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Examination of the lipid profile, antioxidant capacity, and impact of *Sida linifolia* L. extract on the liver and kidney functions of mice infected with malaria

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The study aimed to investigate the impact of treatment with the ethanolic leaf extract of *Sida linifolia* (ELES) in malaria-infected mice and the effect of the extract on tissues and organs. Phytochemical screening was conducted, and the liver and kidney function parameters, lipid profile, antioxidant parameters, and electrolyte status were determined. From the findings of the study, tannins, saponins, flavonoids, steroids, and other phytochemicals were detected. The activities of alkaline phosphatase, aspartate aminotransferase, superoxide dismutase, and total protein, triacylglycerol, high-density lipoproteins, and electrolytes concentrations of the infected animals treated with different doses of the extract were significantly (p <0.05) higher compared to the untreated group (positive control). Conversely, the levels of creatinine, urea, total bilirubin, total cholesterol, low-density lipoproteins, and catalase activity of the treated groups were significantly (p <0.05) lower than those of the positive control. The malondialdehyde and reduced glutathione concentrations of the treated group showed a non-significant (p >0.05) difference compared to the positive control. Overall, these findings suggest that the ethanolic leaf extract of *S. linifolia* exerted an antioxidant effect, but some perturbations in the electrolyte concentrations and the elevated levels of the liver enzymes could mean some potential stress to some tissues and the liver, so care should be taken in the administration of the agent.

Keywords: Biomarkers, Kidney function, Liver function, Malaria, Phytochemicals, Toxicity

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Introduction

The rising resistance of *Plasmodium species* to current malaria interventions necessitates a persistent quest for novel antimalarial agents. A potential avenue for discovering new antimalarial agents lies in natural sources such as *Sida linifolia*. The plant *S. linifolia* is a member of the Malvaceae family, and the genus *Sida* is a widespread weed that can be found in dry woodland areas of West Africa and other parts of the world. The *Sida* genus encompasses over 200 weed species, many of which have various ethnomedicinal uses[1]. The plant is also known by different common names, including flax leaf, fan petals, and balai grand. It has a few synonyms, such as *Malva hirsuta*, *Sida angustissima*, and *Sida campii*.

The liver, one of the body's essential organs, is principally in charge of metabolism and detoxification. Several illnesses are linked to abnormal liver function, including cirrhosis, fibrosis, non-alcoholic fatty liver, hepatitis, and hepatocellular cancer. Thus, to evaluate the severity of liver illnesses, biomarkers are necessary[2]. Increased blood levels of liver enzymes, such as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, signify a disruption in the liver's normal function[3]. Nevertheless, new information linking synthetic drugs to a host of undesirable side effects has accelerated the search for naturally occurring sources of more potent and less dangerous drug candidates- ideally derived from plants[4]. By developing the genetic capacities and essential enzymatic pathways needed to generate a variety of classes of pharmacologically powerful chemicals to support their survival, plants have adapted to flourish in their environment. Medicinal plants are an excellent source of phytoactive chemicals that have a variety of pharmacological applications[4].

Nevertheless, the kidney is another organ that plays vital roles in our body; it is responsible for filtration, balancing the body's fluid, regulating blood pressure, and producing erythrocytes[5]. Sequel to these vital

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roles of the kidney, biomarkers that will assess its proper function are required. These biomarkers, such as creatinine and urinary output, provide valuable information about kidney injury, disease progression, and response to treatment[6]. Plant extracts have been proven to have an ameliorative effect on liver and kidney biomarkers[7-9].

Finding anomalies in blood lipid concentrations, such as triglycerides and cholesterol, is the aim of a lipid profile panel of blood tests[10,11]. It provides vital information about a person's chance of getting cardiovascular disease. Furthermore, several studies have demonstrated that high levels of High-Density Lipoproteins (HDL) relative to Low-Density Lipoproteins (LDL) can prevent coronary heart disease, while increased levels of total or low-density lipoprotein (LDL) cholesterol in the blood are strong risk factors[11]. Nevertheless, the foundation of medicinal herbs is the idea that certain plants have inherent compounds that can both heal and prevent disease. Several herbs, including *Curcuma longa* L., *Coleus forskohlii*, *Fucus vesiculosus, Rosmarinus officinalis* L., and others, have been proven to have ameliorative effects on blood lipid levels[10].

Investigators have provided evidence of the biological effects and therapeutic uses of certain species belonging to the *Sida* genus, such as *S. cordifolia, S. rhombifolia*, and *S. acuta*[12,13,1]. However, studies on the bioactivities of S. *linifolia* are still limited. Based on the information we have collected, traditional healers utilize alcoholic or aqueous mixtures of the leaves for various purposes such as treating malaria, depression, enhancing sexual desire, lowering blood pressure, preventing abortions, and managing painful whitlow[14]. Recently, we have conducted preliminary studies on the antimalaria potential of the leaf extracts, but the protection and safety of the agent on vital tissues and organs were not determined. Therefore, building on previous research, the current study aimed to investigate the antioxidant and impact of treatment with ethanolic leaf extract from *S. linifolia* (ELES) on some vital organs of malaria-infected mice.

