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Aqueous extract of *Enantia chlorantha* Oliv. demonstrates antimalarial activity and improves redox imbalance and biochemical alterations in mice

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Abstract

Background Malaria is an infectious disease, which has continued to cause inconceivable loss of lives every year, almost unabatedly. Currently, it has become more difficult to treat the disease due to the emergence and spread of resistance to recommended antimalarial drugs. This situation necessitates an urgent search for antimalarial compounds with unique modes of action. Here, we investigate the antimalarial activity, antioxidant and anti-inflammatory capacity of *Enantia chlorantha* aqueous stem bark extract (*Ec*ASBE) in vivo.

Methods The extract was screened for selected phytoconstituents including alkaloids and flavonoids. We evaluated the antimalarial activity of *Ec*ASBE against *Plasmodium berghei* NK65 infection in mice, using curative, prophylactic, and suppressive antimalarial test models, respectively. In addition, the antioxidant and anti-inflammatory activities of the extract were assessed.

Results The *Ec*ASBE significantly ($p < 0.05$) inhibited parasitaemia dose-dependently, with the highest inhibition (80.4%) and prolonged survival (MST = 20) observed in the curative test. Our findings reveal significant ($p < 0.05$) improvement of serum ALT, AST, ALP, GGT, and levels of TNF-α, creatinine and urea following extract administration. Furthermore, the extract led to a significant ($p < 0.05$) rise in the levels of CAT, SOD, GPx, and GSH, with a concomitant reduction in NO and MDA levels.

Conclusion The antimalarial, antioxidative, antiperoxidative, and inflammatory-inhibiting properties of the plant in infected mice demonstrate its great value for therapeutic intervention, and substantiate its use in traditional medicine for malaria treatment. Hence, further investigation to identify the repertoire of the active antimalarial components is warranted.

Keywords Cytokines, Drug discovery, Malaria, *Plasmodium berghei*, Oxidative stress

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Background

Malaria continues to take an overwhelming toll on human health and economic development globally, causing unimaginable loss of lives annually. Currently, it threatens about 40% of the world population, with the World Health Organization (WHO) African region being the most affected [1, 2]. Over the past twenty years, progress towards malaria control and eradication through a coordinated effort of national and international agencies, as well as private organisations has resulted in a 40% decrease in the incidence of malaria worldwide [3]. However, these improvements have plateaued in recent years, with malaria still killing more than 600000 people yearly, and children under the age of five make up more than three-quarters of these fatalities [2]. Most of these deaths are attributed to *Plasmodium falciparum*, the most virulent of the human malaria parasites, partly because of its potential to cause cerebral malaria, a fatal neurological complication of the disease [4–7]. Furthermore, malaria parasites induce certain biochemical changes and oxidative stress in the host [8–10]. These alterations are usually implicative of disease progression or severity.

Antimalarial chemotherapy can be used for malaria treatment and prevent malaria transmission. The most effective antimalarial drugs remain artemisinin (ART)-based combination therapies (ACTs), have rapid parasitaemia clearance and resolution of malaria symptoms, with high parasite transmission-blocking potential [2]. However, due to the emergence and spread of resistant *Plasmodium* species, most antimalarial drugs, including artemisinin monotherapy and ACTs, are losing their efficacy [11–15]. The mutability of the parasite's genome to evade antimalarial drugs and the human immune system [16, 17], results in low parasite clearance and treatment failure [18], and greatly complicates malaria control and eradication goals [19]. To overcome this challenge, along with drug-counterfeiting, and apparent limitations of malaria vaccines [20, 21], novel antimalarial therapeutics that can act on unexplored parasite targets must be continuously explored, either to complement or serve as alternatives to existing ones. These efforts are critical to achieving the WHO Global Technical Strategy for Malaria goal of reducing the malaria burden globally by 90% in 2030 [22]. The large deposits of bioactive compounds in medicinal plants [23–30], make them important sources for the identification of effective antimalarial therapeutic leads.

Enantia chlorantha Oliv. is an ornamental tree belonging to the Annonaceae family with a wide distribution in Nigeria and other Central and West African nations. *Enantia chlorantha*, also known as African yellow wood, is called "Dokita Igbo" in Yoruba, and "Erenba-vbogo" among the Binis in Nigeria [27, 31], and "Epoue" in Baka in Cameroun [32]. It is widely used to treat and manage malaria in traditional settings in Nigeria [27], Cameroun [33], as well as hepatitis, jaundice, tuberculosis, and urinary tract infections [33, 34]. Oral administration of *E. chlorantha* stem bark decocted in water is used for the treatment of malaria symptoms including chills, fever, and joint pains [27, 35]. The analgesic and antipyretic [36], anticonvulsant and anti-inflammatory [37], antimicrobial [38, 39] and antimalarial activities [40] of the plant have been reported. In addition, Abubakar et al. [41] reported the antimalarial activity of the respective crude, alkaloid and flavonoid extracts of the plant. Furthermore, Boyom et al. [42] demonstrated the antiplasmodial activity of ethanolic extract and solvent fractions of the plant against the *P. falciparum* W2 strain.

To our knowledge, the antimalarial efficacy of *E. chlorantha*, one of the most used plants for malaria treatment by traditional medicine practitioners in Kwara State, Nigeria [27], has not been sufficiently evaluated scientifically. This underscores a knowledge gap due to scarcity of evidence-based data. Consequently, this study demonstrates the antimalarial activity of *E. chlorantha* aqueous stem bark extract (*Ec*ASBE) in *P. berghei*-infected mice. In addition, we evaluated the effect of the extract on several haematological and biochemical indices in infected animals. We show evidence that *Ec*ASBE possesses potent antimalarial and anti-inflammatory activities, mitigates oxidative imbalance and biochemical alterations in vivo. Our data suggest that this effect is a consequence of the plant suppressing both the activity of *P. berghei* and the excessive host inflammatory response through the synergistic activities of the phytochemicals, especially alkaloids and flavonoids, present in the plant. Taken together, our results contribute critical insights into identifying new antimalarial therapy.

Methods

Collection and authentication of plant

Fresh stem bark of *E. chlorantha* was collected from a forest in Calabar, Cross-River State, Nigeria, with permission. It was identified and authenticated by Prof. E.I. Aigbokhan, a botanic expert in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Dr. H.A. Akinnibosun prepared and deposited the voucher specimen (UBH-E485) at the University of Benin Herbarium for future reference. Additionally, the "Plant List database" [43] was used to validate the identification and scientific name of the plant.

Plant extraction procedure

Fresh *E. chlorantha* stem bark was thoroughly rinsed under running water to remove all debris from the surface and air-dried for four weeks in a well-ventilated area

under shade at ambient temperature (about 25℃). The dried plant materials were pulverised using an industrial machine (Armfield Ltd., Hampshire, England) and sieved to obtain a fine plant powder. A kilogram (1 kg) of fine powdered plant material was macerated in distilled water at room temperature (25℃) for 2 days. The filtrate from the mixture was lyophilised using a freeze-dryer (LTE Scientific Ltd., Oldham, UK), and stored in screw-capped glass vials at 4℃ until further use.

Experimental animals

One hundred and twenty-six (126) male Swiss albino mice of 8 to 12 weeks old weighing between 20 and 24 g were used for the study. The animals were sourced from the Institute for Advanced Medical Research and Training (IAMRAT), University of Ibadan, Ibadan, Nigeria. They were kept in standard plastic cages under standard laboratory conditions (25 ± 5℃, 12-h light/dark cycle) in the Animal Holding Unit at IAMRAT. The mice were treated humanely and provided with standard pelletised mouse feed and water *ad libitum*.

Phytochemical screening of *E. chlorantha* aqueous stem extract (*Ec*ASBE)

The preliminary qualitative phytochemical screening was performed to identify the phytoconstituents in *Ec*ASBE following standard protocols [44, 45]. The extract was screened for alkaloids, flavonoids, glycosides, coumarin, tannins, phenols, triterpenes, saponins, and terpenoids. The phytochemicals present in the extract were further quantified following previously reported protocols [44, 46], but with slight modification. Positive results for these tests were indicated by a change in colour or the formation of precipitates. The procedures are explained below.

Qualitative phytochemical screening

Alkaloids: In each test tube, 2 mL of 5% HCl was added to 1 mL of *Ec*ASBE filtrate in a test tube and heated in a water bath. After mixing thoroughly and filtering, aliquots were taken. Drops of Wagner (solution of iodine in potassium iodide), and Mayer (potassium mercuric iodide solution) reagents were added to each. The formation of brownish red (Wagner), and cream colour precipitates (Mayer), respectively, indicate the presence of alkaloids.

Flavonoids: 1 mL of NaOH was added to 3 mL of *Ec*ASBE filtrate in isopropyl alcohol. The formation of a yellow colouration indicates the presence of flavonoids.

Cardiac glycosides: 2 mL of 50% H_2SO_4 was added to 1 mL of the *Ec*ASBE filtrate, the mixture was heated in boiling water for 15 min. 10 mL of Fehling's solution was added and the mixture boiled. The formation of a brick red precipitate indicates the presence of glycosides.

Tannins: 1 mL of 10% KOH was added to 1 mL of *Ec*ASBE filtrate in a test tube. The formation of a white precipitate indicates the presence of tannins.

Phenolics: Two drops of 5% $FeCl_3$ was added to 1 mL *Ec*ASBE filtrate in a test tube. The presence of a greenish precipitate indicates the presence of phenolics.

Triterpenes: Five drops of acetic anhydride $[(CH_3CO)_2O]$ was added to 1 mL of *Ec*ASBE filtrate. Thereafter, a drop of concentrated H_2SO_4 was added. The mixture was heated for 1 h and neutralized with NaOH followed by the addition of chloroform. The formation of a blue green colour indicates the presence of triterpenes.

Saponins: 5 mL of *Ec*ASBE filtrate was mixed with 5 mL distilled water and shaken vigorously. The formation of a stable froth indicates the presence of saponins.

Terpenoids: 5 mL of *Ec*ASBE filtrate was mixed with 2 mL of chloroform in a test tube. 3 mL of concentrated H_2SO_4 was carefully added to the mixture to form a layer. The formation of a reddish-brown interface indicates the presence of terpenoids.

Steroids: 2 mL acetic anhydride was added to 1 ml of the *Ec*ASBE filtrate in a test tube. Thereafter, 2 mL of H_2SO_4 was added. The formation of blue colouration from green indicates the presence of steroids.

Anthocyanin: 2 mL of HCl and 1 ml ammonia was added to 1 mL of *Ec*ASBE filtrate. The change in colour from pinkish red to bluish violet indicates the presence of steroids.

Phlobatannins: 2 mL of *Ec*ASBE filtrate was added to 1% aqueous HCl and was then boiled with the help of Hot plate stirrer. Formation of red colored precipitate indicates a positive result.

Quantitative phytochemical evaluation

Quantification of alkaloid

20 g of *Ec*ASBE sample was weighed in a beaker into which 250 mL of 10% acetic acid in ethanol was added and kept standing for 4 h at room temperature. The beaker with the extract was placed in a steamy water bath and allowed to concentrate to one quarter of its original volume. Thereafter, concentrated ammonium hydroxide (NH_4OH) was added in a dropwise manner until precipitation was complete. Following sedimentation of the solution, the supernatant was discarded, and the precipitates collected. The precipitate was washed with 20 mL of dilute NH_4OH and filtered with a 125 mm Whatman No.1 filter paper. The filter paper containing the precipitates was left to dry to constant weight. The percentage of alkaloid was calculated using the formula below:

$$\%\text{Alkoloid} = \frac{\text{Weight of filter paper with crude alkaloid} - \text{Initial weight of filter paper}}{\text{Weight of plant sample}} \times 100$$

Quantification of flavonoid

Into a beaker containing 2 g of *Ec*ASBE sample 50 mL of 80% aqueous methanol was added and left to stand for 24 h at room temperature. The supernatant was discarded, and the residue was re-extracted (three times) with 50 mL of ethanol. The solution was afterwards filtered with 125 mm Whatman No.1 filter paper. The filtrate was transferred into a crucible and allowed to dry in a water bath. The percentage of flavonoid was calculated using the formula below:

$$\%\text{Flavonoid} = \frac{\text{Weights of crucible with crude flavonoid} - \text{Initial weight of crucible}}{\text{Weight of plant sample}} \times 100$$

Quantification of glycosides

200 mL of 70% ethanol was added into a 250 mL sterile bottle containing 25 g of *Ec*ASBE sample and shaken vigorously at 25℃ and 300 rpm for 6 h in a water bath shaker. The resulting solvent from the bottle was filtered through 125 mm Whatman No.1 filter paper. The filtrate was transferred into 1 L volumetric flasks after which 500 mL of distilled water was added followed by 100 mL of 12.5% lead acetate. The resultant solution was made to 800 mL with distilled water and vigorously shaken at 300 rpm for 10 min. 200 mL of 4.77% disodium phosphate solution was added to the 800 ml solution to precipitate the excess Pb^{2+} and filtered using 125 mm Whatman No. 1 filter paper. The filtrate was left to evaporate to dryness. 50 mg of the dried filtrate was then dissolved in 2 mL of glacial acetic acid containing 2 drops of 2% ferric chloride solution. The percentage of glycosides was calculated using the formula below:

$$\%\text{Glycosides} = \frac{\text{Weight of dried filtrate}}{\text{Weight of plant sample}} \times 100$$

