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Rectification of Haematological Profile by Leaf Ethanolic Extracts of Carica papaya L. and Faidherbia albida (Del.) A. Chev to Treat Plasmodium berghei Malaria Infected Swiss Albino Mice

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Research Article

Keywords: Lymphocytes, Malaria, Neutrophils, Plant Extracts

Posted Date: June 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1690323/v1

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Abstract

Background: Malaria remains the major cause of morbidity and mortality in Nigeria; the most densely populated country in the African continent. The current economic depression and rising cost of conventional drugs call for urgent alternative therapy. A study was carried out to investigate the effects of *Carica papaya* and *Faidherbia albida* leaf ethanolic extracts on haematological profiles of Swiss albino mice infected with *Plasmodium berghei*.

Methods: Fresh leaves of the test plants were collected and air dried, milled into powder and extracted using soxhlet apparatus with ethanol as the solvent. Thirty Swiss albino mice of both sexes weighing between 140 and 260g were used for the work. The animals were acclimatized for fourteen (14) days and divided into five groups. The animals were inoculated intraperitoneally with infected blood suspension containing about $1x10^6$ paracetamia of *P. berghei*. Animals in group 1 were administered 0.2 ml normal saline, those in group 5 were administered Chloroquine diphosphate at 5 mg/kg body weight while those in groups 2, 3 and 4 were administered 100, 200 and 400 mg/kg of the ethanolic leaf extract respectively. Suppressive test last for 4 days and toxicity of the extracts on the infected mice was monitored. Haematological profiles of the infected mice were studied before infection, during infection and after treatment periods and average paracetamia counts using standard methods. Data obtained were analysed using Analysis of Variance with Duncan's New Multiple Range Test used to separate significant means at 5%%.

Results: Significant differences ($P \leq 0.05$) in the values of PCV, RBCs and WBCs were observed among the treated groups. The extracts stimulate increase in the number of Lymphocytes, Neutrophils, Monocytes, Eosinophils and Basophils with increase in concentration. The high doses of the extracts significantly ($P \leq 0.05$) reduce the number of parasitized erythrocytes among infected mice treated with *C. papaya* showing the highest (75.04%) suppression.

Conclusions: The antiplasmodial activity of the extracts could be attributed to the active constituents such as: Flavonoids, alkaloids, anthraquinones, tannins, saponins, terpenoids/steriods, diterpenoids and cardiac glycosides in the extracts. We therefore recommended 400 mg/kg of *C. papaya* extract against malarial infections.

Introduction

Malaria remains an important public health concern in countries where transmission occurs regularly, as well as in areas where transmission has been largely controlled or eliminated (WHO, 2019). Malaria is responsible for one to three million deaths and 300 to 500 million infections annually (Verma et al., 2011). Children under five and expecting mothers are particularly susceptible to malaria. The disease claims the life of a child every two minutes (WHO, 2018). Nigeria accounted for 27% of malaria cases and 24% of malaria deaths globally in 2016 (WHO, 2018). Malaria remained the leading cause of death in Nigeria with approximate 227,645 deaths in 1990 and 192,284 deaths recorded in 2015. Malaria is a

mosquito borne disease in humans and animals (Ukaegbu et al., 2014). It is caused by parasitic protozoans of the genus Plasmodium with species *P. falciparum, P. vivax* and *P. berghei*. These parasites were transmitted via female *Anopheles funestus*, *Anopheles moucheti*, *Anopheles gambiae* and *Anopheles arabiensis* (Kar et al., 2014).

A number of drugs have been developed over the years for the treatment of the disease, but the newly introduced artemisinin-based combination therapy (ACT) is the main stay drug used today. There is presently a deep concern that the Plasmodium parasite will soon develop total resistance to the existing antimalarial drugs due to the recent reports of increasing resistance of the parasite to the ACT, in many endemic areas. In addition, the mosquito vector has also developed resistance against the insecticides. The high cost as well as the adverse effects associated with some of the existing drugs also limits their usefulness in malarial treatment (Uzor et al., 2020). Thus, the recent reports of artemisinin-resistant and artemisinin-tolerant *Plasmodium falciparum* parasites call for more innovative methods to control and eventually eliminate malaria (Menard and Dondorp, 2017; Cudjoe et al., 2020).

