Full Length Research Paper

Malaria parasite clearance rate of crude methanol extract of Cryptolepis sanguinolenta in mice infected with chloroquine sensitive strain of Plasmodium berghei

Eze, Chinelo C.1*, Attama, Anthony A.2*, Ibezim, Emmanuel C.1, Berebon, Dinebari P.1 and Agbo, Martina C.1

1Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
2Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

Received 17 July, 2018; Accepted 16 August, 2018

This study aims to determine the malaria parasite clearance rate of crude methanol extract of Cryptolepis sanguinolenta in mice infected with chloroquine sensitive strain of Plasmodium berghei. P. berghei was injected in mice and left for 3 days for establishment. Blood sample collected and diluted with phosphate buffer saline was used for infection. Five (5) groups of animals (mice) were used in this study each containing 5 animals each. The body weights of the entire animal were recorded before and after treatment. Group 1 (normal control), Group 2 (positive control, untreated malaria-passaged mice), Group 3 (standard control, malaria-passaged mice treated with 25 mg/kg body weight of chloroquine), Group 4 (malaria-passaged mice treated with 200 mg/kg body weight of extract), and Group 5 (malaria-passaged mice treated with 400 mg/kg body weight of extract). Hematological assessments were carried out before the experiment, 5 days after infection and after treatment. The percentage of parasite load in malaria-passaged mice was found to be significantly (p < 0.05) lower in animals treated with mid and high doses of the extract when compared to control groups. Before treatment, no significant (p > 0.05) elevation was observed in the body weight of mice. On day 5 after infection, dose-dependent significant (p < 0.05) decrease was observed in the test groups. After treatment period, the body weights of the animals exhibited dose-dependent increase. The study thus revealed that Cryptolepis sanguinolenta root extracts possesses antimalarial activity in the in vivo mice model and has the ability of re-establishing the blood cells by boosting and stabilizing the blood parameters.

Key words: Cryptolepis sanguinolenta, chloroquine, Plasmodium berghei, malaria and clearance rate.

INTRODUCTION

Malaria caused by one of five protozoan parasites belonging to the genus Plasmodium spp: Plasmodium

*Corresponding author. E-mail: chinelo.eze@unn.edu.ng. Tel: +234 803 775 9532.

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vivax, Plasmodium malariae, Plasmodium falciparum, Plasmodium ovale, or Plasmodium knowlesi, though treatable or curable has plagued humanity throughout known history and human prehistory accounting for high mortality statistics in the tropics. In 2015, malaria was responsible for 212 million clinical cases, 429,000 deaths globally with most of the deaths estimated to have occurred in the African Region (92%), followed by the South-East Asia Region (6%) and the Eastern Mediterranean Region (2%) (World Health Organization (WHO), 2016). Malaria is not restricted to low and middle-income countries, but endemic in poor populations globally. In a report by (World Health Organization (WHO), 2011), P. falciparum causes the most severe malaria and is predominantly endemic in Africa; P. vivax causing life-threatening symptoms, is the prevalent species in South-East Asia, Latin America, Western Pacific and the Eastern Mediterranean, while P. malariae and P. ovale are less prevalent, and cause less severe disease in human hosts.

However, despite the geometric increasing threat of malaria parasites to lives globally, successful control of the disease is achievable, which includes vector (anopheles’ mosquito) control methods and evaluation of traditionally used herbal remedies (Akuodor et al., 2017) as well as effective case management.

Chloroquine has been a highly effective medicine for treatment and prevention of malaria. However, use of the classical drugs of chloroquine and primaquine in the control of the main causative agents of malaria; P. falciparum and P. vivax, has been exasperated by the resistance of the malarial parasites to these drugs (Olorunniyi and Morenikeji, 2014). This has necessitated the use of “novel” phytomedicines as alternative and effective antimalarial agents from natural products.

The root of the plant Cryptolepis (Cryptolepis sanguinolenta (Lindl.) Schlecter, Asclepiadaceae or Periploceae is derived from the root of C. periploceae; Syn C.triangularis N.E Br., and Pergularia sanguinolenta Lindl. The plant was named by a man called Kanyanga Cimanga. The anti-plasmodial efficacy of aqueous root extract C. sanguinolenta in the treatment of malaria is well-known in West African ethnomedicine. The root has a bitter taste and has been in use in traditional herbal medicine for treatment of malaria in Eastern Nigeria for several decades. In Nigeria, the Vernacular names of C. sanguinolenta is Paran pupa (Yoruba), Gangnamau (Hausa) (Osafo et al., 2017) and Akpa-oku (Igbo).

