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ENHANCING IMPACT OF *PROSOPIS AFRICANA* FRACTION ON BIOCHEMICAL INDICES AND OXIDATIVE BALANCE IN *PLASMODIUM BERGHEI*-INFECTED MICE

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ABSTRACT
This study addresses the ongoing global health challenge of malaria by exploring the therapeutic potential of the *Prosopis africana* fraction in mice infected with *Plasmodium berghei*. Using 42 male mice divided into six groups, including a control group, infected groups without treatment, infected treated with 20 mg/kg bw chloroquine, and infected groups treated with different doses of *Prosopis africana* fraction at 6.25 mg/kg,12.5mg/kg and 25 mg/kg bw respectively. The mice were induced by NK-65 chloroquine sensitive *Plasmodium berghei* and administered oral treatment for four days. After the treatment period, biochemical analysis of blood samples and tissues was conducted, focusing on liver and kidney function markers, as well as oxidative stress indicators. Mice infected with *Plasmodium berghei* exhibited significant alterations in biochemical indices and oxidative balance compared to the control group. However, treatment with *Prosopis africana* fraction mitigated these changes, restoring biochemical parameters and reestablishing oxidative balance. Notably, the dose at 12.5 mg/kg bw of *Prosopis africana* fraction demonstrated superior efficacy in reversing the alterations induced by malaria infection. These findings suggest that *Prosopis africana* fraction holds therapeutic potential in mitigating the biochemical disruptions and oxidative stress associated with malaria infection.

Keywords: Malaria, *Plasmodium berghei*, *Prosopis africana* fraction, biochemical indices, oxidative balance.

INTRODUCTION

Malaria, caused by the Plasmodium parasites, continues to pose a significant worldwide health challenge, impacting millions of individuals, particularly in sub-Saharan Africa, Southeast Asia, and South America (WHO, 2020). The emergence and proliferation of drug-resistant Plasmodium strains have compromised the efficacy of conventional antimalarial medications, compelling the urgent exploration of alternative therapeutic approaches (Ashley and Phyo 2018; White, 2018).

In this pursuit, natural products derived from medicinal plants have emerged as promising reservoirs of innovative antimalarial agents. One such botanical contender is *Prosopis africana*, an indigenous African tree species celebrated for its historical role in traditional medicine, offering relief for various ailments, including fever and malaria (Nwidu *et al.,* 2018). Extensive research has illuminated its pharmacological mastery, showcasing its abilities as an antioxidant, anti-inflammatory, and antimicrobial agent (Nwidu *et al.,* 2018; Odukoya *et al.,* 2019).

However, despite its rich heritage and potential, there is a limited scientific exploration of the specific effects of *Prosopis africana* on malaria infection and the related biochemical changes. In the face of the growing threat of drug resistance, the search for alternative malaria therapies becomes increasingly crucial (Amelo and Makonnen, 2021).

This study focus on investigating the impact of *Prosopis africana* fractions on biochemical markers and oxidative balance in mice infected with *Plasmodium berghei*. The research aims to make a meaningful contribution to the ongoing efforts against malaria, providing optimism for the development of effective interventions to reduce the global burden of this debilitating disease.

MATERIALS AND METHODS

Plant materials

The stem bark part of *Prosopis africana* was obtained from Igbo-owu in Ifelodun Local Government Area, Kwara State of Nigeria, during raining seasons around May- June, 2016. They were identified at the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria. Voucher number UIH 473 was issued and the sample was deposited at the University herbarium.

Malaria parasites

Plasmodium berghei NK65 choloroqunie sensitive strain was obtained from the Institute of Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Ibadan Nigeria, and was sustained in the laboratory by continuous passage of parasitized blood into mice.

Ethical Clearance

Ethical clearance for the study was obtained from the University of Ilorin Ethical Review Committee (UERC), with the UERC Approval number: UERC/ASN/2018/1416

Solvent partitioning

Solvent-solvent partition extracts of the crude aqueous extract was obtained by the method described by Hu *et al*. (2014).

