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Antiplasmodial Activity and Amelioration of Altered Haematological Indices by Methanol Extract of *Peltophorum pterocarpum* in *Plasmodium Berghei*-Infected Mice

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Abstract: The present study evaluated the antimalarial activity of methanol extract of *Peltophorum pterocapum* stem bark and its amelioration of *plasmodium*-induced changes in haematological indices. Phytochemical screening and acute toxicity studies on the plant extract were evaluated. Haematological indices such as packed cell volume (PCV), total white blood cell (WBC) and red blood cell (RBC) counts and haemoglobin (Hb) concentration were also determined using classical methods. A total of 36 mice consisting of six groups were used for the study. Groups 1, 2 and 3 (malaria - infected mice) were treated with 100, 200 and 300 mg/kg (p.o.) body weight of *P. pterocarpum* methanol stem bark extract respectively. Group 4 (standard control) was also infected and treated with 28 mg/kg body weight of artemether/lumefantrine, group 5 (positive control) was infected and left untreated while group 6 (normal control) was uninfected and untreated. The presence of alkaloids, flavonoids, saponins, terpenoids, tannins, carbohydrates, fats and oils, reducing sugars, phenols, resins, protein and steroids were detected in the extract. There was neither clinical sign nor mortality after 24 hours post-treatment observation, even at a dose of 5000 mg/kg body weight. Malaria infection increased percentage parasitaemia but reduced RBCs, PCV and Hb. However, treatment of malaria- infected mice with methanol extract of *P. pterocapum* stem bark reduced percentage malaria parasitaemia in a dose-dependent manner. The extract also restored haemotological changes produced by malaria infection. These findings suggest that methanol extract of *P. pterocarpum* stem bark possesses antimalarial activity exhibits excellent haematopoietic property.

Key words: *Peltophorum pterocarpum* · *Plasmodium berghei* · Malaria · Haematological Indices

INTRODUCTION

Malaria is widespread in tropical and subtropical regions. Plasmodium parasite, is this highly infectious disease has been one of the greatest causes of human illness and death [1]. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis [2]. The emergence and spread of multidrug-resistant *Plasmodium falciparum* has become problematic in undermining malaria control programs in most endemic regions of the world [3]. Since anti-malarial drug resistance compromises the effective treatment of the disease. One important approach in anti-malarial drug discovery and development is investigation of potential anti-malarial candidates from natural products [5]. Nigeria is a country which is rich in a wide range of tropical habitats, remarkable biodiversity and medicinal herbs [5]. In developing countries of the world, a good number of the people in rural communities depend on herbal medical care [6]. The quinoline-based antimalarial quinine isolated from the bark of *Cinchona* species (*Rubiaceae*) is the first anti-malarial drug of plant source and later served as template for the synthesis of the antimalarial drugs; chloroquine and mefloquine [7]. The second major group of antimalarial drugs from a natural source is artemisinin and derivatives. Artemisinin is isolated from the Chinese plant *Artemisia Annua Linn* [8]. Artemisinin-based combination therapies are currently recommended by the World Health Organization (WHO) as the most effective medicines for the treatment of multidrug-resistant *P. falciparum* malaria [9]. However, evidences on artemisinin resistance have been accumulating particularly in Southeast Asia [10, 11]. *P. berghei* is one of the four species that infect mammals other than humans [12]. It is one of the plasmodium species that has been described in Africa murine rodents; others are *P. chabaudi, P.* vinckei and *P. yoeli* [13].

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Fig. 1: Peltophorum pterocarpum plant

Peltophorum pterocarpum belongs to the family *Leguminosae* native to tropical South-eastern Asia and it is an ornamental tree widely distributed around the world including India and Nigeria. It is a deciduous tree growing to 15–25 m tall, with a trunk diameter of up to 1 m. The leaves are bi-pinnate, 30-60 cm long, with 16-20 pinnae, each pinna with 20-40 oval leaflets 8-25 mm long and 4-10 mm broad. The flowers are yellow, 2.5-4 cm diameter, produced in large compound racemes up to 20 cm long (Fig. 1). The fruit is a pod 5-10 cm long and 2.5 cm broad and containing one to four seeds [14]. Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation, ringworm and malaria [15]. In Southeast Nigeria, the stem bark is used in the management of malaria and bacterial infections.