Detailed morphology and pharmacological active alkaloids of C. sanguinolenta have been described elsewhere (Osafo et al., 2017; Barku et al., 2012). C. sanguinolenta is native to West Africa and is found in countries like Ghana, Nigeria, Cote d’Ivoire, Guinea, Guinea-Bissau, Mali, Senegal, Sierra Leone, Angola, Congo, Uganda, and Cameroon (Ajayi et al., 2012). Cryptolepine, the major bioactive alkaloid in the root bark of C. sanguinolenta is basically responsible for antiplasmodial activity against P. falciparum chloroquine sensitive strain D-6 (Barku et al., 2012) and is presently a potential antimalarial lead.

Abay et al. (2015) observed that currently, promising results has been obtained with clinical research conducted on Qing hao (Artemisia annua, Democratic Republic of Congo trials), Totaquina (Cinchona spp., Multicounty trials) and Phyto-liria (C. sanguinolenta, Ghana trial) showing a parasite clearance at days 5 – 7 after treatment of 70 - 100, 92 - 100 and 100%, respectively.

In a prospective descriptive open trial on Ghanaian patients with acute uncomplicated falciparum malaria, Bugyei et al. (2010) appears to report the only antimalarial clinical trial of C. sanguinolenta roots when used as a single herb formulation conveniently packaged as tea-bag sachets (PHYTO-LARIA®). More than half of the patients were cleared of P. falciparum parasitaemia within 72 h, with mean clearance of 82.3 h (Bugyei et al., 2010). However, the use of root and root bark decoction of C. sanguinolenta by traditional medical practitioners in Nigeria and other parts of West Africa in antiplasmodial (antimalarial), anticancer, antihyperglycaemic, antifungal, antihypertensive, antibacterial, anti-inflammatory, tuberculosis, hepatitis and anti-diabetic treatment has been reported (Forkuo et al., 2016; Osafo et al., 2017).

Researchers uses Plasmodium berghei infection of laboratory mouse strains as a model to simulate human malaria because of its similarity to the Plasmodium species (P. falciparum, P. vivax, P. malaria and P. ovale) which cause human malaria, distinctive similarity of its life-cycle to the species that infect humans, and its potentials to causes disease in mice which have symptoms similar to those observed in human malaria (Oluyemi and Folyale, 2017).

The present study investigates the malaria parasite clearance rate (antimalarial potential) of crude methanol extract of C. sanguinolenta in mice infected with Chloroquine sensitive strain of P. berghei by assessing the percent reduction in parasitaemia and changes in haematological parameters.

MATERIALS AND METHODS

Collection and authentication of plant material

C. sanguinolenta roots were collected from Orba in Udenu Local Government Area of Enugu State during the rainy season (June - July, 2017). Identification and authentication were done by Mr Alfred Ozoiko, a taxonomist with the International Center for Ethnomedicine and Drug Development (Inter CEDD) Nsukka with Voucher no. interCE D 042.

Processing of Cryptolepis sanguinolenta root

The freshly harvested roots of C. sanguinolenta were chopped into smaller pieces, washed thoroughly with clean water and sun-dried.
for up to one month to reduce the moisture content. The roots were milled powdered and sieved through a 2.0 mm sieve size to remove larger particles and fiber. The processed root was then stored in sterile and air-tight container for further use.

**Animals and parasite used for the study**

Male Swiss albino mice (25 ± 2 g body weight and 6 – 8 weeks old) were used for this study. The animals were obtained from the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, University of Nigeria animal house. The mice were housed in a wooden cage with wire netting for proper ventilation. Saw dust was used as beddings. The mice were allowed to acclimatize to the laboratory conditions for 2 weeks and given food and water ad libitum.

The use of animals in this study was in accordance with the guidelines approved by the Animal Ethical Committee, University of Nigeria, Nsukka.

Chloroquine sensitive strain of *P. berghei* was obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. The *P. berghei* parasite was maintained for the duration of this study by continuous serial passage of blood from infected to uninfected mice which served as donor mice on a weekly basis.