Quantification of saponin

20 g of *Ec*ASBE sample was taken into a beaker and 100 mL of 20% aqueous ethanol was added. The solution was heated in a water bath at 55℃ for 4 h with continuous stirring. Thereafter, the solution was filtered and the residue re-extracted with 100 mL of 20% aqueous ethanol. The filtrate was left to evaporate over a water bath at about 90℃ to obtain 40 mL of the filtrate which was transferred into a 250 mL-separating funnel to which 20 mL of diethyl ether was added and thoroughly mixed. The aqueous layer was recovered and discarded the diethyl ether layer. The filtrate was further purified using 60 mL of n-butanol, and subsequently washed two times with 10 mL of 5% NaCl. The NaCl layer was discarded, and the remaining solution was heated in a water bath to dryness. The percentage of saponin was calculated using the formula below:

$$\%\text{Saponin} = \frac{\text{Weight of saponin}}{\text{Weight of plant sample}} \times 100$$

Quantification of terpenoids

100 mg of *Ec*ASBE sample was macerated in 9 mL of ethanol for 24 h. After filtration, the mixture was extracted with 10 mL of petroleum ether using separating funnel and then left to dry. The percentage of terpenoids was calculated using the formula below:

$$\%\text{Terpenoids} = \frac{\text{Weight of plant sample used for extraction} - \text{ weight after complete drying}}{\text{Weight of plant sample used for extraction}} \times 100$$

In vivo acute oral toxicity test (median lethal dosage, LD_{50}) of EcASBE

The acute toxicity of *Ec*ASBE was assessed in non-infected, nulliparous and non-pregnant female Swiss albino mice adopting an up-and-down procedure consistent with the Organization for Economic Cooperation and Development (OECD) Guideline No. 425 [47]. Five female mice (8–12 weeks old, 22–24 g) were dosed singly in sequence at 48 h intervals. A single dose of 175 mg/kg body weight (bw) extract was administered to a mouse orally. Food was withheld for 2 h following administration of extract. The mouse was closely observed for gross changes such as changes in feeding pattern, diarrhoea, hair erection, lacrimation, mortality, and other morphological and behavioural indicators of toxicity.

Since death was not observed after 48 h, the second animal was given 550 mg/kg bw extract. This was

followed by 1750 and 5000 mg/kg bw extract for subsequent rounds of administration. The body weight of the animals was taken, 48 h after extract administration, and compared with the initial body weight. The animals were observed for indications of toxicity and mortality over the next fourteen days.

Grouping and dosing of experimental animals

Forty-two mice were used for each of the three antimalarial test models (Curative, Prophylactic and Suppressive). They were randomly placed into six groups for each test model (n = 7). The treatment groups (Groups IV-VI) were treated with 125, 250, and 500 mg/kg bw extract. All treatments were administered using oral gavage with a stainless metallic feeding cannula. The groupings are summarised in Table 1.

In vivo investigation of antimalarial activities of *Ec*ASBE

Preparation of standard inoculum and parasite inoculation

Chloroquine-sensitive *Plasmodium berghei* NK65 strain sourced from the Drug Research Laboratory, IAMRAT, University of Ibadan, Ibadan, Nigeria was inoculated intraperitoneally into mice to induce experimental malaria. Parasitised blood was collected from four donor *P. berghei*-infected mice with parasitaemia levels of 20–30% [48, 49]. Following the determination of parasitaemia of donor mice, they were euthanised with 2% isoflurane. Their blood was collected through retro-orbital venipuncture with a heparinised hematocrit capillary tube [50, 51] into a falcon tube treated with 4 mL anticoagulant citrate dextrose (ACD) solution, Solution A, USP (2.13% free citrate ion). Afterwards, the blood was diluted with physiological isotonic saline (0.9%) based on the level of parasitaemia of the donor mice [52], resulting in a 1 mL of blood containing 5×10^7 *P. berghei*-infected red blood cells (iRBCs). Afterwards, the mice were injected intraperitoneally with 0.2 mL of this diluted blood containing about 10^7 *P. berghei*-iRBCs.

Table 1 Experimental grouping and dosage

Groups (n = 7)	Treatments
Group I	Normal control –administered with the vehicle (distilled water)
Group II	Infected and untreated (administered with the vehicle)
Group III	Infected and treated with standard drug – 10 mg/kg chloroquine (CQ) phosphate
Group IV	Infected and treated with 125 mg/kg bw *Ec*ASBE
Group V	Infected and treated with 250 mg/kg bw *Ec*ASBE
Group VI	Infected and treated with 500 mg/kg bw *Ec*ASBE

Curative (Rane's) test

Following the establishment of malaria in the mice, we proceeded to evaluate the curative ability of *Ec*ASBE using the Rane's test as described by Ryley and Peters [53]. Briefly, the mice were inoculated on the first day (D_0). After the determination of baseline parasitaemia, oral administration of *Ec*ASBE started 72-h post-infection. Treatment was administered once a day for five consecutive days (D_3 to D_7) with 24-h gap between the doses.

Prophylactic (Repository) test

The prophylactic activity of *Ec*ASBE was evaluated following the technique described by Peters [54] (1965). For 4 days (D_0 to D_3), the mice were given *Ec*ASBE once a day orally. On the fifth day (D_4), all mice were inoculated with iRBCs intraperitoneally.

Four-day suppressive test

*Ec*ASBE was evaluated for its schizonticidal efficacy on early *P. berghei* infection in mice adopting a 4-day suppressive test [55]. Beginning on the first day (D_0), three hours after infection, treatments were given orally in single doses throughout the course of four days (D_0 to D_3).

Parasitaemia measurement

The schematic representation of the antimalarial tests is represented in Fig. 1. Parasitaemia was monitored by Giemsa-stained thin blood smears from the tail snip of each mouse on: D_4 for the 4-day suppressive test; after 72 h of infection on D_7 in prophylactic test; and from D_3 after the establishment of infection to D_7 for the curative test. The slides were viewed under a light microscope (×100 oil immersion objective) by an experienced and experiment-blinded microscopist. Survival of the animals was observed using Kaplan–Meier survival analysis and compared using the log-rank (Mantel–Cox) test. The formulae below were used to compute percentage parasitaemia and inhibition [56].

$$\%\text{Parasitaemia} = \frac{\text{Number of parasitised erythrocytes}}{\text{Total number of erythrocytes}} \times 100\%$$

Monitoring of body weight and temperature changes

The measurement of changes in rectal temperature and body weight are important to evaluate the effectiveness of medicinal plant extracts against malarial infection [57]. A sensitive digital weighing balance (Want Balance Instrument Co., Ltd., Jiangsu, China) was used to measure the body weight of each mouse before infection, during, and after the course of treatment. The rectal temperature of the mice was measured with a digital rectal thermometer (Omron Healthcare Co., Ltd., Japan). In the curative

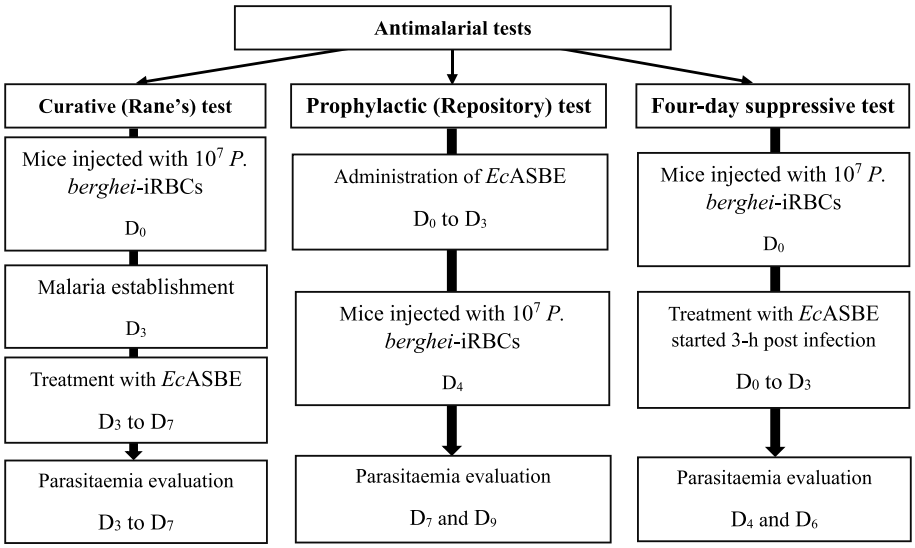

Fig. 1 Schematic representation of the antimalarial tests

test, the weight and temperature of the mice were taken on D_0, D_4 and D_7. The body weight and rectal temperature were taken on D_0, D_4, D_7 and D_9 in the prophylactic test. While the body weight and rectal temperature of the mice were taken on D_0, D_4 and D_6 post-inoculation, in the suppressive test.

Packed cell volume (PCV) measurement

The PCV was measured to assess the efficacy of the test extract in preventing haemolysis due to the multiplication of the parasites in the RBCs. Blood was collected from the tail of each mouse in heparinised capillary tubes, and centrifuged in a microhematocrit centrifuge (Hawksley and Sons Ltd., Lancing, Sussex, England) for 7 min at 11,000 rpm. Afterwards, the PCV was determined using a standard MicroHematocrit Reader (Hawksley and Sons Ltd., Lancing, Sussex, England). The formula [58] below was used to estimate PCV on D_0, D_4 and D_7 in the curative; D_0, D_4, D_7 and D_9 in the prophylactic; and D_0, D_4 and D_6 in the 4-day suppressive tests, respectively:

$$\% \text{ Inhibition} = \frac{(\text{Mean parasitaemia of untreated group} - \text{Mean parasitaemia of treated group})}{\text{Mean parasitaemia of untreated group}} \times 100$$

Determination of haematological, biochemical indices and proinflammatory marker in experimental mice

Blood was collected from the representative animals into 5 mL EDTA (for haematological assays) and plain bottles, respectively, through retro-orbital venipuncture after euthanising them with 2% isoflurane. The blood collected in plain bottles was allowed to clot for 30 min and centrifuged at 5000 rpm for 15 min to separate the serum which was collected and stored at −20℃. The serum was used for biochemical assays and determination of proinflammatory marker.

Biochemical assays

Serum biochemical assays

Sera and tissue homogenates from animals in the curative test (the antimalarial test model showing the highest antimalarial activity of *Ec*ASBE) were used to evaluate biochemical assays. Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), as well as concentrations of albumin and bilirubin were measured spectrophotometrically (VWR International Ltd., Leicestershire, UK) using commercially available diagnostic kits (Fortress Diagnostics Ltd., Antrim, UK) to evaluate liver biochemical function. Using commercially available diagnostic kits (Atlas Medical Ltd., Cambridge, UK), serum creatinine and urea concentrations were measured to evaluate kidney function. While for serum lipid profile, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol levels were measured by using reagent assay kits (Fortress Diagnostics Ltd., Antrim, UK).

Tissue homogenate total protein and redox assays

The tissues (liver and kidneys) were excised from euthanised representative animals across the groups and homogenised by a micro tissue homogeniser (Carrier Transicold Ltd., Palm Beach Gardens, United States) in phosphate buffered saline (PBS) (0.01 M, pH 7.4, 1:5 w/v) in ice-cooled plastic tubes. The homogenised samples were centrifuged at 5000 rpm for 15 min at 4℃ with the resultant tissue homogenates stored in plastic vials at −4℃ until use. Tissue homogenates were used for the determination of liver and renal redox parameters, and total protein concentrations.

Total protein concentration was determined using the Biuret method as outlined in the assay kit's manufacturer instructions (Fortress Diagnostics Ltd., Antrim, UK). The activity of catalase (CAT) and concentration of reduced glutathione (GSH) were evaluated using colorimetric method as described in the guidelines by the assay kit's manufacturer (Elabscience Biotechnology Inc., Houston, Texas, USA). The concentration of malondialdehyde (MDA) was evaluated using colorimetric method, following the guidelines outlined by the manufacturers (Oxford Biomedical Research, Inc., Rochester Hills, Michigan, USA). Nitric oxide (NO) concentration was determined colorimetrically using the Griess method according to the guidelines outlined by the manufacturer (Oxford Biomedical Research, Inc., Rochester Hills, Michigan, USA). Glutathione peroxidase (GPx) activity was evaluated following the protocol describe by the assay kit's manufacturer (Fortress Diagnostics Ltd., Antrim, UK). Additionally, superoxide dismutase (SOD) activity was measured using the protocol outlined by Marklund and Marklund [59].