Chromatographic separation of the partition extract

The methanol partition extract was fractionated by the method described by Patil *et al.* (2011). The extract was fractionated using column chromatography with a column size of (50cm X 5cm) and silica gel (Mesh 60-120).

Animal handling

Thirty-five adult albino mice, with average weight of 21.5 ± 1.4 g

obtained from Animal Holding Unit, Central Research Laboratory, University of Ilorin, Ilorin, Nigeria were used for the experiment. The mice were placed on standard pellet feed (top feeds) and were allowed free access to water. They were acclimatized for 2 weeks before the commencement of the study at standard laboratory conditions (12-hrs light/dark cycle, 25 ± 2^0C).

Inoculation of mice
The mice were inoculated intraperitoneally with *P. berghei* NK65, a chloroquine-sensitive strain. Tail blood was obtained from a donor mouse of known parasitaemia into a sample bottle containing 2 mL citrate/glucose solution. This was then diluted appropriately to obtain an inoculum size of 1×10^7 infected red blood cells in 200 µl which was used to infect each mouse.

Antimalarial Studies
The evaluation of the antimalarial activity of *P. africana* stem bark fractions was carried out using curative test by the method described by Rayley and Peters (1970).

Preparation of serum and tissue homogenates
The procedures described by Yakubu et *al.* (2006) were employed for the preparation of the serum. The procedure described by Akanji and Yakubu (2000) was used for the preparation of liver, kidney, and spleen homogenates.

Liver Function Tests
Determination of Alkaline Phosphatase (ALP) Activity
The procedure described by Wright *et al*. (1972b) was employed for the determination of alkaline phosphatase activity.

Determination of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) Activities
The procedure described by Reitman and Frankel (1957) was used for assaying the activities of alanine aminotransferase and Aspartate aminotransferase.

Determination of Serum Albumin Concentration
The procedure described by Doumas *et al.* (1971) was used for the determination of albumin in the serum of the mice.

Determination of serum bilirubin concentration
Bilirubin was determined by adopting the procedure described by Sherlock (1951).

Determination of serum protein concentration
The procedure described by Zaia *et al*. (2005) was used for the determination of total protein concentration in the serum of the mice.

Kidney Function Test
Determination of serum urea concentration
The method that was used for the determination of urea in the serum was that described by Veniamin and Vakirtzi (1970).

Determination of serum creatinine concentration
Serum creatinine concentration was determined spectrophotometrically by the method described by Kaplan and Pesce (1996).

Determination of serum uric acid concentration
The concentration of Uric Acid was determined using the procedure described by Fossati *et al*. (1980).

Determination of Plasma Sodium-Ion Concentration
Plasma sodium ion concentration was determined using the method of Tietz (1995).

Determination of Plasma Potassium Ion Concentration
The method described by Thomas (1998) was used in the determination of serum phosphate ions.

Determination of Plasma Chloride Ion Concentration
The method described by Thomas (1998) was used in the determination of plasma chloride ion concentration.

***In vivo* antioxidant study**
Determination of concentration of nitric oxide
The assay of nitrite/nitrate in organs (liver, kidney, and spleen) homogenate was done according to the method of Berkels *et al*. (2004).

Determination of concentration of Malnodialdehyde
The rate of lipid peroxidation in tissues was assessed by quantifying malondialdehyde (MDA) levels as described by Varshney and Kale (1990).

Determination of reduced glutathione
The levels of reduced glutathione (GSH) in the tissues were estimated by the method of Beutler and Yeh Mary (1963).

Determination of superoxide dismutase (SOD) activity
The method of Misra and Fridovich (1972) was used to determine SOD activity in tissues.

Determination of catalase (CAT) activity
The method of Sinha (1972) was used to determine catalase (CAT) activity.

Statistical analysis
Data were presented as means of five determinations ± standard error of the mean (SEM). The significance of difference among groups was determined by one-way Analysis of Variance (ANOVA); Duncan's test was used for the Post Hoc analyses and $p < 0.05$ was accepted as significant (Mahajan, 1997). The graph pad prism 5 and SPSS 16.0, SPSS Inc., Chicago, Illinois, USA, was used for analyzing the data.