Up to now, malaria treatment relies on certain drugs that easily confer side effects and easily become resistant by the parasites to the extent that, there is no single antimalarial drug in clinical use to which the parasite has not yet developed resistance (Adebayo and Krettli, 2011; Abdillah et al., 2015; Chandra and Arbor, 2016; Alebie et al., 2017). Furthermore, the most successful malaria vaccine was only partially efficient and short lived (Kayano et al., 2011). There is therefore the need for alternative therapy in the treatment of malaria in Nigeria. Recent research has focused on natural plant product as alternatives to the existing drugs for disease remedy in developing countries (Aiyegoro et al., 2007). Plant derived medicines have been part of traditional health care in most parts of the world for ages and there is increasing interest in them as sources of agents to fight microbial diseases (Mohana et al., 2008: Ghaleb et al., 2009; Ajayi and Akintola, 2010). Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potential for introducing new templates into modern medicine (Akinyemi et al., 2005). The use of plant preparations for the treatment of diseases still holds a strong potential since the influence of natural products upon drug discovery is impressive and a number of clinically active drugs are either natural products or have a natural product pharmacophore (Koehn and Carter, 2005). Ethnopharmacological reports revealed a number of tropical plants with certain antimalarial potentials (Onaku et al., 2011; Rasoanaivo et al., 2011). *Carica papaya* L. and *Faidherbia albida* (Del.) A. Chev (syn: *Acacia albida*) were two medicinal plants used in Nigeria for the traditional treatment of malaria in Nigeria. Studies (Tijani et al., 2008; Oluwakanyinsola et al., 2010; Salawu et al., 2010; Usman et al., 2013; Okpe et al., 2016; Airaodion et al., 2019; Atanu et al., 2021; Oraebosi and Good, 2021) have shown the efficacy of these plants in the treatment of malaria, even though the mechanism of action against the malarial parasites is not yet well explored. This study therefore aimed at studying the effects of leaf ethanolic extracts of the plants on the haematological characteristics of infected mice to bring insight on their mode of action.

Materials And Methods

Plant Samples Collection

Fresh leaves of *Carica papaya* and *Faidherbia albida* were collected from the Botanical Garden, Ahmadu Bello University, Zaria, Nigeria. The collected samples were carried to the Herbarium unit of the Department of Botany, Ahmadu Bello University Zaria for authentication. A herbarium voucher number was assigned to *C. papaya* as ABU01203 and *F. albida* as ABU0900394.

Extraction of Plant Ethanolic Extracts

The extraction methodology followed the protocols of Sasidharan et al. (2011). The collected samples were thoroughly washed and air-dried under shade at room temperature for 2 weeks. The dried leaves were then ground into fine powder with mortar and pestle, and were stored in dry containers until needed. The ethanolic extracts were prepared by soaking 100 g of each powder in 150 ml of 95% ethanol and shaken in orbital shaker at 120 rpm. The preparations were left to stand for another 24 hours and then filtered through a gauze and then Whatman No 1 filter paper. The filtrates were concentrated to dryness at 40°C under reduced pressure on a rotary evaporator and were stored in a refrigerator at − 20°C until use. Different concentrations of 100, 200 and 400mg/kg were prepared from each of the leaves extracts.

Phytochemical Analysis

The phytochemical screening of the ethanolic extract of *C. papaya* and *F. albida* was carried out according to the methods described by Sofowara (1993) and Adegoke et al. (2010) to determine the presence of active constituents in the plant leaves and their composition. The aqueous extracts from each of the test plant was subjected to qualitative test for the presence of bioactive components that include Molisch's test for detection of Carbohydrates, Wagner's test for detection of Alkaloids, Lead subacetate test for detection of Tannins, Keller- Killiani's test for detection of Cardiac Glycosides, Frothing/Foaming test for detection of Saponins, Libermann-Burchard's test for detection of Steroids and Triterpenoids, Copper Acetate's test for detection of Diterpenoids, Ferric chloride test for dsetection of phenols, Alkaline test for the detection of Flavonoids, Xanthoproteic test for the detection of Protein and Borntrager's test for the detection of Anthraquinones. Quantitative analysis of the active phytochemicals present in the two plant extracts followed the method described by Tamasi et al. (2021).

Experimental Animals

Ethical clearance was obtained from Kano State Ministry of Agriculture. The protocol employed met the guidelines of the Good Laboratory Practice (GLP) regulations of World Health Organization. Apparently thirty (30) healthy white Swiss albino mice of both sexes weighing between 140 and 260 g were used for the work and were obtained from the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The mice were housed in metal cages and kept in a ventilated room with 12 hours dark/light cycle. They were fed with standard feed pellets and tap water *ad libitum*. The animals were acclimatized for 2 weeks before proceeding with the experiment.

Animals Inoculation

Plasmodium berghei infected blood was obtained from the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Parasites were harvested from the blood of a donor rat at peak parasitemia (10^9 parasites/ml) and were diluted with normal saline in proportion as indicated by Akuodor *et al*. (2010). Each mouse was inoculated intraperitonealy with infected blood suspension (0.2 mL) containing $1x10^6$ *P. berghei* parasitized red blood cells.

Determination of Extracts Toxicity

The lethal dose (LD_{50}) of the extracts was determined for estimating acute toxicity of the *C. papaya* and *F. albida* extracts according to the method of Lorke (1983). The method involved intraperitoneal administration of different doses (100, 200 and 400 mg/kg) of the extracts to three groups of Swiss albino mice. Afterwards, mortality and manifestation of physical signs in mice for possible toxicity was monitored.

