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Sida acuta Burm.f. leaves ethanol extract ameliorates haematological and biochemical alterations induced by *Plasmodium berghei* ANKA-65 in mice

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Abstract

Background: Malaria has continued to be a threat to man and his wellbeing, especially Africans and Asians. New antimalarial drugs are urgently needed to mitigate malaria treatment failure due to resistant *Plasmodium* species. Medicinal plants used by indigenous Nigerians for treating fever and malaria such as *Sida acuta* Burm.f. (Malvaceae) could be a promising source of lead compounds for developing new generations of antimalarial drugs. The effects of ethanol extract of *S. acuta* leaves (EESAL) on malaria parasitemia, haematological and biochemical status of *P. berghei*-infected mice were investigated, using the 4-day curative test.

Methodology: EESAL was prepared by maceration method. The phyto-constituents and acute toxicity profile of the extract were evaluated using standard protocols. In addition, malaria parasitemia and chemo-suppression, and indicators of haematological and biochemical status of *P. berghei*-infected mice treated with EESAL were assessed.

Results: At 200, 400 and 600 mg/kg/d b.w., p.o doses for 4 consecutive days, EESAL significantly ($p < 0.05$) decreased parasitaemia and suppressed malaria parasite by 89.64%, 95.95% and 97.38%, respectively comparable to negative control. The reduction in percentage malaria parasitemia by EESAL is comparable to Artemether (140 mg/kg/d b.w., p.o) used as standard antimalarial drug in this study. The packed cell volume (PCV), haemoglobin (Hb) concentration, and red blood cell (RBC) and white blood cell (WBC) counts of negative control are significantly ($p < 0.05$) higher than normal control. However, parasitized-EESAL-treated mice have significantly ($p < 0.05$) higher PCV value, Hb concentration and RBC and WBC counts than negative control. Similarly, treatment of parasitized mice with EESAL restored some indicators of the antioxidant, lipid peroxidation, lipid profile and liver status altered by malaria. In addition, EESAL was tolerable up to 5000 mg/kg b.w., p.o.

Conclusion: These results indicate that the EESAL possesses antimalarial activity and normalizes alterations in haematological and biochemical status of malaria-infected mice.

Keywords: *Sida acuta* Burm.f., *Plasmodium berghei*, Malaria, Haematological indices, Biochemical parameters

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Introduction

Malaria is a parasitic disease caused by *Plasmodium species* that causes high annual numbers of hospital visits and deaths globally with higher impact in Sub-Saharan Africa and South-East Asia [1]. Out of the 228 million cases of malaria globally in 2018, 213 million are from the WHO African Region, and Nigeria contributed 25% of this number [2]. This high burden of the disease demands concerted interventions to reduce the impact on man to barest minimum. Current efforts to eliminate malaria have been hampered by antimalarial resistance [3, 4], and worrisome adverse effects of current antimalarials [5]. This situation necessitates the hunt for new, cheap and effective antimalarial drugs especially from natural products such as plants to avoid getting to an era when malaria will become incurable.

Alterations in the haematological profile such as lymphopenia, anaemia, thrombocytopenia, eosinopenia, neutropenia, monocytosis and leucocytosis are prominent indicators of severity of malaria infection [6]. Recently, oxidative stress has been identified as one of the complications in malaria [7]. Furthermore, the existence of dyslipidemia in malaria is well recognized. Mohanty et al. [8] reported significant elevation in total cholesterol (CHOL) and low-density lipoproteins (LDL) while high density lipoproteins (HDL) and triacylglycerols (TAGs) decreased significantly. It was also reported that the extent of dyslipidemia is dependent on the severity of the disease [9, 10]. Similar studies in both vivax and falciparum malaria patients reported that concentrations of HDL, LDL, and CHOL decreased significantly but TAGs increased in malaria-infected patients than in healthy control [11–15]. These reports generally support the opinion that malaria induces dyslipidaemia and hence, a good antimalarial agent should, in addition to clearing malaria parasite, restore homeostasis in lipid status.

Medicinal plants employed in treating fever and malaria by traditional healers is suggested to be potential sources of a new generation of antimalarials. *Sida acuta* Burm.f. (Malvaceae), also known as Wirewood and *Udo* in English and Igbo languages, respectively, is among the common plants traditionally used for treating malaria in Nigeria and other parts of Africa [16–19]. Extracts from the plant have been shown to have larvicidal effects on *Anopheles stephensi* [20], anti-plasmodial [21], analgesic [22], antibacterial [23], antioxidant [24], anxiolytic and neuromodulatory [25] activities. The present research was designed to reveal some of the phytochemical constituents and investigate the acute toxicity profile and in vivo malaria-curative activity of the ethanol extract of *S. acuta* leaves (EESAL). The effect of ESAL on some altered haematological and biochemical (lipid profile, antioxidant and lipid peroxidation and liver status) parameters of *P. berghei*-infected mice was also investigated.

Materials and methods

Chemicals and instruments

Instruments used were weighing balance (Vickas Ltd., England), centrifuge (Vickas Ltd., England), microscope (SMDM-1030, Ted Pella Inc. USA) and spectrophotometer (E312 Model) (Jenway, UK) while reagents for the biochemical experiments were products of Randox (USA). Solvents used (ethanol, ethyl acetate, chloroform and n-hexane) were products of JHD (India) while other chemicals were products of Sigma-Aldrich (USA). All chemicals used were of analytical grade, received in good condition and were freshly prepared before use.

