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Research Article

Biological Efficacy and Toxicological Evaluation of Ethanolic Extract of *Cassia nodosa* Buch.-Ham. (Leguminosae)

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Medicinal plants are used worldwide to treat various diseases. However, scientific validation of the therapeutic potential of most medicinal plants is absent, and little to no information on their potential toxicity exists. It is therefore important to assess the efficacy and toxicity of various medicinal plants commonly used in traditional herbal medicine. The main goal of this study was to evaluate the efficacy and toxicity of the ethanolic extract of *Cassia nodosa,* a plant used in the treatment of fever, cold, gastric pain, diabetes mellitus, and malaria in Ghana. The stem bark extract of *Cassia nodosa* was obtained by cold maceration. Following phytochemical examination via standard procedures, the extract was screened for antiplasmodial, anti-inflammatory, and antioxidant activities. The extract was also evaluated for acute oral toxicity and its toxicity to the liver and kidney of rats. Phytochemicals present in the extract included sterols, alkaloids, tannins, glycosides, and coumarins. In the *in vitro* antiplasmodial activity test, the *Cassia nodosa* extract showed moderate antiplasmodial activity with an IC_{50} value of 23.98 ± 2.07 μg/ml. An ED_{50} value of 8.52 ± 2.83 mg/kg in the anti-inflammatory test indicated that the *Cassia nodosa* extract administered orally was very active in reducing inflammation. The total antioxidant capacity of the extract was determined to be 48.49 ± 5.24 gAAE/100 g with the total phenolic content being 28.48 ± 3.13 (gGAE/100 g) extract. In the DPPH radical scavenging assay, the concentration required for 50% inhibition of DPPH radical was found to be 78.71 ± 6.84 μg/mL. There was a gradual increase in the body weight of rats in both treated and control groups during the acute toxicity test. Clinical biochemical tests did not show any significant differences between control and treated groups of animals in terms of liver and renal functions, 14 days after administration of *Cassia nodosa* extracts. Taken together, these results provide scientific validation for the use of the plant in traditional medicine.

1. Introduction

Natural product-derived therapeutics have remained the mainstay of many healthcare programs all over the world [1]. In Africa especially, plant-based herbal formulations have been used for the treatment of various symptoms and ailments and are still heavily relied upon even in present times. The World Health Organization (WHO) estimates that plant-based therapeutics support about 70% of the world's population in terms of their primary healthcare needs [2]. Herbal medicines are usually affordable and much more accessible and as such are being integrated into the formal primary healthcare pipeline of many countries [3]. The use of herbal medicines in many African countries is usually without the stringent regulations associated with orthodox medicines. As such, issues of toxicity are rarely addressed. However, reports of suspected toxicity and adverse events exist in the literature [4]. These reactions may be due to side effects, overdose, or the presence of toxic compounds in the plant extracts. Reports of renal and hepatotoxicity of herbal preparations have been on the increase [5]. Thus, toxicological evaluation of plant extracts has become necessary in current research endeavors.

In Ghana, over 1,000 plant species have been certified as being medicinal, and most of these plants are either used alone or in combination for the treatment of ailments such as malaria, gonorrhea, diabetes, hypertension, typhoid, wounds, and sexual weakness [6, 7]. Some of these plants

have been proven scientifically to possess activities such as antimicrobial, antidiabetic, antioxidant, antiplasmodial, and anti-inflammatory [8–11]. One such plant used in Ghana in various traditional herbal preparations is *Cassia nodosa* (synonym: *Cassia javanica*).

Cassia nodosa Buch.-Ham. is an ornamental tree in the Leguminosae family. The plant is a perennial tree with a height of 3–5 m. It is scattered in Burma, India, Bangladesh, and Pakistan. *Cassia nodosa* is widely cultivated all over the tropics and is naturalized in Asia, Australia, America, and tropical West Africa including Ghana [12, 13]. The plant, in Ghana, can be located in Obomeng in Kwahu in the Eastern region. Traditionally, *Cassia nodosa* is useful because of the purgative activity of its pods and leaves. It is used to treat bites of insects, cheloid tumor, rheumatism, and ring worms. The bark of *Cassia nodosa* is used as one of the components in antidiabetic ayurvedic formulations. The leaves are used in preparations for the treatment of herpes simplex infection. In Ghana, *Cassia nodosa* is used in preparations targeted towards the cure of malaria, constipation, cold, fever, gastric pain, and diabetes mellitus [14].

Despite its widespread use, no report on the antiplasmodial activities of extracts of the plant exists in the literature. In addition, only scattered reports of the anti-inflammatory and antioxidant activities as well as the toxicity of the plant extract exist [15]. This work was therefore designed to investigate the biological activities (antiplasmodial, anti-inflammatory, and antioxidant) and the toxicological profile of the stem bark ethanolic extract of *Cassia nodosa*. Chloroquine-sensitive *Plasmodium falciparum* 3D7 strain was used for the antiplasmodial assay, whereas the carrageenan-induced foot edema assay was used for the anti-inflammatory study. The antioxidant activities were tested using the phosphomolybdenum and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. The total phenolic content of the extract was also determined using the Folin–Ciocalteu method. Acute toxicity, liver and kidney function test, and behavioral changes in the murine model were used to evaluate toxicity.

2. Experimental Methods

2.1. Collection of Sample, Preparation, and Extraction. Stem bark samples of *Cassia nodosa* were collected from the Eastern Region of Ghana in November 2018. Herbarium specimen of the sample was authenticated, deposited into the Herbarium Section of the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology (KNUST). The samples were cut into very small pieces and air-dried for 28 days before milling into powder. Seven hundred grams (700 g) of the powdered stem bark was subjected to cold maceration in 2.5 L of ethanol for 72 hours with frequent agitation. The ethanolic extract obtained after filtration was concentrated under vacuum (Cole Parmer Rotary Evaporator N-1110, China) and stored at 4°C. Table 1 provides information about the plant and its local medicinal uses in Ghana.