Materials and Methods

Plant collection and identification

Fresh leaves of *S. linifolia* were collected in April 2023 (during the rainy season) from Nsukka town in Nsukka Local Government of Enugu State, Nigeria. A taxonomist named Alfred Ozioko identified the plant at the Bio-resources Development and Conservation Program Research Centre in Nsukka, Enugu, Nigeria, using fresh leaves of *S. linifolia* that were obtained from Nsukka LGA. The plant sample was preserved in a herbarium (BDCP2021724).

Chemicals and equipments

Standard-grade chemicals and reagents utilized in this research were purchased from reliable suppliers and medical supply outlets both domestically and abroad.

Most of the equipment was sourced from the University of Nigeria, Nsukka's Department of Biochemistry, while some were obtained from scientific shops of reputable dealers of scientific equipment in Nsukka, Enugu State.

Extraction procedure

With a few minor adjustments, the extraction procedure was carried out using the methodology described by Zhang *et al.*[15]. Succinctly, five days of shade drying followed by a cautious washing of *S. linifolia* leaves in clean water. Using an electric blender (High-Speed Grinder, China), a known mass (2000 g) of the ground leaves was combined. In a tightly corked conical flask, the ground leaves were macerated for 48 hours in 3.2 L of 100% ethanol. This was to mimic the practice of traditional healers in the use of the plant for the treatment of ailments such as malaria. Once that was done, Whatman paper was used to filter it. Using a soxhlet extraction setup, the filtrate's ethanol content was further evaporated until a constant mass was achieved, yielding a dark green, viscous mass of *Sida linifolia* (ELES) ethanolic extract. The resulting crude extract was kept chilled at 4°C in a screw-capped tank with a labelled, sterile container until it was required for the investigation.

Experimental animals

For the investigation, a total of eighteen (18) Swiss mice weighing between 22 and 36 g of both sexes were utilized. They were procured from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Subsequently, acclimatization of the animals was performed for 7 days; before the commencement of the experiment, the animals were provided with unrestricted access to food and water in regular settings. The mice were then randomly split into six groups, with three mice each, kept in a separate aluminium cage that was compartmentalized. They were also given access to room-temperature water and standard pelletized feed (Chukun finisher).

Ethical clearance

Permission and ethical approval for the responsible use of laboratory animals and the safe conduct of experimental procedures were provided by the University Ethics Commission of the Department of Biochemistry, University of Nigeria Nsukka with the approval number UNN/BCH/9020 (10/03/2022). International protocols for the Care and Use of Laboratory Animals in Biomedical Research were followed throughout all experimental procedures. The animals were humanly handled throughout the period of the experiment.

Preliminary phytochemical screening

The preliminary phytochemical screening was performed using standard techniques, as described by Trease and Evans[16] and Harborne[17]. The following formulae (Eqns. 1–3) were used to determine the concentration of the different classes of phytochemicals.

$$\text{Yield}\left(\frac{\text{g}}{100\ \text{g}}\right) = \frac{\text{Initial weight}}{\text{Resultant weight}} \times 100 \quad \ldots (1)$$

$$\text{Concentration}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Dilution factor} \quad \ldots (2)$$

$$\text{Dilution factor} = \frac{\text{Total volume}}{\text{Weight of extract}} \quad \ldots (3)$$

Experimental design

Plasmodium berghei parasite injection was used to induce malaria in mice, and the extract (ELES) was given orally at doses of 100, 200, and 400 mg/kg b.w. The mice were separated into six groups (n=3/group 6), each of which received a different combination of treatments. The experimental design is thus stated: Group 1: Administered with normal saline (vehicle) only (Normal control); Group 2: Inoculated with malaria parasite without treatment (Positive control); Group 3: Inoculated with malaria parasite and treated with 80 mg/kg b.w. Artesunate (Standard control); Group 4: Inoculated with malaria parasite and treated with 100 mg/kg b.w. of ELES; Group 5: Inoculated with malaria parasite and treated with 200 mg/kg b.w. of ELES; and Group 6: Inoculated with malaria parasite and treated with 400 mg/kg b.w. of ELES

Sample collection and preparation

For 3 days, the animals in each group received different treatments through oral intubation. Using an inhalation jar of diethyl ether, all the animals were euthanized after twenty-four hours following the final treatment. They were then sacrificed by cervical dislocation, and venipuncture blood samples were taken into specimen bottles that had been heparinized to assess the biochemical status. Additionally, the animals' liver organs were taken and processed to generate liver enzymes.