**Preparation of extract**

1 kg ground powdered roots were weighed subjected to extraction with 80% methanol by cold maceration. 500-ml volume of methanol was poured into the container containing the powdered root and left for 24 h. This was filtered with gauze and a funnel into a vessel. The filtrate in a closed container was poured into evaporating dishes and left for 3 days for drying. The processed root was then stored in a tight container for further use.

Preparation of drug

The test drug (Chloroquine Phosphate) used for this experiment were prepared in aqueous solution and administered orally at single dose of 25 mg/kg body weight for three (3) consecutive days.

**Inoculation of mice**

The mice were injected intraperitoneally with 0.2 ml suspension of 10^5 parasitized erythrocytes (*P. berghei*) and were left for 3 days for establishment. Blood sample was collected and diluted with Phosphate buffer saline (PBS) and used for infection.

**Experimental groups**

Five (5) groups of animals (mice) were used in this study each containing 5 animals each. The body weights of all the animals were recorded before and after treatment.

- **Group 1** - Normal control received no extract but 2 ml per 100 kg body weight of sterile distilled water.
- **Group 2** - Positive control, untreated malaria-passaged mice.
- **Group 3** - Standard control, malaria-passaged mice treated with 25 mg/kg body weight of Chloroquine.
- **Group 4** - Malaria-passaged mice treated with 200 mg/kg body weight of extract, and
- **Group 5** - Malaria-passaged mice treated with 400 mg/kg body weight of extract.

**Measurement of parasitaemia**

The measurement of parasitaemia was determined as described elsewhere (Adetutu et al., 2016). The malaria clearance rate was conducted by preparing a thin blood film of blood. Briefly, a thin blood film stained with Giemsa stain was prepared on the fifth day for each mouse. The stain was allowed to dry completely. The percentage of red blood cells (RBCs) infected with malaria parasites were determined microscopically using the x100 objective with immersion oil in 10 different fields on each slide. The % Parasitemia and % Suppression were calculated using the formula (Birru et al., 2017):

\[
\% \text{Parasitaemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC count} \times 100} \quad (1)
\]

\[
\% \text{Suppression} = \frac{\text{Mean } \% \text{ Parasitaemia of Negative Control} - \text{Mean } \% \text{ Parasitaemia of Treatment Group}}{\text{Mean } \% \text{ Parasitaemia of Negative Control}}
\]

**Determination of body weight**

The animals were sacrificed after 14 days by euthanization using diethyl ether. Body weights before and after administrations were recorded using sensitive electronic balance.

**Red blood cell (RBC) count**

The red blood cell count was conducted using counting chamber (haemocytometer). Briefly, 4 ml of RBC diluting fluid was added to 20 µl of well mixed anti-coagulated blood and was allowed to stand for 5 min for destruction of WBC. The counting chamber and cover slip were assembled making sure they are completely clean and dry. The blood sample was remixed with capillary and the grid filled with the blood sample. It was left undisturbed for 2 min to allow the red blood cell to settle. These were examined microscopically with a 40x objective in 5 different boxes with chambers.

**White blood cell (WBC) count**

The white blood cell count was performed using counting chamber (haemocytometer). Aliquot (380 µL) of WBC diluting fluid (Turk’s solution) is added to 20 µL of well mixed anti-coagulated blood to give a 1 in 20 dilutions. It was allowed to stand for 5 min for the Turk’s solution to destroy the RBC and stain the nuclei of the WBCs, making them easier to see and count. The Neubauer counting chamber and the cover slip were assembled making sure they are completely clean and dry. The blood sample was remixed with capillary and the grid filled with the blood sample. It was left undisturbed for 2 min to allow the white blood cell to settle; thereafter, it was covered with the slide, placed on microscope stage and viewed with 40x objective. The cells in the four large corner squares of the chamber were counted.

**Determination of hemoglobin (Hb)**

Aliquot (20 µl) of well mixed blood in anti-coagulant bottle was carefully measured and dispensed into 4 ml drapkin neutral diluting fluid. It was left at room temperature, protected from sunlight for 4-5 min. The colorimeter was zeroed with drapkin fluid as blank and the absorbance of the test blood sample was read at 540 nm.
Figure 1. Effect of methanol extract of Cryptolepis sanguinolenta roots on percentage of parasite load in malaria-passaged mice. Group 1 = Normal control; Group 2 = Positive control (Untreated malaria-passaged mice); Group 3 = Standard control (Malaria-passaged mice treated with chloroquine); Group 4 = Malaria-passaged mice treated with 200 mg/kg b.w. of extract; Group 5 = Malaria-passaged mice treated with 400 mg/kg b.w. of extract.