RESULT
Tables 1 and 2 present both enzymatic and non-enzymatic activities observed in the serum of mice infected with *P. berghei* and treated with the *P. africana* fraction. The negative control group exhibited a significant ($p<0.05$) increase in serum enzyme activities, a decline in albumin and total protein, and a substantial ($p<0.05$) increase in bilirubin compared to the normal control. In contrast, treatment with 12.5 mg/kg bw of the *P. africana* fraction resulted in a significant ($p<0.05$) reduction in serum enzyme activities and an increase in non-enzymatic activities, resembling the positive control and falling within the range of the normal control.

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Table 1: Effect of *P. africana* Fraction on Enzymatic Liver Function Parameters in *P. berghei*-Infected Mice

	ALP (mmol/min/mg protein)	LDH (U/L)	AST (U/L)	ALT (U/L)
Normal control	19.82 ± 0.42^{a}	22.05 ± 0.69^{a}	16.96± 1.37^{a}	9.47 ± 0.47^{a}
Negative control	35.20 ± 1.62^{b}	46.46 ± 2.66^{b}	32.30 ± 0.90^{b}	33.71 ± 0.98^{b}
Positive control	23.89 ± 0.62^{c}	23.54 ± 2.14^{a}	22.786 ± 0.53^{c}	10.07 ± 0.17^{a}
Infected + 6.25 mg/kg bw PaF treated	25.87 ± 0.62^{d}	38.24 ± 0.14^{c}	21.97 ±0.72^{c}	14.19 ± 0.37^{d}
Infected + 12.5 mg/kg bw PaF treated	20.77 ± 0.23^{a}	24.02 ± 1.18^{a}	18.35 ± 0.43^{a}	10.03 ± 0.75^{a}
Infected + 25 mg/kg bw PaF treated	26.79 ± 0.96^{d}	24.30 ± 1.36^{a}	23.32 ± 0.27^{c}	12.38 ± 0.36^{c}

Key: Uninfected not treated = normal control, infected not treated = negative control, infected treated with 20 mg/kg bw of CQ = positive control, PaF = *Prosopis africana* fraction, CQ = chloroquine, bw= body weight. ALP = alkaline phosphatase, LDH = lactate dehydrogenase, , ALT = alanine aminotransferase, AST = aspartate aminotransferase. The values are mean of five replicate ± standard error of mean, values with different superscripts in the same column are statistically different ($p<0.05$).

Table 2: Effect of *P. africana* Fraction on Non- Enzymatic Liver Function Parameters in *P. berghei*-Infected Mice

	Albumin (g/dL)	Total protein (mg/ml)	Bilirubin (µmol/L)
Normal control	51.39 ± 0.79^{a}	46.76 ± 0.87^{a}	84.93 ±1.10^{a}
Negative control	22.90 ± 0.98^{b}	26.70 ± 0.48^{b}	211.02 ± 5.19^{b}
Positive control	47.34 ± 1.84^{a}	44.99 ± 1.09^{a}	87.53 ± 3.70^{a}
Infected + 6.25 mg/kg bw PaF treated	41.57 ± 2.80^{c}	35.79 ± 0.80^{d}	101.82 ± 2.46^{c}
Infected + 12.5 mg/kg bw PaF treated	48.62 ± 1.10^{a}	45.81 ± 0.96^{a}	85.77 ±1.68^{a}
Infected + 25 mg/kg bw PaF treated	44.49 ± 2.43^{c}	41.01 ± 1.10^{c}	120.30 ±0.76^{d}

Key: Uninfected not treated = normal control, infected not treated = negative control, infected treated with 20 mg/kg bw of CQ = positive control, PaF = *Prosopis africana* fraction; CQ= chloroquine; bw= body weight. The values are means of five replicate ± standard error of mean. Values with different superscripts in the same column are statistically different ($p<0.05$).

Table 3 displays selected renal function parameters in *Plasmodium berghei*-NK65-infected mice treated with the *P. africana* fraction. The negative control group exhibited a significant ($p<0.05$) increase in serum urea, uric acid, and creatinine concentrations, coupled with a significant ($p<0.05$) decrease in sodium, potassium, and chloride concentrations compared to the normal control.