2.2. Phytochemical Screening. The ethanolic extract of *Cassia nodosa* was screened for tannins, steroids, flavonoids, alkaloids, coumarins, and glycosides using established qualitative phytochemical procedures reported elsewhere [16, 17]. The presence of tannins was tested by adding 3 mL of 10% lead acetate solution to 3 mL of extract. Formation of a bulky white precipitate indicates the presence of tannins. To test for steroids, 5 ml of the extract was reconstituted in chloroform. Concentrated sulphuric acid was carefully added down the side of the test tube to form a lower layer. The formation of a reddish-brown/cherry red color at the interface indicates the presence of a steroid with a triterpenoid nucleus. For the flavonoid test, a strip of filter paper was dipped into the liquid extract and dried. The filter paper strip was then exposed to ammonia solution followed by fumes of concentrated hydrochloric acid. The observation of an intense yellow coloration upon exposure to the fumes of concentrated ammonia solution and subsequent disappearance of the color upon exposure to the fumes of concentrated hydrochloric acid indicates the presence of flavonoids. The presence of alkaloids was tested by dissolving extract in ammoniacal alcohol, filtering, and evaporating to dryness. The residue was reextracted with 1% sulphuric acid and filtered, and the filtrate rendered alkaline with dilute ammonia solution. It was then shaken with chloroform; the chloroformic layer was then separated and concentrated to dryness. The residue was dissolved in 1% sulphuric acid. The observation of an orange red precipitate by the addition of one drop of Dragendorff's reagent indicates the presence of alkaloids. The test for coumarins involved dissolving the plant extract in 5 ml of ether and evaporating to dryness. The residue obtained was dissolved in hot water and then cooled, after which 0.5 ml of 10% ammoniacal solution was added to a portion of the dissolved residue. The occurrence of an intense bluish green fluorescence under UV light is a positive test for the presence of coumarins and their derivatives. In the test for glycosides, the extract was dissolved in 5 ml of dilute HCl, followed by warming on a water bath for 2 minutes. The resultant solution was filtered and rendered distinctly alkaline by adding 2–5 drops of 20% ammonium hydroxide solution. One milliliter of Fehling's A and B solution was added to the filtrate and warmed on a water bath for 2 minutes. The formation of a brick-red precipitate indicates the presence of glycosides.

3. Bioassays

3.1. Antiplasmodial Activity. Chloroquine-sensitive *Plasmodium falciparum* 3D7 strain was obtained from the Department of Immunology, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, and used for the *in vitro* antiplasmodial tests. Parasites were introduced to freshly prepared O^+ human erythrocytes suspended in complete parasite medium (RPMI-1640 containing 5 mg/mL AlbuMAX II, 10 μg/mL gentamycin, 0.2 μg/mL hypoxanthine, 2 mML glutamine, 25 mM HEPES, and 23.8 mM $NaHCO_3$), enhanced with 10% normal human serum and gassed with a gas mixture containing 2% O_2, 5.5% CO_2, and 92.5% N_2 (Air Liquide, UK). The culture was incubated at 37°C.

Table 1: Botanical name, family, local name, part used, and local indication of plant.

Botanical name	*Cassia nodosa* (synonym: *Cassia javanica*)
Family	Leguminosae
Common name	Pink cassia
Local name	Sesa dua (twi)
Plant part used	Stem bark
Local indication	Constipation, cold, fever, gastric pain, malaria, and diabetes mellitus
Solvent used	Ethanol
Physical state	Brown crystals
Yield (%)	18.97

The parasite culture was maintained daily by aspirating the medium and replacing it with a fresh one [18].

A stock solution (250 mg/mL) of the extract of *Cassia nodosa* was prepared by dissolving in 70% ethanol. The stock was diluted to obtain six concentrations between 0.586 μg/mL and 600 μg/mL. *Plasmodium* culture was then synchronized with 5% sorbitol to obtain the ring stage of the *Plasmodium falciparum* required for the assay. Working solutions were prepared in complete medium. Aliquot (50 μL) of plant extract was dispensed into flat-bottom 96-well microtiter plate (Thermo Fisher Scientific Nunc™, USA), and 1.5% cell suspension of parasitized erythrocytes containing 0.9–1.5% parasitemia was added to each well in triplicates. Solvent controls were also incorporated into some of the wells. Artemisinin was used as standard drug. The plates were then placed in a desiccator, gassed with a special gas mixture (2% O_2, 5.5% CO_2, and 92.5% N_2), capped, and immediately transferred into an incubator set at 37°C.

After 72 hours, the assay was terminated, and thin smears were made on glass slides from each duplicated well. This was then fixed in methanol for 10 seconds and allowed to dry. The slides were then placed in staining jars containing 10% Giemsa stain (Fluka Chemicals, UK) for 15 minutes. They were then washed, dried, and examined using 100 × magnification oil immersion objective lens of light microscope (Leica 1349522X, USA). Blood films were carefully reviewed for the presence of any blood parasites. The number of infected erythrocytes in a given grid area was counted. These included those with rings, trophozoites, and schizonts. A minimum of 1000 RBCs were counted for each smear [19]. The percentage of parasitemia was computed using the expression as follows:

$$\%\ \text{paracitemia} = \frac{\text{number of infected RBCs}}{\text{total number of RBCs}} \times 100. \quad (1)$$

IC_{50} was then computed from plotted dose-response curves deduced from the data. Microsoft Excel and GraphPad Prism software were used for all data and statistical analysis.

3.2. Effect of Extract on Erythrocytes. To assess the effect of the extract on erythrocytes, 200 μL of erythrocytes was incubated with 600 μg/mL of the extract, which represented the highest extract concentration used in the antiplasmodial assay. A drug control group with artemisinin was also prepared. Experimental conditions were kept same as that for the antiplasmodial assay. After 72 hours of incubation, the reaction was terminated. Thin blood smears were prepared and fixed with methanol on glass slides and air-dried. Smears were stained with Giemsa stain, and morphological variations in erythrocytes, if any, were observed under a light microscope fitted with a camera (Leica 1349522X, USA). These morphological findings were compared with the normal erythrocytes of the control group [20].

4. Anti-Inflammation Assay

4.1. Animals Used. The cockerels (*Gallus gallus*, strain Shaver 5790) used in the study were 1-day posthatched obtained from Akate Farms in Kumasi. They were maintained in an animal house at the Department of Pharmacology, KNUST. The cockerels were kept in stainless steel cages with dimensions of $34 \times 57 \times 18\ \text{cm}^3$ and at a population density of 12–13 chicks per cage. By means of water troughs and 1-quart gravity-fed feeders, the cockerel had free access to water and food. The cockerels were acclimatized for 7 days before being used for the assay. Chicks were grouped into five (5) sample sizes throughout the studies. The National Institute of Health guidelines for the care and use of laboratory animals were used as a guide in handling of chicks.

