indigenous cures in folklore or traditional medical systems. The world is currently moving toward herbal or phytomedicines, which serve to repair and restore body systems, especially the immune system, which can then effectively resist external invaders, and aid to eradicate harmful infections without having negative side effects

(Khan & Ahmad, 2019). Nevertheless, plant such as *Ficus glumosa*, have been found to exhibit medicinal properties. It is commonly known as “African rock fig” and is reputed for the treatment and management of various health conditions in African traditional medicine, including dysentery, oedema, hypertension, headache, stomach ailments, menstrual pains, skin diseases, rheumatism, and diabetes mellitus and to treat female sterility (Orwa *et al.*, 2009; Madubunyi *et al.*, 2012). But its impact on parasite dynamics and protein regulation in malaria infected organisms is not well understood. This study investigated how dietary consumption of *Ficus glumosa* leaves (mountain fig) influences parasite load and protein levels in *Plasmodium berghei* infected mice. The results obtained highlight the potential of *Ficus glumosa* leaf consumption in altering parasite dynamics and protein levels in *Plasmodium berghei*-infected mice. As presented in table 4.1, mice fed with diets containing *Ficus glumosa* leaf exhibited a significant reduction ($p < 0.05$) in parasitemia compared to the untreated group. Compounds that reduce parasitaemia by 30% or more are considered active and are further evaluated for secondary screening (Benjamin, 2016). Thus with the percentage chemosuppression observed in table 4.2 above, *Ficus glumosa* qualifies for further evaluation for its antiplasmodial property. This observation is important as it indicates a potential antimalarial activity of the plant; this aligns with previous studies that have identified various *Ficus* species as sources of bioactive compounds with therapeutic properties. Worthy of note is the observation of Obi-Abang *et al.*, (2020) that revealed the presence of alkaloids and flavonoid as the predominant secondary metabolite in *Ficus glumosa* leaves. Alkaloids are one of the major sources of natural products that exhibit antimalarial activity. Indeed, quinine, the first antimalarial drug, belongs to this class. Previous studies have also shown that plant-derived alkaloids have great potential for antimalarial drug development.

In addition to the reduction in parasite load, the study observed notable changes in protein levels amongst the group that consumed diets containing *Ficus glumosa* leaves. This indicates that *Ficus glumosa* may have enhanced the host's immune system, providing a dual mechanism of action; directly targeting the parasite and boosting the host's natural defenses. The modulation of protein levels suggests that *Ficus glumosa* consumption impacts both clearance and the regulation of the inflammatory response, which is critical in the pathology of malaria.

Table.1 Experimental Design for current study

S.No	Groups	Number of animals	Treatment
1	Normal control	6 mice	Rat chow and water
2	Positive control	6 mice	Inoculated with <i>Plasmodium berghei</i> and Fed Normal rat chow only
3	Standard control	6 mice	Inoculated with <i>Plasmodium berghei</i> , fed normal rat chow and administered 5mg/kg body weight Artesunate.
4	HighDose of Extract	6 mice	Inoculated with <i>Plasmodium berghei</i> and Fed Diets incorporated with 5g <i>Ficus glumosa</i> leaves
5	Extract Medium Dose	6 mice	Inoculated with <i>Plasmodium berghei</i> and Fed Diets incorporated with 10g <i>Ficus glumosa</i> leaves.
6	Extract Low Dose	6 mice	Inoculated with <i>Plasmodium berghei</i> and Fed Diets incorporated with 20g <i>Ficus glumosa</i> leaves

Table.2 Mean parasite count (p/μl) of *Plasmodium berghei*-infected mice

Groups	N	Mean Parasite count (P/μL)	Std. Deviation	Std. Error	Statistics
Group A	3	128950.726667	75453.7991255	43563.2712365	F=1.379
Group B	3	116765.523333	47848.7287021	27625.4763965	p= 0.309
Group C	3	110034.920000	8207.7211234	4738.7300000	NS
Group D	3	117474.166667	8563.8168087	4944.3219398	
Group E	3	279137.923333	220527.5045385	127321.6141090	
Total	15	150472.652000	112169.0061655	28961.9128560	