Previous phytochemical investigations of methanol extract of the flowers of *P. pterocarpum* revealed the presence of bergenin, kaempferol and quercetin [16]. Other studies showed that extracts of *P. pterocarpum* exhibit high antioxidant activity [17], antibacterial activity [15,18], hepatoprotective effect [19], antiglycaemic activity [20] and histoprotective effect [21]. The present study was undertaken to evaluate the anti-malarial activity of *P. pterocapum* stem bark extract and its amelioration of plasmodium-induced changes in haematological indices.

MATERIALS AND METHODS

Plant Collection and Identification: Fresh stem bark of *P. pterocarpum* was collected at the University of Nigeria, Nsukka in Enugu State, Nigeria and was authenticated at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria. Voucher specimen was deposited in the herbarium of the Department.

Chemicals and Reagents: All chemicals used in this study were of analytical grade and products of May and Baker (England); British Drug House- BDH (England) and Merck, Darmstadt (Germany). Reagents used for all the assays were commercial kits and products of Randox, USA.

Care and Management of Experimental Animals: Albino Wistar mice of both sexes weighing between 25-30 g were used for the study. The animals were kept in well- aerated stainless steel wire cages in the Department of Biochemistry animal house and were allowed to acclimatize for two weeks in environmentally normal tropical housing (27±3°C, relative humidity 70–90% with natural light/dark cycles at approximately 12 hr). They had free access to standard animal feeds (Vital Feed Nigerian Ltd.) and drinking water *ad libitum* throughout the study. They received human care throughout the experimental period in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No.85-23, revised 1985).

Methods

Extraction of Plant Materials: The stem bark of *P. pterocarpum* was washed with clean water to remove dirt and sand, drained and chopped. It was dried under shade for 3 weeks and then pulverized into fine powder. A quantity of 500 g of the powdered stem bark was macerated in 1.5 liters of methanol for 48 hr. The suspension was filtered with a chess cloth, followed by Whatman No 1 filter paper and the filtrate was concentrated under reduced pressure using a rotary evaporator at 45°C. A brown concentrate of percentage yield of 7.12% was produced and stored in refrigerator at 4°C until used.

Phytochemical Analysis: Qualitative phytochemical analyses were carried out using standard procedures to identify the constituents as described by Harborne [22].

Acute Toxicity and Lethality Test: The acute toxicity and lethality (LD_{50}) test of methanol extract of the plant were determined using the modified method of Lorke [23].

Parasite Inoculation: Donor mouse blood infected with the *P. berghei* was obtained from the animal farm of Faculty of Veterinary Medicine, University of Nigeria, Nsukka and was used for inoculum preparation. The desired blood volume (3 ml) was drawn from the donor mouse by heart puncture and diluted serially in Alsever's solution. The final suspension would contain

Table 1: Experimental design

Groups	Description	Treatment
Group 1	Treatment group 1	Parasitized with 0.2 ml *Plasmodium berghei* infected blood and treated with 100 mg/kg. b. wt of *P. pterocarpum*
Group 2	Treatment group 2	Parasitized with 0.2 ml *Plasmodium berghei* infected blood and treated with 200 mg/kg. b. wt of *P. pterocarpum*
Group 3	Treatment group 3	Parasitized with 0.2 ml *Plasmodium berghei* infected blood and treated with 300 mg/kg. b. wt of *P. pterocarpum*
Group 4	Standard control	Parasitized with 0.2 ml *Plasmodium berghei* infected blood and treated with 28 mg/kg. b. wt of arthemeter/lumenfantrin
Group 5	Positive control	Parasitized with 0.2 ml *Plasmodium berghei* infected blood and untreated
Group 6	Normal control	Uninfected, untreated

about 1×10^6 infected RBC's in every 0.2 ml suspension. This 0.2 ml suspension was injected into the experimental animals (in groups 1, 2, 3, 4 and 5) intraperitoneally to initiate infection. Infection for malaria was confirmed after 72 hr. After the confirmation of malaria, the mice infected (parasitized) and non-infected (normal) were divided into 6 groups of 6 mice each and treated as shown in Table 1:

Percentage parasitaemia in all the mice were determined on day 3 after which treatment began and administration was done orally using an intragastric tube. The treatment lasted for 5 days. Further analyses (percentage parasitaemia and haematological indices) were done on days 5 and 28.

Determination of Percentage Parasitaemia: The percentage parasitaemia was determined by counting the parasitized red blood cells out of red blood cells (RBCs) in random fields of the microscope, a method described by Ochei and Kolhatkar [24] and calculated as follows:

$$\% \text{ Parasitemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100$$

Determination of Haematological Parameters: Haematological parameters such as packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts and haemoglobin (Hb) concentration were determined using the method described by Ochei and Kolhatkar [24].

RESULTS

Phytochemical Screening of Methanol extract of *Peltophorum pterocarpum* Stem Bark: The result of the qualitative phytochemical study of *P. pterocarpum* stem bark is shown in Table 2. The presence of alkaloids, flavonoids, saponins, terpenoids, tannins, carbohydrates, fats and oils, reducing sugars and phenols were detected in high concentrations. Resins, protein and steroids were found in moderate concentrations while anthocyanin and cyanogenic glycosides were not detected.

Table 2: Qualitative Phytochemical Composition of *Peltophorum pterocarpum*

Phytochemicals	Inference
Alkaloid	++
Terpenoids	+++
Saponins	++
Phenols and Tannins	+++
Steroids	+
Protein	+
Anthocyanin	ND
Oils	++
Carbohydrate	+++
Flavonoid	
Cyanogenic glycoside	+++
ND	

Key: + Slightly present
++ Moderately present
+++ Highly present
ND not detected

Table 3: Results of Phase I and Phase II of the acute toxicity test of the Ethanol Extract of *Dennettia tripetala* Seed in Mice

Groups	Dose of extract administered (mg/kg b.w.)	Number of deaths
PHASE I		
Group 1	100	0/3
Group 2	250	0/3
Group 3	500	0/3
PHASE II		
Group 4	1000	0/3
Group 5	3000	0/3
Group 6	5000	0/3

n= 3

Result of the Acute Toxicity Test on **Methanol Extract of *P. pterocarpum* Stem Bark:** At the all the doses of ethanol extract of *P. pterocarpum* stem bark administered orally to the mice for acute toxicity study, there were neither clinical signs nor mortality after 24 hours post-treatment observation. Therefore, the median lethal dose (LD_{50}) value of the extract was estimated to be above 5000 mg/kg body weight (Table 3).

Effect of Methanol Extract of *Peltophorum pterocarpum* Stem Bark on Percentage Parasitaemia of Malaria-infected and Non-infected Mice: Fig. 2 shows that 3 days after inoculation (day 0), the mean percentage