4.2. Carrageenan-Induced Foot Edema in Chicks. The anti-inflammatory activities of *Cassia nodosa* extract was studied in the cockerels using the carrageenan-induced foot edema assay with little modifications. Diclofenac was used as reference standard. The curative method was employed to evaluate the anti-inflammatory activity. Vernier calipers were used to measure dorsoventral foot sizes. All extracts were prepared in normal saline to enhance bioavailability. The reference drug was also prepared in normal saline solution. The drugs and extracts were prepared in a manner that the volume to be administered was based on the weight of each chick. Groups of 5 chicks were treated with different concentrations of 30 mg/kg, 100 mg/kg, and 300 mg/kg of the extract orally 90 minutes after right foot subplantar injection of 1 ml of 1% w/v carrageenan in normal saline solution. The reference drug diclofenac (10, 30, and 100 mg/kg) was also administered via intraperitoneal injection to another group, 90 minutes after the carrageenan administration. After the carrageenan injection, the dorsoventral foot sizes were measured at hourly intervals for up to six (6)

hours. Another group of chicks received only normal saline as treatment, and this served as the negative control [21, 22].

4.3. Data Analysis. Percentage increase in foot size over the five hours following treatment with *Cassia nodosa* extract and the reference drug was calculated using the following formula:

$$\text{percentage increase in foot size} = \frac{F_t - F_0}{F_0} \times 100, \quad (2)$$

where F_t is the foot volume at time t posttreatment and F_o is the foot volume measured at time o before the challenge. Various groups were averaged.

Total foot volume for each treatment was calculated in arbitrary unit as the area under the curve (AUC) for such treatment, and the percentage inhibition of edema was determined for the treatment groups using the following formula:

$$\%\ \text{inhibition of edema} = \frac{\text{AUC}_{\text{control}} - \text{AUC}_{\text{treatment}}}{\text{AUC}_{\text{control}}} \times 100. \quad (3)$$

Differences in area under the curve (AUC) were analyzed by ANOVA and then followed by Dunnett's test. GraphPad Prism version 5 was used for all statistical analyses. ED_{50} values (dose responsible for 50% of maximal effect) for each extract were determined using an iterative computer least square method with the following nonlinear regression (three-parameter logistics) equation:

$$Y = \frac{a + (b - a)}{1 + 10^{(\log ED50 - X)}}, \quad (4)$$

where X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape. GraphPad Prism for windows version 10.0 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and ED_{50} determinations. $P < 0.05$ was considered statistically significant.

5. Antioxidant Activities

5.1. Phosphomolybdenum Method. The reagent solution for the assay consisted of 28 mM trisodium phosphate, 0.6 M sulphuric acid, and 4 mM ammonium molybdate. Six milliliters (6 mL) each of different concentrations (50, 100, 150, 200, and 250 ppm) of the extracts in 1% DMSO was added to 6 mL of the reagent solution in centrifuge tubes and incubated at 95°C for 90 minutes. After incubation, reaction mixtures were allowed to cool at room temperature for 15 minutes, after which absorbance was measured at 695 nm. As a negative control, a blank determination was prepared by adding 6 mL of 1% DMSO to 6 mL of the reagent solution and taken through same experimental procedures as the test extracts. Five different concentrations of reference drug ascorbic acid (20, 40, 60, 80, and 100 ppm) were also prepared and taken through same procedure as the test samples. The ascorbic acid was used to obtain a calibration curve. The total antioxidant capacity (ascorbic acid equivalence) of the extract was then determined as the number of ppm of ascorbic acid per 100 gram of the crude extract. Antioxidant capacity was expressed as equivalents of ascorbic acid with the following equation:

$$\text{TAC} = \frac{\text{C} \times \text{V}}{\text{M}} \times 100, \quad (5)$$

where TAC is the total antioxidant capacity in gAAE/100 g of the test sample, C is the concentration of ascorbic acid (ppm), V is the volume of the reaction mixture, and M is the mass of the extract in the reaction mixture [23].

5.2. DPPH Assay. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay was used to evaluate the radical scavenging activity of the extract. The DPPH reagent (0.1 mM) was prepared in methanol, and 250 μL aliquots were added to 25 μL of different concentrations of extract in a microtiter plate. The mixture was incubated in the dark at room temperature (27°C) for 30 minutes, and absorbance readings were taken at 517 nm. Different concentrations of the ascorbic acid were treated under the same condition and served at the control. The radical scavenging activity of extract was expressed as

$$\text{activity}\,(\%) = \frac{A_O - A}{A_O} \times 100, \quad (6)$$

where A is the absorbance value of the mixture of DPPH and the test samples and A_o is the absorbance value of DPPH. The IC_{50} (concentration required for 50% inhibition) values were obtained from a dose-response curve obtained by plotting percentage scavenged (% activity) against extract concentrations [24].

5.3. Total Phenolic Content. Spectrophotometric method was used to determine the concentration of the phenolic compounds in the extracts. Three microliters (3 μL) of the extract at different concentrations was added to 3 μL of 7.5% Na_2CO_3 solution and 3 μL of 10-fold dilute Folin–Ciocalteu (FC) reagent in a test tube. The test tubes were then covered with aluminum foil and allowed to stand for 30 minutes at room temperature. Different concentrations of gallic acid solutions were prepared, and 3 μL of each concentration was added to 3 μL of the FC reagent and 7.5% Na_2CO_3 solution. This served as the positive control. Absorbance of the resulting solutions was taken at 760 nm. The phenolic content was obtained from the gallic acid calibration curve and expressed as grams of gallic acid equivalent per gram of dry weight of sample (gGAE/100 g) [23].

6. Toxicity Studies

6.1. Animals. The guidelines for testing of chemicals [25, 26] of the Organization for Economic Cooperation and Development (OECD) were used. Female Wistar albino rats (8–10-week-old and pathogen-free) were used for the study. The female Wistar albino rats were obtained from the Animal Unit of the Centre for Plant Medicine Research

(CPMR), Mampong–Akuapem, in the Eastern Region of Ghana. The rats used were nulliparous or nonpregnant. The animals were acclimated to laboratory conditions for 7 days before experiments were carried out. Standard cages under standard conditions, i.e., at a temperature of 25 ± 3°C, with a 12-hour dark/light cycle were used to house the animals. The animals had free access to standard water and diet [27].

