



Research Article

Comparative Study on the Effect of an Antimalarial Drug and *Ficus capensis* Aqueous Leaf Extract on Some Haematological Indices in Albino Rats

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OPEN ACCESS ABSTRACT

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Artemether and lumefantrine (AL) is a frontline antimalarial drug but has been reported to have various side effects. *Ficus capensis* is a plant used by traditional medicine practitioners in Eastern Nigeria for the management and treatment of different disorders, including kidney, heart, and blood-related diseases. Against these backdrops, the effect of AL and *Ficus capensis* aqueous leaf extract (FCALE) on the concentrations of hemoglobin (Hb), red blood cells (RBC), and packed cell volume (PCV) in albino rats was investigated. Twenty-eight acclimatized albino rats were separated into seven groups of four rats each. Group 1 served as the control group. Groups 2 - 4 were administered 8, 16 and 24 mg/kg body weight (b.w) of AL, respectively; while groups 5 - 7 were administered 200, 400 and 600 mg/kg b.w of FCALE, respectively, twice daily for 7 consecutive days through oral intubation. There were significant ($p < 0.05$) reductions in the concentrations of Hb, RBC, and PCV in groups 2 - 4 given different doses of the AL, with concomitant significant ($p < 0.05$) higher concentrations in groups 5 - 7 given FCALE, in a dose-dependent manner compared to the control. The findings of this study showed that the AL brand caused reductions in RBC, PVC, and Hb, hence, it may not be suitable for individuals with hematological disorders such as anemia or hemorrhage, while *Ficus capensis* caused elevations in RBC, PVC and Hb, and could be used in the management of anemia or other blood-related diseases.

Keywords: Artemether and lumefantrine, *Ficus capensis*; Albino rats; Haemoglobin; Red blood cell; Packed cell volume

INTRODUCTION

Malaria is one of the world's most common protozoan diseases, affecting at least 300 million people annually and causing about 1.0 to 1.5 million deaths. It is one of the common causes of morbidity and mortality in West Africa (World Malaria Report, 2021). About ninety percent of

malaria-related deaths occur in Sub-Saharan Africa. The disease is endemic in sub-Saharan Africa, and children below the age of five suffer the highest burden of the disease (Bhag *et al.*, 2019). Several antimalarial drugs are used for the prophylactic and therapeutic treatment of malaria. Unfortunately, in Nigeria, due to the knowledge that fever and headache are among the common symptoms of malaria, many people take antimalarial drugs without prior medical diagnosis and prescriptions once they have these symptoms. Hence, comorbidities such as anemia and other drug-related diseases may arise due to the consumption of these drugs against medical prescription. Anemia, as well as

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alterations in other hematological factors such as packed cell volume (PCV), red blood cell (RBC), and haemoglobin level, is shown to be generally affected by malaria. This is particularly dangerous because recurrent malaria incidents can cause life-threatening anemia (Imoru *et al.*, 2013).