Suppressive Test

A 4-day suppressive test was conducted according to the method described by Mbah et al. (2012). Thirty (30) albino mice of both sexes weigthing (140-260g) were injected intraperitonealy with standard inocula of *P. berghei* containing $1x10^6$ infected erythrocytes. After four hours of inoculation, the infected mice were randomly divided into 5 groups of 3 mice per cage for each extract. After seventy-two (72) hours of infections, all the *P. berghei* malaria-infected mice were treated by oral administration with the ethanolic extracts from *C. papaya, F. albida* leaves and the controls. The infected mice were treated for four consecutive days. Group 1 received 0.2 mL of normal saline (drug free control). Group 2, 3, 4 received 100, 200, and 400mg/kg of the ethanol leaf extract of *C. papaya* respectively while group 5 received 10mg/kg of chloroquine phosphate. Similar treatments were carried out for *F. albida* extracts. All doses were administered orally. On the fifth day, thin blood film was made from the tail blood of each mouse. Thin blood films were made on a slide and air dried, and then fixed in absolute methanol and stained with 3% Giemsa solution at pH 7.2. Slides were viewed using light microscopy (Vickers Instruments) with oil immersion (X1000 magnification). The percentage of infected RBCs was determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs. A minimum of 500 RBCs total was counted

Percentage of Infected RBCs = Number of infected RBCs x 100

Total Number of RBCs Counted

Average chemo suppression was calculated as follows:

% Suppression = Average parasitemia in Control—Average parasitemia in Treated x 100

Average parasitemia in Control

Determination of Packed Cell Volume

Packed Cell Volume (PCV) was determined using the microhaematocrit method as decribed by Coles (1986). Briefly, heparinized capillary tubes were filled with blood samples to 75% (¾) their length by capillary action. Piece of gause was carefully used to dry the outside of the tubes while the opposite end of the tubes sealed with flame. The tubes were then loaded onto a microhematocrit centrifuge and centrifuge at 3000 rpm for ten minutes. The PCV values were measured using hematocrit reader.

Evaluation of Haematological Parameters

Evaluation of Total Erythrocytes count followed the method described by Coles (1986). Blood sample were drawn carefully by aspiration to 0.5 marks of the pipette and transferred into a clean cuvette containing diluent (0.9% of normal saline). With another pipette the dilution fluid were drawn to 101 mark and mixed gently, the diluted blood were then discharged into haemocytometer counting chamber and allowed to settle. The Total erythrocyte count was determined by viewing under microscope at X40 resolution of a compound microscope. The total number of cells in 20 fields in the centre of the counting chamber was calculated. The value obtained represented the total number of the erythrocyte per microliter (µL). The remaining haematological parameters such as white blood cells (WBCs), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS) and basophils (BAS) count followed the method described by Asangha et al. (2017) using automated hematology analyser. The haematological data were taken before infection, during infection and after complete treatment.

Data Analysis

The data obtained were analyzed using Analysis of Variance (ANOVA) with Duncan's New Multiple Range Test (DNMRT) used to separate the means that were significant at 5% level. The lethal dose of the extracts was determined using probit analysis.

Results

The results for the phytochemical constituents in *C. papaya* and *F. albida* are presented in Table 1. The result revealed the presence of ten (10) active constituents: Flavonoids, proteins, alkaloids, anthraquinones, tannins, saponins, terpenoids/steriods, diterpenoids, cardiac glycosides and carbohydrates in *C. papaya* extract. However, with the exception of diterpenoids and anthraquinones, nine (9) active phytochemicals were found in *F. albida* extracts. Carbohydrates and proteins are the most abundant phytochemicals while cardiac glycosides were the least (0.38%) in papaya and 0.31% in *Faidherbia albida* leaf extracts.

Table 1
Qualitative and quantitative phytochemicals in *C. papaya* and *F. albida* leaf extracts

Extract	Constituent	Test	Result	Percentage Composition (%)
Carica papaya	Flavonoids	Alkaline	+	1.96
	Phenols	Ferric chloride	-	0.00
	Proteins	Xanthoproteic	+	2.91
	Alkaloids	Wagner's	+	19.43
	Anthraquinones	Borntrager's	+	1.07
	Saponins	Frothing/Foami	-	2.46
	Tannins	Lead subacetate	+	12.39
	Terpenoids/Steriods	Libermann-Burchard's	+	4.96
	Diterpenoids	Copper Acetate's	-	0.41
	Cardiac glycosides	Keller-Killiani's	+	0.38
	Carbohydrates	Molisch's	+	54.03
		TOTAL		100.00
Faidherbia albida	Flavonoids	Alkaline	+	1.91
	Phenols	Ferric chloride	+	1.43
	Proteins	Xanthoproteic	+	1.95
	Alkaloids	Wagner's	+	16.96
	Anthraquinones	Borntrager's	+	0.00
	Saponins	Frothing/Foami	-	1.23
	Tannins	Lead subacetate	+	8.91
	Terpenoids/Steriods	Libermann-Burchard's	+	3.82
	Diterpenoids	Copper Acetate's	+	0.00
	Cardiac glycosides	Keller-Killiani's	+	0.31
	Carbohydrates	Molisch's	+	63.48
		TOTAL		100.00

KEY: + = PRESENT -= ABSENT

The results of acute toxicity of the extracts revealed that, the extract caused no mortality within the dose range of 100–400 mg/kg in the initial 24 hours as well as within the successive 14 days. This was a clear indication that doses used were safe. No physical and behavioral signs of overtoxicity such as decreased motor activity, decreased body/limb tone, writhing, respiration, and death amongst others were observed. The result shows that LD_{50} of the extract is greater than 1000 mg/kg.