Determination of liver function biomarkers

Determination of alkaline phosphatase (ALP) activity

This method's basic principle is that phenolphthalein monophosphate, a colourless substrate, interacts with alkaline phosphate to form phenolphthalein and phosphoric acid, which becomes pink at alkaline pH values and can be measured spectrophotometrically. A volume of 0.05 mL of the sample was pipetted into the duplicate test tubes containing the blank and sample. A pipette was used to fill the blank tube with 0.05 mL of distilled water. Pipetting 3.0 mL of the substrate into each tube, mixing it, and measuring the absorbance at 405 nm was the next step. The absorbance of the sample and the blank was measured thrice at one-minute intervals after the stopwatch was set in motion. The following formula was used to compute the alkaline phosphatase activity:

$$\text{ALP activity U/mL} = A \times V \times T$$

Assay of aspartate aminotransferase (AST) activity

The Randox kit's (AS3604) Reitman and Frankel[18] method was used to measure the activity of aspartate aminotransferase (AST).To measure aspartate aminotransferase activity, the following data about oxaloacetate hydrazone with 2, 4-dinitrophenylhydrazine were observed. Pipettes were used to transfer 0.5 mL of the AST substrate phosphate buffer solutions into the test tubes designated for the sample (T) and reagent blank (B). Only T tubes received the 0.1 mL serum sample, which was introduced and well mixed. Then, B received an addition of 0.1 mL of distilled water. After thoroughly mixing the entire reaction media, it was incubated for 30 minutes at 37°C in a water bath.

After the incubation period, T and B were each given 0.5 mL of 2, 4-dinitrophenylhydrazine. It was thoroughly mixed and then allowed to stand at 25°C for exactly 20 minutes. Finally, the test tubes holding the reagent and the blank were filled with 5.0 mL of NaOH solution and carefully mixed. The absorbance of T was measured at 550 nm in comparison to B after 5 minutes.

Both the sample test (T) and sample blank (B) test tubes received 0.5 mL of each of the AST substrate phosphate buffer solutions by pipetting. Test tubes designated for sample test (T) were filled with a 0.1 mL serum sample, which was then well mixed. Subsequently, the B was filled with 0.1 mL of distilled water. The reaction mixture was thoroughly mixed and then incubated for 30 minutes at 37°C in a water bath. The T and B test tubes were filled with 0.5 mL of 2, 4-dinitrophenylhydrazine shortly after incubation. Additionally, B was given 0.1 mL of the sample. The medium was mixed and then allowed to stand at 25°C for exactly 20 minutes. Finally, 50 mL of sodium hydroxide (NaOH) solution was added to the T and B test tubes and thoroughly mixed. Five minutes later, at 550 nm, the absorbance of T was measured about that of B.

Activity assay for alanine aminotransferase (ALT)

The Randox kit's instructions (AL1105) for the Reitman and Frankel[18] method were used to measure the activity of ALT. The amount of pyruvate hydrazone generated by 2,4-dinitrophenylhydrazine can be used to calculate the ALT activity. Just 0.5 mL aliquots of the ALT substrate phosphate buffer solutions were pipetted into two sets of test tubes, labelled B (sample blank) and T (sample test). A 0.1 mL serum sample was added to the T-test tubes alone, thoroughly mixed, and then incubated for 30 minutes at 37°C in a water bath. Test tubes T and B received 0.5 mL of 2, 4-dinitrophenylhydrazine after the incubation. Additionally, only the B received 0.1 mL of the serum sample. After carefully mixing the entire medium, it was left to stand at 25°C for precisely 20 minutes. Subsequently, each test tube was filled with 5.0 mL NaOH solution and thoroughly mixed. The absorbance of the T was measured at 550 nm with regard to the B after 5 minutes.

Determination of total protein concentration

The Lowry *et al.*[19] method was utilized to ascertain the total protein concentration. Bovine serum albumin (BSA) in various concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µL) was placed in eleven sets of test tubes arranged in a rack. Two milliliters of solution D (48 milliliters of 2% $NaCO_3$ in 0.1 N NaOH, one milliliter of 1% NaKTartarate in H_2O, and one milliliter of 0.5% $CuSO_4.5H_2O$ in H2O) in a known volume. Ten minutes were spent letting the mixture settle at room temperature. Each test tube receives an aliquot of 0.2 mL of diluted phenol, which is added right away and vortexed. Following a 30-minute incubation period, the absorbance measurements at 600 nm were made.

Determination of total bilirubin concentration

According to the instructions in the Randox kit (BR3749), the Jendrassik and Grof[20] method was used to calculate the total bilirubin concentration. Sulfanilic acid (0.2 mL) was pipetted into the sample blank tube and the sample tube. Next, 0.05 mL of sodium nitrite was quickly added to the sample tube. Exactly 10 mL of coffee and 0.2 mL of sample were pipetted into each sample blank tube and sample tube. The mixes were mixed and then incubated for 10 minutes at 20 to 25°C. Finally, tartrate (1.0 mL) was pipetted into the sample and blank tubes. The mixes were mixed once more, and after 30 minutes of incubation at 20–25°C, their absorbances at the sample blank wavelength of 578 nm were measured.