Study animals

Fifty-four adult Swiss albino mice of average body weight of 30 ± 5 g were used for this study; 18 and 36 mice for acute toxicity and antimalarial studies, respectively. The mice were maintained on commercial feed (Chukun Feeds Nig. Ltd.) and water ad libitum. The study was approved by the Faculty of Biological Sciences Committee on Research and Bioethics (UNN/FBS/EC/1022). The study animals were ethically handled throughout the experimental period in accordance with the institutional, national and international ethical recommendations for care and use of laboratory animals.

Standard drug

Artemether (a combination of artemether and lumenfantrin, 80:480 w/w, Ajanta Pharma Ltd., India) was the standard antimalarial of choice in this study.

Extraction of plant materials

In June of 2019, fresh leaves of *S. acuta* Burm.f. (Malvaceae) were collected from its natural habitat in Opanda-Nimbo, Enugu State, Nigeria and were authenticated by Mr. Alfred Ozioko, a taxonomist of the Bioresources Development and Conservation Programme (BDPC) Research Centre, Nsukka, Enugu State (voucher number - Intercedd/160). This was compared with features available in the plant database (<http://www.theplantlist.org/>) for confirmation. Two weeks air-dried leaves of *S. acuta* plant were powdered and the powder was extracted thrice with ethanol by cold maceration method. Filtrates from the extraction chambers were dried (rotary evaporator at 45 °C), obtaining crude ethanol leaf extract of *S. acuta* (EESAL) (13.32%). Ethanol was used as a solvent in this study based on the traditional practice where the leaves are soaked in ethanol for 2–3 days and the decoction orally taken for 4–7 days to treat malaria [16, 26, 27]. In addition, results of pilot study conducted using different solvent extracts showed that ethanol extract has significantly higher activity compared to n-hexane, ethyl acetate, water and chloroform extracts (data not shown).

Phytochemical analysis of EESAL

The quantitative secondary metabolites in EESAL were determined using standard procedures described by Trease and Evans [28] and Harborne [29] as per [30]. The procedures are briefly presented below.

Detection and quantification of tannins in EESAL

Using ferric chloride assay, the presence of tannins in the extract was determined using the method of the methods of Trease and Evans (1989). Extract (0.1 g) was stirred with 10 ml of distilled water and then filtered. The appearance of blue-black precipitate when three drops of 1% ferric chloride solution were added to 2 ml of the filtrate indicated the presence of tannins. Thereafter, the amount of total tannins in the sample was quantified using the method of Harborne (1973). To 5 ml of the filtrate above in a test tube, 0.1 N ferric chloride in 0.1 N HCl and 0.008 M potassium ferricyanide were added and mixed. The absorbance of the mixture was read at 720 nm. The tannin content of the extract was extrapolated from a tannic acid standard curve.

Detection and quantification of alkaloids in EESAL

The alkaloids in EESAL were detected by Dragendorff's reagent test following the methods of Trease and Evans (1989). Briefly, 0.1 g of EESAL was boiled with 5 ml of 1% aqueous HCl on a water bath for 10 min and filtered. The appearance of orange-red precipitate on addition of 2 drops of Dragendorff's reagent to 1 ml of the filtrate indicated the presence of alkaloids. Following the method of Harborne (1973), the quantity of total alkaloids in EESAL was determined. EESAL (0.1 g) was macerated in 20 ml of 20% H₂SO₄ in ethanol (1:1) for 30 min and was filtered. To 1 ml of the filtrate, 5 ml of 60% H₂SO₄ was added and after 5 min, 5 ml of 0.5% formaldehyde in 60% H₂SO₄ was also added, mixed and allowed to stand for 3 h. The absorbance of the mixture was read at a wavelength of 550 nm. The alkaloid content was extrapolated from an atropine standard curve.

Detection and quantification of glycosides in EESAL

The presence of glycosides in EESAL was detected using Fehling's test as described by Trease and Evans (1989). Briefly, 0.1 g of EESAL was mixed with 30 ml of distilled water and heated on a water bath for 5 min, cooled and was filtered. To 5 ml of the filtrate, a mixture of 0.2 ml of each of Fehling's solutions A and B was added drop by drop till pH changes to alkaline (pH ~ 8). The appearance of a brick-red precipitate on heating in water bath for 2 min indicated the presence of glycosides. To 0.1 g EESAL, 20 ml of distilled water and 2.5 ml of 15% lead acetate were added, mixed and allowed to stand for 30 min and was filtered. To 2 ml of the filtrate, 2.5 ml of chloroform was added and shaken vigorously and

allowed to stand for 5 min. The lower layer was collected into a beaker and was evaporated to dryness in a water bath. Thereafter, 3 ml of glacial acetic acid, 0.1 ml of 5% ferric chloride and 0.25 ml of concentrated H₂SO₄ were added to the beaker, mixed very well and allowed to stand in a dark chamber for 2 h. Thereafter, absorbance of the resultant mixture was measured at 530 nm against the reagent blank (not containing EESAL).

$$\begin{aligned} \text{Glycoside content (mg/100g)} \\ = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the sample} - \text{Absorbance of the blank}} \times 100 \end{aligned}$$