The effect of *C. papaya* ethanolic extracts on haematological profile of *P. berghei*-infected mice is shown in Table 2. The result indicated significant difference ($P \leq 0.05$) in the effects of various concentrations of the extracts on the blood parameters of infected mice. The extracts cause marked reduction in the PCV values during the periods of infection ranging from 48.33–43.67%. The WBC counts increase during infection period (from $10.73x10^3$ to $11.50\ x10^3/\mu L$). These values were reduced from $10.70x10^3$ to $11.33\ x10^3/\mu L$ after treatment. Similarly, the RBC counts increase during infection period and after treatment with the extracts. The result also showed that neutrophils and lymphocytes were affected significantly by the extracts. The neutrophils were found to reduce from 49.00–53.00% during infection period with decrease in concentration. However, after treatment the values of neutrophils reduce from 48.67–44.33 with decrease in concentration. Similar trend was found in the number of lymphocytes. More so, the number of Eosinophils was found to increase during infection period by the extracts.

Table 2
Effect of *Carica papaya* extracts on blood parameters of *Plasmodium berghei* infected mice

Sample (mg/kg)	PCV (%)	WBCx10^3	RBCx10^6	MON	NEU	LYM	EOS	BAS
$Saline_{BI}$	47.33 ± 3.79^a	10.33 ± 0.85^b	11.53 ± 0.64^{ab}	2.00 ± 1.00^a	50.00 ± 1.00^a	44.00 ± 1.00^a	3.00 ± 1.00^a	1.00 ± 0.00^c
$Saline_{DI}$	44.00 ± 4.86^b	11.17 ± 0.85^a	11.40 ± 0.79^b	1.67 ± 0.58^b	50.07 ± 2.08^a	42.67 ± 2.52^b	2.33 ± 1.15^b	2.67 ± 1.15^b
$Saline_{AT}$	39.33 ± 4.73^c	10.97 ± 0.45^{ab}	11.70 ± 0.62^a	1.67 ± 1.15^b	49.00 ± 1.00^b	44.00 ± 1.00^a	2.33 ± 1.53^b	3.00 ± 1.00^a
100_{BI}	50.67 ± 3.06^a	10.90 ± 0.85^b	12.13 ± 0.50^a	2.67 ± 1.53^a	48.67 ± 3.06^b	44.33 ± 4.04^b	2.33 ± 0.58^b	2.00 ± 0.00^a
100_{DI}	48.33 ± 2.52^b	11.33 ± 0.66^a	11.70 ± 0.44^b	1.67 ± 1.15^b	53.00 ± 6.56^a	40.33 ± 6.03^c	3.67 ± 1.53^a	1.67 ± 0.58^b
100_{AT}	47.00 ± 1.00^c	11.30 ± 0.47^{ab}	12.13 ± 0.23^a	1.67 ± 0.58^b	48.67 ± 1.73^c	48.00 ± 2.65^a	1.67 ± 0.58^c	2.67 ± 0.58^a
200_{BI}	47.33 ± 1.53^a	9.80 ± 0.20^b	11.63 ± 0.12^a	1.33 ± 0.58^b	54.33 ± 5.86^a	39.67 ± 6.80^c	3.33 ± 0.58^a	1.00 ± 0.00^b
200_{DI}	43.67 ± 2.89^b	10.73 ± 0.27^a	11.01 ± 0.57^b	2.33 ± 0.58^a	49.33 ± 2.08^b	43.00 ± 2.65^b	2.33 ± 0.58^b	3.00 ± 1.00^a
200_{AT}	42.67 ± 3.79^c	10.70 ± 0.42^{ab}	11.73 ± 0.56^a	1.33 ± 0.58^b	46.00 ± 2.89^c	44.67 ± 1.53^a	2.33 ± 0.58^b	3.00 ± 1.00^a
400_{BI}	50.67 ± 2.08^a	10.57 ± 0.60^c	12.27 ± 0.55^b	3.67 ± 0.58^a	52.67 ± 6.66^a	40.33 ± 5.69^c	1.00 ± 0.00^b	2.33 ± 0.58^b
400_{DI}	48.00 ± 0.00^b	11.50 ± 0.10^a	12.07 ± 0.15^c	1.33 ± 0.58^b	49.00 ± 1.00^b	47.33 ± 1.53^a	3.33 ± 0.58^a	2.33 ± 0.58^b
400_{AT}	48.33 ± 1.53^b	11.33 ± 0.12^b	12.93 ± 5.66^a	1.67 ± 1.16^b	44.33 ± 1.53^c	43.33 ± 2.08^b	3.33 ± 2.08^a	3.33 ± 1.53^a
Chl_{BI}	47.67 ± 2.52^a	10.20 ± 0.53^b	11.71 ± 0.71^a	1.00 ± 0.00^a	48.33 ± 1.53^a	46.00 ± 2.00^c	2.67 ± 1.53^{ab}	2.00 ± 1.00^b
Chl_{DI}	44.00 ± 1.73^b	10.47 ± 0.30^a	11.27 ± 0.31^b	1.33 ± 0.58^a	45.00 ± 1.00^b	50.00 ± 1.00^a	2.33 ± 0.58^b	2.33 ± 0.58^b
Chl_{AT}	47.00 ± 2.65^a	10.20 ± 0.20^b	11.73 ± 0.55^a	1.33 ± 0.58^a	44.00 ± 2.00^c	49.67 ± 1.15^b	3.33 ± 0.58^a	3.33 ± 0.58^a