Artemether (80 mg) and lumefantrine (480 mg) is an antimalarial drug used in the treatment or prophylaxis of malaria. When hemoglobin breaks down, lumefantrine binds to heme, blocking detoxification to crystalline malaria pigment (hemozoin). The peroxide group in artemether releases harmful free radicals when it binds to heme in the same mechanism (Warhurst *et al.*, 2001). The expression of the multidrug resistance protein PGH-1 is necessary for resistance to both lumefantrine and artemether. This shared resistance mechanism is what causes the co-artemether components to become more potentiated. PGH-1 mutations linked to chloroquine resistance influence sensitivity to lumefantrine and artemether; nevertheless, if PGH-1 is mutated, it is overexpressed (Warhurst *et al.*, 2001). After two parasite asexual cycles have been subjected to artemether, lumefantrine is crucial in removing the remaining parasites, while artemether is linked to the quick parasite clearance (Kloprogge *et al.*, 2015). Rapid absorption converts artemether, a lipid-soluble derivative of dihydroartemisinin, into its active metabolite dihydroartemisinin (DHA). Within two hours of treatment, artemether and DHA reach their maximum concentrations, which causes a rapid decrease in the biomass of asexual parasites and a prompt remission of symptoms (Djimé and Lefèvre, 2009; Marwa *et al.*, 2022). Lumefantrine has a 98% binding rate to fat and plasma lipoproteins, making it a highly lipophilic substance (Chotivanich *et al.*, 2012). Concurrent uptake of fat meals increases lumefantrine's bioavailability (Ashley *et al.*, 2007a; Djimé and Lefèvre, 2009). Artemether and lumefantrine have terminal elimination half-lives of ≤ 1 hour and 3-5 days, respectively (Ashley *et al.*, 2007b; Djimé and Lefèvre, 2009). Compared to malaria patients, healthy volunteers eliminate lumefantrine far more slowly (terminal half-life: 2-3 days and 4-6 days) (Djimé and Lefèvre, 2009; Marwa *et al.*, 2022). After artemether and DHA have been removed, the gradual removal of lumefantrine is crucial in removing any remaining parasites. This helps to prevent recrudescence, which can occur when parasites are exposed to high concentrations of lumefantrine that accumulate over time due to the drug's lengthy half-life (Djimé and Lefèvre, 2009; Marwa *et al.*, 2022).

For ages, people have utilized medicinal plants as a recognized alternative to conventional treatment to enhance their health by reducing the side effects and expenses of synthetic medications (Rouhi-Boroujeni *et al.*, 2015). More than 80 % of people in underdeveloped nations use traditional medicinal herbs to cure a range of illnesses (Rouhi-Boroujeni *et al.*, 2015).

Ficus capensis Thumb (family: Moraceae) is an evergreen tree that is widely distributed in the tropics. Its leaves are broad, greenish and produce fruits all year round. It is

commonly called "bush fig tree." In Nigeria, it is locally referred to as Akokoro (Igbo), Opoto (Yoruba), and Uwaraya (Hausa) (Otitoju *et al.*, 2014). The leaves of *Ficus capensis* are used as a vegetable both in soup and yam pottage in various parts of Nigeria (Otitoju *et al.*, 2014). Traditionally, *Ficus capensis* has been used for the treatment of dysentery and wound dressing (Igoli *et al.*, 2005). It is also used to treat circumcision wounds, leprosy and epilepsy, rickets, infertility, gonorrhea, edema and respiratory disorders (Olowokudejo *et al.*, 2008) and abortion (Owolabi *et al.*, 2009). *Ficus capensis* leaves and roots are traditionally used for the treatment of anaemia, leprosy, tuberculosis, sexually transmitted diseases, convulsions, wounds, diarrhea and dysentery (Ahmadu *et al.*, 2007; Oyeleke *et al.*, 2008; Ibe *et al.*, 2022). Apart from its traditional uses, scientific investigations have reported its blood-boosting (Otitoju *et al.*, 2014; Njoku-oji *et al.*, 2016), anti-sickling (Umeokoli *et al.*, 2013; Mpiana *et al.*, 2008) antibacterial (Oyeleke *et al.*, 2008), anti-abortion (Owolabi *et al.*, 2009), immune-stimulatory (Daikwo *et al.*, 2012), anti-diarrhoea (Owolabi, 2013), antioxidant (Ramde-Tiendrebeogo *et al.*, 2012) effects and pro-fertility in treating azoospermia (Akomolafe *et al.*, 2016). The phytochemical screening, antioxidant and antimicrobial properties of the leaf extracts of *F. capensis* have been reported by Muanda *et al.* (2010) and Igwe *et al.* (2016). The preliminary photochemical screening of leaves and stem bark extracts of *F. capensis* revealed the presence of alkaloids, balsams, carbohydrates, flavonoids, free anthraquinones, tannins, glycosides, terpenes, resins, sterols and saponins using UV-visible spectroscopy. Glycosides were not present in the leaf but present in the stem bark. The presence of alkaloids reveals its activity against pathogenic bacteria (Adebayo-Tayo and Odeniyi, 2012; Ezeigwe *et al.*, 2020).