Total bilirubin (µmol/L) = 185 × sample blank (578 nm)

Normal Range = 17 µmol/L

Determination of direct bilirubin concentration

This was carried out using the Jendrassik and Grof[20] method, which is described in the kits for Randox assays (BR3804). Test tubes were set up for sample and blank, respectively. A volume of 200 µL of Sulphanilic acid (which contains Hydrochloric acid) was dispensed into the appropriate tube. One drop of Sodium Nitrite was dispensed into a sample test tube. Normal saline (2000 µL) was pipette into both tubes and mixed. A volume of 200 µL of sample was dispensed into both tubes. For ten minutes, the mixes were incubated at room temperature. All tubes were filled with distilled water (1000 µL) and then incubated further for five minutes, after which a spectrophotometer was used to measure absorbances at 540 nm.

The bilirubin concentration in milligrams per deciliter was determined by multiplying the absorbance by a factor of 14.4.

Evaluation of renal function indices

Determination of serum creatinine concentration

The Jaffe method of Tietz[21] was used to measure serum creatinine levels with a few modest adjustments. Based on alkaline picrate, the Jaffe creatinine technique was developed. A combination known as creatinine-picrate is created when creatinine in the sample combines with picrate at an alkaline pH. There exists a clear correlation between the concentration of creatinine in the sample and the pace

at which the absorbance increases at 500 nm due to the development of this complex.

Determination of serum urea concentration

In accordance with Tietz[21], the Urease-Berthelot method was also employed to ascertain the serum urea levels. Urea is hydrolyzed by urease to produce ammonia and CO. The resulting ammonia then combines with hypochlorite and a phenolic chromogen to generate a green complex that may be detected by spectrophotometry.

Evaluation of oxidative stress biomarkers

Determination of superoxide dismutase (SOD) and catalase (CAT) activities

The techniques of Fridovich[22] and Xin *et al.*[23] were used to measure the activities of SOD and CAT, respectively. After being cleaned, some of the liver tissue that had been removed from the sacrificed mice was homogenized in a 1:10 (w/v) suspension of ice-freeze Tris buffer (50 mmol/L) at a pH of 7.4. After the combination was spun for 20 minutes at 10,000 × g at 4°C, the activities of important liver antioxidant enzymes were measured in the supernatant.

The Glutathione (GSH) level was ascertained using Ellman's [24] approach.

Evaluation of lipid profile

Whereas the approach provided by Allain *et al.*[25] was used to determine the amount of total cholesterol (TChol), the level of triacylglycerol (TAG) was estimated using the Bucolo and David[26] method. Demacker *et al.*[27] and Allain *et al.*[25] described the Precipitation and Cholesterol technique, which was used to determine the High-density lipoprotein (HDL) level. Using Friedewald's *et al.*[28] equation, the low-density lipoprotein (LDL) level was also determined.

$$\mathrm{LDL-C\ (mmol)} = \mathrm{TC - HDL - VLDL}$$

$$\mathrm{VLDL - C(mmol)} = \frac{\mathrm{TG}}{2.2}$$

Determination of serum electrolytes

Determination of sodium and potassium ion

The method of estimating sodium ions (Na^+) and potassium ions (K^+) through flame emission photometric analysis works based on the following principle: putting a liquid sample through an atomizer or nebulizer causes the atoms to become excited. As they return to their ground state, they emit light with distinct wavelengths, colours, and intensities, which, when passed through a suitable filter and detected by a photosensitive detector, directly correspond to the concentration of the atom (metallic ion) present.

After setting the flame photometer, de-ionized water was used to normalize the measurement, and then standards of Na ions at known concentrations were run through and their readings recorded. The serum samples were sent through the nebulizer one after the other, and readings from each detector were recorded. We always used de-ionized water to reset the meter to zero before getting another reading.

Determination of bicarbonate

Labelled as standard and sample, two test tubes were present. All test tubes were pipetted with 1.0 mL (one millilitre) of bicarbonate reagent. Three minutes at 37ºC were spent incubating the test tubes. Following incubation at 340 nm, the absorbance was measured.

Calculation of bicarbonate activity:

$$CO^2 + H_2O \rightleftharpoons H_2CO_3$$

Determination of chloride ion

A standard chloride calibrator and 10 µL of material were placed into separate test tubes. Each test tube received one (1 mL) of chlorine reagent, which was added, mixed, and incubated for five minutes at 25°C. At 500 nm, the absorbance was measured.

Calculation:

$$NaCl \rightleftharpoons Na^+ + Cl^-$$

Statistical analysis

Version 23 of the Statistical Product and Solution Service (SPSS) was used to analyze the collected data. ANOVA was used to compare the data across the various groups and determine whether the differences were significant at p <0.05. The least significant difference (LSD) test post hoc was used for mean value separation and comparison.

Results

Percentage yield

The percentage yield of ethanol extract from *S. linifolia* leaf was 27.42%.

Phytochemical compositions of *S. linifolia* leaf extract

Table 1 displays the findings on the quantitative phytochemical content of the ethanolic leaf extract of *S. linifolia* (ELES). Important classes of phytocompounds, including tannin, phenolics, flavonoids, cyanogenic chemicals, saponin steroids, alkaloids, glycoside, and terpenoids, were found in varying amounts.