N.B: Values with the same superscript down a column for each sample are not significantly different (P = 0.05)

Sample (mg/kg)	PCV (%)	WBCx10^3	RBCx10^6	MON	NEU	LYM	EOS	BAS
KEY: PCV = Packed Cell Volume, WBC = White Blood Cells RBC = Red Blood Cells NEU = Neutrophils								
LYM = Lymphocytes, BAS = Basophils EOS = Eosinophils MON = Monocytes								
BI = Before Infection DI = During Infection AT = After Treatment								

Similarly, the effect of *F. albida* extracts on the haematological profile of *P. berghei*-infected mice is presented in Table 3. The result indicated significant differences ($P \leq 0.05$) in the effects of the extracts on the blood profiles during infection and after treatment. The extracts caused a reduction in the number of PCV from 48.00–44.00% with decrease in concentrations. However, the amount of WBCs increased with increase concentration from the range of 9.70x10^3-10.57x10^3/ µL before infection to a range of 10.70-11.40x10^3/µL during infection and 11.40-11.77x10^3/µL after infection with increase in concentration. Similar trend was found in the amount of RBCs with increase in concentration. More so, same trend was found for the number of lymphocytes and Basophils. However, the monocytes and neutrophils decreased in number with increase in concentrations. The extracts cause the reduction of Eosinophils amount during infection period which increase after infection.

Table 3
Effect of *Faidherbia albida* extracts on blood parameters of *Plasmodium berghei* infected mice

Sample (mg/kg)	PCV (%)	WBCx10^3	RBCx10^6	MON	NEU	LYM	EOS	BAS
$Saline_{BI}$	47.67 ± 1.53^a	9.97 ± 0.38^b	11.50 ± 0.62^c	1.33 ± 0.58^b	48.00 ± 2.00^b	46.00 ± 1.00^a	3.33 ± 1.53^a	1.33 ± 0.58^c
$Saline_{DI}$	45.33 ± 2.08^b	11.37 ± 0.32^a	13.70 ± 3.72^a	1.33 ± 0.58^b	39.00 ± 28.69^c	36.33 ± 4.16^b	3.00 ± 1.00^a	2.00 ± 1.73^b
$Saline_{AT}$	41.67 ± 2.08^c	11.10 ± 0.26^a	12.53 ± 0.35^b	2.00 ± 0.00^a	56.67 ± 4.16^a	36.33 ± 4.16^b	1.67 ± 0.58^b	3.33 ± 0.58^a
100_{BI}	48.33 ± 1.53^a	9.97 ± 0.35^b	11.67 ± 0.49^b	1.67 ± 1.16^b	53.00 ± 9.64^a	40.33 ± 10.01^b	2.67 ± 0.58^b	2.33 ± 0.58^b
100_{DI}	46.00 ± 2.00^b	11.37 ± 0.45^a	11.83 ± 0.55^b	1.67 ± 0.58^b	48.67 ± 2.08^b	45.00 ± 3.00^a	1.67 ± 1.16^c	3.00 ± 1.00^a
100_{AT}	45.33 ± 2.52^b	11.40 ± 0.53^a	12.23 ± 0.56^a	2.00 ± 1.00^a	46.33 ± 1.53^c	45.33 ± 0.58^a	3.00 ± 1.00^a	3.33 ± 1.53^a
200_{BI}	46.33 ± 2.52^a	9.70 ± 0.30^b	11.17 ± 0.47^b	1.33 ± 0.58^a	47.67 ± 2.08^a	46.00 ± 1.00^a	3.00 ± 1.00^a	2.00 ± 1.73^c
200_{DI}	44.00 ± 2.65^b	10.70 ± 0.52^{ab}	11.63 ± 0.76^{ab}	1.67 ± 1.15^a	47.67 ± 2.52^a	45.33 ± 1.53^b	2.33 ± 0.58^{ab}	3.00 ± 1.00^b
200_{AT}	42.10 ± 0.26^c	11.67 ± 3.21^a	12.00 ± 2.65^a	1.00 ± 0.00^a	47.67 ± 0.58^a	45.00 ± 2.65^b	2.67 ± 0.58^b	3.67 ± 0.58^a
400_{BI}	50.33 ± 1.53^a	10.57 ± 0.47^b	11.80 ± 0.40^b	2.00 ± 1.00^a	53.33 ± 7.57^a	41.00 ± 6.08^c	2.33 ± 0.58^b	1.33 ± 0.58^b
400_{DI}	48.00 ± 1.73^b	11.40 ± 0.20^b	11.87 ± 0.12^{ab}	1.67 ± 0.58^b	48.33 ± 2.08^b	45.00 ± 2.00^b	2.00 ± 1.00^b	3.00 ± 1.00^a

