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Prosopis africana Stem Bark Extract: Effects on Parasitaemia and Haematological Parameters of *Plasmodium berghei* -infected Mice

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ABSTRACT: *Herbal medicine practitioners use Prosopis africana* for the treatment of malaria in endemic regions of sub-Saharan Africa. In this study, the acclaimed antimalarial activity of aqueous extract of *P. africana* stem bark was investigated. Thirty mice (21.05 ± 0.56g) were randomly assigned into six groups. Group A served as uninfected control, whereas animals in groups B, C, D, E and F were infected with *Plasmodium berghei* (NK 65 chloroquine-sensitive), and treated for four days with 0.2ml of distilled water, 20 mg/Kg body weight (bw) of chloroquine, 100, 150, and 200 mg/Kgbw of the extract respectively. Phytochemical analysisof the extractshowed the presence of alkaloid (5.08 mg/g), anthraquinone (1.43 mg/g), flavonoids (4.72 mg/g), glycosides (4.41mg/g), phlobatannins (0.19mg/g), tannins (2.03 mg/g) and saponins (1.22 mg/g). Treatment with the aqueous extract of *P. africana* stem bark at 100, 150, and 200 mg/kg bw produced 89.63, 82.16 and 75.60% chemo-suppression of the parasite respectively, whereas 84.89% was recorded in mice treated with the reference drug, chloroquine. Infection of mice with *P. berghei* significantly ($p<0.05$) reduced white blood cells, red blood cells, haemoglobin, packed cell volume and platelets counts, whereas treatment of the infected mice with the extract significantly ($p<0.05$) increased the level of these haematological indices. This study showed that aqueous extract of *P. africana* stem bark significantly reduced the parasite in the blood of infected mice and enhance the haematological indices associated with *P. berghei* infection, with the best activity at 100 mg/Kg bw of the extract.
Keywords: *Prosopis africana, Plasmodium berghei*, herbal medicine, haematological parameters, parasitaemia

Introduction

Nearly half of the world's populations areat risk of having malaria infection with about91 countries and territories within areas at risk of malaria transmission (WHO, 2018). The sub-Saharan region of Africa continues to carry a disproportionately high share of the global malaria burden, with an estimated 228 million clinical episodes, and 405,000 deaths (WHO, 2018). Six countries from the region account for more than half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (12%), Uganda (5%), Niger (4%), Cote d'Ivoire (4%) and Mozambique (4%) (WHO, 2019).

Complication from severe malaria causes damage to vital organs and tissue such as liver, kidney, spleen, brain and blood.

Haematological parameters are indicativeand predictive values of the association between blood cells and malaria infection (Kimbi *et al*., 2013). Alteration in the haematological parameters in individuals down with malaria wasthe most common complication of the disease. These changes convoluted the primary cell line such as red blood cell, leucocytes and thrombocytes (Maina *et al*., 2010). Haematological changes such as anaemia, thrombocytopenia, and leukocytosis or leukopenia have been reported in malaria. The degree of alterations of the blood parameters varies according to the parasitaemia; the nutritional status, the background hemoglobinopathy, the socio-demographic factors and immunity to disease (Erhart*et al*., 2004) in the affected patients. Severe injury and death may arise from malaria anaemia, particularly in children and pregnant women (Menendez *et al*., 2000) as evident in the world malaria report of the WHO in 2018 and 2019.

Plant products have been part of phytomedicine which can be derived from any part of the plant like bark, leaves, flowers, seed etc. that contain active component (Cragg and David, 2001). Phytochemicals are bioactive compounds present in a different part of the plant and their extracts.

Prosopis africana is a leguminous plant that wildly grows in the savanna region; the tree is mostly found growing wild in Northern and the Middle Belt of Nigeria, in the area like Zamfara, Kaduna, Yobe and Enugu states. The other States where it can be found include Taraba, Kogi, Nassarawa, Benue etc. (Idoko *et al.,* 2013). Previous studies on the plant have established its antioxidant, anti-inflammatory, wound-healing and antimicrobial activities (Ayanwuyi *et al.,* 2010; Ezike *et al*., 2010; Ajiboye *et al*., 2013; Henciya *et al*., 2017). Previously, some commonly used plants for the treatment of malaria in Ilorin metropolishave been screened against *Plasmodium berghei*-infected mice. At doses of 50 mg/Kgbw and 200 mg/Kg bw respectively, *P. africana* was reported to have shown the highest therapeutic activity against the malaria parasite (Abubakar *et al.,* 2016).