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Table 3: Renal function parameters in *Plasmodium berghei*-NK65-infected mice treated with *P. africana* fraction

	Urea (mmol/L)	Uric acid (mmol/L)	Creatinine (mg/dL)	Sodium (Mmol/L)	Potassium (mg/dL)	Chloride (mg/dL)
Normal control	341.70 ± 6.49^{a}	3.167 ± 0.07^{a}	09.57 ±0.37^{a}	67.37 ± 0.49^{a}	3.51 ± 0.10^{a}	53.37 ± 1.45^{a}
Negative control	527.92 ± 4.76^{b}	5.79 ± 0.19^{b}	13.14 ± 0.49^{b}	54.02 ± 1.50^{b}	1.53 ± 0.14^{b}	32.23 ± 1.94^{b}
Positive control	352.50 ± 2.30^{a}	3.73 ± 0.04^{a}	10.60 ± 0.38^{a}	66.16 ± 0.62^{b}	2.95 ± 2.10^{a}	49.89 ± 2.52^{a}
Infected + 6.25 mg/kg bw PaF treated	468.02 ± 10.30^{c}	4.58 ± 0.08^{c}	7.20 ± 0.63^{d}	64.92 ± 0.79^{c}	2.28 ± 0.07^{c}	42.54 ± 0.84^{c}
Infected + 12.5 mg/kg bw PaF treated	334.95 ± 4.86^{a}	3.29 ± 0.04^{a}	10.06 ± 0.29^{a}	67.96 ± 1.04^{a}	3.30 ± 0.14^{a}	50.13 ± 0.26^{a}
Infected + 25 mg/kg bw PaF treated	430.85 ± 0.56^{c}	4.46 ± 0.08^{c}	11.61 ± 0.59^{c}	65.33 ± 0.46^{c}	2.54 ± 0.10^{c}	44.00 ± 1.17^{c}

Key: Uninfected not treated = normal control, infected not treated = negative control, infected treated with 20 mg/kg bw chloroquine = positive control, PaF = *Prosopis africana* fraction; bw= body weight. The values are means of five replicate ± standard error of mean. Values with different superscripts in the same column are statistically different ($p<0.05$).

Table 4 presents the antioxidant status in the liver and kidney of mice infected with *Plasmodium berghei*-NK65 and treated with the *Prosopis africana* fraction. The negative control group exhibited a significant ($p<0.05$) increase in Malondialdehyde (MDA) and nitric oxide (NO) concentrations, along with a significant ($p<0.05$) decrease in reduced glutathione (GSH) concentrations in selected tissues compared to the normal control. Treatment at 12.5 mg/kg bw of the fraction also demonstrated a significant ($p<0.05$) increase in MDA and NO concentration, with a significant ($p<0.05$) decrease in GHS concentration compared to the positive control. Figure 1 and figure 2 show the Superoxide dismutase (SOD) and catalase activities in the liver and kidney of *Plasmodium berghei*-NK65-infected mice treated with the *Prosopis africana* fraction. A significant ($p<0.05$) decrease in SOD activity and significant decreased in catalase activity was observed in the selected tissues of the negative control when compared to the activity observed in the normal control. Furthermore, treatment with the *P. africana* fraction at 12.5 mg/kg bw demonstrated significant ($p<0.05$) activity that was similar to the SOD and catalase activities recorded in the positive control.

Table 4: Concentrations of Malnodialdehyde, nitric oxide and glutathione in selected tissues of *Plasmodium berghei*-NK 65-infected mice treated with Prosopis *africana* fraction