N.B: Values with the same superscript down a column are not significantly different (P = 0.05)

KEY: PCV = Packed Cell Volume, WBC = White Blood Cells RBC = Red Blood Cells NEU = Neutrophils

LYM = Lymphocytes, BAS = Basophils EOS = Eosinophils MON = Monocytes

BI = Before Infection DI = During Infection AT = After Treatment

The result for the effect of *C. papaya* extracts on *P. berghei* paracetamia is shown in Table 4. The result showed marked reduction in the number of infected RBCs from a range of 1.57-1.24x10^6/µL to as low as 1.52-1.03x10^6/µL with increase in concentration. There is also significant reduction in percentage suppression of the paracetamia with increase in concentration with 400 mg/kg showed 67.86% suppression.

Sample (mg/kg)	PCV (%)	WBCx10^3	RBCx10^6	MON	NEU	LYM	EOS	BAS
400_{AT}	50.17 ± 2.08^a	11.77 ± 0.21^a	12.53 ± 0.35^a	1.67 ± 0.58^b	46.33 ± 1.53^c	46.33 ± 1.15^a	3.00 ± 1.00^a	2.67 ± 0.58^a
Chl_{BI}	47.33 ± 1.15^a	10.03 ± 0.55^c	11.00 ± 0.43^b	1.67 ± 0.58^a	47.00 ± 2.65^a	47.00 ± 4.00^c	3.00 ± 1.00^a	1.33 ± 0.58^b
Chl_{DI}	44.33 ± 0.58^b	10.60 ± 0.10^b	12.03 ± 0.31^{ab}	1.00 ± 0.00^b	45.33 ± 0.58^b	48.67 ± 1.53^b	3.00 ± 1.00^a	2.00 ± 1.00^a
Chl_{AT}	47.33 ± 0.58^a	11.60 ± 0.20^a	12.36 ± 0.15^a	1.33 ± 0.58^b	43.67 ± 2.31^c	49.67 ± 1.53^a	3.00 ± 1.00^a	2.33 ± 1.53^a

N.B: Values with the same superscript down a column are not significantly different (P = 0.05)

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Table 4
Effect of *Carica papaya* extracts in reducing paracetamias of *Plasmodium berghei* infected mice

Sample (mg/kg)	Total RBC	Infected RBC	Average Paracetamia	% Suppression
$Saline_{DI}$	448.62 ± 32.93	2.00 ± 0.10	20.95 ± 1.03	-
$Saline_{AT}$	480.35 ± 47.03	1.60 ± 0.22	19.60 ± 2.09	-
100_{DI}	469.77 ± 5.24	1.57 ± 0.08	13.43 ± 1.24	35.89
100_{AT}	498.72 ± 58.16	1.52 ± 0.12	14.83 ± 0.82	24.34
200_{DI}	434.12 ± 13.97	1.44 ± 0.14	10.03 ± 0.53	52.12
200_{AT}	448.87 ± 13.33	1.35 ± 0.13	10.00 ± 0.51	48.97
400_{DI}	443.28 ± 11.62	1.24 ± 0.03	5.23 ± 0.32	75.04
400_{AT}	480.03 ± 72.60	1.03 ± 0.11	6.30 ± 0.54	67.86
Chl_{DI}	437.73 ± 2.93	0.83 ± 0.08	2.92 ± 0.42	86.06
Chl_{AT}	563.19 ± 38.29	0.67 ± 0.06	2.88 ± 0.67	85.31

Furthermore, the effect of *F. albida* extracts in reducing paracetamia of *P. berghei* infected blood is shown in Table 5. The result revealed that, *F. albida* extracts reduce the number of infected RBCs from a range of 1.67-1.48x10^6/ µL to as low as 1.65-1.43x10^6/µL with increase in concentration. The result indicated 400 mg/kg to have the highest effect among the extracts in suppressing the paracetamia to as high as 58.48%.