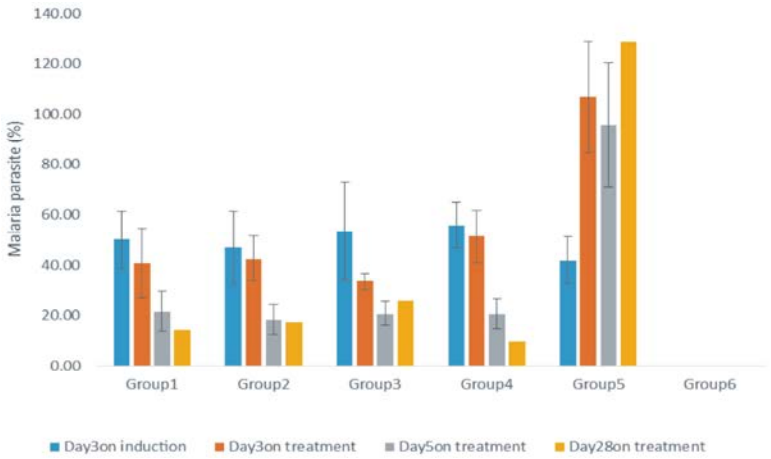

Fig 2: Effect of methanol extract of *Peltophorum pterocarpum* stem bark on percentage parasitaemia of malaria-infected and non-infected mice (n= 6)
Group 1: Malaria infected mice + 100 mg/kg b. wt. of methanol stem bark extract
Group 2: malaria infected mice + 200 mg/kg b. wt. of methanol stem bark extract
Group 3: malaria infected mice + 300 mg/kg b. wt. of methanol stem bark extract
Group 4: malaria infected mice + 28 mg/kg b. wt. standard drug (artemether and lumefantrine).
Group 5: malaria infected mice & untreated (positive control).
Group 6: non-infected group (Normal control)

parasitaemia of mice in groups 1, 2, 3 and 4 (standard control) were significant ($p > 0.05$) when compared to the mean value for the percentage parasitaemia of mice in group 5 (positive control). On day 3 post- treatment, there were significantly ($p < 0.05$) lower mean percentage parasitaemia of mice in groups 1, 2, 3 and 4 compared to those of mice in group 5 (positive control). Same were observed on days 5 and 28.

Effect of Methanol Extract of *Peltophorum Pterocarpum* Stem Bark on Packed Cell Volume of Malaria-infected and Non-infected Mice: Fig. 3 shows that 3 days after inoculation, the mean values for PCV of mice in groups 1, 2, 3 and 4 were not significantly ($p > 0.05$) different from the value obtained for mice in group 5 (positive control). On day 5 of treatment, all the treated and control groups had significantly ($p < 0.05$) higher PCV values when compared to group 5 (positive control). However, all the treated groups had significantly ($p < 0.05$) lower PCV values when compared to group 6 (Normal control). On day 28 post- treatment, all the treated and control groups had significantly ($p < 0.05$) higher PCV values when compared to group 5 (positive control).

Effect of Methanol Extract of *Peltophorum pterocarpum* stem bark on Red Blood Cell Count in Mice: Fig. 4 shows that 3 days after inoculation, the mean RBC count of mice in groups 1, 2, 3 and 4 (standard control) were not significantly different ($p > 0.05$) when compared to the mean RBC count of mice in group 5 (positive control). On day 5 of treatment, the mice in groups 1, 2, 3 and 4 (standard control) had significantly ($p < 0.05$) higher mean RBC count when compared to mice in group 5 (positive control). Same was observed on day 28 post- treatment. Also, only mice in group 2 (infected and treated with 200 mg/kg of the extract) showed a significantly ($p < 0.05$) lower mean RBC count when compared to mice in group 6 (Normal control).

Effect of Methanol Extract *of Peltophorum pterocarpum* on Total White Blood Cell Count in Mice: Fig. 5 shows that 3 days after inoculation, mice in groups 1, 2 and 3 showed significantly ($p < 0.05$) lower total white blood cell (TWBC) count when compared to the mean values of mice in groups 4, 5 and 6 (standard, positive and normal control) respectively. On day 5 after commencement of treatment, only group 4 (standard control) showed a significant increase in mean TWBC count when compared to the value of group 5 (positive control). On day 28 post-treatment, all the mice in groups 1, 2, 3 and 4 (standard control) showed significant increases ($p < 0.05$) in TWBC count when compared to mice in group 5 (positive control). However, no significant ($p > 0.05$) difference was observed when the mean TWBC counts of mice in groups 1, 2, 3 and 4 were compared with that of mice in group 6 (Negative control).