Various drugs have been developed to treat malaria, but the health and financial burden of the disease persist due to the improved resistance to pharmaceutically approved antimalarial drugs. Moreover, the alarming rate at which the parasite develops resistance make it imperative to search for more effective, affordable and safer therapeutic agents that can be used to treat malaria as well as the secondary complications associated with the disease. Thus, the aim of this study was to determine the secondary metabolites present in the aqueous extract of *P. africana* stem bark; evaluate the effects of the extract on the level of parasitaemiaand haematological parameters of *P. berghei*-infected mice.

Materials and Methods

Plant materials and authentication

The plantwas obtained from Igbo-owu in Ifelodun Local Government Area, Kwara State, Nigeria. The plant wasauthenticated at the herbarium Unit of Department of Plant Biology where a Voucher specimen (UIH473) was deposited.

Ethical clearance

Before laboratory and experimental study with animals, the ethical authorisation for this study was obtained from the University of Ilorin Ethical Research Committee and approval number UERC/ASN/2018/1416 was issued. The study adheres strictly and conforms to the Guide for the care and use of laboratory animals. National Academies Press; 2010 (Council NR, 2010).

Laboratory Animals

Thirty albino mice with average weight (21.05 ± 0.56g) obtained from the Animal Holding Unit of Departmentof Biochemistry, University of Ilorin, Nigeria,was used for the experiment. The mice were maintained on standard pellet feed and allowed access to drinking water. They were acclimatised for two weeks before the commencement of the study.

Malaria parasite

NK65 chloroquine-sensitive strain of *Plasmodium berghei*was obtained from the Institute for Advanced Malaria Research and Training (IMRAT), College of Medicine, University of Ibadan, Nigeria, and sustained in the laboratory by continuous passage of parasitised blood into mice.

Extract Preparation

Fresh Prosopis *africana*stem bark waswashed dried under the room temperature (25ºC-35ºC) for two weeksbefore crushed into powder. A known volume (500 mL) of distilledwater was used to exhaustively extract 50 g of the powdered plant sample for two days at room temperature 25 ± 2°C. The filtrate was concentrated using the freeze dryer (ZIRBUS, VaCo 5-11, zirbus technology, Germany) and serial dilutions were made with distilled water to give the appropriate dose that was administered to the infected mice.

Animals grouping

The mice were grouped randomly into six (A-F) of five animals per cage. The mice in group A (control)were not infected but administered with distilled water. The twenty-fivemice in Group B - F were inoculated intravenously with 1×10^6 red blood cells infected with the chloroquine-sensitive *P. berghei* NK65 strain. The day of inoculation was defined as day zero (D0). Group B was infected but also administered with distilled water served as an untreated group. Group C was treated with chloroquine at a dose of 20 mg/Kg body weight (bw). Groups D, E and F received 100, 150 and 200 mg/Kg bw of the extract, respectively.

Antimalarial assay

The antimalarial activity of the extracts was determined using curative antimalarial test model Ryley and Peters (1970). After four days of treatment,tail tips of the micewere cut to prepare the blood smears. The air-dried films were then fixed with methanol,stained with Giemsa solution. The parasitaemia level was examined under the microscope at magnification x 100 oil immersion. The percentage parasitaemiawas computed from the following expression:

Parasitised RBC/ (parasitized RBC + Non- parasitized RBC) x 100

The mean percentage chemo-suppression of parasite was determined using Obih and Makinde (1985) expression:

$$\textbf{Mean percentage chemo-suppression} = \frac{\textbf{mean\% parasitaemia in control} - \textbf{mean\% parasitaemian in test}}{\textbf{mean \% parasitaemia in control}} \times \textbf{100}$$

Determination of secondary metabolites

The phytochemical screening of the consitutent secondary metabolites in the plants extract was carried out following the methods discribed by Trease and Evans (1978) and Santaram and Harborne (1984).