Table 5
Effect of *Faidherbia albida* extracts in reducing paracetamia of *Plasmodium berghei* infected mice

Sample (mg/kg)	Total RBC	Infected RBC	Average Paracetamia	% Suppression
$Saline_{DI}$	467.80 ± 19.28	2.10 ± 0.05	19.18 ± 1.08	-
$Saline_{AT}$	480.06 ± 28.85	1.62 ± 0.20	19.87 ± 0.83	-
100_{DI}	468.00 ± 16.63	1.67 ± 0.24	12.50 ± 1.52	34.83
100_{AT}	458.84 ± 70.16	1.65 ± 1.33	17.32 ± 1.33	12.83
200_{DI}	509.27 ± 59.88	1.38 ± 0.02	10.92 ± 0.64	43.07
200_{AT}	447.87 ± 11.34	1.47 ± 0.13	12.18 ± 0.26	38.70
400_{DI}	448.68 ± 42.37	1.48 ± 0.11	11.77 ± 0.42	38.63
400_{AT}	458.24 ± 29.14	1.30 ± 0.10	8.25 ± 1.39	58.48
Chl_{DI}	476.98 ± 58.67	1.19 ± 0.82	2.72 ± 0.16	36.76
Chl_{AT}	501.98 ± 25.74	0.70 ± 0.13	3.25 ± 0.78	83.64

Discussion

Carica papaya and *F. albida* leaves contained some active phytochemicals which probably played significant role in suppressing *Plasmodium berghei* paracetamia. The variations in quantity and quality of these phytochemicals among the two plant species agrees with the finding of Jahan et al. (2008) who reported that phytochemical constituents vary quantitatively and qualitatively in different plant species and even in plant parts of the same species. Phytochemical analysis is very vital in the evaluation of some active biological components of some plants as reported by Anwar et al. (2007). The occurrence of this wide range of phytochemicals shows the antiplasmodial potentials of *C. papaya* and *F. albida*. Alkaloids, saponins, tannins, flavonoids, and steroids have been known to be biologically active (Nethathe and Ndip 2011). The presence of alkaloids, flavonoids, saponins, tannins, glycosides, and steroids in the leaves of *C. papaya* agrees with the finding of Alorkpa et al. (2016) who reported similar phytochemicals in two varieties of papaya leaves. Ohouko et al. (2020) also reported similar phytochemical constituents in *F. albida* leaves. The antiplasmodial activity of the extracts reported by this study could be attributed to these phytochemicals (Abdulelah and Zainal-Abidin, 2007).

The phytochemicals present in the two test plants such as Tannins (Asres et al., 2001), alkaloids (Saxena et al., 2003) and phenols (Hilou et al., 2006) were reported to have antiplasmodial properties. The alkaloids consist of quinine, which is anti-malarial (Baskaran et al., 2012). Li et al. (2011) reported that several drugs have been obtained from alkaloid-containing plants because of its phamacological

importance. More so, previous studies (Ene et al., 2009; Musa et al., 2010; Kayano et al., 2011) have attributed the antimalarial activities of plants to the presence of flavonoids, tannins and triterpenes which could be acting singly or in synergy with one another to exert the observed antimalarial activity as stressed by Okokon et al. (2005). Phytosteroids and flavonoids are metabolites that have been proved to possess potential immunomodulatory effects in plants (Aherne et al., 2007) which as a consequence might have some impact on the host-parasite interrelationships (Gebrehiwot et al., 2019). Similar finding was also reported by the work of Ibrahim et al. (2011) from *Indigofera pulchra* leaves.

The non-toxic effects of the doses of two plant extracts reported by this study which according to Jayasinghe et al. (2008) is the main concern of indigenous therapeutic preparations have demonstrated the relative potential of the extracts in the treatment of malaria fever; thereby fulfils the criteria set for lack of acute toxicity by CDER (1996).

The present study revealed reduction in PCV values due to treatment with *C. papaya* and *F. albida* extracts. This finding agrees with that of Atanu et al. (2021) who reported low PCV values in the blood profile of malaria-infected mice treated with *C. papaya* extracts. However, this finding contradicts that of Airaodion et al. (2019) who reported reduction in PCV of P. *berghei* infected untreated mice (negative control) and infected treated with 100 mg/kg of *C. papaya* after 4 days of treatment. This variation might probably be attributed to the effect of extraction protocol on the phytochemical constituents of the plants. PCV was measured to evaluate the effectiveness of the extracts in preventing hemolysis due to a rising parasitaemia level. In untreated mice, the parasite count increased and the hematocrit PCV decreased markedly from day to day as reported by Okokon et al. (2005).