Herein, the present study aimed to compare the effects of an antimalarial drug comprising of artemether (80 mg) and lumefantrine (480 mg), and *Ficus capensis* aqueous leaf extract on hemoglobin (Hb), red blood cell (RBC) and packed cell volume (PCV) in albino rats.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used for this study include: chloroform (99%) (BDH, chemical Ltd. Poole England), sodium citrate, Drabkin's reagent (catalog number D5941 containing sodium bicarbonate, potassium ferricyanide, and potassium cyanide). The antimalarial drug comprising of artemether (80 mg) and lumefantrine (480 mg) (AL) was purchased from Octovia Pharmacy in Abakaliki, Ebonyi State,

Collection and preparation of drug samples

The six tablets of antimalarial drugs containing Artemether and Lumefantrine (80/480 mg) were placed in a clean beaker containing 200 ml of distilled water and allowed to dissolve for 30 minutes. The mixture was stored in room temperature during the seven days of administration.

Collection and preparation of plant sample

Fresh leaves of *Ficus capensis* were collected from a local farm in Umuigboke in Ohaozara Local Government Area of Ebonyi State. The leaves were identified in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. Two hundred grams of fresh *F. capensis* leaves were cut into pieces and pounded into a paste with a mortar and pestle. Afterwards, 500 ml of distilled water was added to it and allowed to soak for 1 hour, then sieved with a muslin cloth to get a gold-brown aqueous extract. The extract was concentrated using a rotary evaporator.

Animal handling

Thirty (30) male adult albino rats weighing 78 to 93 g were purchased from the Animal Science Department, Ebonyi State University, Abakaliki, and transferred in steel cages to the Department of Biochemistry animal house, Ebonyi State University. Animals were allowed to acclimatize for seven days before administration of drug samples. They received feed and water throughout the study period. The animals were weighed daily using a weighing balance to determine the actual volume of drug solution and plant extract to be administered.

Experimental design

Twenty-eight (28) albino rats were divided into seven groups of four rats each as follows: Group 1, Normal control rats; Group 2, 8 mg/kg b.w of AL; Group 3, 16 mg/kg b.w of AL; Group 4, 24 mg/kg b.w of AL; Group 5, 200 mg/kg b.w of *F. capensis* aqueous leaf extract (FCALE); Group 6, 400 mg/kg b.w of FCALE; Group 7, 600 mg/kg b.w of FCALE.

The weight determinations and treatment lasted for seven days; after which the rats were sacrificed under mild anesthesia using chloroform and blood samples were collected via ocular and cardiac puncture in EDTA-anticoagulant bottles for haematological analyses.

Determination of red blood cells (RBC) count

The method of Ochei and Kolhatkar (2008) was used. In brief, an aliquot (0.02 ml) of blood was added to 3.98 ml of sodium citrate and mixed well. After 5 minutes, the first few drops were discarded by holding the pipette vertical and the counting chamber was charged with the fluid. It was allowed to settle for 3 minutes. By Switching to low power (10 X) objective, the centre large square with 25 small squares were adjusted to light and then adjusted to high power (40 X) objective. The red blood cells in the four corner squares and one central square were counted using the formula below:

$$\text{Total RBC/cu mm (mm}^3\text{)} = \frac{\text{number of cells counted (N) x dilution factor}}{\text{Area counted x depth of fluid}}$$

Where:

Dilution = 1:200

Area counted = 1/5 sq.mm

Depth of fluid = 1/10mm

Number of red blood cells counted = N x 200

Total RBC = N x 200 x 50 = N x 10,000 (1/5x1/10) (mm³)

Determination of the packed cell volume (PCV)