	MDA (U/mg protein)		GSH (mg/dL)		NO (d/L)	
	Kidney	Liver	Kidney	Liver	Kidney	Liver
Normal Control	2.50 ± 0.11^{a}	2.30 ± 0.02^{a}	93.12 ± 3.14^{a}	80.34 ± 2.35^{a}	320.07 ± 10.07^{a}	217.11 ± 3.53^{a}
Negative control	3.67 ± 0.16^{b}	3.96 ± 0.07^{b}	45.94 ± 1.02^{b}	56.44 ± 2.36^{b}	641.02 ± 4.52^{b}	523.36 ± 7.07^{b}
Positive control	2.87 ± 0.12^{c}	2.19 ± 0.02^{a}	64.25 ± 0.75^{c}	80.38 ± 0.97^{a}	328.46 ± 4.12^{a}	223.78 ± 9.06^{c}
Infected + 6.25 mg/kg bw PaF treated	2.80 ± 0.08^{c}	2.28 ± 0.06^{a}	53.99 ± 1.30^{d}	63.51 ± 1.01^{c}	363.72 ± 21.21^{c}	239.11 ± 5.73^{c}
Infected + 12.5 mg/kg bw PaF treated	2.83 ± 0.05^{c}	2.27 ± 0.05^{a}	75.52 ± 0.84^{ac}	80.51 ± 0.71^{a}	331.85 ± 4.22^{a}	217.50 ± 4.61^{a}
Infected + 25 mg/kg bw PaF treated	2.94 ± 0.09^{c}	2.55 ± 0.12^{ab}	54.58 ± 0.64^{d}	67.61 ± 1.64^{c}	339.88 ± 5.63^{a}	275.84 ± 13.1 9^{c}

Key: Uninfected not treated = normal control, infected not treated = negative control, infected treated with 20mg/kg bw chloroquine = positive control, PaF = *Prosopis africana* fraction, bw= body weight, MDA= Malnodialdehyde, GSH= reduced glutathione, NO= nitric oxide. The values are means of five replicates ± standard error of mean, values with different superscripts in the same column are statistically different ($p<0.05$).

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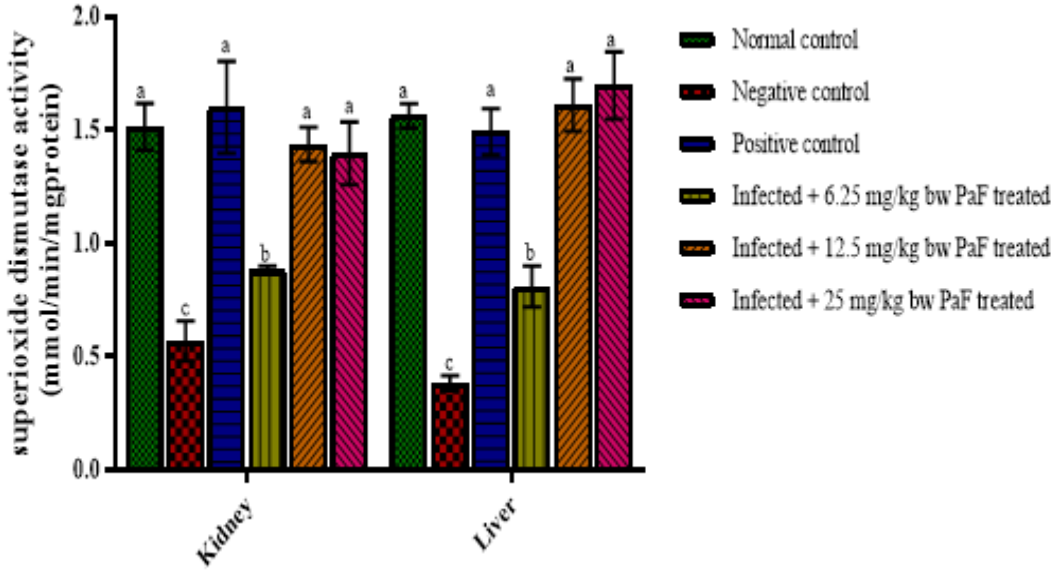

Figure 2: Catalase activities in selected tissues of *Plasmodium berghei*-NK65-infected mice treated with the *Prosopis africana* fraction

Key: Non-infected = normal control, infected not treated = negative control, infected treated with 20 mg/kg bw of chloroquine = positive control, PaF = *Prosopis africana* fraction, bw = body weight. The bars are means of five replicate ± standard error of mean, bars for each tissue with different superscripts are statistically different at $p<0.05$.