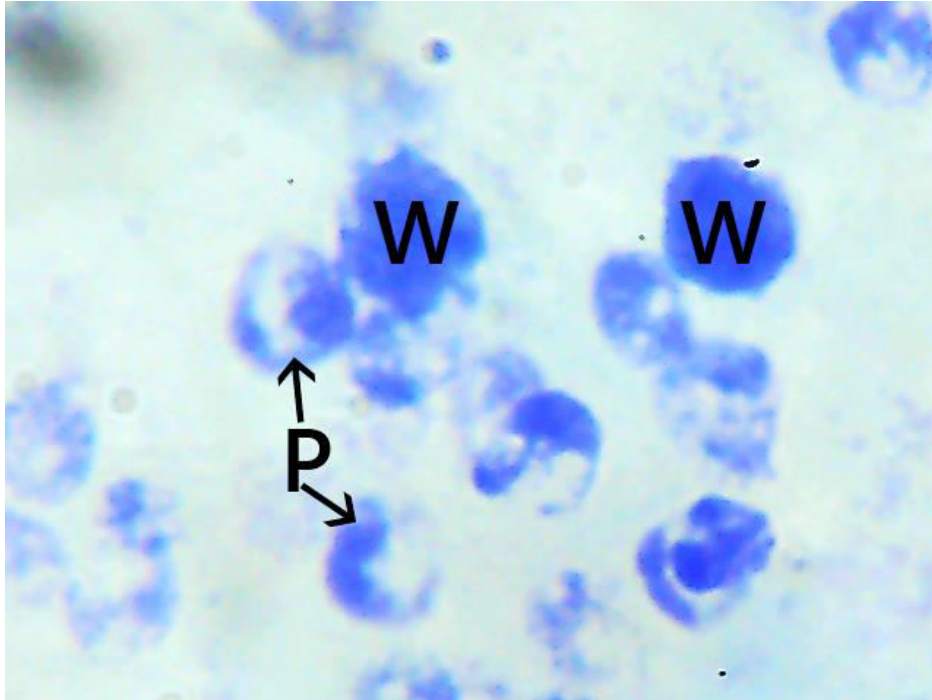
Table.3 Results for Chemo-suppression

Groups	Chemosuppression(%)	Statistics	
Group A	54	X ² =0.429	
Group B	58	P=0.934	
Group C	61		NS
Group D	58		

Table.4 Results for serum total protein of *Plasmodium berghei* infected mice

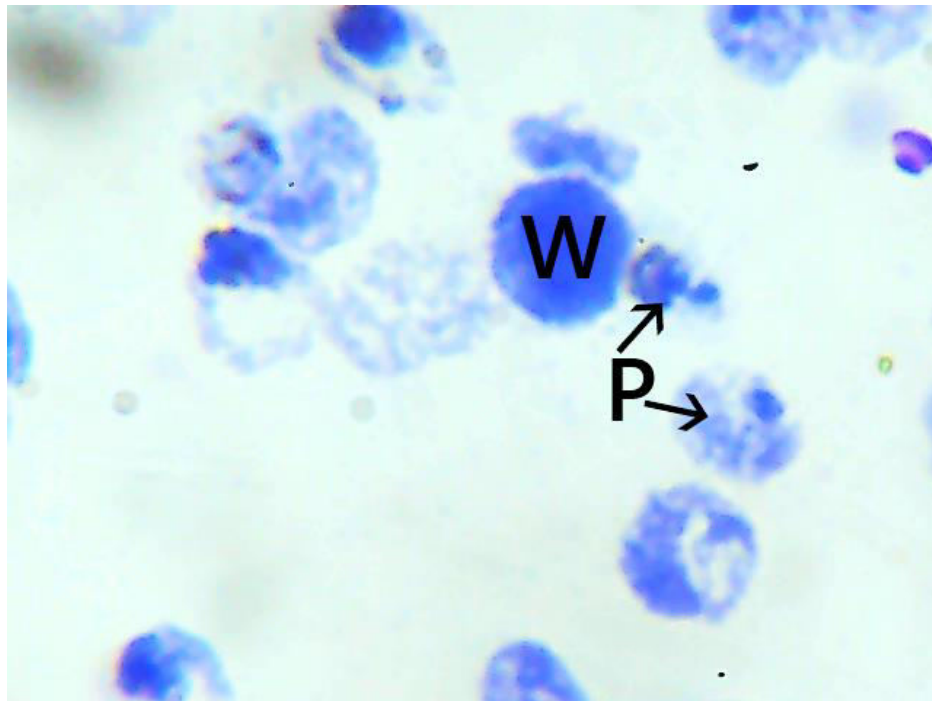
Groups	Serum total protein	Standard Deviation
Group 1	5.7635	41741
Group 2	5.5884	17546
Group 3	5.6192	22537
Group 4	5.8348	00000
Group 5	6.1142	52435
Group 6	6.3034	52118
Total	5.8773	44846