PCV was estimated as described by Ochei and Kolhatkar (2008). Briefly, blood sample was taken with heparinised capillary tube, cleaned and sealed with plasticine. The filled tubes were placed in the microhaematocrit centrifuge and spun at 12,000 ×g for 5 minutes. The spun tubes were placed in microhaematocrit reader and the PCV was read as a percentage.

$$\text{PCV (\%)} = \frac{\text{Packed RBC column height} \times 100}{\text{Total blood volume height}}$$

Determination of haemoglobin (Hb) concentration

Hb concentration was determined using cyanomethaglobin method as described by Ochei and Kolhatkar (2008). Whole blood (0.02 ml) was added to 5 ml of Drabkin's solution in a test tube (1:250 dilution). This was well mixed, allowed to stand for 10 minutes and the absorbance was read at 540 nm with Drabkin's solution as a blank. The Hb concentration was calculated using the formula hereunder:

Heamoglobin (HB) = Reading of test /Reading of standard x standard conc./4

Statistical analysis

Results were analyzed with statistical package for social sciences (SPSS) version 23 using one way analysis of variance (ANOVA). Duncan's test was used to compare the means at 5 % probability level and values were considered significant at P < 0.05. The results were given as mean ± standard error of the mean (SEM) of samples' triplicates.

RESULTS

Effects of AL and FCALE on body weight of rats

Table 1 shows the effects of AL and FCALE on the average body weight of the rats during the seven days of administration. There was a significant (p < 0.05) increase in the average body weight of the normal control group from day 1 (87.50 ± 8.66 g) to the day 7 (100.50 ± 10.12 g) of the experiment. The average body weight of the group 2 that was given 8 mg/kg b.w of AL showed significant (p < 0.05) increase from day 1 (83.15 ± 9.12 g) to day 5 (89.10 ± 7.80 g) before a gradual weight loss on day 6 (89.00 ± 5.47 g) and day 7 (88.20 ± 5.95 g). The average body weight of group 3 given 16 mg/kg b.w of AL increased significantly (p < 0.05) from day 1 (86.12 ± 6.24 g) to day 3 (87.50 ± 8.75 g), but decreased from day 4 (86.40 ± 8.85 g) to day 7 (79.18 ± 4.90 g). While group 4 given 24 mg/kg b.w of AL showed non-significant (p > 0.05) increase in the average body weight on day 1 (87.15 ± 7.12 g) and day 2 (87.50 ± 6.10 g), but showed a significant (p < 0.05) reduction in average body weight from day 3 (86.10 ± 6.56 g) to day 7 (73.20 ± 8.65 g). However, there were significant (p < 0.05) increases in the average body weights of group 5 (85.38 ± 9.95 - 101.71 ± 8.61 g), group 6 (85.46 ± 5.76 - 103.15 ± 8.12 g), and group 7 (84.90

$\pm 8.63 - 104.53 \pm 5.01$ g) administered 200, 400 and 600 mg/kg b.w of FCALE, respectively from day 1 to day 7.

Table 1: Effect of Artemether-Lumefantrine (AL) and *Ficus Capensis* Aqueous Leaf-Extract (FCALE) on Average Body Weight (G) of Rats during the Seven Days of Administration.