Determination of pack cell volume (PCV)

A plane capillary tube was filled up to two-third with well mixed EDTA anti coagulated blood and the unfilled end was sealed with a sealant material. The packed cell volume (PCV) was determined by centrifuging heparinized blood in a micro hematocrit tube at 10,000 rpm for 5 min to separate the blood into a layer of volume of packed red blood cells which when divided by the total volume of the blood sample gives the PCV. The % PCV was read on the hematocrit reader before infection, after infection and after treatment. The PCV of each mouse was determined using the formula:

$$PCV = \frac{\text{Volume of packed RBC per volume of blood}}{\text{Total volume of blood}} \times 100 \quad (3)$$

White cell count

The Leishman staining technique was used. A drop of the fluid was placed on one end of the glass slide using an applicator. Another glass slide was used to make a smear of the fluid on the glass slide using the push wedge technique. The stain was made to cover the film and then left to stand for 2 min. Thereafter, distilled water, twice the quantity of stain was used to flood the thin film; the setup was rocked gently for 2 min and then allowed to stand for another 15 min before rinsing the stain. The slide was left to air-dry and then examined on the microscope using the oil immersion objective lens of x100 magnification. The cells were counted and differentiated on morphology basis using a tally counter.

Statistical analysis

The data were expressed as the mean ± standard deviation of the mean (SD). Statistical analysis was carried out employing t – test and one-way ANOVA following Dunnett’s multiple comparison test.

RESULTS

Effect of methanol extract of Cryptolepis sanguinolenta roots on percentage of parasite load in malaria-passaged mice

On day 5 after infection, significantly (p < 0.05) higher percentage of parasite was observed in all groups except Group 1 (normal control group). On day 1, 2 and 3 post treatment, significant (p < 0.05) reduction were observed in the parasite of Groups 4 and 5 passaged mice treated with 200 mg/kg b.w and 400 mg/kg b.w. of the extract, respectively when compared to the parasite of Groups 2 (malaria-passaged untreated) and 3 (malaria-passaged treated with Chloroquine) mice. There were no significant (p > 0.05) differences between Groups 4 and 5 malaria-infected mice administered low (200 mg/kg b.w.) and high (400 mg/kg b.w.) doses of the extract, respectively (Figure 1).

Effect of methanol extract of Cryptolepis sanguinolenta roots on the body weight of malaria-passaged mice

Before treatment, the weights of all the animals in all groups were observed and recorded. The results on day
5 after infection revealed relative decrease in their weights except in Group 1 (normal control). After the treatment period, the body weights of rats from Groups 2 to 5 exhibited dose-dependent increase. Significantly (p < 0.05) higher body weights of mice in all test groups were observed compared to the body weights of mice in the control groups. However, non-significant difference (p > 0.05) was observed across all groups except Group 2 mice that were passaged but untreated (Figure 2).

**Effect of methanol extract of *Cryptolepis sanguinolenta* roots on blood parameters of malaria-passaged mice**

Figure 3A shows the result of the effect of methanol extract of *C. sanguinolenta* roots on packed cell volume of malaria-passaged mice. Non-significant difference (p > 0.05) was observed in the packed cell volume (PCV) of all the groups before the treatment period. Dose-dependent significant (p < 0.05) reduction was observed in all groups except in Group 1 (normal control), on day 5 after infection. Results obtained after the treatment period showed significantly (p < 0.05) higher PCV of Group 5 mice compared to the PCV of mice in all the groups except Group 2. On the other hand, Group 5 mice had PCV that was found to be significantly (p < 0.05) lower than the PCV of Group 1 mice. The PCV of Group 4 mice was found to be significantly (p < 0.05) lower and higher than the PCV of Groups 1 and 2 mice, respectively.

Figure 3B shows the result of the effect of methanol extract *C. sanguinolenta* roots on red blood cell volume of malaria-passaged mice and dose-dependent decrease and increase in red blood cell (RBC) count were observed for day 5 after infection and after treatment periods in Groups 4 and 5 administered 200 and 400 mg/kg b.w. of the extract, respectively as shown in Figure 3. Before treatment, non-significant (p > 0.05) reduction was witnessed in RBC count in all the groups compared to the normal control (Group 1) mice. After the treatment period, it was observed that the RBC counts in Groups 4 and 5 exhibited significant (p < 0.05) elevation compared to the RBC counts of Group 2 mice that were malaria-passaged but untreated. On the other hand, Group 5 mice had significantly (p < 0.05) higher RBC count than the RBC counts of Groups 2 and 3 mice representing untreated and that treated with chloroquine, respectively.