Liver function parameters

Effect of ELES on liver enzymes' activities and protein concentration

Table 2 displays the impact of ELES on the concentrations of total protein, total bilirubin (TBIL), and direct bilirubin (DBIL), as well as the liver enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total protein.

The ALP activity was highest (32.33±0.331 IU/L) in the group that was not inoculated with the malaria parasite and received normal saline treatment, also referred to as the normal control. The lowest activity (25.67±3.528 IU/L) was observed in the group that received the malaria parasite and was not treated, also known as the positive control. All groups receiving extract therapy had significantly (p <0.05) greater ALP activity on the third day post-treatment compared to the positive control.

The standard control group, which consisted of infected mice treated with 80 mg/kg b.w. of artesunate, had the lowest AST activity (5.00±2.52 IU/L), while group 6 (infected mice treated with 400 mg/kg b.w. of ELES) had the highest AST activity (12.33±0.67 IU/L). On the third day following treatment, all groups treated with extract exhibited significantly (p <0.05) increased AST activity in comparison to the positive control.

Group 4 (infected mice treated with 100 mg/kg b.w. of ELES) had the highest ALT activity (77.00±6.03 IU/L), while the normal control group had the lowest activity (9.33±0.67 IU/L). On the third day following treatment, Group 4's ALT activity was significantly (p <0.05) higher than that of the positive control.

Group 4 had the highest TP concentration (4.40±0.00g/dL), whereas the normal control group had the lowest concentration (3.77±0.09 g/dL). On day three post-treatment, the TP concentration of infected mice treated with 100, 200, and 400 mg/kg b.w. of ELES, respectively, was considerably (p <0.05) greater than that of the positive control.

Group 3 (standard control) had the greatest TBIL concentration (0.997±0.122 mg/dL), whereas Group 5 had the lowest concentration (0.153±0.267 mg/dL). On the third day after treatment, the TBIL concentration in all groups that received extract therapy was significantly (p <0.05) lower than that of the positive control. Group 6 had the greatest DBIL concentration (2.023±0.477 mg/dL), whereas the positive control group had the lowest concentration (1.037±0.012 mg/dL). On the third day after treatment, the DBIL concentration in all groups that received extract therapy was significantly (p <0.05) greater than that of the positive control.

Effect of ELES on renal indices

Fig. 1 shows the impact of ELES on renal indices (creatinine and urea concentrations) in malaria-

Table 1 — Phytochemical compositions of ELES

Phytochemical constituents	Conc. (mg/g)
Tannin	0.733±0.002
Saponin	0.418±0.007
Flavonoids	1.166±0.016
Steroids	0.593±0.017
Cyanogenic compounds	0.302±0.008
Glycoside	0.375±0.005
Alkaloids	0.471±0.003
Phenols	1.043±0.006
Terpenoid	0.590±0.005

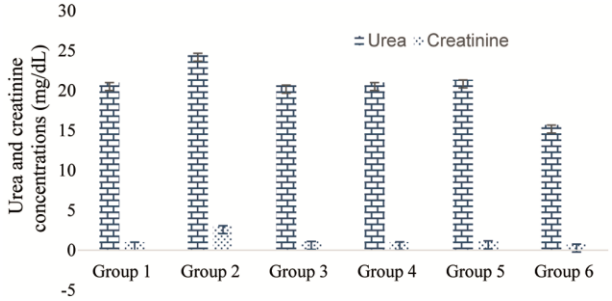

Fig. 1 — Effect of treatment with ELES on urea and creatinine concentrations in malaria-infected mice.

Table 2 — Effect of ELES on ALP, AST, ALT activity, and TP, TBIL and DBIL concentrations of malaria-infected mice

Group	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	TP (g/dL)	TBIL (mg/dL)	DBIL (mg/dL)
1	32.33±0.331^{b}	7.67±2.84^{a}	9.33± 0.67^{a}	3.77±0.09^{a}	0.970±0.040^{b}	1.257±0.059^{a}
2	25.67±3.528^{c}	6.00±1.53^{c}	26.00±10.58^{b}	4.10±0.00^{e}	0.943±0.299^{b}	1.037±0.012^{a}
3	27.67±3.93^{d}	5.00±2.52^{b}	65.67±15.07^{c}	4.20±0.00^{b}	0.997±0.122^{b}	1.043±0.023^{a}
4	31.00±2.00^{a}	6.67±0.88^{c}	77.00±6.03^{d}	4.23±0.03^{b}	0.210±0.095^{a}	1.383±0.338^{a}
5	31.33±1.33^{a}	10.00±2.30^{d}	54.00±18.00^{e}	4.33±0.03^{c}	0.153±0.267^{a}	1.897±0.532^{a}
6	31.33±1.45^{a}	12.33±0.67^{e}	53.33±18.67^{f}	4.40±0.00^{d}	0.523±0.267^{a}	2.023±0.477^{b}

The result is displayed as the mean ± standard error of the mean (S.E.M). Values with different letter superscripts down the column differ significantly (p <0.05).