DISCUSSION

The study's findings suggest that malaria parasite infection caused an increase in assayed enzyme activities in the serum, likely due to leakage from the tissue resulting from organ injury. This aligns with the conclusions of Woodford *et al*. (2020) and Enechi *et al.* (2019), attributing the derangement in liver function enzyme activities to the hepatotoxic effect or membrane disruption activities of the parasite during its extra-erythrocytic life cycle in the liver. However, the observed ameliorative effect of the fraction at 12.5 mg/kg bw suggests improved enzyme activities, approaching normal control values. This effect may be linked to membrane stabilization and the maintenance of hepatocyte integrity, preventing the leakage of liver enzymes into the circulatory system (Oyedapo and Famurewa, 1995).

Moreover, the reduction in total protein and albumin concentrations observed may be attributed to the fraction's ability to restore abnormalities caused by the parasite to normal values after treatment. In this study, the decrease in serum bilirubin concentration in the treated group may result from the reduction in red blood cell destruction by *Plasmodium berghei* due to parasite clearance and the antioxidant effect of the extract, as bilirubin is a derivative of RBC destruction (Trampuz *et al.,* 2003; Enechi *et al*., 2019).

The rise in these serum parameters in the negative control during malaria infection is likely due to inflammation and interstitial nephritis induced by the malaria parasite, aligning with findings from Essien-Baidoo *et al.* (2019). However, treatment with *Prosopis africana* fraction at 12.5 mg/kg bw led to significant ameliorative effects, restoring the altered levels of serum urea, creatinine, and uric acid. This outcome is consistent with the observations of Anigboro (2018), who noted a normalization of these parameters in malaria-infected mice treated with various plant extracts.

In addition, a significant ($p<0.05$) alteration in serum electrolyte levels (Na, K, Cl) observed in the negative control after the establishment of malaria infection aligns with the work of Arise *et al*. (2013) and Kiru *et al*. (2018), who reported similar electrolyte imbalances in untreated *P. berghei*-infected mice. However, the extract's ameliorative effect suggests interference with the electrolyte imbalance induced by the malaria parasite.

The degradation of host hemoglobin by the malaria parasite induces continuous oxidative stress on red blood cells (RBCs), leading to the generation of reactive oxygen species (ROS). To counteract the harmful effects of reactive species, the body relies on antioxidant defense mechanisms such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). GSH plays a crucial role in protecting cells against oxidative stress and toxins. MDA, a product of lipid peroxidation, serves as an indicator of oxidative damage and is associated with various pathological conditions. Nitric oxide (NO) functions as a redox signal, limiting oxidative injury by scavenging ROS or inducing the antioxidant system.

Studies by Luersen *et al*. (2000) found decreased levels of antioxidant enzymes (CAT and SOD) and proteins, along with increased lipid peroxidation (MDA) during malaria infection, consistent with the significant increase in antioxidant enzyme activities observed in this study. Additionally, Nwankwo *et al.* (2017) reported a decrease in MDA concentration in parasitized mice treated with Picralima nitida Seed extract. Similarly, this study observed a significant reduction in MDA concentration with treatment at 12.5 mg/kg bw of the *P. africana* fraction, aligning with Nwankwo *et al.* (2017)'s findings.

Conclusion

In conclusion, the findings of this study underscore the complex interplay between malaria parasite infection and host physiological parameters. However, treatment with the *Prosopis africana* fraction at 12.5mg/kg bw showed promising results, suggesting membrane stabilization and restoration of hepatocyte integrity, which may mitigate enzyme leakage. Furthermore, the fraction demonstrated the ability to restore abnormal levels of total protein, albumin, bilirubin, and serum electrolytes, indicating its potential in alleviating the systemic impact of malaria infection. Notably, the reduction in lipid peroxidation and enhancement of antioxidant enzyme activities highlight the extract's antioxidative properties, crucial in mitigating oxidative stress induced by the malaria parasite. These findings provide valuable insights into the therapeutic potential of natural extracts in combating malaria-associated complications and warrant further investigation into their mechanisms of action..

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