Days	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	87.50 $\pm$ 8.66 ^a	83.15 $\pm$ 9.12 ^a	86.12 $\pm$ 6.24 ^d	87.15 $\pm$ 7.12 ^f	85.38 $\pm$ 9.95 ^a	85.46 $\pm$ 5.76 ^a	84.90 $\pm$ 8.63 ^a
2	88.14 $\pm$ 9.45 ^b	84.13 $\pm$ 8.45 ^b	87.10 $\pm$ 6.10 ^e	87.50 $\pm$ 6.10 ^f	85.97 $\pm$ 5.75 ^a	86.33 $\pm$ 5.63 ^b	85.15 $\pm$ 9.96 ^b
3	91.20 $\pm$ 5.80 ^c	86.41 $\pm$ 8.33 ^c	87.50 $\pm$ 8.75 ^e	86.10 $\pm$ 6.56 ^e	88.35 $\pm$ 7.39 ^b	89.12 $\pm$ 4.95 ^c	88.53 $\pm$ 9.56 ^c
4	93.45 $\pm$ 13.42 ^d	88.14 $\pm$ 8.69 ^d	86.40 $\pm$ 8.85 ^d	83.30 $\pm$ 6.33 ^d	92.15 $\pm$ 8.04 ^c	92.69 $\pm$ 4.95 ^d	91.98 $\pm$ 9.95 ^d
5	95.19 $\pm$ 14.90 ^e	89.10 $\pm$ 7.80 ^e	84.18 $\pm$ 7.75 ^c	80.50 $\pm$ 10.75 ^c	95.13 $\pm$ 5.68 ^d	96.57 $\pm$ 4.86 ^e	94.50 $\pm$ 9.57 ^e
6	98.10 $\pm$ 10.64 ^f	89.00 $\pm$ 5.47 ^e	82.20 $\pm$ 9.46 ^b	77.30 $\pm$ 12.83 ^b	98.66 $\pm$ 9.67 ^e	99.15 $\pm$ 10.23 ^f	99.20 $\pm$ 5.71 ^f
7	100.50 $\pm$ 10.12 ^g	88.20 $\pm$ 5.95 ^d	79.18 $\pm$ 4.90 ^a	73.20 $\pm$ 8.65 ^a	101.71 $\pm$ 8.61 ^f	103.15 $\pm$ 8.12 ^g	104.53 $\pm$ 5.01 ^g

Values are mean $\pm$ SEM; n = 4. Values with different superscript alphabets down the columns are significant at P < 0.05. Group 1 = normal control; Groups 2, 3, and 4 = 8, 16, and 24 mg/kg b.w. of Artemether-Lumefantrine (AL), respectively; Groups 5, 6, and 7 = 200, 400, and 600 mg/kg b.w of *Ficus capensis* aqueous leaf-extract (FCALE), respectively.

Effects of AL and FCALE on red blood cell (RBC) count

The RBC counts ($\times 10^{12}/\mu\text{L}$) of groups 2 (280.75 ± 5.94), 3 (249.57 ± 7.39), and 4 (219.16 ± 10.51) given 8, 16 and 24 mg/kg b.w of AL, respectively decreased significantly ($p < 0.05$) in a dose-dependent pattern compared to that of the normal control group (314.08 ± 9.29). However, significant ($p < 0.05$) increases in a dose-dependent pattern was seen in the RBC counts ($\times 10^{12}/\mu\text{L}$) of groups 5 (329.66 ± 9.28), 6 (359.59 ± 4.74), and 7 (387.58 ± 6.39) administered respectively with 200, 400 and 600 mg/kg b.w of FCALE, when compared to the normal control group (Figure 1).

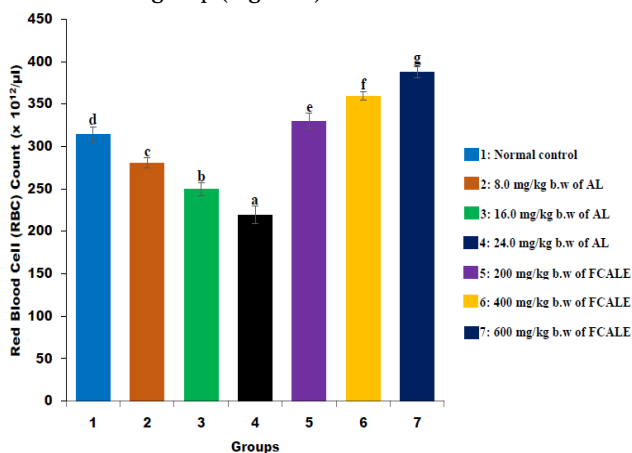


Figure 1: Effect of AL and FCALE on the red blood cell (RBC) count in albino rats. Values are mean $\pm$ SEM; n = 4. Values with different superscript alphabets over the bars are significant at P < 0.05. AL = Artemether and Lumefantrine; FCALE = *Ficus capensis* aqueous leaf extract.