Determination of serum level of proinflammatory marker

Serum level of tumour necrosis factor-alpha (TNF-α) was measured using a commercially available Sandwich-enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (Elabscience Biotechnology Inc., Houston, Texas, USA), based on a biotinylated monoclonal antibody specific for mouse TNF-α, with a threshold sensitivity of 9.38 pg/mL. Plates were read with a Victor3® Multilabel Plate Reader (Perkin-Elmer) at 450 nm.

Evaluation of the relationship between parasitaemia and antioxidant enzyme activities and inflammation Pearson correlation coefficients (r) was conducted to estimate the relationship between parasitaemia and antioxidant enzyme activities and inflammation in infected animals in the curative antimalarial test.

Haematological analysis

Haematological parameters were determined following previously described methods [60, 61]. The haematological parameters evaluated were haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), neutrophils (Neut), and Lymphocytes (Lymph).

Statistical analysis

Data were analysed using One-way analysis of variance (ANOVA) and represented as mean ± standard error of the mean (mean ± SEM). All grouped data in antimalarial activities, inflammation, and biochemical assays were statistically analysed using GraphPad Prism v9 (GraphPad Software Inc., San Diego, California, USA), while those in weight, temperature, and haematological parameters were statistically analysed with SPSS data analysis software v25 (IBM SPSS Statistics for Windows, IBM Corporation, NY, USA). Tukey's post hoc test was used to evaluate statistical significance for grouped data at $p < 0.05$. Pearson correlation coefficients (r) using GraphPad Prism v9 was used to calculate the relationship between parasitaemia and antioxidant enzymes and inflammation in infected animals, at 95% confidence interval (CI).

Results

Phytochemical constituents of *Ec*ASBE

Phytochemical screening of *Ec*ASBE was carried out to determine the presence or absence of secondary metabolites in the extract. The extract contained phytoconstituents including alkaloids, flavonoids and saponins (Table 2). Alkaloids (194.17 ± 3.97) and flavonoids (111.12 ± 1.06) were found to be the most abundant phytoconstituents in the extract.

Acute oral toxicity of *Ec*ASBE

The acute oral toxicity of the extract was carried out so as to define the safety limit and select suitable doses for further experiments in mice. Following the acute oral toxicity test, no death was recorded within the first 24 h and the observation period of 14 days. Furthermore, a thorough physical and behavioural examination of the experimental animals revealed no obvious symptoms of acute toxicity induced by the extract. The animals showed no outward symptoms of abnormalities such as posture, movement, salivation, loss of appetite, tremors, diarrhoea, depression, and lachrymation. The findings indicate that the LD_{50} of the extract is greater than 5000 mg/kg. Hence, the doses of 125 ($^1/_{40}$th of the LD_{50}), 250 ($^1/_{20}$th of the LD_{50}), and 500 ($^1/_{10}$th of the LD_{50}) mg/kg bw extract were selected and used for the antimalarial testing.

Table 2 Phytochemical constituents of *Ec*ASBE

S/n	Qualitative phytochemical screening	Detected	Quantitative phytochemical screening	Value (mg/100 g)
1	Alkaloids	+	Alkaloids	194.17 ± 3.97
2	Flavonoids	+	Flavonoids	111.12 ± 1.06
3	Glycosides	+	Glycosides	0.23 ± 0.01
4	Saponin	+	Saponin	0.06 ± 0.00
5	Terpenoids	+	Terpenoids	0.03 ± 0.01
6	Tanin	-		
7	Phenolics	-		
8	Steroids	-		
9	Triterpene	-		
10	Anthocyanin	-		
11	Phlobatannins	-		

+ Detected;—Not detected

Table 3 Effects of *Ec*ASBE on body weights, rectal temperatures and PCV of *P. berghei*-infected mice in the curative test

Group	Weight (g)			Temperature (℃)			PCV (%)		
	D0	D4	D7	D0	D4	D7	D0	D4	D7
Normal control	20.71 ± 0.57^{a}	21.57 ± 0.92^{a}	23.00 ± 0.69^{b}	36.27 ± 0.18^{a}	36.27 ± 0.05^{a}	35.71 ± 0.11^{a}	50.09 ± 0.55^{a}	49.86 ± 0.35^{b}	49.47 ± 0.59^{b}
Untreated	19.86 ± 0.67^{a}	19.29 ± 0.57^{a}	17.00 ± 0.44^{a}	36.46 ± 0.40^{a}	36.80 ± 0.25^{a}	37.46 ± 0.11^{b}	49.96 ± 0.21^{a}	46.74 ± 0.44^{a}	42.84 ± 0.50^{a}
Standard drug (CQ)	20.14 ± 0.80^{a}	21.14 ± 0.91^{a}	21.71 ± 1.02^{b}	35.84 ± 0.23^{a}	36.34 ± 0.11^{a}	36.33 ± 0.12^{a}	55.11 ± 0.83^{c}	55.04 ± 0.23^{e}	53.33 ± 0.68^{c}
125 mg/kg bw *Ec*ASBE	20.43 ± 0.90^{a}	22.29 ± 1.08^{a}	23.43 ± 1.34^{b}	36.09 ± 0.32^{a}	36.59 ± 0.16^{a}	35.86 ± 0.24^{a}	53.77 ± 0.89^{bc}	53.35 ± 0.41^{de}	52.98 ± 0.65^{c}
250 mg/kg bw *Ec*ASBE	20.57 ± 0.97^{a}	20.00 ± 1.45^{a}	21.00 ± 1.09^{b}	36.34 ± 0.28^{a}	36.71 ± 0.16^{a}	35.64 ± 0.21^{a}	52.03 ± 0.88^{ab}	51.65 ± 0.58^{c}	49.80 ± 0.67^{b}
500 mg/kg bw *Ec*ASBE	20.57 ± 0.61^{a}	20.71 ± 0.81^{a}	22.00 ± 0.53^{b}	36.07 ± 0.35^{a}	36.67 ± 0.15^{a}	36.17 ± 0.18^{a}	53.34 ± 0.64^{bc}	52.81 ± 0.27^{cd}	49.02 ± 0.24^{b}

Values are expressed as mean ± SEM (n = 5). Values with different superscript letters along a column for a given parameter are significantly different ($p < 0.05$) from each other

Effects of *Ec*ASBE on body weights, rectal temperatures and PCV of mice

In Rane's curative test, treatment of *P. berghei*-infected mice with *Ec*ASBE exerted significant ($p < 0.05$) effect on body weights and rectal temperatures of the treated mice in comparison with that of the untreated group on D_7 (Table 3). Also, the extract exerted significant ($p < 0.05$) effect on the PCV of the treated mice in comparison with the untreated group on D_4 and D_7 (Table 3). These data indicate that therapeutic intervention with the plant can avert weight loss, improve body temperature and PCV in malaria-infected mouse model.

In the prophylactic test, administering *Ec*ASBE to *P. berghei*-infected mice had a significant ($p < 0.05$) effect on their body weights on D_7 and D_9, as well as on their rectal temperatures and PCV on D_9, relative to the untreated group (Table 4). The extract exerted significant ($p < 0.05$) effect on the PCV of the treated mice in comparison with the untreated group on D_9. These data suggest that prophylactic treatment with the plant can prevent weight loss, temperature fluctuations and PCV reduction in infected animals.

In the suppressive test, following treatment of *P. berghei*-infected mice with *Ec*ASBE, a significant ($p < 0.05$) effect on body weights, rectal temperatures and PCV of the treated mice was recorded when compared with untreated group on D_6 (Table 5). These results indicate the plant can significantly suppress weight loss, temperature fluctuations and PCV reduction in infected animals at higher doses.

Antimalarial activity of *Ec*ASBE in *P. berghei*-infected mice

We investigated the Rane's curative effect of *Ec*ASBE on established malaria in vivo. Administration of the extract significantly ($p < 0.05$) reduced parasitaemia level dose-dependently, in comparison with the untreated group

Table 4 Effects of *Ec*ASBE on body weights, rectal temperatures and PCV of *P. berghei*-infected mice in the prophylactic test

Group	Weight (g)				Temperature (℃)				PCV			
	D0	D4	D7	D9	D0	D4	D7	D9	D0	D4	D7	D9
Normal control	20.29 ± 0.61^{a}	22.71 ± 0.75^{bc}	23.43 ± 0.37^{c}	24.71 ± 0.81^{b}	36.06 ± 0.30^{a}	35.64 ± 0.16^{b}	36.03 ± 0.06^{a}	35.96 ± 0.15^{ab}	48.25 ± 0.77^{a}	49.71 ± 0.89^{a}	50.31 ± 0.10^{ab}	51.37 ± 0.41^{bc}
Untreated	20.14 ± 0.99^{a}	20.00 ± 0.72^{ab}	19.43 ± 0.48^{a}	17.71 ± 0.47^{a}	35.53 ± 0.18^{a}	35.50 ± 0.14^{ab}	36.17 ± 0.22^{a}	37.50 ± 0.11^{c}	52.69 ± 0.29^{b}	52.18 ± 0.18^{bc}	49.87 ± 0.28^{a}	47.72 ± 0.80^{a}
Standard drug (CQ)	20.70 ± 1.08^{a}	24.14 ± 0.46^{c}	24.14 ± 0.70^{c}	24.86 ± 1.30^{b}	35.40 ± 0.15^{a}	35.57 ± 0.11^{ab}	36.23 ± 0.28^{a}	36.49 ± 0.20^{b}	51.66 ± 0.46^{b}	51.37 ± 0.09^{b}	51.90 ± 0.20^{bc}	50.07 ± 0.80^{ab}
125 mg/kg bw *Ec*ASBE	20.86 ± 0.88^{a}	23.71 ± 0.68^{c}	24.14 ± 0.59^{bc}	25.14 ± 0.74^{b}	35.96 ± 0.27^{a}	35.30 ± 0.14^{ab}	36.01 ± 0.23^{a}	36.00 ± 0.19^{ab}	51.10 ± 0.38^{ab}	51.57 ± 0.23^{bc}	52.61 ± 0.52^{c}	54.70 ± 0.89^{d}
250 mg/kg bw *Ec*ASBE	20.14 ± 0.80^{a}	22.14 ± 0.59^{abc}	22.43 ± 0.78^{c}	23.71 ± 0.81^{b}	35.39 ± 0.17^{a}	35.04 ± 0.07^{a}	36.50 ± 0.09^{a}	35.64 ± 0.21^{a}	52.44 ± 1.00^{b}	52.54 ± 0.11^{c}	52.38 ± 0.46^{c}	51.38 ± 0.62^{bc}
500 mg/kg bw *Ec*ASBE	20.29 ± 0.89^{a}	19.86 ± 0.67^{a}	20.57 ± 0.78^{ab}	22.57 ± 0.87^{b}	35.83 ± 0.12^{a}	35.34 ± 0.14^{ab}	36.19 ± 0.18^{a}	36.11 ± 0.16^{ab}	52.11 ± 0.56^{b}	52.11 ± 0.96^{bc}	52.69 ± 0.58^{c}	53.85 ± 0.48^{cd}

Values are expressed as mean ± SEM (n = 5). Values with different superscript letters along a column for a given parameter are significantly different ($p < 0.05$) from each other

Table 5 Effects of *Ec*ASBE on body weights, rectal temperatures and PCV of *P. berghei*-infected mice in the suppressive test

Group	Weight (g)			Temperature (℃)			PCV		
	D0	D4	D6	D0	D4	D6	D0	D4	D6
Normal control	19.29 ± 0.61^{a}	20.86 ± 0.40^{a}	22.14 ± 0.70^{b}	35.63 ± 0.18^{a}	36.37 ± 0.07^{ab}	36.33 ± 0.05^{a}	51.87 ± 0.82^{ab}	51.46 ± 0.31^{b}	50.87 ± 0.63^{d}
Untreated	20.00 ± 0.69^{a}	19.00 ± 0.58^{a}	17.86 ± 0.40^{a}	36.3 ± 0.16^{a}	35.80 ± 0.24^{ab}	36.90 ± 0.13^{b}	49.76 ± 0.40^{a}	47.25 ± 0.16^{a}	44.58 ± 0.31^{a}
Standard drug (CQ)	20.14 ± 1.01^{a}	20.86 ± 1.01^{a}	23.00 ± 1.50^{b}	35.8 ± 0.10^{ab}	35.66 ± 0.11^{a}	36.09 ± 0.10^{a}	51.36 ± 0.48^{ab}	51.02 ± 0.43^{b}	49.85 ± 0.99^{cd}
125 mg/kg bw *Ec*ASBE	20.00 ± 0.58^{a}	19.29 ± 0.87^{a}	20.14 ± 1.16^{ab}	35.89 ± 0.14^{ab}	36.57 ± 0.34^{b}	36.24 ± 0.09^{a}	52.96 ± 0.66^{b}	51.30 ± 0.12^{b}	47.71 ± 0.62^{bc}
250 mg/kg bw *Ec*ASBE	20.29 ± 0.81^{a}	20.57 ± 1.09^{a}	22.14 ± 1.24^{b}	36.10 ± 0.13^{ab}	35.66 ± 0.21^{a}	36.39 ± 0.09^{a}	53.80 ± 0.94^{b}	52.42 ± 0.44^{b}	45.87 ± 0.44^{ab}
500 mg/kg bw *Ec*ASBE	19.98 ± 0.30^{a}	19.57 ± 0.78^{a}	21.29 ± 0.52^{ab}	35.56 ± 0.15^{b}	35.87 ± 0.18^{ab}	36.31 ± 0.10^{a}	52.48 ± 0.60^{ab}	52.00 ± 0.30^{b}	50.68 ± 0.39^{d}