Detection and quantification of terpenoids in EESAL

The existence of terpenoids in EESAL was detected by acetic anhydride test following the procedure of Trease and Evans (1989). EESAL (0.1 g) was dissolved in 5 ml of ethanol and 1 ml of acetic anhydride and drops of concentrated H₂SO₄ were added. A change in colour from pink to violet indicated the presence of terpenoids. To determine the quantity of terpenoids in the sample, a quantity (0.1 g) of EESAL was macerated in 20 ml of ethanol for 10 min and was filtered. To 1 ml of the filtrate, 1 ml of 5% phosphomolybdic acid solution was added and shaken, followed by gradual addition of 1 ml of concentrated H₂SO₄. The mixture was left to stand for 30 min and thereafter, 2 ml of ethanol was added and absorbance was measured at 700 nm against the reagent blank. The terpenoid content was estimated as follows;

$$\begin{aligned} \text{Terpenoid content (mg/100g)} \\ = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the sample} - \text{Absorbance of the blank}} \times 100 \end{aligned}$$

Detection and quantification of flavonoids in EESAL

Using the lead acetate test, the presence of flavonoids in EESAL was detected as per Trease and Evans (1989). To 5 ml of the filtrate from the tannins test, 3 ml of lead acetate solution was added. The formation of yellowish precipitate indicated the presence of flavonoids. To quantify the amount of total flavonoids in EESAL, 0.1 g of the sample was macerated in 20 ml of ethyl acetate, and the suspension was filtered. To 5 ml of the filtrate, 5 ml of dilute ammonia was added, mixed and allowed to separate into two layers. The upper layer of the mixture, containing the flavonoids was collected and the absorbance was read at 510 nm using ethyl acetate as the blank. The concentration of flavonoids in the extract was extrapolated from a catechin standard curve.

Detection and quantification of steroids in EESAL

To 0.1 g of EESAL, 2 ml of acetic acid was added and mixed thoroughly. The solution was cooled in ice followed by the addition of concentrated tetraoxosulphate (VI) acid

(H₂SO₄). The formation of a violet ring which turns bluish on addition of acid indicated the presence of steroids. To quantify the content of steroids in EESAL, 0.1 g of the sample was macerated in 20 ml of ethanol and filtered. To 2 ml of the filtrate, 2 ml of chromogen solution (4 ml ferric chloride stock made up to 24 ml with concentrated H₂SO₄) and 1 ml of 0.5% potassium hexacyanoferrate (III) solution were added, mixed and the mixture was left to stand for 30 min in a water bath set at 70 °C. Thereafter, the mixture was cooled and made up to 10 ml with distilled and the absorbance was measured at 780 nm against the reagent blank (prepared by adding all the reagents except in the sample test tube except the extract).

$$\begin{aligned} \text{Steroid content (mg/100g)} \\ = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the sample} - \text{Absorbance of the blank}} \times 100 \end{aligned}$$

Detection and quantification of phenols in EESAL

The presence of phenols in EESAL was detected by ferric chloride test following the method of Trease and Evans (1989). To 2 ml of the filtrate from tannin test, few drops of 10% ferric chloride solution were then added. The appearance of a greenish-blue or violet colour indicated the presence of a phenolic hydroxyl group. To determine the quantity of phenolic compounds in EESAL, the Folin-ciocalteu's reagent test method of Harborne (1973) was adopted. To quantify the amount of phenols in EESAL, 0.1 g was macerated in 20 ml of 80% ethanol and filtered. To 5 ml of the filtrate, 0.5 ml of Folin-ciocalteu's reagent was added and allowed to stand for 3 min. This was followed by the addition of 2 ml of 20% sodium carbonate (IV) and the mixture was left to stand for 10 min. The absorbance of the resultant mixture was measured at 765 nm against the reagent blank.

$$\begin{aligned} \text{Phenol content (mg/100g)} \\ = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the sample} - \text{Absorbance of the blank}} \times 100 \end{aligned}$$

Acute toxicity test

The acute toxicity profile of the plant extract was determined in two phases using a method described by Lorke [31]. A total of 18 mice were used for this test. In phase I, nine mice were divided into three groups of three mice each (groups 1–3 received 10, 100 and 1000 mg/kg b.w of EESAL, respectively). The mice were observed for 24 h for signs of toxicity and lethality. Thereafter, the remaining nine mice were randomized into three groups of three mice each (groups 4–6 received 1600, 2900 and 5000 mg/kg b.w of EESAL, respectively). These mice were again observed for 24 h for signs of toxicity and lethality. Body weight of the mice 24 h post-EESAL administration were measured and compared with the initial body weight.

Parasite inoculation

Plasmodium berghei-infected blood from a donor mouse was used for inoculum preparation. The blood was diluted serially in Alsever's solution to make a suspension containing about 1×10^7 infected RBCs in every 0.2 mL suspension. The mice in test groups (groups 2–6) were intraperitoneally inoculated with 0.2 mL of this suspension to initiate infection. Malaria parasite infection was confirmed 72 h post-infection.

Experimental design for malaria curative study

Thirty-six adult Swiss mice used for this study were divided into six groups of six mice each as follows:

Group 1 = Normal control (administered with the vehicle, normal saline)

Group 2 = Negative control (inoculated with malaria parasite and untreated)

Group 3 = Standard drug control (*P. berghei*-parasitized + 140 mg/kg b.w. of Artemether).

Group 4 = *P. berghei*-parasitized + 200 mg/kg b.w. of EESAL.