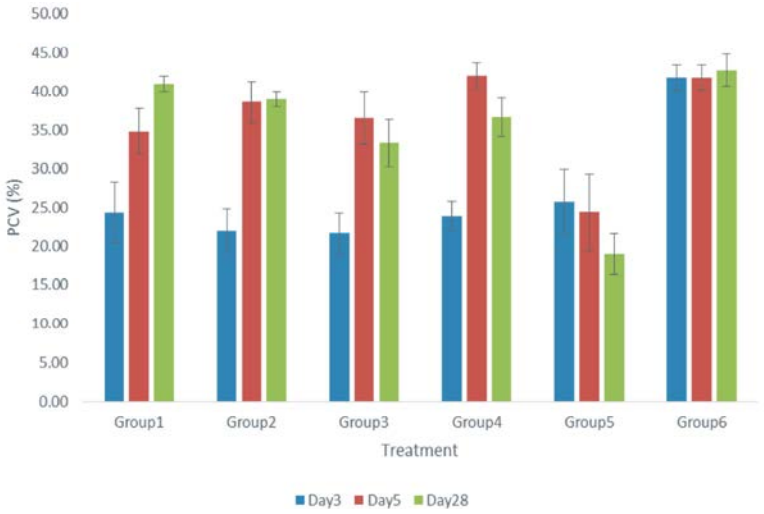

Fig. 3: Effect of methanol extract of *peltophorum pterocarpum* stem bark on packed cell volume of malaria-infected and non-infected mice (n= 6).
Group 1: malaria infected mice + 100 mg/kg b. wt. of methanol stem bark extract
Group 2: malaria infected mice + 200 mg/kg b. wt. of methanol stem bark extract
Group 3: malaria infected mice + 300 mg/kg b. wt. of methanol stem bark extract
Group 4: malaria infected mice + 28 mg/kg b. wt. standard drug (artemether and lumefantrine)
Group 5: malaria infected mice & untreated (positive control)
Group 6: non-infected group (Normal control)

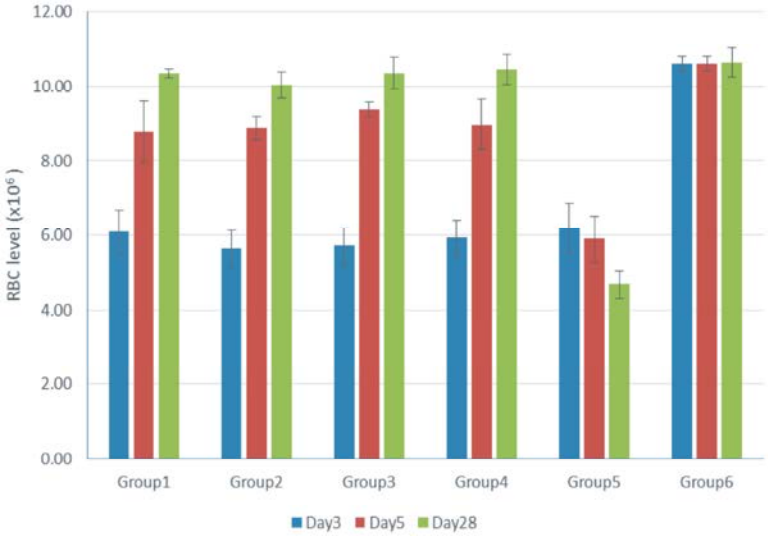

Fig. 4: Effect of Methanol Extract of *Peltophorum pterocarpum* Stem Bark on Red Blood Cell Count of malaria-infected and non-infected mice (n= 6).
Group 1: malaria infected mice + 100 mg/kg b. wt. of methanol stem bark extract
Group 2: malaria infected mice + 200 mg/kg b. wt. of methanol stem bark extract
Group 3: malaria infected mice + 300 mg/kg b. wt. of methanol stem bark extract
Group 4: malaria infected mice + 28 mg/kg b. wt. standard drug (artemether and lumefantrine).
Group 5: malaria infected mice & untreated (positive control).
Group 6: non-infected group (Normal control)