Table 3 — Effect of ELES on antioxidant and lipid profile parameters in malaria-infected mice

Groups	CAT (U/mg)	SOD (U/mg)	GSH (mg/dL)	MDA (mg/ml)
1	3.27±0.42^{d}	20.27±0.91^{b}	1.46±0.77^{d}	1.27±0.22^{a}
2	3.27±0.64^{d}	19.25±0.82^{a}	0.68±0.50^{a}	1.04±0.01^{a}
3	1.37±0.42^{a}	20.08±0.52^{b}	1.11±0.20^{c}	1.04±0.01^{a}
4	3.43±0.56^{b}	18.19±0.27^{c}	1.00±0.06^{b}	1.03±0.01^{a}
5	1.55±0.93^{a}	20.92±0.11^{b}	0.68±0.22^{a}	1.06±0.03^{a}
6	1.50±0.67^{a}	20.82±0.20^{b}	0.64±0.08^{a}	1.03±0.00^{a}
	TCHOL (mmol/L)	TAG (mmol/L`)	HDL (mmol/L)	LDL (mmol/L)
1	3.77±0.54^{b}	2.00±0.21^{b}	2.50±0.21^{a}	2.07±0.43^{b}
2	5.60±1.11^{c}	1.20±0.10^{a}	0.27±0.15^{b}	4.03±1.23^{c}
3	3.13±0.22^{b}	1.27±0.33^{a}	2.57±0.30^{a}	2.27±0.09^{b}
4	3.67±0.84^{b}	1.30±0.06^{a}	2.70±0.36^{a}	3.67±1.20^{a}
5	2.90±0.50^{a}	1.27±0.03^{a}	2.20±0.64^{a}	3.40±0.32^{a}
6	2.33±0.38^{a}	1.27±0.09^{a}	2.27±0.64^{a}	3.67±0.32^{a}

The result is displayed as the mean ± standard error of the mean (S.E.M). Values with different letter superscripts down the column differ significantly (p <0.05).

infected mice. The findings showed that, in comparison to the positive control, the measured values of urea and creatinine were significantly (p >0.05) lower in each of the treatment groups.

Effect of ELES on antioxidant parameters

Table 3 displays the outcome of ELES's impact on antioxidant parameters in malaria-infected mice. The findings indicated that, in comparison to the positive control, mice given the extract and infected with the malaria parasite had lower serum levels of the antioxidant enzyme catalase (CAT). Furthermore, mice given different doses of ELES showed comparable levels of the antioxidant enzyme superoxide dismutase (SOD) and increased activity of the enzyme when compared to the positive control group. However, non-significant (p >0.05) differences were found when the levels of reduced glutathione (GSH) and malondialdehyde (MDA) in the treatment groups were compared to the positive control.

Effect of ELES on the lipid profile

Table 3 shows the effect of ELES on the lipid profile in malaria-infected mice. The results demonstrated that each treatment group's concentrations of total cholesterol (TCHOL) and low-density lipoproteins (LDL) were significantly (p <0.05) lower than those of the positive control. Conversely, there was a significant (p <0.5) increase in the levels of triacylglycerol (TAG) and high-density lipoproteins (HDL) compared to the positive control.

Effect of ELES on serum electrolytes concentration

Fig. 2 shows that the normal control group had the lowest sodium ion (Na^+) concentration (180.00± 10.693 mmol/L), and the standard control group had

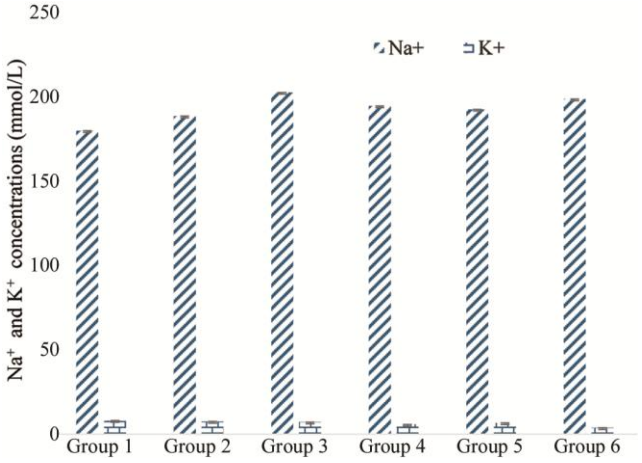

Fig. 2 — Effect of treatment with ELES on serum Na^+ and K^+ concentrations of malaria-infected mice.

the highest concentration (202.67±12.387 mmol/L). On the third day post-treatment, the Na^+ concentrations in all groups that received extracts did not differ significantly (p >0.05) from the reference control.

Group 6 had the lowest potassium ion (K^+) concentration (3.833±0.1764, mmol/L), whereas the normal control had the greatest concentration (8.100±0.2082 mmol/L) (Fig. 2). All groups treated with extract had significantly (p <0.05) lower K^+ concentrations than the positive control.