Figure.1



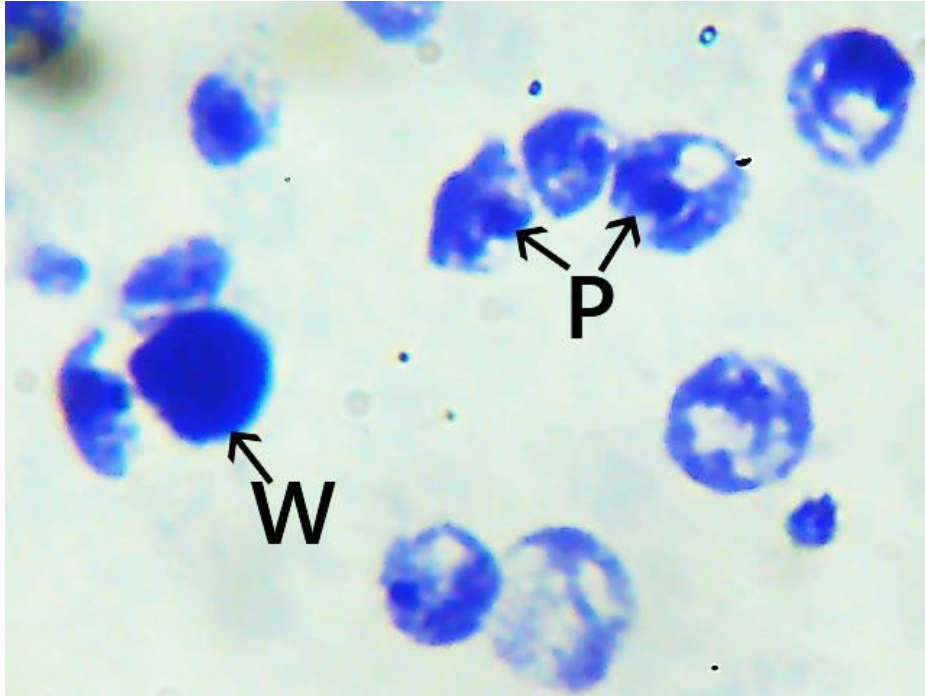
Gp1- There are white blood cells (W) and numerous ring form trophozoites of malaria parasite (P) in the thick blood film. Giemsa x1000 magnification.

Figure.2



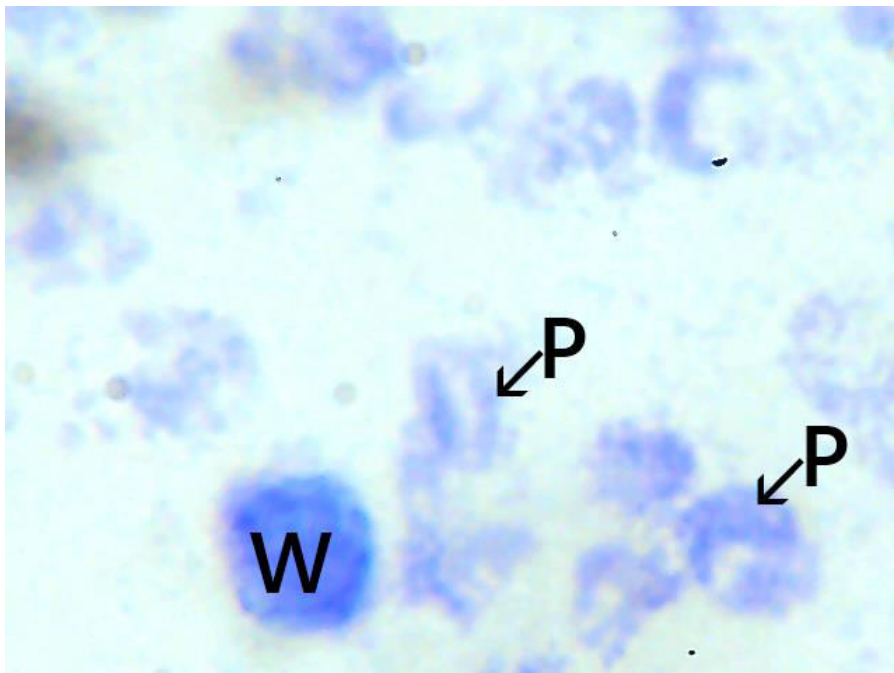
GP2- There are white blood cells (W) and numerous ring form trophozoites of malaria parasite (P) in the thick blood film. Giemsa x1000 magnification.