6.2. Acute Toxicity and Behavioral Changes. Twenty female Wistar albino rats were fasted overnight but allowed free access to water. They were randomly divided into four groups of 5 animals in each cage. To a control group, dimethyl sulfoxide (DMSO, 1% in distilled water) was administered, whereas the 3 other groups received *Cassia nodosa* extract at doses of 50 mg/kg, 300 mg/kg, and 2,000 mg/kg, as prescribed by OECD. The animals were observed immediately after extract or vehicle administration and at 30 minutes, 60 minutes, 4 hours, and 24 hours post-extract or vehicle administration and then once a day for the next 14 days for any signs or symptoms of toxicity. Their autonomic (salivation, defecation, perspiration, piloerection, and lacrimation), behavioral (walking patterns, grooming, and rearing), and neurological (drowsiness, convulsion, tremors, and gait) profiles were noted. Also, weight loss (daily) was monitored. Final weights of rats were taken on day 15th, followed by clinical physical examinations. At the end of the study, records were taken on the survived rats [28].

6.3. Serum Analysis. The rats were anesthetized (3% pentobarbital sodium; dose: 50 mg/kg), and blood (2–3 mL) was collected from their chest cavity into gel tubes with no anticoagulants. Blood was centrifuged at 3,000 rpm for 10 minutes to obtain sera. Blood chemistry (urea, creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), total bilirubin (TB), indirect bilirubin (IB), and direct bilirubin (DB)) was studied in the sera to test hepatic and renal functions. Serum analyses were carried out using the Automated Clinical Chemistry Analyzer (Le Scientific Medfuture LCC, USA) following standard procedures [29].

6.4. Statistical Analysis. The results of the serum analyses were expressed as mean ± SEM of three replicates tests. All the grouped data were statistically evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test (P values < 0.05 were considered to be statistically significant). Body weight measurements were expressed as mean ± SEM for 5 animals in each group.

7. Results

7.1. Extraction and Phytochemical Screening. The herbarium specimen of *Cassia nodosa* was authenticated at the Department of Herbal Medicine, KNUST, and a voucher number (KNUST/HM1/2018/SB009) was assigned. Cold maceration of the stem bark sample of *Cassia nodosa* with ethanol afforded a brown solid in about 19% yield (Table 1). Biological activities of most medicinal plants are due to the presence of different phytochemicals. Phytochemical screening of the crude ethanolic extract of the stem bark of *Cassia nodosa* indicated that alkaloids, tannins, and glycosides were moderately present. Sterols and coumarins were also present. Flavonoids were however absent in the extract. Table 2 summarizes the results of the phytochemical analyses.

7.2. In Vitro Antiplasmodial Activity. *Cassia nodosa* extract showed appreciable antiplasmodial activity with an IC_{50} value of 23.98 ± 2.07 μg/ml, as shown in Table 3. The artemisinin standard showed antiplasmodial activity with an IC_{50} of 0.014 μg/mL [30]. Classify the antiplasmodial activity of plant extracts as follows: $IC_{50} < 5$ μg/mL (very active), 5 μg/mL < IC_{50} < 10 μg/mL (active), 10 μg/mL < IC_{50} < 25 μg/mL (moderately active), 25 μg/mL < IC_{50} < 50 μg/mL (weakly active), and $IC_{50} > 50$ μg/mL (inactive) [30]. Based on this classification, the ethanolic extract of *Cassia nodosa* can be described as possessing moderate antiplasmodial activity. Incubation of infected red blood cells with *Cassia nodosa* extracts resulted in no noticeable changes in the morphology of the red blood cells even after 72 hours, indicating that these extracts are not harmful to the red blood cells (Figure 1).

7.3. Inhibition of Carrageenan-Induced Foot Edema in Chicks. The time course curve in Figure 2 shows the change in foot volume of the chicks from induction of inflammation to a couple of hours postintervention. The foot volume of chicks increased from carrageenan administration all the way to the 90^{th} minute where maximum foot volume was recorded. At this point, drug or extract was administered. Negative control group received only saline. A reduction in foot volume was observed for all groups. However, a noticeable reduction in foot volume was observed for groups that received diclofenac (Figure 2(c)) and *Cassia nodosa* extract (Figure 2(a)). The area under each curve, which represents the total edema, was computed and plotted as bar charts (Figures 2(b) and 2(d)). A dose-dependent relationship is observed for both standard diclofenac and *Cassia nodosa* extract groups. The dose-dependent effect was also evident in the % inhibitions of all the *Cassia nodosa* extract (Figures 2(b) and 2(d)).

The dosage required to reduce edema by 50% (ED_{50}) was 8.52 ± 2.83 mg/kg for *Cassia nodosa* extract and 10.08 ± 1.23 mg/kg for the standard diclofenac treatment. However, no significant difference existed between these two groups (Table 3).

7.4. Antioxidant Activities. The antioxidant activities of the extract of *Cassia nodosa* were studied by the phosphomolybdenum (PM) and DPPH radical scavenging assays. The total antioxidant capacity (TAC) obtained from the PM assay was 48.49 ± 5.24 g/100 g AAE. From the DPPH assay, the concentration of the extract required to scavenge 50% of

Table 2: Phytoconstituents in the stem bark extract of *Cassia nodosa*.

Phytochemical test	Result
Flavonoids	–
Sterols	+
Alkaloids	++
Tannins	++
Glycosides	++
Coumarins	+

–, absent; +, present; ++, strongly present.

Table 3: Antiplasmodial and anti-inflammatory activities of *Cassia nodosa* extract.

Extract or standard drug	Antiplasmodial activity (IC_{50} in μg/mL)	Anti-inflammatory activity (ED_{50} in mg/kg)
Cassia nodosa	$23.98 \pm 2.07^{*}$	$8.52 \pm 2.83^{\#}$
Artemisinin	0.014 ± 0.005	NA
Diclofenac	NA	$10.08 \pm 1.23^{\#}$

*Antiplasmodial activity was described as moderate based on classification by Ramanandraibe and coworkers [30]. #No significant difference between values ($P < 0.05$). NA, not applicable (test was not performed). Results were expressed as mean ± SEM of 3 replicate experiments.