Blood preparation and determination of haematological parameters

The mice were sacrificed on the four days post-treatment by anaesthetizing with diethyl ether. Blood was collected in well labelled sample bottles containing EDTA for the hematological analysis. The automated haematologic analyzer, Sysmex KX- 21(SYSMEX Corporation JAPAN) was used to analyze the haematological parameters.

Statistical analysis

Data were presented as mean of seven determinations ± standard error of mean (SEM). The significance of difference among groups were determined by the one-way Analysis of Variance (ANOVA), the Tukey's Test was used for the Post Hoc analyses and $p < 0.05$ was accepted as significant level (Mahajan, 1997).

Results

Phytochemical consitutents from the crude aqueous extract of *Prosopis africana* stem bark was presented inTable 1. Alkaloid was the most abundant (26.63%) class of secondary metabolites found in the extract, followed by flavonoids (24.74%) and cardiac glycosides (23.11%) whilephlobatanninswas the least (1.00%).

Figure 1 shows parasitaemia level of infected mice treated with aqueous extract of *P. africana* stem bark. Treatment with various doses (100, 150 and 200 mg/Kg bw) of *P. africana* extract for four days significantly ($p<0.05$) reduced parasitaemia level when compared with the infected mice administred distilled water to serve as the untreated group. Post-treatment assessment showed that percentage parasitaemia gradually decreased in treated groups, while it continued to increase in the untreated group. On the 8th day (4 days after treatment), the highest reduction in parasitaemia level among the *P. africana* treated groups was observed in those treated with 100 mg/Kg bw of the aqueous extract and values was not significantly different from infected mice treated with the standard drug (choloriquine).

Percentage inhibition of *Plamodium berghei* in infected mice by *P. africana* extract is shown in Figure 2. Treatment for four days with either chloroquine or various doses of the extract exhibited significant inhibition of the malaria parasite (75 -90%) and the suppresive effect was sustained during the post-treatment periods. On the 8th day (four days after treatments were discontinued), groups treated with 100, 150 and 200 mg/kg bw of aqueous extract of *P. africana* stem bark recorded 89.63, 82.16 and 75.60% chemo-suppression of the parasite respectively while 84.89% inhibition was observed in chloroquine-treated group.

Figure 3 shows the red blood cells (RBC) and white blood cells (WBC) counts of *P. berghei* infected mice treated with aqueous extract of *P. africana.* Other relevant haematological parameters (packed cell volume (PCV), haemoglobin (Hb) and platelets count) are shown in Table 2.

Blood indices such as RBC, Hb, PCV, WBC and platelet counts significantly ($p < 0.05$) decreased in mice infected with the malaria parasite when compared with the uninfected control group. However, these haematological parameters were significantly ($p < 0.05$) increased in infected groups treated with various doses of *P. africana* extract and chloroquine, when compared with values determined in the untreated group. The haematological indices of mice treated with100 mg/Kg bwwere within the normal range determined in the uninfected control group.

Table 1: Secondary metabolite of aqueous extract of *Prosopis africana* stems bark

Secondary metabolite	Aqueous extract of *P. africana* (mg/g) (percentage composition in the extract)
Alkaloid	5.08± 0.06 (26.63%)
Anthraquinone	1.43 ± 0.01 (7.50%)
Flavonoids	4.72± 0.07 (24.74%)
Cardiac glycosides	4.41±0.24 (23.11%)
Phlobatannins	0.19 ± 0.00 (1.00%)
Tannins	2.03± 0.01 (10.64%)
Saponins	1.22 ± 0.01 (6.38%)