Values are expressed as mean ± SEM (n = 5). Values with different superscript letters along a column for a given parameter are significantly different ($p < 0.05$) from each other

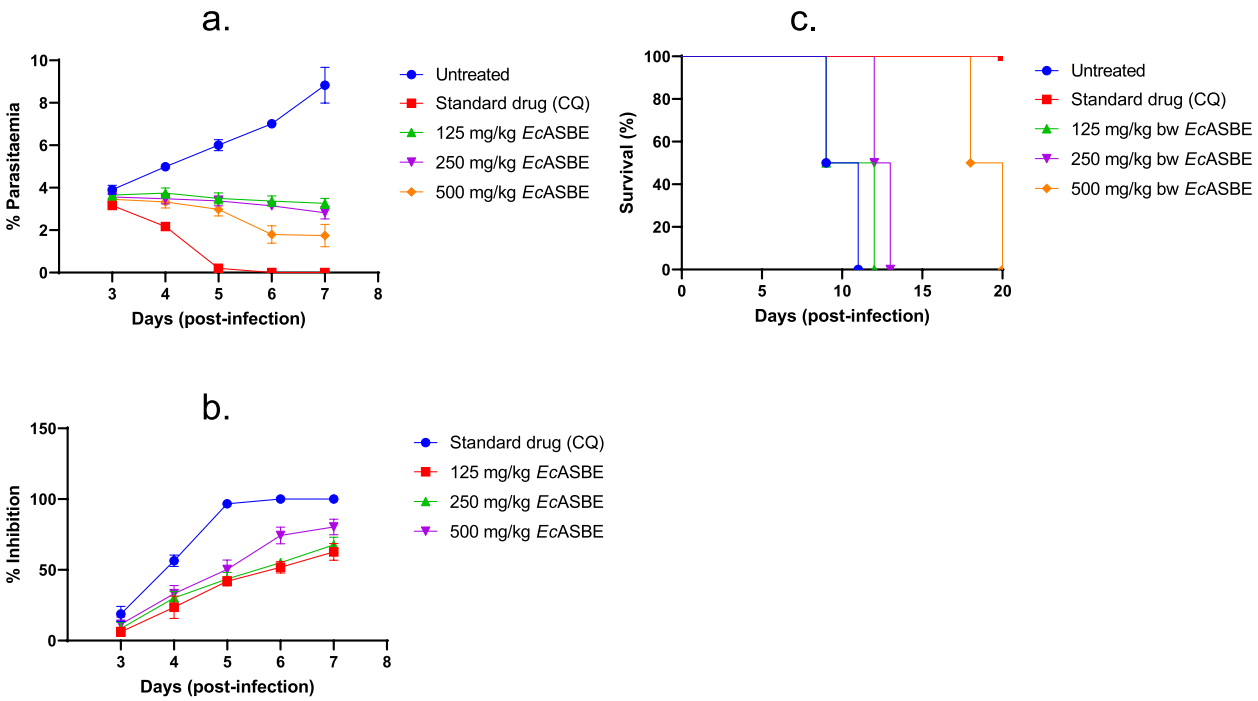

Fig. 2 Curative antimalarial efficacy of *Ec*ASBE in *P. berghei*–infected mice. (**a**) percentage parasitaemia and (**b**) percentage inhibition. Values are expressed as mean ± SEM (n = 5). (**c**) Survival of mice was monitored using the Kaplan–Meier survival analysis and data were compared by a Log-rank (Mantel–Cox) test

(Fig. 2a). Parasitaemia in the extract–treated groups measured at D_7 was lower than parasitaemia measured on previous days, showing that continued treatment with the extract caused a reduction of parasitaemia from the peak measured at D_4. The analysis of the inhibition activity of the extract showed that the extract exhibited the highest curative effect at dose 500 mg/kg bw on D_7 with a parasitaemia inhibition effect of 80.4%, but lower than that of the standard drug chloroquine (100%) (Fig. 2b). The mean survival time (MST) analysis revealed an improvement in the survival of animals treated with 500 mg/kg bw *Ec*ASBE (MST = 20 days) when compared with the untreated animals (MST = 11 days) (Fig. 2c). These results strongly support that therapeutic intervention with the extract can significantly reduce and inhibit parasitaemia, as well as prolong the survival of infected animals in an established murine *Plasmodium* spp. infection.

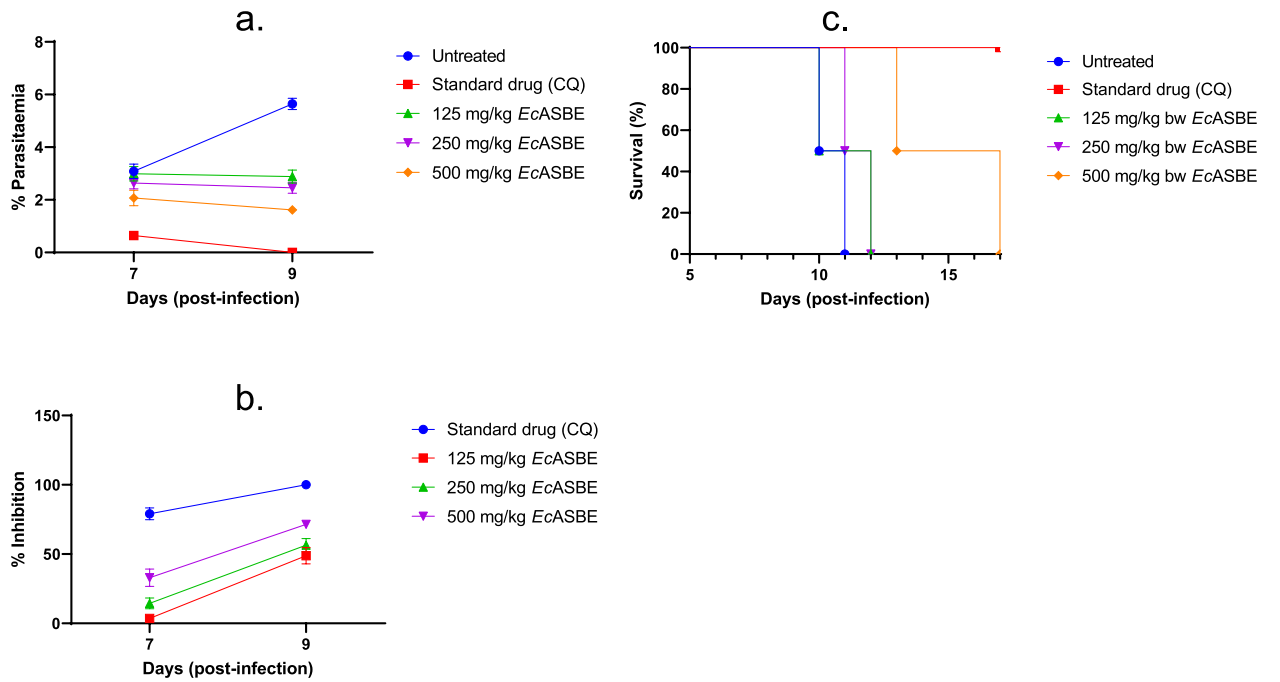

Fig. 3 Prophylactic antimalarial efficacy of *Ec*ASBE in *P. berghei*–infected mice. (**a**) percentage parasitaemia and (**b**) percentage inhibition. Values are expressed as mean ± SEM (n = 5). (**c**) Survival of mice was monitored using the Kaplan–Meier survival analysis and data were compared by a Log-rank (Mantel–Cox) test

We tested the prophylactic effect of *Ec*ASBE on the animals. Following pretreatment of infected mice with the extract, the level of parasitaemia reduced significantly ($p < 0.05$) dose-dependently, in contrast to the untreated group (Fig. 3a). As shown in Fig. 3b, the extract exerted the highest prophylactic potential and the highest inhibition effect (71.4%) at the highest dose 500 mg/kg bw on D_9, but lower than that of the standard drug chloroquine (100%). The administration of *Ec*ASBE dose-dependently increased the survival of infected animals, with 500 mg/kg bw *Ec*ASBE improving survival of infected animals (MST = 17 days) as against (MST = 11 days) recorded for untreated animals (Fig. 3c). Together, the data demonstrate the preventive effect and potential of *Ec*ASBE to improve survival in experimental animals.

Finally, we evaluated the suppressive effect of *Ec*ASBE on experimental animals. The 4-day suppressive test is meant to assess the antimalarial efficacy of the extract during the initial stages of infection [62]. Evaluation of the suppressive activity of the extract revealed that the extract caused a reduction in parasitaemia dose-dependently in treated mice, with the highest parasitaemia reduction recorded at 500 mg/kg bw on D_6 (Fig. 4a). The observed reduction was statistically significant ($p < 0.05$) in comparison with the untreated group. The extract exerted parasitaemia inhibition effect of 71% at the highest dose 500 mg/kg bw, but lower than 94.3% recorded for the standard drug chloroquine (Fig. 4b). The administration of the extract caused a significant ($p < 0.05$) improvement in the MST of infected animals in a dose-dependent manner (Fig. 4c). The MST of extract-treated animals (MST = 14 days) recorded at 500 mg/kg bw *Ec*ASBE significantly ($p < 0.05$) differs from (MST = 9 days) of the untreated animals. Altogether, these results suggest that the extract acts to suppress the onset of *P. berghei*-induced malaria in the animals and improves their survival.

Effects of *Ec*ASBE on *P. berghei*-infected host biochemical alterations

Effects of EcASBE on serum liver enzymatic activities, and serum bilirubin and albumin levels in P. berghei-infected mice

We conducted experiments to investigate the ability of *Ec*ASBE to mitigate liver dysfunction induced by *P. berghei* infection in the liver of the animals. The effect of the extract on the activities of serum liver enzymes ALT, AST, ALP, and GGT in *P. berghei*- infected mice is presented in Fig. 5a–d. The activities of ALT, AST, ALP, and GGT were significantly ($p < 0.05$) increased in untreated mice. Following treatment with the extract, there was a significant ($p < 0.05$) reduction in the activities of these enzymes in the infected mice to levels that

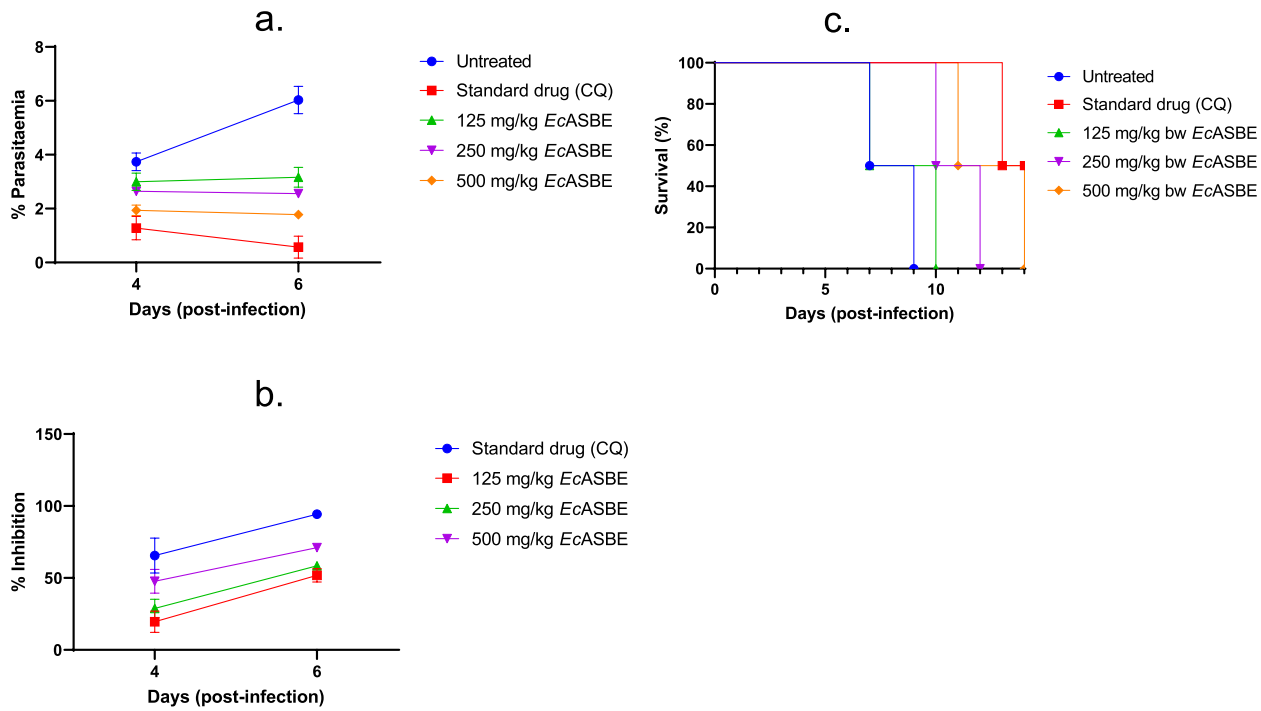

Fig. 4 Suppressive antimalarial efficacy of *Ec*ASBE in *P. berghei*–infected mice. (**a**) percentage parasitaemia and (**b**) percentage inhibition. Values are expressed as mean ± SEM (n = 5). (**c**) Survival of mice was monitored using the Kaplan–Meier survival analysis and data were compared by a Log-rank (Mantel–Cox) test