Figure 3C shows the results of the effect of methanol extract *C. sanguinolenta* roots on white blood cell count of malaria-passaged mice and the total white blood cell (WBC) on day 5 after infection recorded the highest count across the groups except Group 2 which represented positive control group, while the lowest WBC count was recorded after treatment in all the groups. Observation before treatment period recorded non-significant (p > 0.05) decreases in WBC count of the mice in the test groups (Groups 4 and 5) malaria-passaged mice administered 200 and 400 mg/kg b.w. of the extract compared to all the control groups.

After treatment, significant (p < 0.05) reduction was found in the total WBC counts of Groups 4 and 5 mice compared to the total WBC counts of Groups 1, 2 and 3 mice that represented normal control mice, malaria-passaged untreated and Chloroquine-treated groups, respectively.
Figure 3D shows the result of the effect of methanol extract of *Cryptolepis sanguinolenta* roots on haemoglobin concentration of malaria passaged mice and the highest and lowest haemoglobin (Hb) concentrations were observed in mice of all the groups except normal control mice (Group 1) before the treatment period and 5 days after infection, respectively. Non-significant differences (p > 0.05) were witnessed in the haemoglobin concentration of mice Groups 4 and 5 treated with low and high doses of the extract, respectively compared to the haemoglobin concentration of mice in normal, positive and standard control groups.

Results recorded after treatment period showed significantly (p < 0.05) lower level of Hb concentration in Groups 4 and 5 mice compared to the Hb concentration of mice in the normal control group. Conversely, significantly (p < 0.05) higher concentration of Hb was witnessed in the test animals compared to that of Group 3 mice (positive control).

**DISCUSSION**

The aqueous methanol extract of *C. sanguinolenta* showed a high percentage of malaria parasite clearance rates in Chloroquine sensitive strain of *P. berghei* comparable to the standard Chloroquine drug. Initially, percentage of parasite clearance was very low during treatment with the methanolic extract of *C. sanguinolenta* roots but higher during the final stage of treatment. Importantly, our results indicated that the inhibition by the extract of *C. sanguinolenta* roots was effective than the positive control (untreated malaria-passaged mice) and standard control (malaria-passaged mice treated with chloroquine) used for treatment of malaria. It was remarkable to observe that the parasite clearance by the extract was better than the positive control, an established drug (chloroquine) commonly used for treatment of malaria. Similar observation was reported by Bugyei et al. (2010) in which *C. sanguinolenta* was better at fever and malaria parasite clearance than chloroquine treated patients in a clinical study. The results showed that the percentage of parasite load in malaria passaged mice was found to reduced significantly in animals treated with mid (200 mg/kg body weight of extract) and high doses (400 mg/kg body weight of extract) of the extract when compared to control groups. This rapid parasite clearance by the extract may be due to its early
Tmax, quick clearance from the plasma, possibly accumulating in vital organs and extending plasma residence time (Donkor, 2016). This suppressive antiplasmodial effect of the methanol extract of *C. sanguinolenta* root on the multiplication of *P. berghei* portends that these extracts are a potential source for new antimalarial drugs.

Evaluation of the methanol extract of *C. sanguinolenta* roots on the body weight of malaria-passaged mice showed that 5 days after infection, the body weights of the malaria-passaged mice administered with chloroquine, 200 and 400 mg/kg b.w. of the extract witnessed relative decrease. The reduction in body weight gain is a simple and sensitive index of toxicity after exposure to toxic substances (Pillai et al., 2011).

A dose-dependent decrease was observed in treated mice compared to that of normal control mice. However, the result showed that after the treatment period, the body weights of mice from all the groups except the normal control exhibited dose-dependent increase. Plants with antimalarial activity are expected to prevent body weight loss in infected mice resulting from rise in parasitaemia (Nardos and Makonnen, 2017).

Thus, higher body weights of mice in all test groups were observed compared to the body weights of mice in the control groups. However, non-significant difference was observed across all groups except Group 2 mice that were passaged but untreated. This result is consistent with (Birru et al., 2017), in which neither the extract doses nor the standard drug-treated group demonstrated statistically significant difference in body weight compared to the control group. The discrepancy was attributed to imbalance of the extract to potentiate protective effect and the cumulative pathophysiologic changes associated with the infection (Birru et al., 2017).