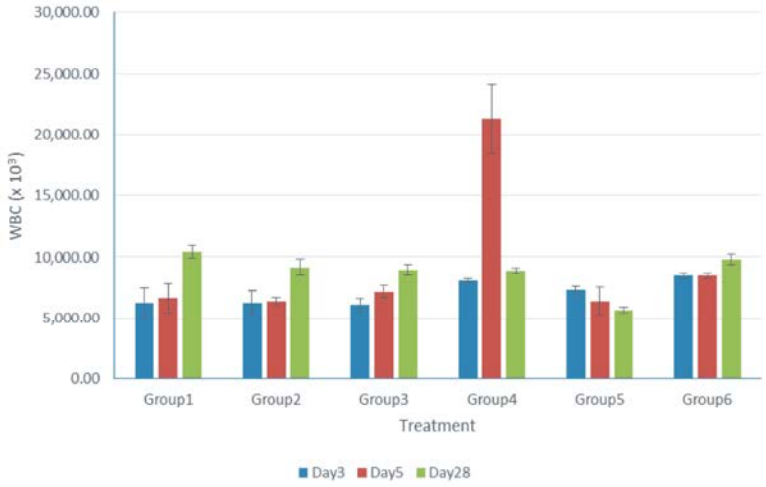

Fig. 5: Effect of Methanol Extract of *Peltophorum pterocarpum* Stem Bark on Total White Blood Cell Count of malaria-infected and non-infected mice (n= 6).
Group 1: malaria infected mice + 100 mg/kg b. wt. of methanol stem bark extract
Group 2: malaria infected mice + 200 mg/kg b. wt. of methanol stem bark extract
Group 3: malaria infected mice + 300 mg/kg b. wt. of methanol stem bark extract
Group 4: malaria infected mice + 28 mg/kg b. wt. standard drug (artemether and lumefantrine).
Group 5: malaria infected mice & untreated (positive control).
Group 6: non-infected group (Normal control)

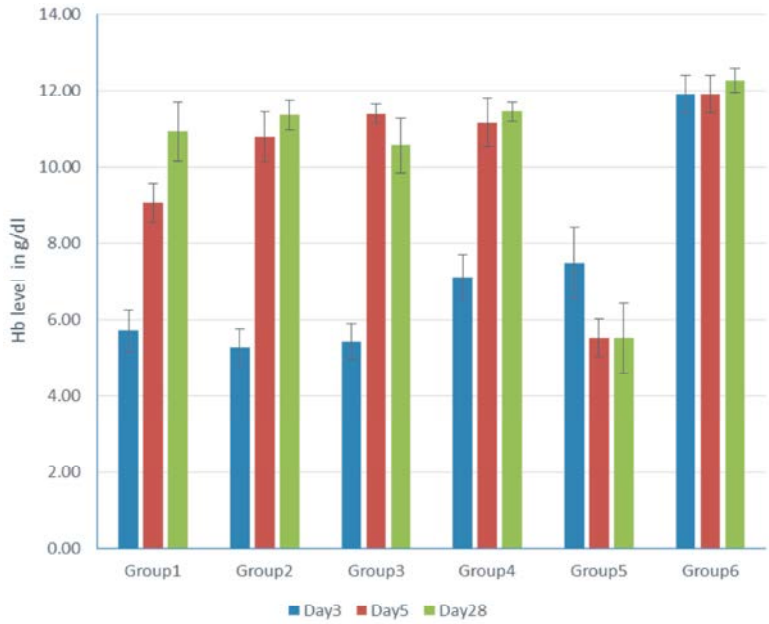

Fig. 6: Effect of Methanol Extract of *Peltophorm Pterocarpum* Stem Bark on Haemoglobin Concentration of malaria-infected and non-infected mice (n= 6).
Group 1: malaria infected mice + 100 mg/kg b. wt. of methanol stem bark extract
Group 2: malaria infected mice + 200 mg/kg b. wt. of methanol stem bark extract
Group 3: malaria infected mice + 300 mg/kg b. wt. of methanol stem bark extract
Group 4: malaria infected mice + 28 mg/kg b. wt. standard drug (artemether and lumefantrine).
Group 5: malaria infected mice & untreated (positive control).
Group 6: non-infected group (negative control)