Group 5 = *P. berghei*-parasitized + 400 mg/kg b.w. of EESAL.

Group 6 = *P. berghei*-parasitized + 600 mg/kg b.w. of EESAL.

Treatment lasted for 4 days (from day 0-day 3) and on day 4, overnight fast blood sample was collected from each of the mouse for parasitaemia count, haematological and biochemical (lipid peroxidation, antioxidant and liver status, and lipid profile) analyses. The three doses were selected based on activity-guided dose selection from pilot study.

Determination of malaria parasitemia in parasitized mice

Malaria parasitaemia was determined by methods described earlier [32, 33] and the percentage malarial parasitemia and chemosuppression were calculated using the relations below:

$$\begin{aligned} \% \text{Malaria Parasitemia (MP)} \\ = \frac{\text{Number of parasitized RBCs}}{\text{Total number of RBCs counted}} \times 100 \end{aligned}$$

$$\begin{aligned} \% \text{Malaria Chemosuppression} \\ = \frac{\text{Day0 MP} - \text{Day3 MP}}{\text{Day0 MP}} \times 100 \end{aligned}$$

Determination of haematological and some biochemical status of experimental mice

Haematological indices were determined by previous methods [34, 35]. Aspartate and alanine aminotransferases (AST and ALT) and alkaline phosphatase (ALP)

activities were assayed using previously described methods [36, 37] as contained in the Randox commercial kits. Serum total bilirubin [38], CHOL [39], and HDL and TAG concentrations were determined using colorimetric methods [40] as contained in Randox commercial kits. Serum LDL level was determined as the difference between CHOL and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene glycol monomethyl ether. The extent of lipid peroxidation (level of malondialdehyde (MDA)) was measured as per known protocol [41]. Superoxide dismutase (SOD) and catalase (CAT) activities were assayed using methods earlier described [42, 43], respectively while the reduced glutathione (GSH) concentration was determined according to method described by Kings and Wootton [44].

Statistical analysis

Primary data from the laboratory were analyzed using one way analysis of variance in Statistical Product and Service Solution version 20.0 (IBM Inc., USA) and Dunnett's post hoc test was used to compare means across the groups. The results were presented as mean \pm standard deviation (SD) in tables and mean values with $p < 0.05$ were considered significant.

Results

Phytochemical composition of EESAL

As shown in Table 1, the phytochemical compositions of EESAL include tannins (573.28 ± 30.74 mg/100 g), terpenoids (161.75 ± 3.12 mg/100 g), steroids (0.41 ± 0.08 mg/100 g), phenols (3115.16 ± 143.23 mg/100 g), alkaloids (4.62 ± 1.04 mg/100 g), flavonoids (2051.44 ± 84.32 mg/100 g) and glycosides (108.54 ± 12.71 mg/100 g).

Acute toxicity profile of the extract

The result of acute toxicity test (Table 2) shows that EESAL was not lethal even at the highest dosage (5000 mg/kg b.w., *p.o.*) administered. Similarly, there was no

significant body weight and behavioural changes within the 24 h observation period post-extract administration. This shows that the extract is tolerable and hence, the median lethal dose (LD_{50}) of the extract was therefore assumed to be higher than 5000 mg/kg body weight.

Effect of EESAL on malaria parasitemia of *Plasmodium berghei*-infected mice

After 72 h post inoculation (before the administration of the extract), there was no significant ($p > 0.05$) difference among the percentage parasitemia of the test mice. However, after the treatment of parasitized mice with artemether (group 3) and graded doses of EESAL (groups 4–6), the percentage malaria parasitemia were significantly ($p < 0.05$) lower than negative control (Table 3). Similarly, EESAL and standard drug suppressed malaria parasite count by comparable percentage, suggesting that EESAL has good antimalarial activity.

Effect of EESAL on some haematological indices of *Plasmodium berghei*-infected mice

The pack cell volume (PCV), red blood cell (RBC) and white blood cells (WBC) counts, and haemoglobin (Hb) concentration of negative control mice were lower than normal control. However, administration of graded doses of EESAL to parasitized mice caused significantly ($p < 0.05$) higher PCV, RBC count and Hb concentration when compared with negative control (Table 4).

Effect of EESAL on liver status in *Plasmodium berghei*-infected mice

The ALT, ALP and AST activities and total bilirubin concentration of *P. berghei*-infected and untreated mice (negative control) were significantly ($p < 0.05$) higher than normal control. After 4 consecutive days oral treatment with graded doses of EESAL and artemether, *P. berghei*-infected mice in groups 3–6 normalized the liver status (by returning the values of the liver function markers of parasitized-EESAL-treated mice to levels of mice in normal control) (Table 5).