The effect of methanol extract of *C. sanguinolenta* roots on various blood parameters such as pack cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and haemoglobin concentration (Hb) of malaria-passaged mice were evaluated. A reduction in concentration of PCV, RBC and Hb are useful indicators of clinical malarial anemia and frequently monitored as drug efficacy against Plasmodium infection (Yeo et al., 2017).

In the present study, PCV was measured to assess the efficacy of the extract and chloroquine in preventing haemolysis due to increasing parasitemia level. There was no observable difference in the packed cell volume (PCV) of all the groups before the treatment period.

However, dose-dependent reduction was observed, on day 5 after infection among Groups 4 and 5 malaria-infected mice treated with 200 and 400 mg/kg b.w. of the extract compared to the PCV of normal control mice (Group 1). This suggests that the malaria parasite actually attacked the erythrocyte (RBC) and reduced their PCV which upon treatment with extract, regenerated the blood cells leading to improved PCV. We observed a relative reduction in the PCV of the test mice compared to that of Groups 2 and 3 mice which were found to be non-significant. This finding is consistent with Ajayi et al. (2012) and Adetutu et al. (2016) who observed that neither the extract nor the chloroquine significantly prevented the reduction of PCV as compared to the untreated group. The inability of the extract to prevent the reduction of PCV may be attributed to the anti-haemolytic potential of saponin found in the *C. sanguinolenta* roots.

Non-significant differences were observed in the haemoglobin concentration of mice treated with low and high doses of the extract respectively compared to the haemoglobin concentration of mice in normal (Group 1), positive (Group 2) and standard (Group 3) control groups.

Several researchers (Ajayi et al., 2012; Donkor, 2016; Osonwa et al., 2017; Madukaku et al., 2015) have reported similar findings that haemoglobin concentrations were not significantly different from the parasite control treated groups. Conversely, higher concentration of haemoglobin witnessed in the test animals compared to that of Group 3 mice (positive control) agreed with other reports (Ekaidem and Akpan, 2016; Parker et al., 2016).

Plasmodium parasite strains have high affinity for RBCs and feeds on it. Initially, a dose-dependent decrease in red blood cell (RBC) count were observed for day 5 after infection but gradual increase was recorded after treatment periods in Groups 4 and 5 administered 200 and 400 mg/kg b.w. of the extract respectively. In an earlier report, Ajayi et al. (2012) observed that *C. sanguinolenta* did not alter red cells (RBCs) and its related indices in rats treated with 250 mg/kg b.w. Changes in RBC are the most typical feature of malarial infections (Yeo et al., 2017).

The lowest WBC count was recorded after treatment in all the groups. After 5 days of infection, the total WBC count of Group 4 malaria-passaged mice that were treated with 200 mg/kg b.w. of the extract was found to be elevated significantly compared to the total WBC counts of the mice in the Group 5 mice treated with 400 mg/kg b.w. of the extract and control groups. After treatment, significant reduction was found in the total WBC counts of Groups 4 and 5 mice compared to the total WBC counts of Groups 2 and 3 mice that represented malaria-passaged untreated and chloroquine-treated groups respectively.

These findings support earlier reports by (Ajayi et al., 2012) that aqueous root extracts of *C. sanguinolenta* and its major alkaloid cryptolepine had little or no effect on RBC, WBC, Hb concentration, and platelets when administered to rats for 3 or 7 days compared to the control.

In a similar study but with different extracts, Adetutu et al. (2016) and Parker et al. (2016) observed an insignificant difference in the WBC counts of all groups. The results of the present study imply that the *C. sanguinolenta* root extracts enhanced the normal status of the WBC.
Conclusion

The study confirmed that *C. sanguinolenta* root extracts possesses anti-malarial activity in the *in vivo* mice model and has the ability of re-establishing the blood cells by boosting and stabilizing the blood parameters. The results of this investigation, thus, likely support the ethnomedicinal use of this plant in Eastern Nigeria for the treatment of malaria.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

REFERENCES


Adetutu A, Olorunnisola OS, Owode AO, Adegbola P (2016). Inhibition of *in vivo* growth of *Plasmodium berghei* by *Launaea taraxacifolia* and *Amaranthus viridis* in Mice. Malaria Research and Treatment.