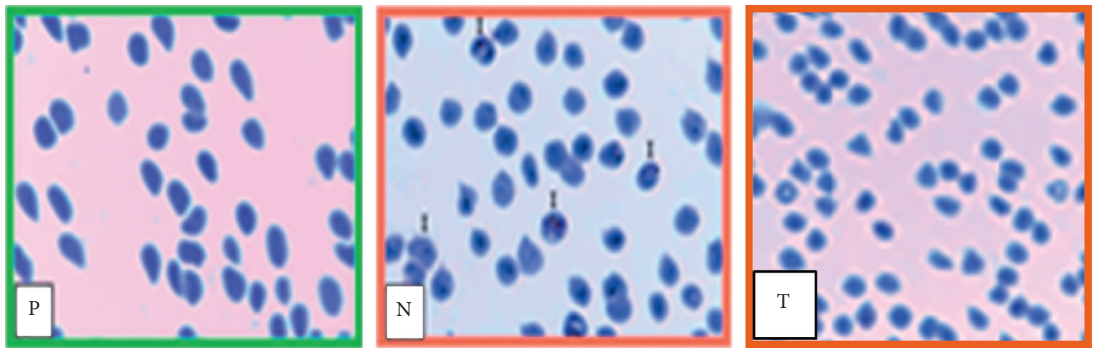


Figure 1: Micrographs of *Plasmodium falciparum*-infected red blood cells after extract or standard drug treatment for 72 hours and staining with Giemsa. P, positive control (artemisinin treated); N, negative control (untreated); T, *Cassia nodosa* treated. Infected red blood cells are denoted as "I" in erythrocytes.

the DPPH radicals (IC_{50}) was estimated to be $78.71 \pm 6.84\,\mu$g/mL. In the DPPH assay, the standard ascorbic acid yielded an IC_{50} of $20.71 \pm 2.39\,\mu$g/mL. The total phenolic content (TPC) of the extract was also determined since phenolic compounds have been suggested as potent antioxidants. The TPC of the *Cassia nodosa* extract was determined to be 28.48 ± 3.13 g GAE/100g, indicating that the phenolic content in the extract was about one-fourth that of pure gallic acid. Table 4 summarizes the results of the antioxidant activity assays.

7.5. Toxicological Evaluations

7.5.1. Acute Toxicity and Behavioral Changes. To evaluate acute toxicity, 50, 300, and 2000 mg/kg of *Cassia nodosa* extracts were administered to groups of Wistar female albino rats. Control group received only saline. Rats were then studied for 14 days. All animals did not manifest any visible signs of toxicity at all doses studied. No deaths were also recorded. No tremors, diarrhea convulsion, and salivation signs were observed. Morphological characteristics (skin, nose, eyes, and fur) were observed to be normal, whereas unusual behaviors such as walking backward and self-mutilation were absent. Grip strength, reactivity to handling or sensory stimuli, gait, and posture were all normal. Thus, clinical observations did not show any significant differences between treated groups and the control.

The body weight of the treated and control rats gradually increased during the duration of the study (Figure 3). The average body weight of control rats at the beginning of the experiment was 120.65 ± 9.85 g. This increased to 164.4 ± 16.68 g after 14 days. On average, the body weight gain for the control rats group was 43.75 g. For the treatment groups, the initial average body weights of rats administered with 50 mg/kg, 300 mg/kg, and 2000 mg/kg doses were 94.72 ± 9.73 g, 121.4 ± 15.64, and 163.04 ± 8.38, respectively.