Effect of EESAL on lipid peroxidation and antioxidant parameters of *Plasmodium berghei*-infected mice

It was observed that negative control had significantly ($p < 0.05$) higher MDA concentration than normal control. Conversely, negative control had significantly ($p < 0.05$) lower SOD and CAT activities, and GSH concentration than normal control. When malaria-parasitized mice were administered EESAL and artemether, there was a significant ($p < 0.05$) reduction in MDA concentration and elevation in SOD and CAT activities and GSH concentration than negative control. Furthermore, compared to normal control, the lipid peroxidation and

Table 1 Phytochemical composition of the extract

Phytochemical constituents	Concentration (mg/100 g)
Tannins	573.28 ± 30.74
Terpenoids	161.75 ± 3.12
Steroids	0.41 ± 0.08
Phenols	3115.16 ± 143.23
Alkaloids	4.62 ± 1.04
Flavonoids	2051.44 ± 84.32
Glycosides	108.54 ± 12.71
Saponins	ND

Results are expressed in Mean \pm SD ($n = 3$)

ND not detected

Table 2 Acute toxicity profile of the extract

Groups (Treatment)	Mortality	Behavioral and body weight changes
Phase I		
Group 1 (10 mg/kg b.w. EESAL)	0/3	Not significant
Group 2 (100 mg/kg b.w. EESAL)	0/3	Not significant
Group 3 (100 mg/kg b.w. EESAL)	0/3	Not significant
Phase II		
Group 4 (1600 mg/kg b.w. EESAL)	0/3	Not significant
Group 5 (2900 mg/kg b.w. EESAL)	0/3	Not significant
Group 6 (5000 mg/kg b.w. EESAL)	0/3	Not significant

antioxidant parameters of parasitized-EESAL-treated mice were not significantly different, suggesting that EESAL has reversed the alterations in lipid peroxidation and antioxidant status (Table 6).

Effect of the EESAL on lipid profile of *Plasmodium berghei*-infected mice

Malaria induced significantly ($p < 0.05$) higher serum concentrations of CHOL, LDL, cholesterol and TAGs, but significantly ($p < 0.05$) lower serum HDL concentration in negative control compared to normal control. When administered graded doses of EESAL and artemether for 4 days, parasitized-EESAL-fed mice had significantly ($p < 0.05$) reduced CHOL, LDL and TAGs but significantly ($p < 0.05$) increased HDL concentration compared to negative control (Table 7).

Discussion

The use of plants as a source of nutrients and drugs is as old as man. Plant products have provided many therapeutic agents, and many conventional drugs have their roots traced from natural sources [45]. The isolation of these natural products is usually based on their uses in traditional practice. *S. acuta* Burm.f. is used by rural Nigerian dwellers for treating malaria, fever among others [46]. This study examined the ability of crude ethanol leaf extract of *S. acuta* (EESAL) to reduce malaria parasite load and modulate alterations in haematological and

biochemical status markers of *P. berghei*-infected mice. The presence of phytochemicals such as alkaloids, terpenoids, glycosides, flavonoids, phenols, steroids and saponins in EESAL suggest that the extract might be bioactive and elicit some pharmacological effects like antioxidant and antimalarial activities [47]. In addition, the lack of any morphological, behavioural and body weight change, and mortality in mice orally administered EESAL up to 5000 mg/kg b.w after 24 h showed that the extract is tolerable and safe for consumption. This suggests that the LD₅₀ of EESAL is above 5000 mg/kg b.w., *p.o.* This result differs from a report by Konaté et al. [22] that aqueous-acetone extract of *S. acuta* has LD₅₀ of 3.2 g/kg b.w. The variation between our result and that of Konaté et al. [22] could be attributed to differences in the solvent of extraction and location of source of the plant. It is documented that substances whose LD₅₀ are above 5000 mg/kg b.w. *p.o.* are considered safe and non-toxic [48].

The antimalarial activity of EESAL, characterized by significant ($p < 0.05$) dose-dependent decrease in percentage malaria parasitemia and increase in parasite chemosuppression in parasitized-EESAL-treated mice when compared with negative control could be due to the phytoconstituents of the extract. Extracts of some plants (such as *Fagara zanthoxyloides* and *Erythrina senegalensis* leaves and *Peltophorum pterocarpum* stem bark) that are rich in phytochemicals also present in EESAL have been shown to have antimalarial effects, although with

Table 3 Effect of EESAL on malaria parasitemia of *Plasmodium berghei*-infected mice

Groups	Day 0 Malaria parasitaemia (%)	Day 4 Malaria parasitaemia (%)	Chemosuppression (%)
Normal control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	–
Negative control	45.75 ± 8.18 ^b	81.75 ± 2.06 ^f	–
MP + 140 mg/kg b.w. of Artemether	42.50 ± 6.45 ^b	10.50 ± 4.12 ^e	75.29 ± 3.61 ^a
MP + 200 mg/kg b.w. EESAL	48.25 ± 6.34 ^b	5.00 ± 0.82 ^d	89.64 ± 5.74 ^b
MP + 400 mg/kg b.w. EESAL	49.50 ± 4.20 ^b	2.00 ± 0.22 ^c	95.95 ± 3.71 ^{bc}
MP + 600 mg/kg b.w. EESAL	47.75 ± 3.86 ^b	1.25 ± 0.50 ^b	97.38 ± 1.87 ^c

Results are expressed in Means ± SD (n = 6); At $p < 0.05$, mean values with different letters of the alphabets (a, b, c, d ...) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different

Table 4 Effect of the EESAL on some haematological indices in *Plasmodium berghei*-infected mice