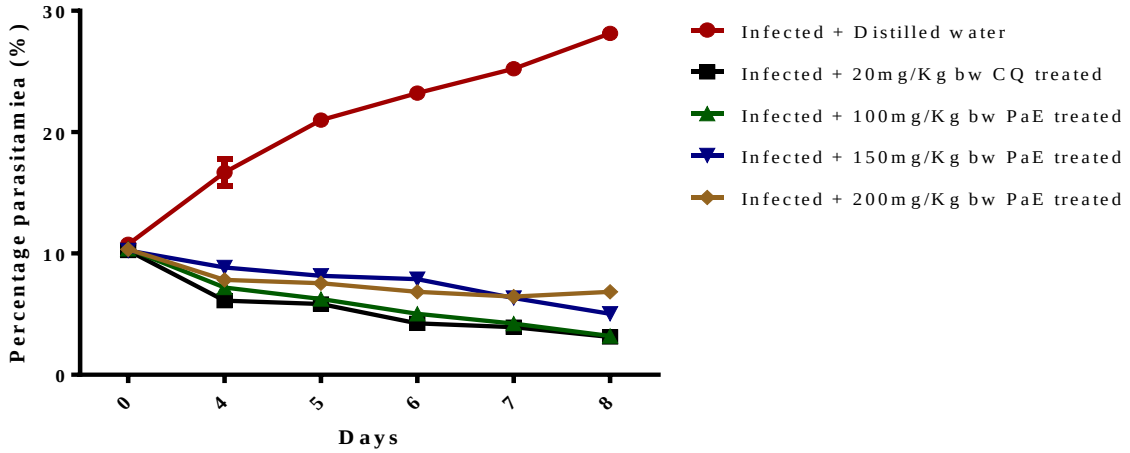

Figure 1:Percentage parasitaemia of infected mice treated with aqueous extract of *Prosopis africana*stem bark for four days
*PaE = *Prosopis africana* extract

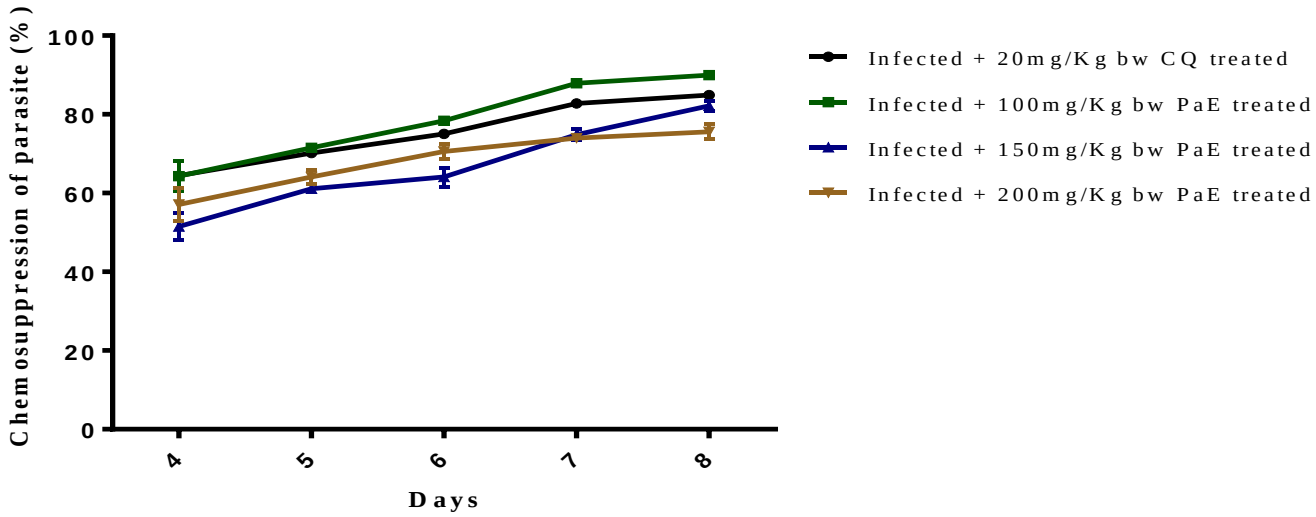

Figure 2: Percentage chemosuppression (inhibition) of parasite in *Plasmodium berghei*-infected mice treated with aqueous extract of *Prosopis africana*stem bark
*PaE = *Prosopis africana* extract