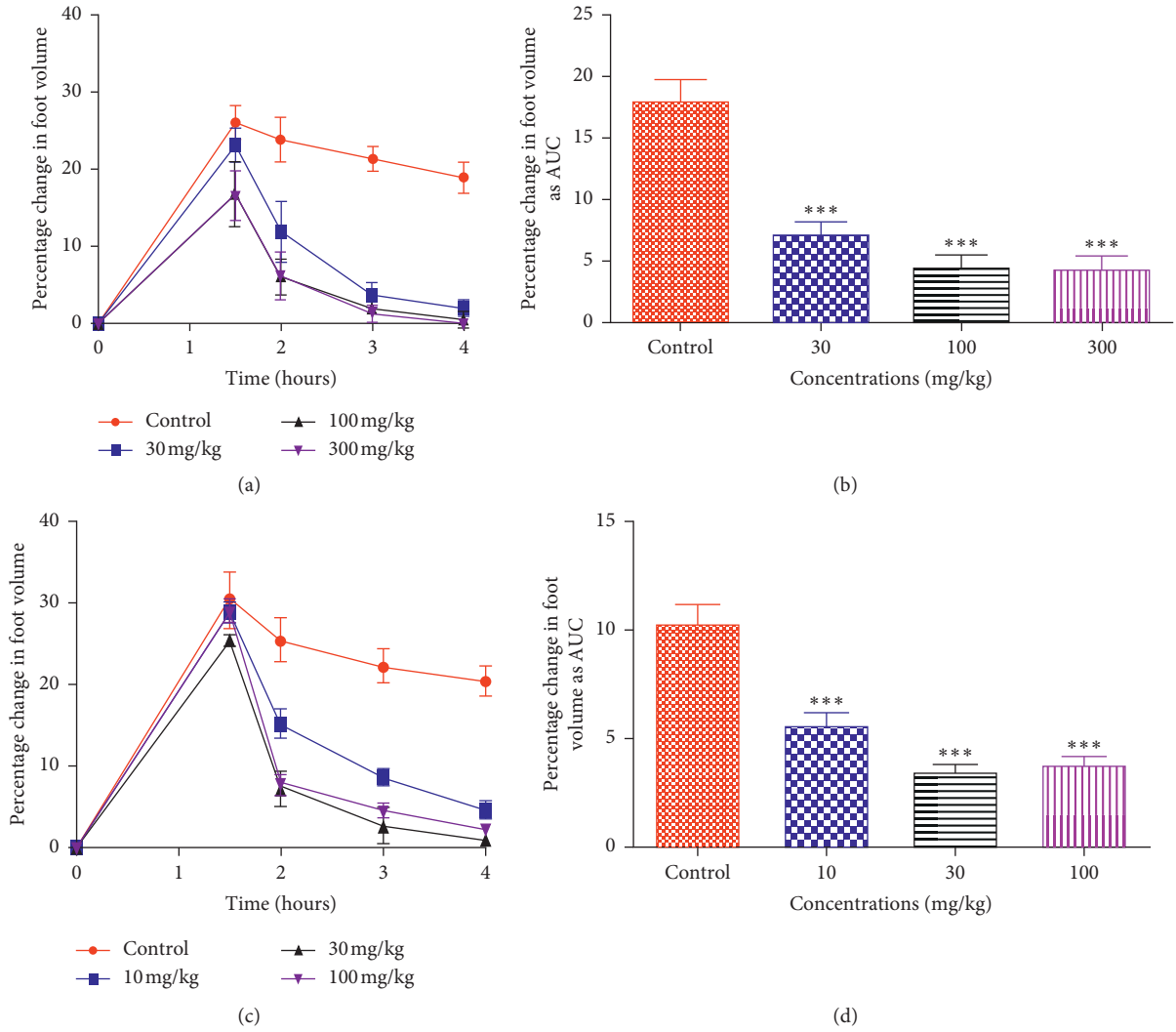


FIGURE 2: Effects of varying concentrations of *Cassia nodosa* ethanolic extract (a) and diclofenac (c) on carrageenan-induced edema in chick foot expressed on a time course curve, and the total edema response was expressed as area under the curve for *Cassia nodosa* ethanolic extract (b) and diclofenac (d). All values represent mean ± SEM for five replicates ($n = 5$). $P > 0.05$ (ns), $P < 0.05^{*}$, $P < 0.01^{**}$, and $P < 0.001^{***}$, compared to control group treated with normal saline (one-way ANOVA followed by Dunnett's post hoc test).

After 14 days, the average weights recorded were 144.14 ± 20.66, 161.53 ± 25.44 g, and 192.72 ± 18.30 and 50 mg/kg, 300 mg/kg, and 2000 mg/kg in treatment groups, respectively. The average body weight gain for rats treated at these three doses (50 mg/kg, 300 mg/kg, and 2000 mg/kg) was 49.42 g, 40.13 g, and 29.68 g, respectively.

7.5.2. Serum Analysis. To evaluate the effect of *Cassia nodosa* extract on renal and hepatic function, key enzyme activities related to the proper functioning of the liver and kidney were measured. Urea and creatinine levels were used in the kidney function test, whereas alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), total bilirubin (TB), indirect bilirubin (IB), and direct bilirubin (DB) were used for liver function tests. The levels of ALT, ALP, AST, TB, IB, and DB serum samples drawn from rats treated with different concentrations of extract are presented in Figure 4, whereas the levels of urea and creatinine for the same treatments are presented in Figure 5. For all parameters measured, no significant difference existed between values obtained for the treatment groups and those for the control group ($P < 0.05$).

8. Discussion

The stem bark of *Cassia nodosa* was chosen for this study primarily because of its widespread use in folkloric medicine for the treatment of various diseased conditions. *Cassia nodosa* is used to treat ear infection, fever, stomach aches, malaria, headache, and sores [14]. Cold maceration was chosen as the method of extraction to protect the integrity of the thermolabile active principles. Additionally, it was chosen to reflect the method used by many traditional herbal practitioners in the preparation of many herbal medications. After concentration, the extract was obtained in 18.9% yield.

Table 4: Antioxidant activities of *Cassia nodosa* extract.

Extract or standard drug	DPPH scavenging activity (IC_{50} in μg/mL)	Total antioxidant capacity (g AAE/100g)	Total phenolic content (g GAE/100g)
Cassia nodosa	78.71 ± 6.84	48.49 ± 5.24	28.48 ± 3.13
Ascorbic acid	20.71 ± 2.39	NA	NA

Results were expressed as mean ± SEM of 3 replicate experiments; NA, not applicable (test was not performed for that assay).

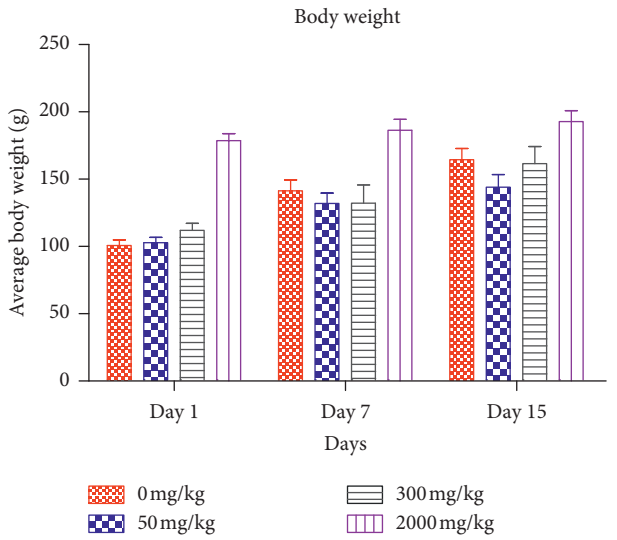


Figure 3: Average body weight of rats treated with *C. nodosa* extracts in acute toxicity study. Each bar represents mean ± SEM ($n = 5$).

This is similar to the yields of ethanolic extracts obtained from medicinal plants [9]. Biological activities of most medicinal plants are as a result of the presence of secondary metabolites. These secondary metabolites have been implicated in the anticarcinogenic, antileprosy, antioxidant, antidiabetic, anti-inflammatory, anticholinergic, hypoglycemic, antimalarial, and antimicrobial activities of many medicinal plant extracts [31]. From the phytochemical screening, the ethanolic extract of *Cassia nodosa* stem bark was found to contain tannins, which are known to have impressive biological properties and are widely used in wound healing formulations [14]. Alkaloids were also present in the extract. Alkaloids have been found useful in the treatment of malaria, neuralgia, rheumatism, and hypertension. Also, alkaloids can be used to reduce abdominal pains, boils, septic wounds, eye conditions, and headaches by acting as analgesics [32]. Glycosides have been proven to be useful as emetics and diuretics [33]. Steroids can be used during pregnancy to enhance easy delivery and improve libido in men and fertility in women [34]. The crude extract was positive for the presence of both sterols and glycosides. Coumarins, which were also present in the *Cassia nodosa* extract, are known to exhibit anti-inflammatory and antimicrobial activities [35].