Groups	Haematological indices			
	PCV (%)	RBC ($\times 10^6$)	WBC ($\times 10^3 \text{ mm}^3$)	Hb (g/dl)
Normal control	40.75 \pm 1.26 ^{bc}	10.43 \pm 0.32 ^b	10,525.00 \pm 25.00 ^{ab}	10.86 \pm 0.35 ^c
Negative control	33.75 \pm 3.50 ^a	8.16 \pm 0.65 ^a	10,025.00 \pm 573.73 ^a	9.53 \pm 0.21 ^a
MP + 140 mg/kg b.w. of Artemether	36.75 \pm 3.30 ^{ab}	9.82 \pm 0.69 ^b	11,025.00 \pm 741.08 ^b	10.06 \pm 0.29 ^b
MP + 200 mg/kg b.w. EESAL	41.75 \pm 2.36 ^c	10.64 \pm 0.70 ^b	10,275.00 \pm 95.74 ^{ab}	11.13 \pm 0.34 ^c
MP + 400 mg/kg b.w. EESAL	43.70 \pm 3.50 ^c	10.25 \pm 0.06 ^b	10,325.00 \pm 95.74 ^{ab}	11.33 \pm 0.46 ^{cd}
MP + 600 mg/kg b.w. EESAL	43.50 \pm 1.29 ^c	10.61 \pm 0.34 ^b	14,025.00 \pm 602.07 ^c	11.70 \pm 0.20 ^d

Results are expressed in Means \pm SD ($n = 6$); At $p < 0.05$, mean values with different letters of the alphabets (a, b, c, d ...) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different
RBC red blood cells, PCV packed cell volume, WBC white blood cells and Hb hemoglobin concentration

lower chemosuppression than *S. acuta* [49–51]. For example, plant-derived flavonoids, alkaloids and terpenoids have been shown to exhibit potent antimalarial activities [52–54].

The observed significant ($p < 0.05$) reduction in total haematological status (PCV value, Hb concentration, and RBC and WBC counts) in this study agrees with the results of previous studies both in human [55–57] and rodent [58] malaria. This suppression in RBC count, PCV and Hb concentration could be linked to the invasion of erythrocytes by malaria parasites, mechanical devastation of parasitized red cells and the use of Hb as nutrient source during erythrocytic stage of the parasite life cycle [59]. These haematological aberrations may have resulted from the presence of malaria parasites in the host; malaria parasites release adhesion molecules on the surface of parasitized erythrocytes leading to vascular adhesion. It also leads to the adhesion of both parasitized and non-parasitized erythrocytes, increasing RBCs destruction by splenic clearance [60]. In addition, malaria induces dyserythropoiesis, mitigating restoration of erythropoietic balance [61]. Therefore, the elevation in haematological indices of parasitized-EESAL-treated mice showed that the administration of EESAL ameliorated alterations in haematological status induced by malaria. This ameliorative effect suggests that one or

more of the phytoconstituents of the extract is haematopoietic. It could also mean that the clearance of malaria parasite boosted the drive to maintain haematological homeostasis in the host.

Findings of this study showed that mice in the negative control group had significant ($p < 0.05$) elevation in serum activities of AST, ALP and ALT, and concentration of total bilirubin when compared with normal control. This suggests that malaria infection induces hepatotoxicity that may have resulted from the disruption of hepatocyte membrane by inflammatory events associated with byproducts of parasite metabolism. This disruption in the hepatocyte membrane leads to the leakage of intracellular cytosolic enzymes of the hepatocytes into circulation, increasing their activities in the serum. The above result is in line with an earlier report [62]. Similarly, the significantly ($p < 0.05$) higher level of serum total bilirubin in negative control when compared with normal control could be a product of elevated destruction of haemoglobin by the malaria parasite [63]. Bilirubin is a breakdown product of haemoglobin that is usually metabolized in the liver and its accumulation in serum suggests hepatic dysfunction; hence, the estimation of bilirubin level in serum is a useful marker of liver status [64]. The ability of the extract to normalize hepatic status in parasitized mice when compared to control

Table 5 Effect of the EESAL on the liver function parameters in *Plasmodium berghei*-infected mice

Groups	Liver function markers			
	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	T. bil (mg/dl)
Normal control	20.25 \pm 2.63 ^a	64.50 \pm 3.42 ^a	37.00 \pm 2.58 ^a	0.45 \pm 0.12 ^b
Negative control	36.50 \pm 3.42 ^c	95.25 \pm 6.70 ^b	73.25 \pm 10.63 ^b	0.65 \pm 0.08 ^c
MP + 140 mg/kg b.w. of Artemether	25.50 \pm 1.91 ^b	62.25 \pm 4.35 ^a	41.25 \pm 0.96 ^a	0.52 \pm 0.06 ^b
MP + 200 mg/kg b.w. EESAL	20.50 \pm 1.73 ^a	67.25 \pm 4.35 ^a	36.75 \pm 2.99 ^a	0.41 \pm 0.08 ^{ab}
MP + 400 mg/kg b.w. EESAL	26.25 \pm 2.63 ^b	64.75 \pm 3.50 ^a	34.50 \pm 3.11 ^a	0.44 \pm 0.02 ^{ab}
MP + 600 mg/kg b.w. EESAL	20.00 \pm 3.74 ^a	62.25 \pm 2.63 ^a	35.25 \pm 2.50 ^a	0.37 \pm 0.06 ^a

Results are expressed in Means \pm SD ($n = 6$); At $p < 0.05$, mean values with different letters of the alphabets (a, b, c, d ...) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different
AST aspartate aminotransferase, ALT alanine aminotransferase, ALP alkaline phosphatase, T. bil total bilirubin

Table 6 Effect of the EESAL on lipid peroxidation and antioxidant parameters of *Plasmodium berghei*-infected mice