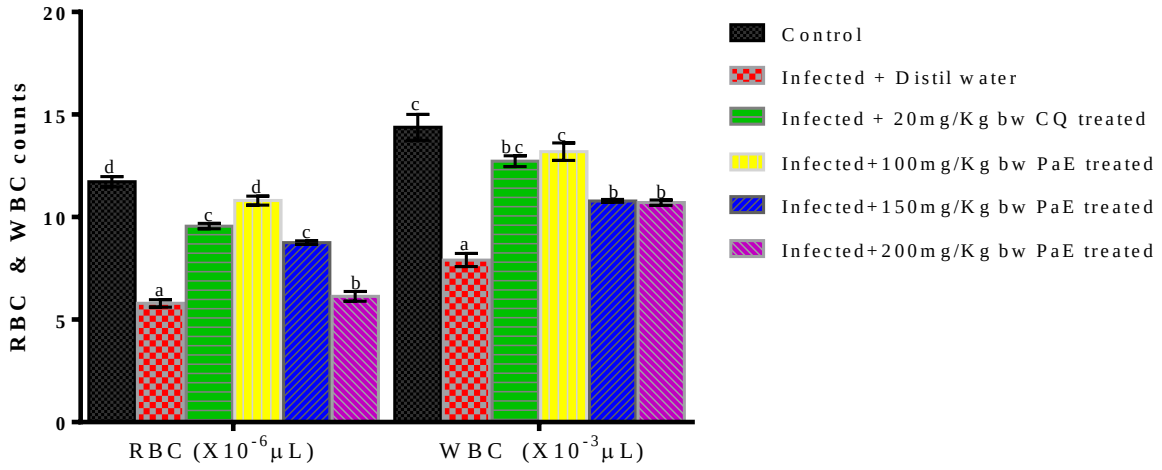

Figure 3: Red blood cell and white blood cell countsof infected mice treated with aqueous extract of *Prosopis africana*stem bark

*PaE =*Prosopis africana* extract; RBC= red blood cell; WBC = white blood cell. The bars are mean of five replicate ± standard error of mean, bars with different superscripts are statistically different at p>0.05

Table 2: Hemoglobin count,Packed cell volume and Platelet countof infected mice treated with aqueous extract of *Prosopis africana* stem bark

	Hb (g/dL)	PCV (%)	PLT (x10^3/µL)
Control (uninfected)	12.30 ± 0.55c	53.80 ± 1.42^c	1045 ± 30.60^d
Infected not treated	7.63 ± 0.22^a	35.25 ± 0.14^a	725 ± 14.43^a
Infected + CQ treated	12.50 ± 0.12^c	42.40 ± 1.39b	895 ± 20.21^b
Infected + 100 mg/kg PaE treated	12.19 ± 0.60^c	52.25 ± 0.87^c	989 ± 25.24^c
Infected + 150 mg/kg PaE treated	11.27 ± 0.66^b	49.84 ± 0.32^{bc}	812.00 ± 18.48^b
Infected + 200 mg/kg PaE treated	11.90 ± 0.27^b	47.47 ± 1.74^b	983.00 ± 16.17^c

*PaE = *Prosopis africana* extract; Hb = Hemoglobin count; PCV = packed cell volume; PLT = platelet count.The bars are mean of five replicate ± standard error of mean,values with different superscripts are statistically different at p>0.05

Discussion

Alkaloids, saponins, tannins, phlabatannins, flavonoids, anthraquinone and glycosides found in the aqueous extracts of *P. africana* stem bark was in agreement with the finding of (Kolapo*et al.*, 2009; Ayanwuyi*et al.*, 2010; Ezike*et al.*, 2010; Ajiboye*et al*., 2013;Dhananjaya*et al.*, 2014).Alkaloids have pronounced effects and are classed as pharmacologically active (Trease and Evans 1989).