Effects of AL and FCALE on parked cell volume (PCV)

Figure 2 shows the PCV (%) of 51.02 ± 0.91 (group 2), 47.42 ± 1.97 (group 3) and 35.96 ± 3.59 (group 4) in the rats given 8, 16 and 24 mg/kg b.w of AL, respectively, which decreased significantly ($p < 0.05$) in a dose-dependent order when compared to that of the normal control group (52.05 ± 3.16). Conversely, significant ($p < 0.05$) elevations in a dose-dependent pattern was seen in the PVC (%) of groups 5 (55.58 ± 3.58), 6 (56.98 ± 6.56), and 7 (60.39 ± 6.61) administered respectively with 200, 400 and 600 mg/kg b.w of FCALE, when compared to the normal control group.

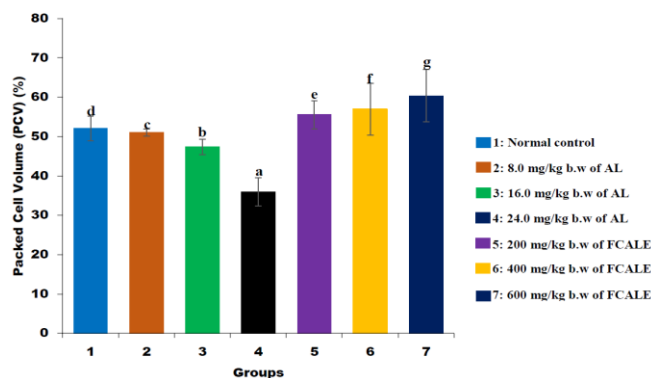


Figure 2: Effect of AL and FCALE on the packed cell volume (PCV) in albino rats. Values are mean $\pm$ SEM; n = 4. Values with different superscript alphabets over the bars are significant at P < 0.05. AL = Artemether and Lumefantrine; FCALE = *Ficus capensis* aqueous leaf extract.

Effects of AL and FCALE on hemoglobin (Hb) level

There was a significant ($p < 0.05$) decrease in the Hb levels (g/dl) of groups 2 (11.32 ± 0.81), 3 (10.21 ± 0.86), and 4 (7.38 ± 0.78) administered respectively with 8, 16 and 24 mg/kg b.w of AL, when compared to the normal control group (11.73 ± 0.67). However, significant ($p < 0.05$) increase was observed in the Hb levels (g/dl) of groups 5 (12.72 ± 0.10), 6 (13.52 ± 0.28), and 7 (14.21 ± 0.61) administered respectively with 200, 400 and 600 mg/kg b.w of FCALE, when compared to the normal control group. These trends were dose-dependent (Figure 3).

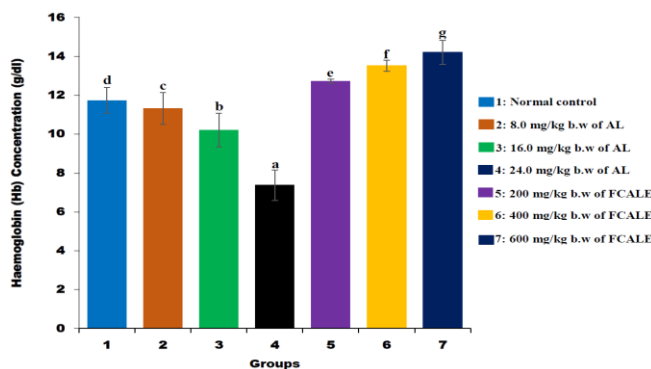


Figure 3: Effects of AL and FCALE on the haemoglobin (Hb) in albino rats. Values are mean $\pm$ SEM; n = 4. Values with different superscript alphabets over the bars are significant at P < 0.05. AL = Artemether and Lumefantrine; FCALE = *Ficus capensis* aqueous leaf extract.