Fig. 3 shows that the normal control had the highest concentration of chloride ions (Cl^-) at 96.33±0.33 mmol/L, while group 4 and the standard control had the lowest concentration at 95.33±0.33 mmol/L. Groups 5 and 6 had significantly (p <0.05) greater Cl^- concentrations on the third day following treatment compared to the positive control.

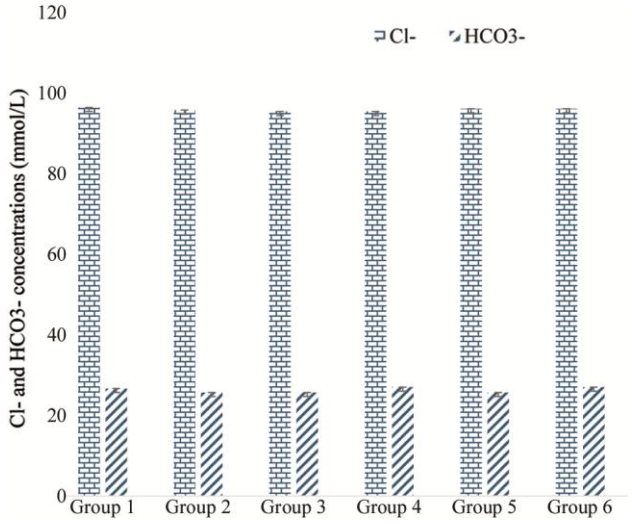

Fig. 3 — Effect of treatment with ELES on serum Cl^- and $HCO3^-$ concentrations of malaria-infected mice.

The highest bicarbonate ion (HCO_3^-) concentration (Fig. 3) was found in Groups 4 and 6 (27.00±0.00 mmol/L), while the lowest concentration was in positive control (25.67±0.33 mmol/L). Comparing Groups 4 and 6 to the positive control, the HCO_3^- concentration was significantly (p <0.05) higher.

Discussion

Medicinal plants are excellent sources of phytocompounds with pharmacological relevance and can serve as models for creating new pharmacologic agents and contemporary pharmaceutics due to the rich composition of their bioactive secondary metabolites[29]. These explain why there has been a recent upsurge in the search for plant-based medicine candidates to replace synthetic treatments, which are nearly always associated with side effects when used in the treatment of illnesses. Despite having numerous uses in African traditional medicine, *Sida linifolia* L. is an underappreciated medicinal weed with few published scientific studies on its bioactivities. Herein, we evaluated *S. linifolia's* bioactive ingredients, ability to scavenge free radicals *in vivo*, and potential for ameliorating lipid profile markers, as well as liver and kidney function enzymes.

The presence of relevant phytochemicals, such as alkaloids, tannins, steroids, and polyphenols, among others, in their required concentrations plays a vital role in biological activities. Significant concentrations of phenols, saponins, steroids, glycosides, flavonoids, alkaloids, tannins, terpenoids and cyanogenic compounds were found in the phytochemical screening of the ELES. Also, Nwankwo *et al.*[1] reported that considerable concentrations of saponins, cyanogenic compounds, phenols, alkaloids, flavonoids, steroids, glycosides, and tannins were also found in the ethanolic leaf extract of *S. linifolia*. The pharmacological potentials of phenolic compounds such as flavonoids, tannins, etc., are widely recognized by their anti-inflammatory and antioxidant properties; the authors also showed a good correlation between the phenolics and flavonoids contents of *Grewia optiva* extracts which exerted free radical scavenging potentials[30]. Consequently, the high concentration of phenolics, flavonoids, and tannins in ELES suggests that the plant may have a superior pharmacological profile.

Increased activities of AST, ALP, and ALT are signs of injury to organs or tissues in the body, including red blood cells, parenchymal cells, and other cells[31]. On day 3 post-treatment, the extract-treated groups outperformed the usual control in terms of AST activity. All the extract-treated groups showed higher levels of liver enzymes (AST, ALP, and ALT) than the group that was infected but without treatment. This could either be a result of an adaptive response by the hepatocytes or stress impacted by the extract. This does not conform with the findings of Francis *et al.*[32], who found that mice treated with varying doses of *Senna alata* extract after contracting malaria had reduced ALT and AST activity in comparison to the group that was infected but did not receive treatment. Another study by Ahmad *et al.*[33] averred that the administration of *Zizyphus oxyphyla* resulted in a significant (p <0.05) decrease and restoration of normalcy in the concentrations of some haematological indices, as well as ALT, AST, and ALP. Chronic liver and kidney illnesses, including cirrhosis and nephritic syndrome, are associated with decreased amounts of albumin, the primary component of total protein that is excreted in the urine[34]. On the third day post-treatment, the results indicated that Group 6 had the highest total protein (TP) content while the normal control group had the lowest concentration. Groups 4, 5, and 6 had significantly (p <0.05) greater TP concentrations than the positive control. The data demonstrating a dose-dependent rise in total protein following the extract's administration indicated that the liver's integrity, which is crucial in the production of total proteins, was preserved by the extract. The findings of this study agreed with that of Ahmad *et al.*[34], which showed that rats treated with a flora spike of *P. vulgaris* extract had a significantly

higher total protein level (p <0.01) than the harmful control group.