The determination of percentage inhibition or chemosupression of parasitaemia is the most dependable parameter in antimalarial screening, and results of this study clearly shown that *P. africana* extract at the various doses suppressed the parasite multiplication by 70-90% after four days of treatment. This correlated with the work of Isha*et al.* (2003) who reported that an extract is considered active against *P. berghei* if it demonstrated 50% growth inhibition of the parasite following a 4-day curative test in mice. The high content of alkaloids in the extract maybe responsible for the observed antimalarial activity. Alkaloids acts as a flood schizonticdal and weak gamatocide against plasmodium when accumulated in food vacuoles of plasmodium, it acts by inhibiting the hemozoin crystallisation, thus

facilitating aggregation of cytotoxic heme (Isabelle and Leshe, 2008). Quinine, an alkaloid extracted from the bark of cinchona tree and has been used to treat malaria since 1632 until it was no longer recommended as the first line of treatment by the WHO in 2006 due to its adverse effect and resistance by the parasite. Similarly, in recent years, the antimalarial properties of flavonoids have been reported. (Lehane and Saliba, 2008; Penna-Coutinho& Aguiar 2018). A recent review by Boniface and Ferreira (2019) showed that more than 220 flavonoids with various degrees of *in vitro* bioactivities against infectious diseases had been isolated and identified from different parts of plants. The researchers highlighted the challenges of reproducibility of the *in vitro* capacity of the flavonoids *in vivo* models to allow for better understanding of their bioactivity. In this study, the inhibition of the parasite that was observed by the extract of *P. africana* stem bark at 100 mg/Kg bw which was higher than that of the reference drug (chloroquine) after the treatment was discontinued, could be attributed to the high concentration of both alkaloids and flavonoids in the *P. africana* stem barkextract. Potential synergism of flavonoids from *Artemisia annua* with artemisinin against malaria have been postulated (Ferreira *et al.*, 2010). Potentiation of antimalarial activity of Artemisinin by flavonoids from *A. annua* was reported byLiu*et al*., 1992. The authors reported that casticin and artemetin of *A. annua* did not show *in vitro* antiplasmodial activity when tested as monotherapy but showed synergistic effects when tested in combination with artemisinin. Currently, antimalarial combination therapies (ACT) is recommended by the WHO for the treatment of uncomplicated malaria in order to preventresistance and ameliorates the secondary complications arising from Plasmodium infection such as oxidative stress, anaemia and immune-suppression.

One of the features of malaria mortality in sub-Saharan Africa is severe anaemia. Haematological function parameters play a major role in malaria pathology (Maina *et al*., 2010).Very low level of RBC, haemoglobin and PCV can predispose to anaemia (Muhammad and Oloyede, 2009). In this study, significant decreased ($p<0.05$) observed in RBC, Hb and PCV count after infection with *P. berghei* parasite, could be an indication of anaemia which might be due to hemolysis. The increase in RBC, Hb and PCV counts observed in the uninfected control group after the treatment with the *P. africana* stem bark extract to near normal range shows that the extract can not only inhibit parasite but can also revert the anaemic condition of the infected mice. Similar improvement inWBC and platelets counts were observed in the *P.berghei*-infected mice treated with the *P. africana* extract. WBC is the leukocytic cells that serve as the first line of the immune response against xenobiotics and infectious diseases, while platelets are thrombocytic cells that help in repairs of damaged blood vessels preventing bleeding. Haematological changes such as anaemia, leukocytosis or well-known thrombocytopenia features in malaria patients (Kotepui *et al.*, 2014). The amelioration effected by administration of *P. africana* extract is an indication that the immune system of the *P. bergehi* mice was also boosted.

Conclusion

In conclusion, data from this study showed that *P. africana* extract demonstrated inhibitory potentials of the malaria parasite when administered at 100mg/kg bw to *P. berghei*-infected mice. The extract also improved the haematological function indices, ameliorating anaemia, improving blood-oxygen carrying capacity, and boosting the immune system of the *Plasmodium*-infected mice. This establishes the possible use of the plant in herbal medicine forthe treatment of malaria and showsthat aqueous extracted could be further refined and explored as a repository of new antimalarial pharmaceuticals.

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