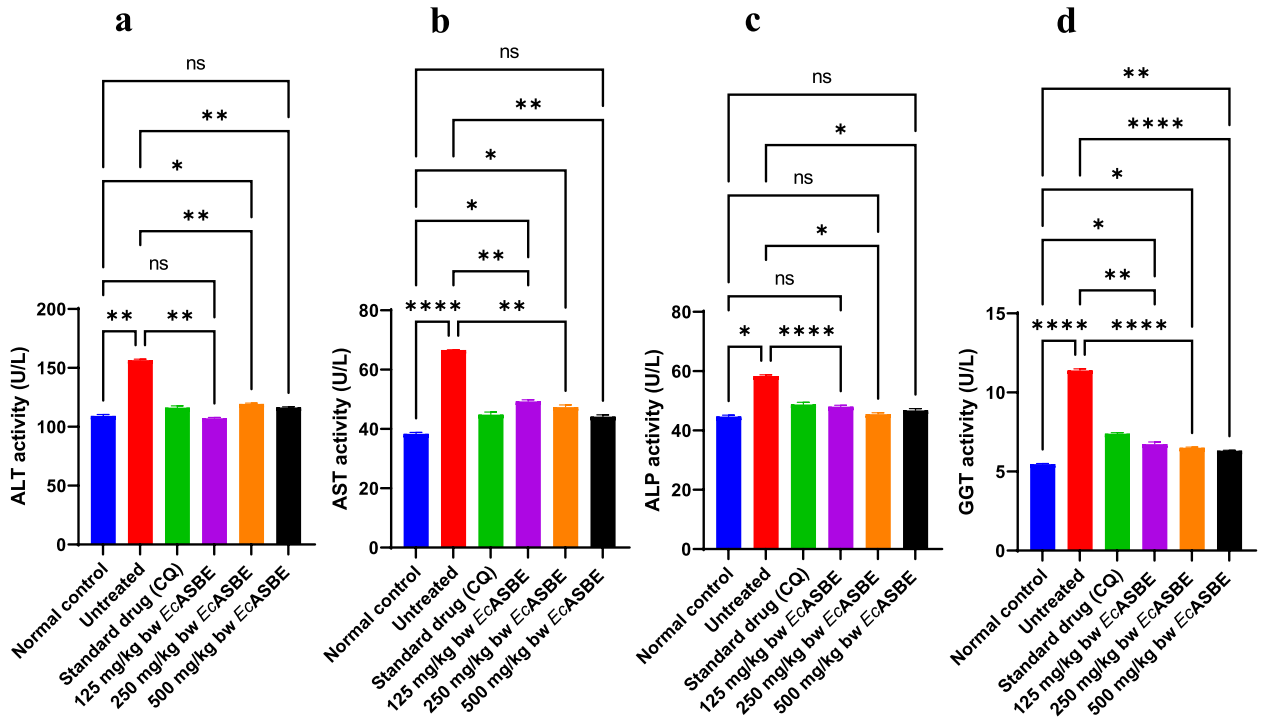

Fig. 5 Effects of *Ec*ASBE on liver indices in *P. berghei*-infected mice. (**a**) alanine aminotransferase (ALT), (**b**) alkaline phosphatase (ALP), (**c**) aspartate aminotransferase (AST) and (**d**) γ-glutamyl transferase (GGT) activities. Values are expressed as mean ± SEM (n = 5). ns: not significant; *,**, and ****: significant at $p < 0.05$

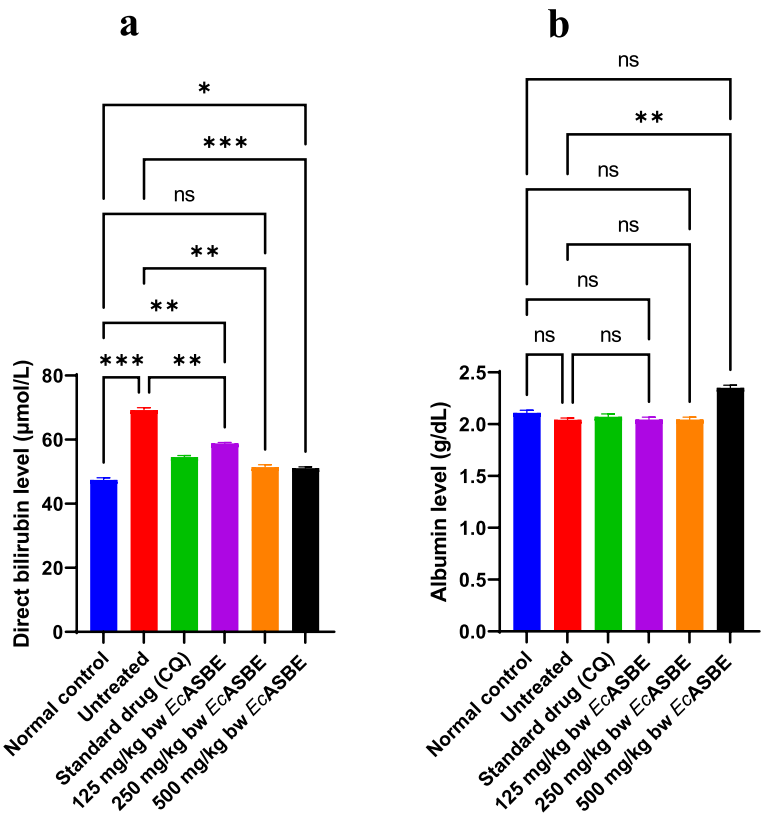

Fig. 6 Effects of *Ec*ASBE on the levels of serum in *P. berghei*-infected mice. (**a**) Direct bilirubin and (**b**) Albumin. Values are expressed as mean ± SEM (n = 5). ns: not significant; *,**, and ***: significant at $p < 0.05$

are identical to the normal control group, especially as concentration increase. These data show that the extract can significantly avert biochemical alterations induced by *P. berghei* infection in the activities of liver enzymes in the animal model.

We also measured the levels of serum albumin and direct bilirubin to evaluate the effect of the extract on *P. berghei*-induced liver dysfunction and the results are presented in Fig. 6. There was an increase in the level of serum direct bilirubin in the untreated mice (Fig. 6a) which was significantly ($p < 0.05$) reduced upon treatment with the extract dose-dependently to a level that is similar to the normal control group. However, comparing the levels of albumin in the treatment groups (125 and 250 mg/kg bw, respectively) and the untreated group, there was no significant ($p > 0.05$) difference (Fig. 6b). These results further validate the ability of the extract to avert biochemical alterations by mitigating liver dysfunction.

Effects of EcASBE on serum kidney indices in P. berghei-infected mice

We investigated the effect of on parasite-induced kidney dysfunction by measuring the levels of serum urea and creatinine in *P. berghei*-infected mice. We observed a significant ($p < 0.05$) increase in the levels of these kidney indices in the untreated mice as depicted in Fig. 7a and b, an outcome which was reversed significantly ($p < 0.05$) upon treatment with the extract in a dose-dependent manner to near normal. These data reflect the potential of the extract to enhance renal function in *Plasmodium* infection.

Effects of EcASBE on the lipid profile and inflammation in P. berghei-infected mice

In a dose-dependent experiment, we evaluated the effect of *Ec*ASBE on lipid profile and inflammation following *P. berghei* infection in vivo. As presented in Fig. 8a-d, there was an elevation in the levels of LDL cholesterol, total cholesterol, triglyceride, and a decrease in the level of HDL cholesterol in the untreated group. Administration of the extract caused a significant ($p < 0.05$) improvement of these parameters dose-dependently to levels that are comparable to the normal control. In a similar vein, the level of TNF-α, a proinflammation marker increased in untreated mice (Fig. 8e). Upon administration of the extract, TNF-α level was significantly ($p < 0.05$) reduced to a level similar to the normal control

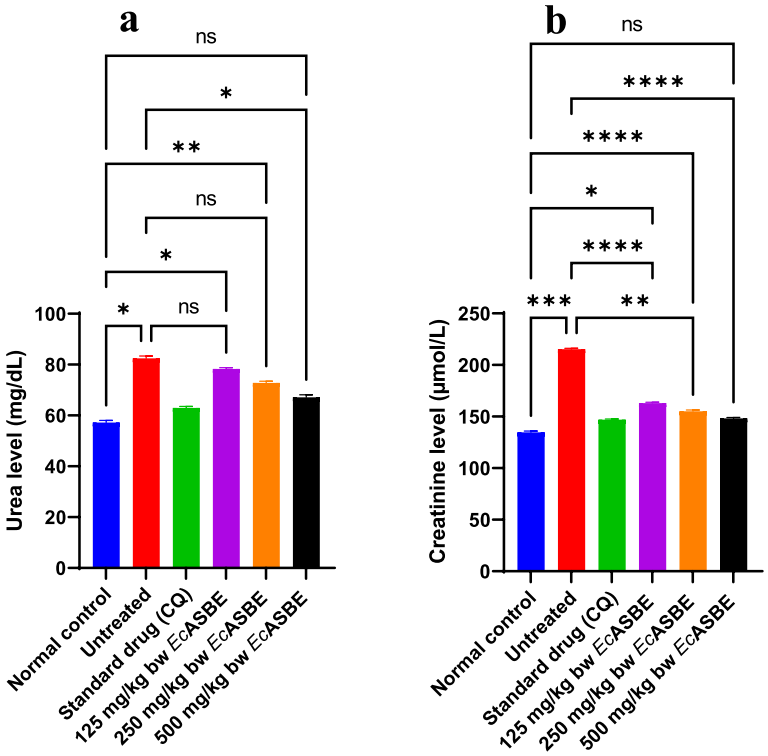

Fig. 7 Effects of *Ec*ASBE on kidney indices in *P. berghei*-infected mice. (**a**) Urea and (**b**) Creatinine levels. Values are expressed as mean ± SEM (n = 5). ns: not significant; *,**,***, and ****: significant at $p < 0.05$

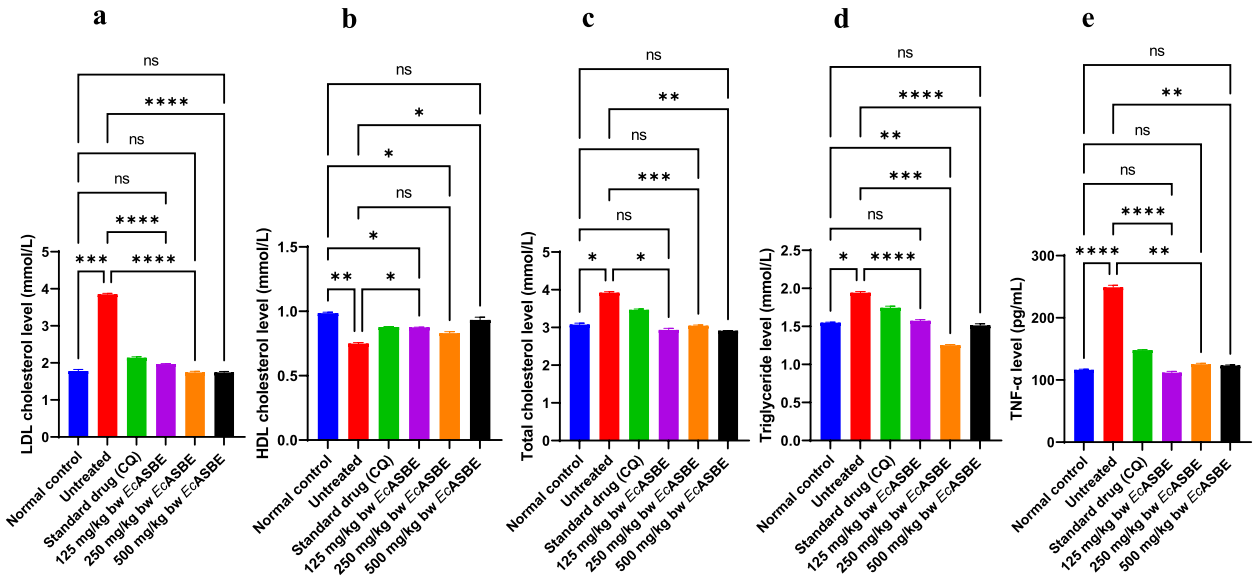

Fig. 8 Effects of *Ec*ASBE on lipid profile (**a**-**d**), and inflammation (**e**) in *P. berghei*-infected mice. (**a**) Low density lipoprotein (LDL) cholesterol, (**b**) High density lipoprotein (HDL) cholesterol, (**c**) Total cholesterol, (**d**) Triglyceride and (**e**) Tumour necrosis factor-alpha (TNF-α) levels. Values are expressed as mean ± SEM (n = 5). ns: not significant; *,**,***, and ****: significant at $p < 0.05$

group. Taken together, these data strongly suggest that the extract has antidyslipidaemic properties and can attenuate inflammatory responses by lowering the levels of proinflammatory cytokine, which is a key factor in pathogenesis of malaria in mouse model.