DISCUSSION

Some antimalarial drugs are associated with known common side effects such as headache, loss of appetite, weakness, joint pain, or muscle pain (Obasi *et al.*, 2022). The observed decrease in feed intake following AL administration could be due to the aforementioned common side effects of antimalarial drugs. The mechanism that causes loss of appetite may also be responsible for weight loss. However, Samuel *et al.* (2018) reported that treatment of male rats with both artesunate-amodiaquine (ATS-AMQ) and artemether-lumefantrine (ART-LUM) for 3 and 6 days caused no statistically significant difference in body weight between the treatment groups and the control. The weight gain seen in the FCALE-treated group is consistent with another study that found that diabetic rats treated with herbal mixture experienced a significant increase in weight (Obasi and Ogugua, 2024; 2021; 2020).

Administration of AL caused significant reductions in the PCVs, RBC counts, and Hb levels in the rat groups, in a dose-dependent manner (Figures 1 – 3). Similar effects of chloroquine and coartem on haematological parameters in rats have been reported after 7 days of administration (Ofem *et al.*, 2013). In addition, Osonugal *et al.* (2013) found that while artemether significantly decreased the animals' hematological profiles in a dose-dependent manner, the depressed blood parameter indices gradually recovered when the drug use was stopped. Another study reported significant elevations in the RBC count, Hb, and PCV in chloroquine (CQ)-administered rats after three days, but no significant alterations were observed after seven days of administration; whereas, the RBC count, Hb, and PCV were significantly reduced in artequin (AQ) recipients compared with the control (Ugwu *et al.*, 2021). The current study supports reports from preclinical data as well as the documented pharmacology of artemisinins, indicating that prolonged or repeated exposure to ACT medications may alter blood cell counts and increase the risk of anemia (Obianime and Aprioku, 2011). According to Elsharkawy *et al.* (2013), the decrease in RBC count occurred due to excessive damage to the erythrocytes or inhibition of erythrocyte formation.

The effect of *Ficus capensis* on PCV, RBC count, and Hb level reveals its blood-boosting capacity by significant elevated levels of these hematological parameters. Our result is similar to the result of a previous study, which showed that a poly-herbal drug (Ruzu herbal bitters) caused a significant increase in the hematological indices of diabetic rats (Obasi and Ogugua, 2020). Plant effect that enhances the blood indices, represented by the increase in RBC count, Hb and PCV in the present study, may be related to its phytochemicals, such as flavonoids, which have an antioxidant and health promoting activities (Agbafor *et al.*, 2017, Ezeigwe *et al.*, 2020, Okoro *et al.*, 2021). Additionally, our results are in agreement with those of Hossen *et al.* (2017), who found that co-administration of turmeric and *Ficus capensis* in rats increases the RBC count and HB level, indicating the immune-stimulatory activity of the extract, rendering turmeric a potential alternative armamentarium

against anaemia. The blood-boosting activity observed in this study also aligns with a previous study that ethanolic leaf extract of *Ficus capensis* showed a protective effect against phenylhydrazine-induced anaemia in Wistar rats by improving the levels of haematological indices such as PCV, RBC count, and Hb (Ibe *et al.*, 2022).

CONCLUSION

In this study, administration of AL causes reduction in hemoglobin level, red blood cell count, and packed cell volume, and may not be suitable for patients with hematological disorders, such as anemia or hemorrhage. Moreover, *Ficus capensis* induces elevation in the level of hemoglobin, red blood cell count, and packed cell volume and could be suitable for managing anemia or other blood-related diseases.

AUTHORS' CONTRIBUTIONS

DCO: collection of samples, analyses, interpretation of data, statistical analysis and writing of the manuscript: KNA: conceptualization, design of the work, interpretation of data and proof-reading of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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