Figure.3



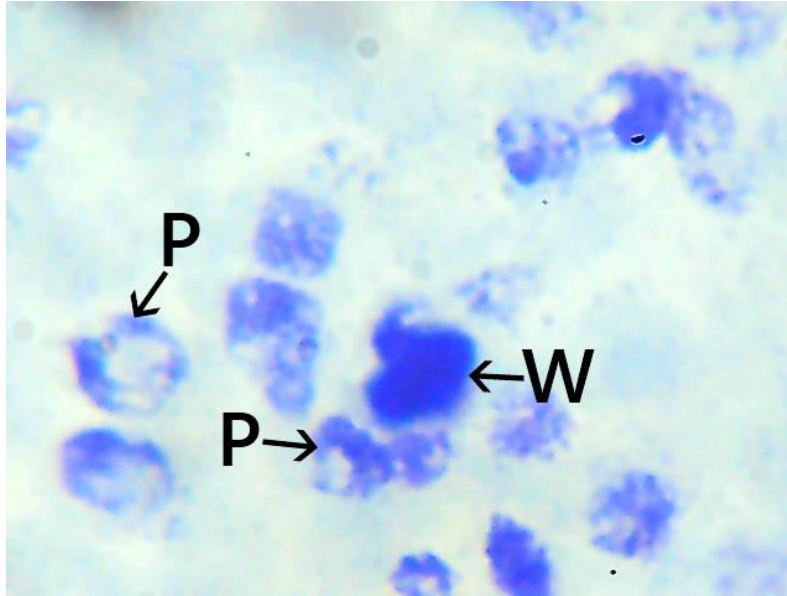
Gp3- There are white blood cells (W) and numerous ring form trophozoites of malaria parasite (P) in the thick blood film. Giemsa x1000 magnification.

Figure.4



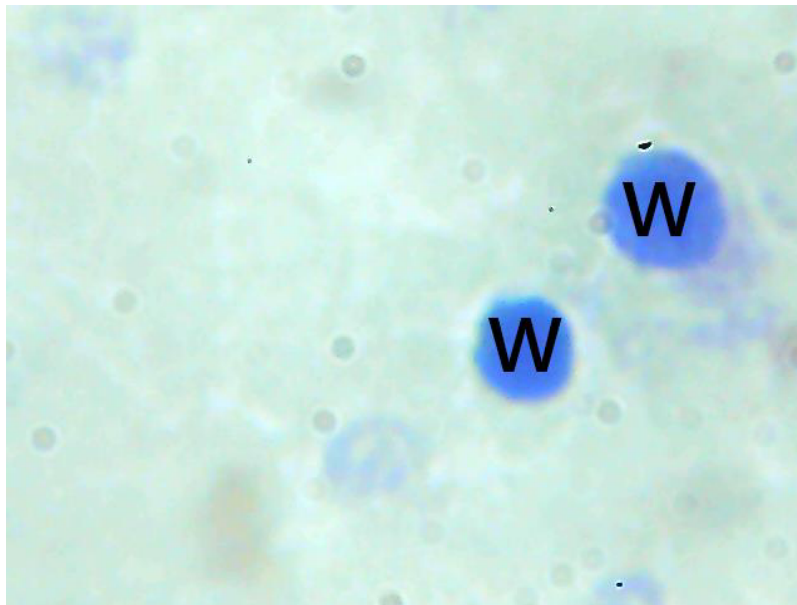
GP4- There are white blood cells (W) and few ring form trophozoites of malaria parasite (P) in the thick blood film. Giemsa x1000 magnification.