Effects of EcASBE on redox balance and protein level in tissues of P. berghei-infected mice

Malaria induces oxidative stress in the host. Hence, we investigated the antioxidative effect of *Ec*ASBE in the liver and kidney of infected mice. In this study, oxidative stress was induced in the liver (Fig. 9a-e)

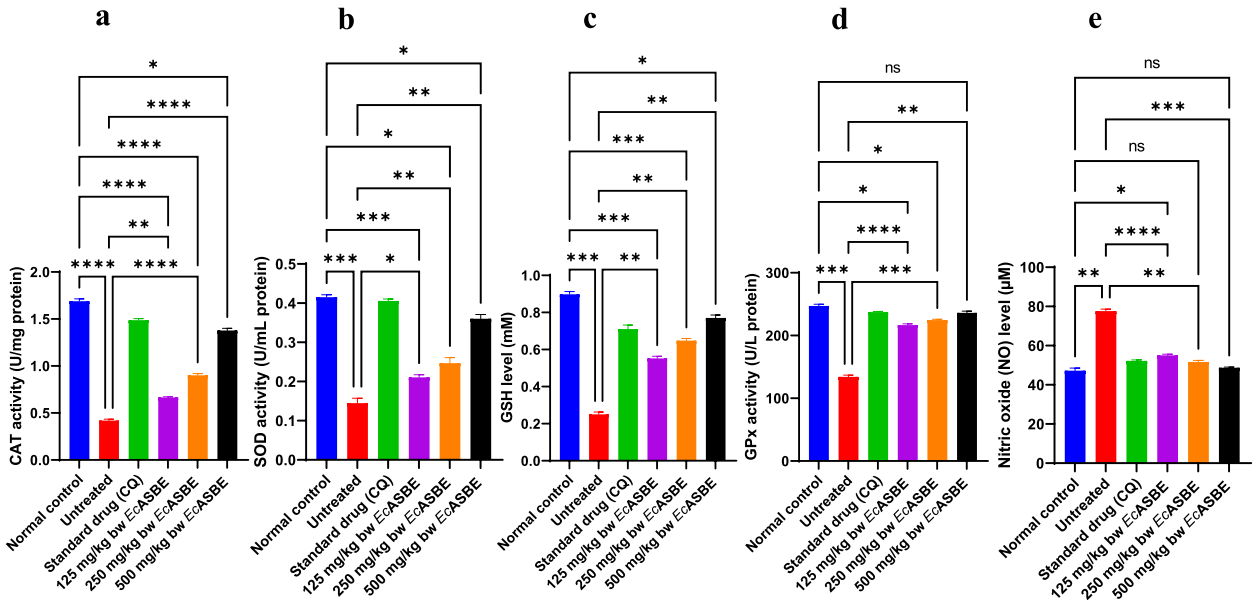

Fig. 9 Effects of *Ec*ASBE on antioxidant status in the liver of *P. berghei*-infected mice. (**a**) Catalase (CAT) activity, (**b**) Superoxide dismutase (SOD) activity, (**c**) Reduced glutathione (GSH) level, (**d**) Glutathione peroxidase (GPx) activity and (**e**) Nitric oxide (NO) level. Values are expressed as mean ± SEM (n = 5). ns: not significant; *,**,***, and ****: significant at $p < 0.05$

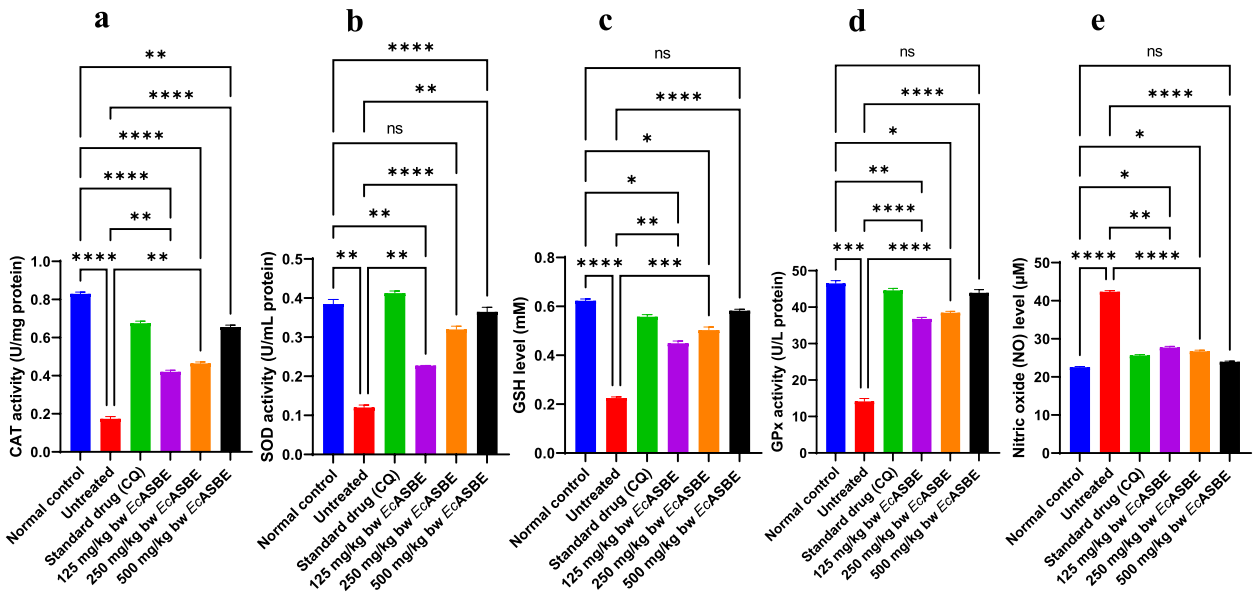

Fig. 10 Effects of *Ec*ASBE on antioxidant status in the kidney of *P. berghei*-infected mice. (**a**) Catalase (CAT) activity, (**b**) Superoxide dismutase (SOD) activity, (**c**) Reduced glutathione (GSH) level, (**d**) Glutathione peroxidase (GPx) activity and (**e**) Nitric oxide (NO) level. Values are expressed as mean ± SEM (n = 5). ns: not significant; *,**,***, and ****: significant at $p < 0.05$

and kidney (Fig. 10a-e) of *P. berghei*-infected mice. This caused a significant ($p < 0.05$) reduction in the levels of CAT, SOD, GPx, and GSH, with a concomitant elevation of NO level, in the untreated group. Following treatment with the extract, the levels of the antioxidants significantly ($p < 0.05$) improved in a dose-dependent manner to levels similar to those of the normal control group.

To further investigate the ability of the extract to improve redox balance, we evaluated the level of lipid peroxidation and protein level in the in the tissues of *P. berghei-infected* mice. Our results show a significant ($p < 0.05$) elevation in MDA level, a biomarker of lipid peroxidation, in the liver and kidney of the untreated group (Fig. 11a and 12a), which is connotative of an increased oxidative stress in the animals in this group. Treatment with *Ec*ASBE significantly ($p < 0.05$) depleted MDA level dose-dependently to levels similar to the normal control group. In addition, an elevation in the protein level was significantly ($p < 0.05$) reversed on treatment with *Ec*ASBE especially at 125 and 250 mg/kg bw *Ec*ASBE, respectively, comparable to the normal control group (Fig. 10b and 11b). But, at the highest dose of *Ec*ASBE (500 mg/kg bw), there was no significant ($p < 0.05$) effect on the protein level. Altogether, these data suggest the antioxidative and antiperoxidative effect of the extract, as well as its ability to maintain protein level in murine model, triggering an enhanced resolution of malaria in the animals.

Relationship between parasitaemia and the activities of the antioxidant enzymes and levels of proinflammation cytokine

Using Pearson correlation coefficients (r), we explored the relationship between parasitaemia and enzyme antioxidant activities in the liver of *P. berghei*-infected mice in the curative antimalarial test, and the results are presented in Fig. 13. The results of the r analysis indicate a negative linear relationship (r = −0.7737, 95% CI −0.9842 to 0.3420) between parasitaemia and the antioxidant enzyme CAT in the liver of *P. berghei*-infected mice (Fig. 13a). In a similar manner, there was a negative linear relationship (r = −0.5606, 95% CI −0.9654 to 0.6364) between parasitaemia and the antioxidant enzyme SOD in the liver of *P. berghei*-infected mice (Fig. 13b). A positive linear relationship (r = 0.2689, 95% CI −0.8042 to 0.9304) between parasitaemia and the level of TNF-α in *P. berghei*-infected mice is shown in Fig. 13c.

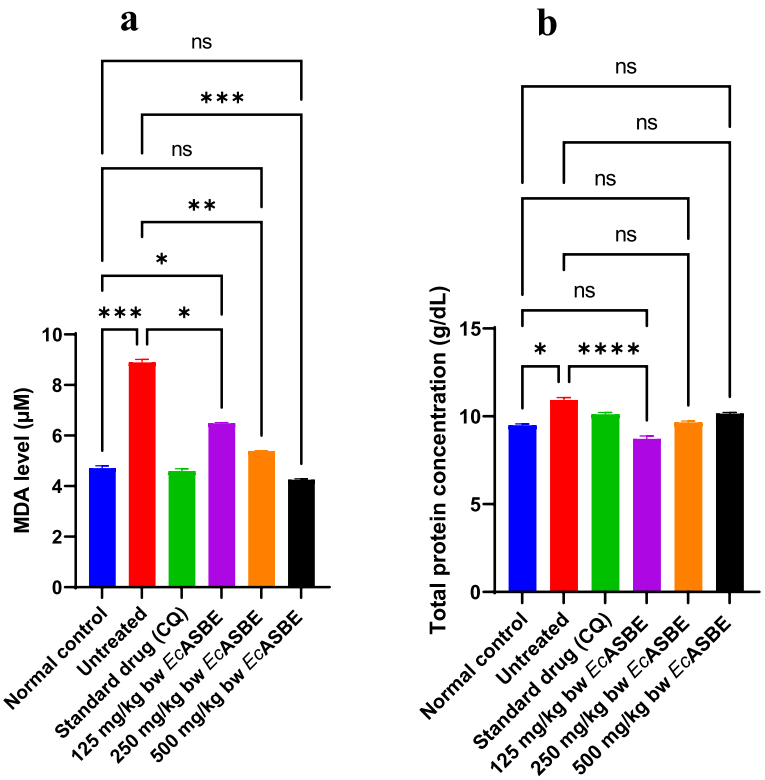

Fig. 11 Effects of *Ec*ASBE on lipid peroxidation and protein level in the liver of *P. berghei*-infected mice. (**a**) Malondialdehyde (MDA) and (**b**) Total protein levels. Values are expressed as mean ± SEM (n = 5). ns: not significant; *,**,***, and ****: significant at $p < 0.05$

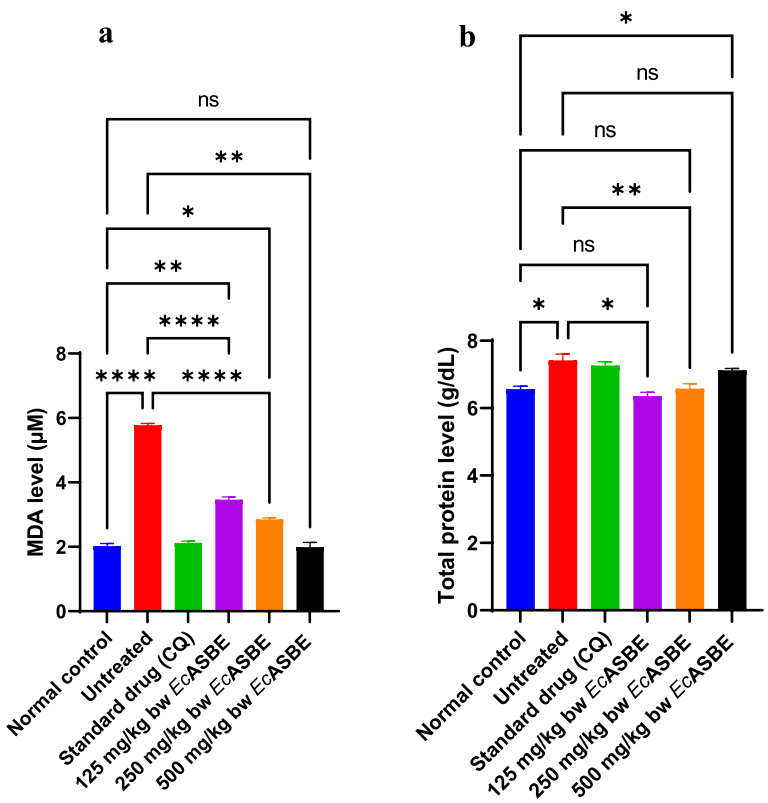

Fig. 12 Effects of *Ec*ASBE on lipid peroxidation and protein level in the kidney of *P. berghei*-infected mice. (**a**) Malondialdehyde (MDA) and (**b**) Total protein levels. Values are expressed as mean ± SEM (n = 5). ns: not significant; *,**,***, and ****: significant at $p < 0.05$