Increased blood bilirubin concentrations are a sign of parenchymal or biliary liver injury[35]. Our study's findings indicated that the extract enhanced the liver's capacity to eliminate blood's unconjugated hydrophobic bilirubin by combining it with glucuronic acid and employing the enzyme UDP-glucuronide transferase to convert it into water-soluble conjugated bilirubin. This was supported by the evidence that the extract treatment resulted in a dose-dependent drop in total bilirubin levels. This also correlated with the observation of Mogaka *et al*.[36], who pointed out that root extract of *Senna occidentalis* had an ameliorative effect on bilirubin concentration in malaria-infected mice.

The significant increases in the superoxide dismutase and catalase activities of the treated groups indicate that the antioxidant enzyme systems were able to scavenge the free radicals due to the illness, but the non-significant reductions in the reduced glutathione and malondialdehyde concentrations could be attributed to shortness in the treatment period or that the extract did not influence the antioxidant effect through such pathways.

Sidiki *et al.* demonstrated with an *in vivo* study that variations in serum lipid concentrations are a hallmark of malaria; individuals suffering from malaria have hypocholesterolemia, reduced levels of HDL, elevated levels of LDL, and elevated TAG levels. These lipid anomalies are temporary and can occur in both complex and non-complex instances of the most common *Plasmodium* species[37]. The underlying mechanisms causing the observed dyslipidemia in malaria cases might be attributed to either the parasite, the host, or a combination of the two[31]. Nevertheless, our result showed that malaria infection-induced dyslipidemia could be normalized by the treatment of mice with ELES. The mechanism of normalization of dyslipidemia can be by reduction of lipogenesis, increase of lipolysis, and lipid β-oxidation which are associated with the downregulation of AKT serine/threonine kinase 1 (AKT 1), cyclin D 1 (CCND 1), and the upregulation of vascular endothelial growth factor A (VEGFA) and estrogen receptor 1 (ESR 1) in the liver and adipose tissue[38]. The results of our study correlate with the study of Francis *et al.*[32], which reported that the extract of *Senna alata* possessed an ameliorative effect and normalized dyslipidemia.

Serum urea and creatinine concentrations were determined to evaluate the extract's impact on the renal dysfunction brought about by malaria infection treatment. The concentration of serum urea and creatinine in the treated groups was relatively lower compared to the infected but untreated group. This could mean that the extract exerted nephroprotective effectively; however, there is a need for histopathological analysis of the kidney tissues to corroborate these findings to make sure there is no underlying damage. The current study's findings are in line with the report by Ahmad *et al*.[34], which showed that infected mice administered *Zizyphus oxyphyla* had an ameliorative effect on the kidney by lowering serum urea and creatinine levels.

Electrolytes such as potassium, chloride, sodium, magnesium, bicarbonate, etc., help to maintain electrical neutrality in cells, generating and conducting potentials in the neurons and muscles[39]. High or low levels of these electrolytes disrupt normal bodily functions and can lead to life-threatening complications. For example, a low level of sodium can lead to delirium, and a high level of potassium can lead to arrhythmias. When comparing the extract-treated groups to the standard control group on the third post-treatment day, the concentrations of sodium ion electrolyte were lower in the former. A drop in potassium and sodium ions concentrations following extract therapy suggests that administering the extract could result in dehydration or injury to kidney tissues[40]. The serum electrolytes (bicarbonate, chloride, sodium, and potassium) demonstrated significant reductions.

Conclusion

The hepato-protective impact of the extract of *S. linifolia* is indicated by the reduction in the activities of liver function enzyme indicators, elevated blood bilirubin concentration, and increased total protein of the treated groups. The hepatoprotective impact of the *S.linifolia* extract is indicated by the elevated blood bilirubin and increased concentration of the total protein of the treated groups. However, there were marked increases in the levels of liver enzyme activities, which could be attributed to stress conditions contributed either by the doses of the extracted administered or the duration of treatment. The kidney's functionality was improved by the extract's treatment, resulting in a considerable decrease in the mice's serum creatinine and urea

concentrations. Changes in the serum electrolyte levels indicated that the extract disrupted the body's equilibrium of electrolytes (K and Na ions). As a result, caution should be exercised when administering the extract because excessive loss of these ions could seriously tamper with the balance of these electrolytes in the body and can result in serious health hazards. Overall, the study depicted nephroprotective, antioxidant, and dyslipidemia modifying effects, but the apparent toxic effects of the extract on the liver and somewhat imbalance in the concentrations of the electrolyte call for further research before the agent could be declared a very safe one.

Conflict of interest

There is no conflict of interest, according to the authors.

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