Groups	Lipid peroxidation and antioxidant parameters			
	MDA Conc. (mg/dl)	SOD activity (IU/L)	CAT activity (IU/L)	GSH Conc. (mg/dl)
Normal control	2.68 ± 0.99 ^b	9.63 ± 0.42 ^b	0.65 ± 0.02 ^b	0.21 ± 0.05 ^a
Malaria control	6.05 ± 0.68 ^c	4.18 ± 0.38 ^a	0.16 ± 0.05 ^c	0.10 ± 0.01 ^b
MP + 140 mg/kg b.w. of Artemether	2.25 ± 0.70 ^{ab}	9.52 ± 0.74 ^b	0.66 ± 0.06 ^b	0.26 ± 0.07 ^a
MP + 200 mg/kg b.w. EESAL	2.31 ± 0.39 ^{ab}	9.94 ± 0.58 ^b	0.68 ± 0.18 ^b	0.22 ± 0.14 ^a
MP + 400 mg/kg b.w. EESAL	2.28 ± 0.30 ^{ab}	9.95 ± 0.39 ^b	0.72 ± 0.10 ^b	0.24 ± 0.05 ^a
MP + 600 mg/kg b.w. EESAL	1.54 ± 0.59 ^a	10.19 ± 0.19 ^b	0.68 ± 0.06 ^b	0.25 ± 0.03 ^a

Results are expressed in Means ± SD (n = 6); At $p < 0.05$, mean values with different letters of the alphabets (a, b, c, d ...) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different
MDA malondialdehyde, SOD Superoxide dismutase, CAT catalase, GSH reduced glutathione

suggests that EESAL has normalization effect on hepatocyte integrity of parasitized mice. Hepatic damage from malaria has been linked with oxidative stress, suggesting that the modulation of hepatocytes in this study by the extract could be linked with its antioxidant phytoconstituents.

The involvement of lipids in the development and complications associated with malaria is a matter of recent scientific discussion. Alterations in lipid metabolism in malaria infection, characterized by variation in lipid profile of malaria infected persons when compared with uninfected, have been well documented [8, 11–13]. The reduction in HDL concentration and elevation in CHOL, LDL and TAGs concentration in Negative control when compared with normal control agrees with the reports of earlier studies [50, 58]. In mice infected with *P. chabaudi*, Kluck et al. [65] reported higher CHOL and lower TAGs levels when compared to control. It was further shown that this dyslipidaemia in malaria is exhibited by the inhibition of the AMPK-ACC pathway via reduced 5'AMP-activated protein kinase (AMPK) phosphorylation. The significantly lower HDL level in parasite control mice when compared with normal control might be attributed to a reduction in cholesterol transport, cholesterol esterification by lecithin cholesterol acyl

transferase, and inhibition of lipid metabolizing liver enzyme by a parasite factor such as using lipids in the synthesis of haemozoin [66]. In addition, parasite activities might have inhibited HDL-synthesizing enzymes. On the other hand, the elevation in TAGs level might be attributed to an elevation in VLDL synthesis in the liver, due to liver damage as observed in this study. In addition, the hypertriglyceridemia could be attributed to elevated secretion of TAGs in serum and a decline in its clearance by cholesterol ester transfer proteins. As suggested by Khovidhunkit et al. [67], it can be deduced from the above observations that alterations in lipid metabolism in malaria are essentially due to disease-associated mechanisms. In general, the effects of malaria infection in serum lipid status are dependent on malaria parasite species, severity of infection, history of parasite infection, and other host genetic factors. The reduction in levels of CHOL, LDL and TAGs and elevation in HDL levels of parasitized-EESAL-treated mice towards normal control mice showed that the phytoconstituents of the extract might have normalized the lipid status of the parasitized mice.

The increase in the extent of lipid peroxidation which was estimated by the concentration of MDA in negative control when compared with control in this study agrees

Table 7 Effect of the EESAL on lipid profile of *Plasmodium berghei*-passaged mice

Groups	Concentration of lipid profile panels			
	LDL (mg/dl)	HDL (mg/dl)	CHOL (mg/dl)	TAG (mg/dl)
Normal control	13.75 ± 3.86 ^{ab}	63.75 ± 2.63 ^b	77.25 ± 2.50 ^a	94.50 ± 5.26 ^b
Malaria control	72.25 ± 3.10 ^d	29.25 ± 2.50 ^a	99.25 ± 7.46 ^b	110.75 ± 2.75 ^c
MP + 140 mg/kg b.w. of Artemether	20.25 ± 2.63 ^c	68.00 ± 4.32 ^{bc}	95.75 ± 4.79 ^b	82.00 ± 6.32 ^a
MP + 200 mg/kg b.w. EESAL	16.50 ± 1.00 ^{abc}	73.50 ± 8.19 ^c	94.25 ± 4.03 ^b	77.75 ± 8.34 ^a
MP + 400 mg/kg b.w. EESAL	18.00 ± 2.16 ^{bc}	70.75 ± 5.10 ^c	92.00 ± 3.56 ^b	81.00 ± 4.08 ^a
MP + 600 mg/kg b.w. EESAL	13.00 ± 2.94 ^a	73.75 ± 5.44 ^c	94.25 ± 5.12 ^b	82.25 ± 9.12 ^a