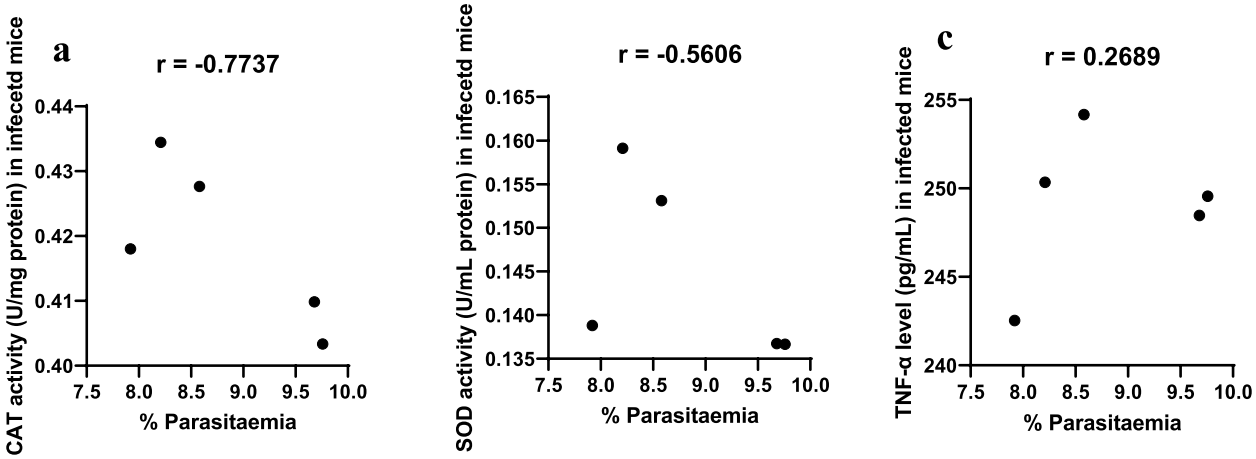

Fig. 13 Correlation between parasitaemia and activities of antioxidant enzymes in the liver and levels of TNF-α in *P. berghei*-infected mice. (**a**) Correlation between parasitaemia and activities of CAT, and (**b**) SOD in the liver of *P. berghei*-infected mice. (**c**) Correlation between parasitaemia and the level of TNF-α in *P. berghei*-infected mice

Effects of EcASBE on haematological parameters in P. berghei-infected mice

Various manifestations of malaria are associated with the asexual blood stage of *Plasmodium* life cycle and the subsequent destruction of the RBC. Generally, *Plasmodium* infection causes haematological alterations in the host [63]. Hence, we directly evaluated the effect of *Ec*ASBE on mouse hematological parameters in an established infection. Mice in the untreated group showed decreased RBC counts (1.16 ± 0.12) compared with the normal

control group and a lower Hb content (7.57 ± 0.21) as against 6.88 ± 0.09 and 11.58 ± 0.18 recorded for both RBC and Hb in the normal control group. The findings indicate the presence of malarial-induced anaemia in mice in the untreated group (Table 6). On treatment with the extract, these parameters were significantly ($p < 0.05$) improved dose-dependently. Also, animals in the untreated group had decreased WBC counts (2380.00 ± 60.83) in contrast to the normal control group (2846.67 ± 68.07). The percentage of other WBC differentials neutrophils and lymphocytes decreased significantly ($p < 0.05$) in the untreated group, and on treatment with the extract these parameters improved significantly ($p < 0.05$) to near normal.

We further investigated the ability of *Ec*ASBE to prevent alterations in haematological indices in *P. berghei* infection. Untreated mice showed decreased RBC counts (1.31 ± 0.05) and a lower Hb content (5.63 ± 0.32) in comparison with 3.07 ± 0.59 and 9.95 ± 0.30 recorded for both RBC and Hb respectively in the normal control group (Table 7). On treatment with the extract, there was a significant ($p < 0.05$) restoration and normalisation of the RBC and Hb levels. Table 7 also showed a significant ($p < 0.05$) increase in the WBC count of animals in the untreated group (4350 ± 132.29) in comparison with the normal control (2333.33 ± 59.38), and a decrease in lymphocyte percentage (23.00 ± 1.00) was observed in the untreated group comparable to the normal control (28.33 ± 1.53). These changes were significantly ($p < 0.05$) improved following extract administration. Malaria infection caused a significant ($p < 0.05$) decrease in neutrophils in the untreated group (59.00 ± 2.00) relative to the normal control (64.67 ± 1.53), with the extract having no significant ($p < 0.05$) effect on neutrophil level.

Furthermore, in the suppressive test, we evaluated if *Ec*ASBE can suppress haematological changes in *P. berghei*-induced malaria in vivo. The data showed a significant ($p < 0.05$) reduction in RBC counts (1.11 ± 0.05) and Hb level (6.13 ± 0.76) in the untreated group, contrary to 2.75 ± 0.33 and 11.00 ± 0.44 recorded for both RBC and Hb in the normal control group (Table 8). A significant ($p < 0.05$) improvement on these parameters was recorded following treatment with the extract, as the dose increased. In addition, malaria infection significantly ($p < 0.05$) raised the WBC count (4733.33 ± 28.87), and decreased the percentage of neutrophils (54.67 ± 0.58) and lymphocytes (21.00 ± 1.00) in the untreated group relative to the normal control group (3016.67 ± 76.38) (Table 8). These alterations were significantly ($p < 0.05$) improved to near normal upon treatment at varying doses. Altogether, the findings may indicate the erythropoietic effect and the ability of the extract to ameliorate alterations in

Table 6 Effects of *Ec*ASBE on haematological indices in *P. berghei*-infected mice in the curative test

Group	Hb (g/dL)	RBC (100/µL)	WBC (µL)	Neut (%)	Lymph (%)
Normal control	11.58 ± 0.18^{e}	6.88 ± 0.09^{e}	2846.67 ± 68.07^{d}	58.00 ± 1.00^{d}	32.67 ± 1.15^{b}
Untreated	7.57 ± 0.21^{a}	1.16 ± 0.12^{a}	2380.00 ± 60.83^{a}	48.33 ± 0.57^{a}	26.67 ± 0.58^{a}
Standard drug (CQ)	11.27 ± 0.60^{de}	6.17 ± 0.56^{d}	2813.33 ± 55.08^{d}	57.00 ± 1.73^{cd}	32.67 ± 0.58^{b}
125 mg/kg bw extract	9.23 ± 0.15^{b}	4.41 ± 0.06^{b}	2386.68 ± 20.22^{ab}	51.00 ± 1.00^{ab}	35.00 ± 1.00^{bc}
250 mg/kg bw extract	9.77 ± 0.38^{bc}	4.48 ± 0.02^{bc}	2563.33 ± 15.28^{bc}	54.33 ± 1.15^{bc}	34.33 ± 1.15^{bc}
500 mg/kg bw extract	10.67 ± 0.21^{cd}	5.12 ± 0.11^{c}	2643.33 ± 70.24^{cd}	55.67 ± 1.53^{cd}	35.33 ± 0.58^{c}

Hb Haemoglobin, *RBC* Red blood cell, *WBC* White blood cell, *Neut* Neutrophils, *Lymph* Lymphocytes. Values are expressed as mean ± SEM ($n = 5$)

Values with different superscript letters along a column for a given parameter are significantly different ($p < 0.05$) from each other

Table 7 Effects of *Ec*ASBE on haematological indices in *P. berghei*-infected mice in the prophylactic test

Group	Hb (g/dL)	RBC (100/µL)	WBC (µL)	Neut (%)	Lymph (%)
Normal control	9.95 ± 0.30^{b}	3.07 ± 0.59^{c}	2333.33 ± 59.38^{a}	64.67 ± 1.53^{d}	28.33 ± 1.53^{c}
Untreated	5.63 ± 0.32^{a}	1.31 ± 0.05^{a}	4350 ± 132.29^{d}	59.00 ± 2.00^{bc}	23.00 ± 1.00^{a}
Standard drug (CQ)	9.77 ± 0.40^{b}	2.07 ± 0.15^{b}	2216.67 ± 57.74^{a}	62.67 ± 1.15^{cd}	28.67 ± 1.15^{c}
125 mg/kg bw extract	9.87 ± 0.49^{b}	2.20 ± 0.08^{b}	3150.00 ± 50.00^{b}	46.33 ± 2.08^{a}	24.67 ± 1.53^{ab}
250 mg/kg bw extract	11.23 ± 0.06^{b}	4.19 ± 0.04^{d}	3650 ± 100.00^{c}	59.33 ± 1.53^{bc}	28.00 ± 1.00^{bc}
500 mg/kg bw extract	11.13 ± 0.49^{b}	3.44 ± 0.19^{c}	3333.33 ± 125.83^{b}	55.33 ± 1.53^{b}	29.33 ± 1.53^{c}

Values are expressed as mean ± SEM ($n = 5$). Values with different superscript letters along a column for a given parameter are significantly different ($p < 0.05$) from each other

Hb Haemoglobin, *RBC* Red blood cell, *WBC* White blood cell, *Neut* Neutrophils, *Lymph* Lymphocytes

Table 8 Effects of *Ec*ASBE on haematological indices in *P. berghei*-infected mice in the suppressive test

Group	Hb (g/dL)	RBC (100/μL)	WBC (μL)	Neut (%)	Lymph (%)
Normal control	11.00 ± 0.44^{c}	2.75 ± 0.33^{bc}	3016.67 ± 76.38^{a}	65.67 ± 3.06^{d}	29.33 ± 1.53^{d}
Untreated	6.13 ± 0.76^{a}	1.11 ± 0.05^{a}	4733.33 ± 28.87^{e}	54.67 ± 0.58^{ab}	21.00 ± 1.00^{a}
Standard drug (CQ)	8.83 ± 0.55^{b}	2.94 ± 0.34^{c}	2900.00 ± 50.00^{a}	59.33 ± 1.53^{bc}	27.00 ± 1.00^{abc}
125 mg/kg bw extract	8.67 ± 0.61^{b}	2.16 ± 0.18^{b}	4100.00 ± 50.00^{d}	52.67 ± 1.53^{a}	28.00 ± 1.00^{cd}
250 mg/kg bw extract	10.20 ± 0.89^{bc}	4.09 ± 0.34^{d}	3533.33 ± 104.08^{c}	58.67 ± 0.58^{bc}	24.67 ± 0.58^{b}
500 mg/kg bw extract	9.73 ± 0.21^{bc}	2.52 ± 0.13^{bc}	3200.00 ± 50.00^{b}	60.67 ± 2.08^{c}	25.67 ± 1.53^{bc}

Hb Haemoglobin, *RBC* Red blood cell, *WBC* White blood cell, *Neut* Neutrophils, *Lymph* Lymphocytes

Values are expressed as mean ± SEM ($n = 5$). Values with different superscript letters along a column for a given parameter are significantly different ($p < 0.05$) from each other

haematological indices in malaria infection in murine model.

Discussion

Malaria continues to pose significant global health challenges especially in Nigeria and other endemic countries, largely because of the spread of drug-resistant *Plasmodium* species, and high cost and inaccessibility of recommended antimalarial drugs [2, 14, 15]. These factors make it urgent to search for cheap and potent antimalarial compounds with unique multiple mechanisms of action to counteract the growing menace of drug-resistant malaria infections.

The phytochemical analysis of *Ec*ASBE revealed an array of phytochemical constituents with flavonoids and alkaloids predominantly present in the extract. Flavonoids have been reported to inhibit the transportation of myoinositol and L-glutamine into *Plasmodium*-infected RBCs during the intraerythrocytic stage of the life cycle of the parasite [64]. Both myoinositol and L-glutamine play significant roles that are critical to the survival and proliferation of the parasite [65–68]. On the other hand, alkaloids act as antimalarial agents by inhibiting the production of protein and halting the conversion of toxic haem which results from the destruction of haemoglobin from being converted into haemozoin, a non-toxic pigment [69]. Collectively, the antimalarial effect of the plant may be attributed to its phytoconstituents.