Effect of Methanol Extract of *Peltophorm pterocarpum* Stem Bark on Haemoglobin Concentration: Fig. 6 shows that 3 days after inoculation, the mean haemoglobin (Hb) concentrations of mice in groups 1, 2 and 3 were significantly ($p < 0.05$) lower compared to mice in groups 4, 5 and 6 (standard, positive and negative control) respectively. On day 5 of treatment, mice in groups 2, 3 and 4 (Standard control) showed significant increases ($p < 0.05$) in the mean Hb concentrations when compared to mice in groups 1 and 5 (positive control) respectively. However, mice in groups 1, 2 and 4 showed significant ($p < 0.05$) decreases in the mean Hb concentrations when compared to values of mice in group 6 (normal control). On day 28 post- treatment, there were significant ($p < 0.05$) increases in mean Hb concentrations of mice in groups 1, 2, 3 and 4 (standard control) when compared to that of mice in group 5 (positive control).

DISCUSSION

The study evaluated antimalarial activity and effect of methanol extract of *Peltophorum pterocarpum* stem bark on some haematological indices in *Plasmodium berghei*-infected mice. Acute toxicity study of methanol extract of *P. pterocarpum* stem bark showed that this extract has a median lethal dose (LD_{50}) > 5000 mg/kg/day. This suggests that the extract is relatively safe at the doses used in this study. Quantitative phytochemical analysis of the plant stem bark revealed the presence of alkaloids and flavonoids in high concentrations; tannins and terpenoids in moderate concentrations, while saponins were present in low concentrations. This is in accordance with previous report on the phytochemical investigation of hexane extract of stem bark of *Peltophorum pterocarpum* [25].

Plasmodium berghei parasite is used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to chloroquine; making it the appropriate parasite for this study [26]. *Plasmodium berghei* has been used in studying the activity of potential antimalarials in mice [27] and in rats [28]. It produces diseases similar to those of human plasmodium infection [29]. Antimalarial activity evaluation showed that methanol extract of *P. pterocarpum* evoked remarkable antimalarial activity. Mice infected with *P. berghei* were characterized by parasitaemia which continued to rise steadily in untreated infected mice. This is in accordance with previous reports on antiplasmodial activities of medicinal plants [30]. Methanol extract of *P. pterocarpum* stem bark was able to significantly ($p < 0.05$) reduce the parasitaemia at the doses used in this study, indicating that the extract has potent antimalarial effect. The antimalarial activity exhibited by the plant extract was similar to that of the standard antimalarial drug (artemether/lumefantrine) [31]. The observed antimalarial activity is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as an antimalarial agent. Although the mechanism of action of the leaf extract has not been evaluated in the present study, some of constituents detected such as alkaloids, flavonoids and tannins and terpenoids have been implicated in antiplasmodial activities [32, 33].

Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. Malaria-infected patients tended to have lower platelets, WBC, lymphocytes, eosinophils, RBC counts and Hb concentration when compared to non-malaria-infected patients [2]. Our study found that RBC, PCV, Hb were significantly ($p < 0.05$) lower in the infected and untreated mice compared with the infected and treated and the uninfected mice (normal control). This is in line with the findings of Igbeneghu and Odaibo [34] that showed that humans with acute malaria have lower RBC count, PCV and Hb concentration compared with apparently-healthy individuals. Malaria parasites in their erythrocytic stage invade RBCs and feed on haemoglobin, generating haemoglobin metabolites such as bilirubin in the plasma [35, 36]. These result in lower RBC count, PCV value and Hb concentration in malaria infection as observed in the present study. However, treatment of malaria-infected mice with the plant extract restored the altered haematological indices as seen in groups 1, 2, 3 and 4.

CONCLUSION

The results obtained from this study show that the methanol extract of *P. pterocarpum* stem bark possesses antimalarial activity. The results also show that the extract exhibits excellent haematopoietic property by reversing and restoring the altered plasmodium-induced changes in haematological indices.

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