Results are expressed in Means ± SD (n = 6); At $p < 0.05$, mean values with different letters of the alphabets (a, b, c, d ...) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different
CHOL total cholesterol, HDL high density lipoprotein, TAGs triacylglycerols, LDL low density lipoprotein

with an earlier study in human malaria patients. In agreement with our findings, a reduction in antioxidant status (activities of SOD and level of vitamin C) when compared to healthy control was reported in *P. falciparum* infected Yemen patients [68]. Similarly, Fabbri et al. [69] showed that *P. vivax* malaria patients have elevated lipid peroxidation status (MDA level) and reduced antioxidant status than healthy control. Similarly, the lower SOD and CAT activities in Negative control mice than normal control is consistent with the findings of D'Souza et al. [70] who also reported that the reduction in the activities of the above antioxidant enzymes was more in *P. falciparum* malaria patients than *P. vivax* malaria patients. It was also reported that the increase in MDA level in *P. falciparum* malaria patients was much more when compared to *P. vivax* malaria patients [71]. In this study, the significant ($p < 0.05$) decrease in the MDA concentration and elevation in the activities of antioxidant enzymes of parasitized mice treated with EESAL compared with Negative control mice could be explained in two ways. Firstly, the extract might be rich in mineral contents that serve as cofactors to these enzymes and hence, increasing their activities. In addition, the phytoconstituents of the extract such as flavonoids which have antioxidant effects could have limited the level of lipid peroxidation. Selenium has been shown to boost antioxidant status and reduce the level of lipid peroxidation in malaria [72]. This is also supported by an earlier study [73] that vitamin E ameliorated *P. berghei*-induced anaemia and pathological alterations in serum biochemistry.

In a recent study, Ajayi et al. [74] showed that extracts of *Vernonia amygdalina* and *Tithonia diversifolia* at 400 mg/kg suppressed *P. berghei* in mice by 75% and 66%, respectively which were lower than 95.95% recorded in the present study. In addition, Muluye et al. [75] showed that root extract of *Euphorbia abyssinica* at 200, 400 and 600 mg/kg respectively gave 66.87%, 84.94% and 93.69% chemosuppression of *P. berghei* infection in mice which were however, lower than the suppression by *S. acuta* extract in the present study. A similar lower chemosuppression malaria parasite (71.93%, 80.97% and 85.64% at 100, 200, and 400 mg/kg) was recorded by *Terminalia mantaly* stem bark water extract [76]. Furthermore, Biruk et al. [77] reported that at 400 mg/kg each, aqueous-methanol and water extracts of *Terminalia brownie* bark had lower chemosuppression (61.2 and 51.1%, respectively) compared with the results of this study. The higher malaria chemosuppression by EESAL relative to many other plant extracts, and its ability to abrogate alterations in hematology and biochemical status of malaria-passaged mice has made a potential source of new antimalarial agent(s). Interestingly, some *Sida* secondary metabolites including stigmasterol and

its derivative, stigmasterol-3-O- β -D-glucopyranoside, have been shown to exhibit antimalarial properties [53, 78]. Similarly, the presence of high levels of flavonoids, alkaloids, terpenoids and tannins which have been known to have antimalarial and chemoprotective activities could be responsible for the findings of the present study [27, 79, 80]. This study has some limitations such as the inability to track the specific compounds responsible for the antimalarial activities of EESAL and characterize them and inability to evaluate the specific mechanism of killing the parasites by EESAL. Hence, this study is considered a preliminary study which has opened the door for further in-depth investigation of the active ingredients through activity-guided fractionation and characterization using chromatographic and spectroscopic techniques which are currently going on in our laboratory. However, findings of this study generally demonstrate that EESAL has antimalarial property, modulates oxidative stress, and rescues haematological aberrations and abrogated dyslipidemia and hepatic dysfunction associated with malaria.

Conclusion

From the above results, it can be concluded that by reducing parasite density in infected mice, EESAL exhibits antimalarial properties. The findings also showed that EESAL normalized the haematological and some biochemical status altered by malaria. This suggests that the plant is a potential reserve of novel antimalarial agents; hence, isolation of specific components of the extract responsible for the above biological activities using bioassay-guided fractionation is warranted. In addition, the specific molecular mechanism(s) of action of the extract and its active compounds are unknown and hence, recommended for further investigations.

Abbreviations

EESAL: Ethanol extract of *Sida acuta* leaves; PCV: Packed cell volume; Hb: Haemoglobin; RBC: Red blood cell; WBC: White blood cell; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; MDA: Malondialdehyde; CHOL: Cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TAG: Triacylglycerol/triglyceride; LD₅₀: Median lethal dose; CAT: Catalase; GSH: Reduced glutathione; SOD: Superoxide dismutase; PVS: Polyvinyl sulphate; SD: Standard deviation; PK: 5'AMP-activated protein kinase

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Authors' contributions

Conceptualization, OCE and CCA; data curation and analysis, IUO, PCO, CAN, TCU, EAA, ACN and BCC; writing—original draft preparation, OCE, CCA and IUO; writing—review and editing, IUO, and CCA. All authors have read and agreed to the published version of the manuscript.

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Declarations

Ethics approval and consent to participate

The study protocols were approved by the Faculty of Biological Sciences Committee on Research Ethics, University of Nigeria, Nsukka (UNN/FBS/EC/102). Other national and international guidelines for the care and use of animals for research were strictly followed in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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