To evaluate the biological activity of the ethanolic extract of *Cassia nodosa*, 3 major classes of assays were used: antiplasmodial, antioxidant, and anti-inflammatory activities. These assays were chosen to reflect the traditional use of the plant. Thus, *in vitro*-based assays were used to evaluate antioxidant and antiplasmodial activities, whilst the *in vivo*-based carrageenan-induced edema in the chick model was used to assess anti-inflammatory activity.

The *in vitro* antiplasmodial activity of the *Cassia nodosa* extract was studied by testing it against chloroquine-sensitive (3D7) *Plasmodium falciparum*, a parasite strain commonly used in antiplasmodial drug sensitivity research. Microscopy was then employed in quantification of the percentage inhibition. The result from the study showed that the plant has an antiplasmodial activity with an IC_{50} value of 23.98 μg/ml. The plant was thus considered to possess moderate antiplasmodial activity [30]. The results of this study show that *Cassia nodosa* has comparable antiplasmodial activity to other plant extracts such as *Bidens pilosa, Paspalum scrobiculatum*, and *Mitragyna ciliata* [9]. All these plants possessed moderate antiplasmodial activity against a chloroquine-sensitive *Plasmodium falciparum* strain. It has been reported that chloroform extracts of the stem bark and fruit pod of *Cassia fistula* also possessed moderate antiplasmodial activity [36]. Interestingly, other plant species in the genus *Cassia* have been validated as possessing antiplasmodial activities. *Cassia siamea* [37], *Cassia occidentalis* [38], *Cassia nigricans* [39], *Cassia singueana*, [40] and *Cassia alata* [41] have all been validated as possessing promising antiplasmodial activities. For some of these plants, some compounds responsible for the antiplasmodial activities have been isolated [42]. As shown in Figure 1, artemisinin (P) was able to completely clear all *Plasmodium* parasites from the infected red blood cells after 72 hours of treatment. In contrast, the negative solvent control clearly showed the presence of parasites in the red blood cells. In the *Cassia nodosa* treatment, most of the parasites were cleared, confirming the antiplasmodial activity recorded. No injury to red blood cells was also observed. This study thus provides scientific support for the antiplasmodial capabilities of the stem bark of *Cassia nodosa*.

In screening new anti-inflammatory drugs, carrageen-induced acute foot edema in animal models is commonly used [8, 22]. The main action of anti-inflammatory agents is to block the activity of cyclooxygenase (COX) enzymes and reduce the production of prostaglandins (PG). Cytokines, chemokines, and eicosanoids (such as PG) are inflammatory mediators that stimulate inflammatory response during tissue damage [37, 39]. The extract of *Cassia nodosa* exhibited dose-dependent anti-inflammatory activities in this study by reducing the induced edema. The development of edema is biphasic. The first phase is attributable to the

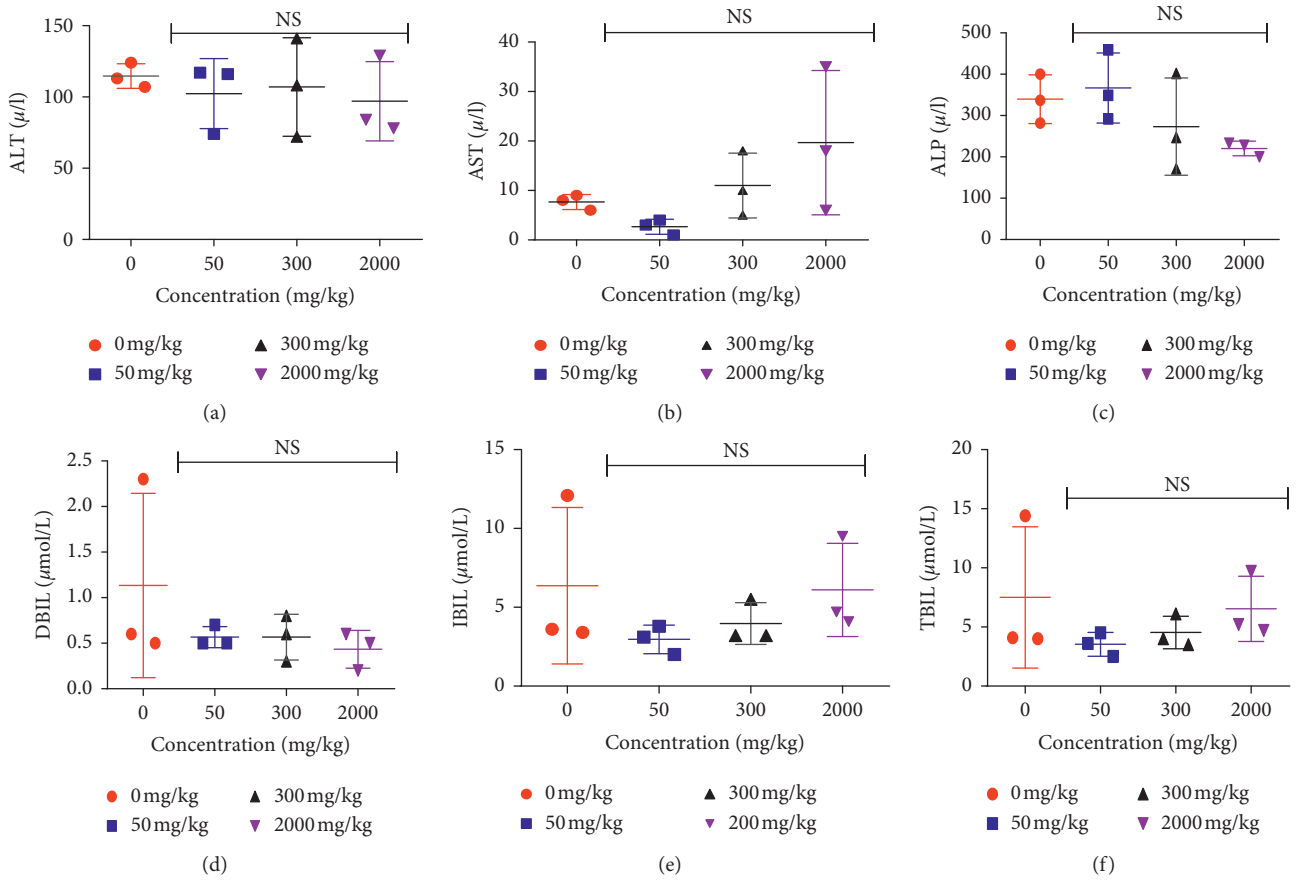


Figure 4: Scatter plot showing effect of *C. nodosa* extract on some key liver parameters: (a) alanine aminotransferase (ALT); (b) aspartate aminotransferase (AST); (c) alkaline phosphatase (ALP); (d) direct bilirubin (DB); (e) indirect bilirubin (IB); (f) total bilirubin (TB). Sample size for each treatment is 3 ($n = 3$). $P < 0.05$ was considered to be statistically significant (one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test).