The increased in number of different WBCs above the normal range (4.29-7.77x10^3/µL, 2.45-5.47x10^3/µL, 1.52-3.53x10^3/µL, 0.02-0.13x10^3/µL, 0.01-0.08x10^3/µL and 0.00-0.04x10^3/µL for leucocytes, lymphocytes, neutrophils, monocytes, eosinophils and basophils respectively) in the blood profile of albino mice reported by Ihedioha et al. (2012). This increase in the number of different WBCs can be attributed probably to the immuno-stimulatory effects of the extracts against the *P. berghei* parasitized blood. This finding agrees with that of Silitonga and Silitonga (2017) who reported increase in the leucocytes counts of the hematology profile on rats induced BCG and leaf extract of *Plectranthus amboinicus*. The present study reported that *C. papaya* and *F. albida* extracts modify the immune system by increasing the amount of lymphocytes, monocytes and neutrophils to fight *P. berghei* paracetamia in the blood of infected mice. This finding is also in consistence with that of Fatmawaty et al. (2017) who reported high efficacy in rectifying immune conditions by a combination of *Delonix regia* and *Carica papaya* with ratio of 1:1 in *Plasmodium berghei*-infected mice.

The reduction of parasitemia in *Plasmodium berghei*-infected mice with increased in the concentration of the ethanolic leaf extracts indicated the significant role played by the extracts in controlling *P. berghei* infection. This is in agreement with the work of Atanu et al. (2021) who reported decreased in parasitemia of *Plasmodium berghei*-infected mice due to treatment with *Alstonia boonei* and *Carica papaya* extracts compared with the malaria negative control group. The antiplasmodial effect of *C. papaya* and *F. albida*

leaves extracts suggests that the extracts contain some phytochemicals that could interfere with the survival of the parasites *in vivo*. The antiplasmodial ability of the extract in the higher dose of the leaf extracts indicated that the active compounds are concentrated in the higher doses. This agrees with the finding of Krettli et al. (2009) who reported highest suppression of *P. berghei* parasitemia at the maximum dose as the active compounds, responsible for the antimalarial activity, mostly occur in low levels in natural products and activity may not be detected in lower doses.

Akuodor *et al*. (2017) reported that, the 4-day suppressive test is a standard test that is commonly used for the evaluation of antimalarial, and the determination of the percentage inhibition of parasitemia density is the most reliable parameter. The present study revealed a significant suppression of the parasites by the extracts. The antimalarial activity shown by the extracts is consistent with the traditional use of the plants as herbal remedy against malaria fever. Comparing the activity of the test plants with chloroquine, which has been used for suppressive and curative antiplasmodial activities (Ajaiyeoba et al., 2006; Builder *et al*., 2011) indicated that, even though chloroquine exhibited higher suppressive and curative antiplasmodial activities by the extent of decrease in parasitemia, the higher doses of the extracts showed similar antiplasmodial activities (above 50%).

Thus, treatment with the ethanolic extracts of *C. papaya* and *F. albida* leaves significantly inhibited parasitemia compared to the controls indicating their efficacy in malaria treatment. Similarly, Francois et al. (1996) reported more than 50% suppression due to the effects of leaf extracts of *Neutolaena lobata*. More so, previous studies (Thomas et al., 2004; Fatmawaty and Astuti, 2013 and Okpe et al., 2016) have shown the potential antimalarial properties of *C. papaya in vitro* and *in vivo.* Tahir et al. (1999), Ali et al. (2010) and Malviya et al. (2011) individually reported the antiplasmodial activity of the root extracts of *Acacia nilotica* (*F. albida*) against *Plasmodium berghei* and *Plasmodium falciparum* in mice. Ali et al. (2002) also reported suppressive activity of methanolic and chloroform extracts of *Salvadora persica* on *P. berghei.*

Conclusion

The study concluded that *C. papaya* and *F. albida* leaf ethanolic extracts have antiplasmodial potentials. They cause significant increase in the amount of various WBCs such as Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils; thereby stimulating immune-stimulatory responses. There is an increase in the amount of lymphocytes. neutrophils and monocytes among the treated groups. High doses of the extracts showed high percentage suppression to *P. berghei* paracetamia with the effect of *C. papaya* as the highest (75.04%). The antiplasmodial activity of the extracts could be attributed to the active constituents: Flavonoids, proteins, alkaloids, anthraquinones, tannins, saponins, terpenoids/steriods, diterpenoids, cardiac glycosides and carbohydrates present in *C. papaya* extract. We therefore recommended 400 mg/kg of *C. papaya* extract against malarial infections due to Plasmodium spp as it shows no toxicity to the host.

Declarations

- **Ethics approval and consent to participate**

Ethical approval sought from the Department of Veterinary Services, Kano state Ministry of Agriculture, Nigeria.

- **Consent for publication**

Not Applicable.

- **Availability of data and materials**

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

- **Competing interests**

The authors declare that they have no competing interest.

- **Funding**

Institution Based Research grant from Tertiary Institutions Trust Fund (TETFUND)

- **Authors' contributions**

NS designed the experiment, supervised the experiment and wrote original draft of the manuscript. BSM conducted the experiment, analyzed the data obtained and helped in literature search while ZSI participated in conducting the experiment and writing—review and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

- **Acknowledgements**

The authors wish to acknowledge the receipt of financial support in the form of Institution Based Research grant from TETFUND and the management of College of Education and Preliminary Studies, Kano state, Nigeria. The authors also appreciate the technical support of Mal. Usaini of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

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