Figure.5



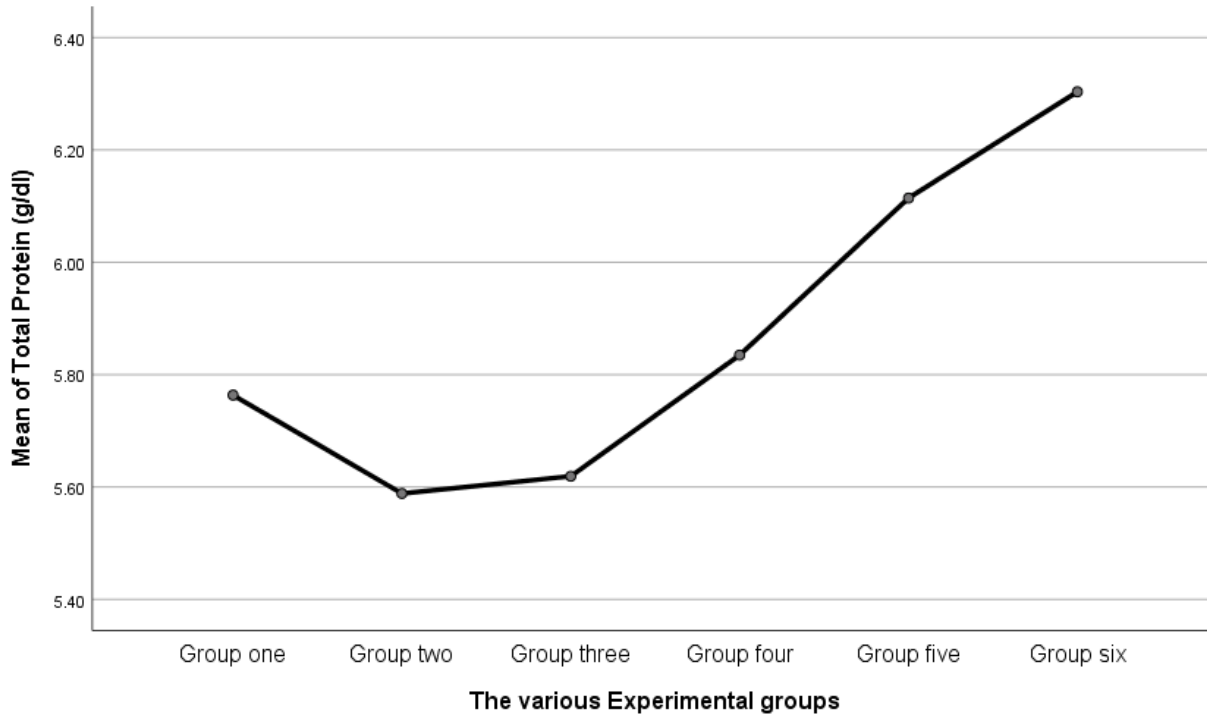
Gp5- There are white blood cells (W) and numerous ring form trophozoites of malaria parasite (P) in the thick blood film. Giemsa x1000 magnification.

Figure.6



GP6- White blood cells (W) only present in thick blood film. Giemsa x1000 magnification

Figure.7 Graphical presentation of the mean of total protein



However, the exact mechanisms behind these effects remain unclear and require further biochemical analyses. The study reveal that dietary in-cooperation of *Ficus glumosa* leaf produces significant variability in mean parasite count, chemo-suppression rates, and serum total protein levels of *Plasmodium berghei*-infected mice. These results pave the way for more detailed investigations into its mechanism of action, safety and efficacy as an adjunct or alternative therapy in malaria treatment.

Author Contributions

Magdalene Obi-Abang: Investigation, formal analysis, writing—original draft. Jonathan Osine Enyike: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

Not applicable

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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