Alterations in body weight and temperature are important parameters in *P. berghei*-infected mice [70]. The loss of body weight is one of the common characteristics of rodent malaria [71], probably due to loss of appetite, disruption of metabolic activities and hypoglycemia in infected mice [70]. In the three antimalarial tests, *Ec*ASBE significantly averted weight loss and prevented adverse temperature alterations corroborating previous reports [71, 72]. These findings indicate that the plant has the ability to attenuate weight loss and malaria-induced body temperature fluctuations.

The experimental data presented herein underscores a promising and superior antimalarial activity of *Ec*ASBE. In Rane's curative test, the extract reduced the parasitaemia dose-dependently. The parasitaemia inhibition activity of the extract in the curative test was higher than those recorded for both prophylactic and suppressive tests, an indication that it possesses high schizonticidal activity on the proliferative phase of the parasite. Invasion of RBCs alone does not necessarily cause malaria; the production of free radicals [73], activation of phospholipase cascade [74–76], and production of prostaglandins [77, 78], in response to invading pathogens also contribute to disease development. Consequently, the reduced parasite burden in the curative test may be connected with the inhibitory effects of the extract on parasite-growth supporting pathways including the haem detoxification pathway, that are critical to the parasite survival and growth, as well as its production of free radicals [79]. In addition, our data support the preventive ability of the extract, with our findings showing significant parasitaemia reduction. Furthermore, our findings showed that the extract significantly suppressed and inhibited parasitaemia dose-dependently, indicating that the plant could be used in suppressing malaria infection in its early stages. This is suggestive of the ability of the plant to inhibit parasite proliferation and RBC infectivity. Our findings extend those of Abubakar et al. [41] in documenting the antimalarial activities of *E. chlorantha* alkaloids and flavonoids. The lower inhibition activity of the plant in both prophylactic and suppressive tests in comparison with the curative test may be connected to rapid metabolism and clearance of the bioactive ingredients in the extract in the liver before malaria establishment [80]. In addition to inhibiting intraerythrocytic proliferation of the malaria parasite, the extract also improved the survivability of infected mice.

The parasitaemia reduction could be linked to the phytoconstituents, especially alkaloids and flavonoids,

present in the plant, which have been implicated in the inhibition of growth and multiplication of malaria parasites [41, 81–83]. Another plausible explanation is that the extract prevented RBC invasion, inhibited the parasite access to essential nutrients, and/or exerted cytotoxic effect on the parasite [79]. The extract could also have reduced parasite burden by indirectly stimulating the host immune response, thereby altering the properties of iRBCs [84]. Altogether, these data suggest that the extract possesses promising active antimalarial agents and its potential use in developing new antimalarial compounds, while also improving the survival of the animals.

Haematological anomalies are unique features of malaria. The significant reduction in total haematological parameters including PCV percentage, RBC and Hb concentrations, as well as WBC counts and other WBC differentials (lymphocytes and neutrophils) in the untreated group in this study extends the findings of earlier studies in rodent [85, 86], and human [63, 87] malaria. The reduction in RBC indices (RBC count, PCV percentage and Hb concentration) could be linked to invasion and haemolysis of infected RBCs by *Plasmodium* spp., destruction of uninfected RBCs, and the use of Hb as a source of nutrient during the intraerythrocytic stage of *Plasmodium* spp. life cycle [88, 89]. Also, malaria induces defective erythropoiesis, hindering the maintenance of erythropoietic equilibrium [90]. The reduction in WBC count and other WBC differentials in the uninfected group in the Rane's curative test is indicative of weakening of the immune system of the rodent or localisation of WBCs away from the peripheral circulation to the spleen and other marginated pools, instead of outright reduction or inactivity due to the disease [91]. But in the suppressive and prophylactic tests, an elevation in the WBC count was observed. A probable assumption is that the host immune system is still very active in these tests since the disease condition has not been established before the commencement of treatment. In all tests, the restoration of haematological parameters in *P. berghei* infection following treatment with *Ec*ASBE indicates its ability to decrease or avert the invasion of uninfected RBCs by the parasite, eliminate the parasite from iRBCs before haemolysis, improve erythropoiesis, and inhibit or decrease intraerythrocytic development of the parasite [92]. Considered together, the results imply that the plant can ameliorate haematological alterations caused by malaria infection.

Malaria is a highly inflammatory and oxidative disease. As a result of increased production of reactive oxygen and nitrogen species (ROS and RON) during *Plasmodium* spp. infection, the endogenous antioxidant defense of the host is compromised, resulting in redox imbalance and oxidative stress, a generalised occurrence in *Plasmodium* spp. infection [93, 94]. Increased ROS/RNS production occurs due to the degradation of haemoglobin of the host by the parasite [95, 96], and also through the host immune response which leads to the production of ROS/RNS inside the phagosomes through a mechanism known as respiratory or oxidative burst [10]. The extracellular release of ROS generated during the respiratory burst also contributes to an increase in the oxidative state in the infected host [97].

The depleted levels of GSH, SOD, CAT and GPx, and a simultaneous rise in NO level, depicts an occurrence of oxidative damage in the tissues and/or proinflammation following *P. berghei* infection in mice. This is consistent with earlier findings on the dysregulation of these oxidative indicators in malaria [98–100]. The induction of oxidative stress leads to increased production of superoxide ($O_2^{\bullet-}$) through the Fenton (or Haber–Weiss) reaction [94]. In order to detoxify the microenvironment of the generated $O_2^{\bullet-}$, the host increases the activity of SOD, the key $O_2^{\bullet-}$ antioxidant defense system [101, 102]. However, if the elevated $O_2^{\bullet-}$ are not converted by SOD to hydrogen peroxide (H_2O_2) it can react with NO to generate peroxynitrite ($ONOO^-$), a potent radical which promotes oxidation [103] and plays a significant role in proinflammation [104]. Thus, the increased NO level with a simultaneous depleted SOD activity underscores induction of oxidative stress in the untreated group. Also, the rise in MDA level in the untreated group indicates an incidence of lipid peroxidation, following the induction of oxidative damage. Hence, the improvement of these parameters on treatment with *Ec*ASBE indicates that the plant possesses the potential to mitigate lipid peroxidation and restore redox imbalance, supporting earlier reports on the antioxidant protective effect of plants [29, 105, 106]. Altogether, these findings showed the antioxidative and antiperoxidative effect of the extract which could be connected with the phytochemicals, especially flavonoids and alkaloids, present in the plant [107, 108].

Abnormalities in liver function are relatively frequent findings in malaria mainly due to its involvement in the life cycle of *Plasmodium* spp. These abnormalities are usually transient and are primarily represented by elevations in the serum or plasma level of liver enzymes ALT and AST activities, and to a lesser extent, by elevations in the activities of ALP and GGT [8]. Increased activities of these enzymes can indicate irritation, injury or damage to the liver, associated with cellular integrity, hepatic inflammation, and cellular obstruction, which may have caused the enzymes to leak out into the blood [109]. The current study shows elevated activities of ALT, AST, ALP

and GGT in *P. berghei*-infected untreated mice, an indication of hepatocellular dysfunction, supporting earlier reports [8, 93, 110, 111].

Bilirubin, a major breakdown product of haemoglobin, is usually metabolised in the liver with its accumulation in serum indicates hepatocellular dysfunction; hence, measuring the level of serum bilirubin also serves as a useful marker of liver function [112]. Hepatocellular dysfunction induced by *P. berghei* infection was further confirmed by the significantly hyperbilirubinemia in the untreated group while the infection had no effect on the level of serum albumin in the animals. The hyperbilirubinemia in the untreated group could be a product of elevated haemoglobin catabolism [113] or hepatocellular damage by the parasite [114], with a resultant impairment in bilirubin uptake and excretion. Hepatocellular damage from malaria has been linked with oxidative stress. Therefore, the evidential improvement of the hepatic status in *Ec*ASBE-treated animals implies that the plant has hepatoprotective effect, which may be connected with its antioxidant phytoconstituents.

Impairment of renal function in malaria is adequately reported [115, 116], and this could be linked to vascular dysfunction as a result of the degradation of the endothelial glycocalyx mediated by *Plasmodium*-induced oxidative stress [117, 118]. In the current study, the incidence of renal dysfunction was confirmed through elevated levels of serum creatinine and urea in the untreated group. Such dysfunctions are connected with fatal complications in malaria, contributing to high morbidity and mortality [116, 119]. The antioxidant potential of *Ec*ASBE with its ability to scavenge initiators of lipid peroxidation, preserving the structural integrity of the kidney membrane and enhancing renal function, are demonstrated by the improvement in serum creatinine and urea levels of *Ec*ASBE-treated groups. These findings support the renoprotective activity of the plant during malaria.

Dysregulation of lipid metabolism is connected with infection, inflammation and oxidative stress [120]. In the present study, a significant change in *P. berghei*-infected untreated mice was the augmentation of serum total cholesterol, LDL cholesterol, and triglyceride, with a depletion in HDL cholesterol level, suggesting a dysregulation of the host lipogenesis because of the parasitic infection. Evidence show that malaria parasites can manipulate the lipid metabolism pathways of the host, since they cannot synthesise several classes of lipids that are essential for their development and replication [121, 122]. Dyslipidaemia could have been caused by increased lipoprotein synthesis and its absorption, and/or a decrease in its breakdown. Elevated serum levels of total cholesterol, LDL cholesterol, and triglycerides may be the result of increased synthesis and absorption to make up for the host serum lipids that the parasite uses for metabolic functions. The reduced HDL level in the untreated mice may have resulted from a decrease in the transfer of cholesterol, and/or from the parasite inhibiting the liver enzymes that metabolise lipids, including the use of lipids in the synthesis of haemozoin [121]. The plant demonstrates antidyslipidaemic properties by significantly improving the lipid profile relative to the untreated group; a finding that is consistent with previous reports [123, 124]. Antidyslipidaemic properties of the plant may be linked to the phytoconstituents in the extract, which may have modulated oxidative damage on the hepatocytes [125–127].

The pathophysiology of malaria is usually connected with a polyclonal activation of the immune system which manifests in exacerbated production of proinflammatory cytokines including IFN-γ, TNF-α, IL-1, IL-6, IL-8 and IL-12 [128, 129]. In agreement with the above, our findings show that malaria infection markedly raised the level of TNF-α in the untreated group; evidence of inflammation in the infected animals. TNF-α is produced by various cells including T and B lymphocytes, macrophages, and mast cells [130], and its expression is critical in the pathogenesis and pathophysiology of malaria, with levels of the proinflammatory cytokine correlating with disease severity [131]. The increased TNF-α level in the untreated group was significantly improved after extract administration, supporting earlier findings on the anti-inflammatory activity of plants [84, 106]. The result is implicative of the anti-inflammatory property of the plant, which may be attributed to its phytoconstituents [132, 133].

Conclusion

The emergence and spread of drug-resistant malaria severely threaten malaria management globally, making it more imperative to develop novel antimalarial compounds that are cheap and effective in the treatment of drug-sensitive and drug-resistant malaria, respectively. Our findings not only demonstrate the antimalarial action of *Ec*ASBE, but the antioxidative, anti-inflammatory, hepatoprotective and nephroprotective properties in a murine malaria model. Collectively, our findings warrant further studies to explore the antimalarial compounds of the extract.

Abbreviations

ACTs	Artemisinin (ART)-based combination therapies
*Ec*ASBE	*Enantia chlorantha* aqueous stem bark extract
CAT	Catalase
GPx	Glutathione peroxidase
GSH	Glutathione
MDA	Malondialdehyde
NO	Nitric oxide
SOD	Superoxide dismutase
ALP	Alkaline phosphatase

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GGT	Gamma-glutamyl transferase
IAMRAT	Institute for Advanced Medical Research and Training
OECD	Organization for Economic Cooperation and Development
ARRIVE	Animal Research: Reporting of In Vivo Experiments
iRBCs	Infected red blood cells
Hb	Haemoglobin
PCV	Packed cell volume
RBC	Red blood cell
WBC	White blood cell
Neut	Neutrophils
Lymph	Lymphocytes
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
TNF-α	Tumour necrosis factor-alpha
IFN-γ	Interferon gamma
IL	Interleukin
MST	Mean survival time
r	Pearson correlation coefficients

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Authors' contributions
IOE, OSA, OMO: Methodology, Conceptualization, Project administration, Investigation, Writing–original draft, Writing—review & editing. OSA, OMO: Supervision. All the authors listed in this paper have read and approved the final version of the submitted manuscript, and agreed to be accountable for the content of the work.

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Data availability
The data generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The collection of plant followed relevant guidelines and regulations outlined in the International Union for Conservation of Nature (IUCN) Policy Statement on Research Involving Species at Risk of Extinction, the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora. All animal studies were performed humanely and consistent with the Guidelines for the Care and Use of Laboratory Animals [47], and the National Research Council Guide for the Care and Use of Laboratory Animals [134]. Approval for the study was obtained from the Landmark University Research Ethical Review Committee (LUAC/BCH/2023/0002A). Consent to participate was not applicable. The study was reported in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0 [135].

Consent for publication
Not applicable.

Competing interest
The authors declare no competing interests.

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