release of kinins, histamine, and serotonin in the first 60 minutes after the injection of carrageenan. The second phase which is more pronounced is related to the release of PG within 2–3 hours [43]. Although this study did not explore the principal mechanism by which the extracts inhibited carrageen-induced inflammation, the possible anti-inflammatory activity of *Cassia nodosa* extract may be due to inhibitory effect on release of inflammation mediators since a rapid reduction in edema was observed upon extract administration. The low ED_{50} of the *Cassia nodosa* extract was comparable to the diclofenac used in this study. This result points to the presence of promising anti-inflammatory agent(s) in the extract which could be isolated. In crude extracts, active principles may work in synergy to elicit the desired response [44]. Even though the high activity of the extract in reducing inflammation may be attributed to single component in the mixture, it is also possible the synergistic action may play an important role in the observed anti-inflammatory activity [45].

Cassia nodosa is traditionally used to treat many diseases whose pathogenesis is linked to oxidative stress. The antioxidant activities of the leaf extract of *Cassia nodosa* have been reported [46]. The ability of the stem bark extract to scavenge free radicals is however absent in the literature. This study reported the antioxidant potentials of *Cassia nodosa* stem bark extract. The total antioxidant capacity was determined to be 48.49 ± 5.24 gAAE/100 g crude extract. This indicates that the extracts of antioxidant potential are about half that of standard ascorbic acid. This is very impressive given that only the crude extract was evaluated. About 78 μg/mL of the extract was needed to scavenge 50% of DPPH radicals in the radical scavenging assay. Polyphenolic constituents have been associated with high radical scavenging capabilities. Detection of tannins in the extract and the presence of kaempferol derivatives in the plant support the antioxidant properties of the *Cassia nodosa* extract [46]. The high TPC value determined in the extracts supports the suggestion that antioxidant activity may be due to phenolic compounds present and could explain the traditional use of the plant to cure oxidative-based diseases.

To determine the safety of drugs and medicinal plant products for human use, a toxicological assessment is conducted to predict toxicity and provide guidelines for the selection of a "safe" dose [47]. Changes in body weight during treatment provide an index of the animal general health status, which may be important for assessing their health [48]. The body weight of all animals increased gradually throughout the duration of the study (Figure 3).

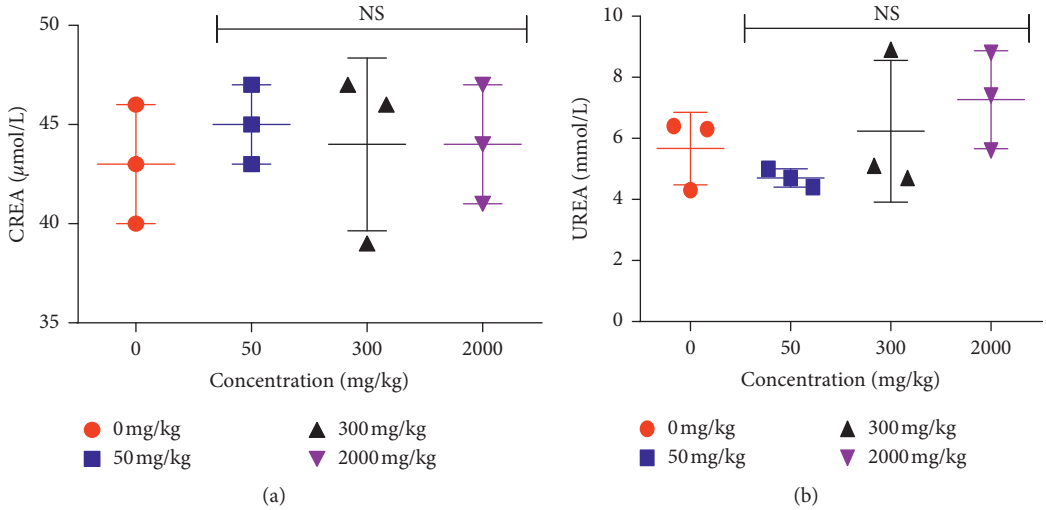


Figure 5: Scatter plots showing the effect of *C. nodosa* extract on some key kidney parameters: (a) creatinine; (b) urea. Sample size for each treatment is 3 ($n = 3$). $P < 0.05$ was considered to be statistically significant (one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test).

The average weight gain for the control rats was 43.75 g. Average weights of rats administered with 50 mg/kg, 300 mg/kg, and 2000 mg/kg doses were 49.42 g, 40.13 g, and 29.68 g respectively. These study results are in agreement with the results of Loha and coworkers, where a methanol extract of *Syzygium guineense* leaves were evaluated for their effects on body weights of rats [29]. The mean weight increase recorded in the Loha work is comparable to those observed in this study. Since clinical observations did not show any significant differences between treated groups and control, *Cassia nodosa* stem bark extract was nontoxic at doses at or below 2000 mg/kg. *Cassia nodosa* ethanolic extract showed no mortality even at an oral dose of 2000 mg per kg body weight in the female Wistar rats. Therefore, the median acute lethal dose value (LD_{50}) is more than 2000 mg/kg [49].

Disease or response to toxic substances can be indicated by changes in key biochemical parameters which are sensitive indicators of metabolic defects or organ function. Detoxification and metabolism of compounds are the main function of the liver. The liver is thus a specific target organ for drugs and toxic substances. Biochemical assessment is important as there are several reports of kidney and liver toxicity associated with phytotherapeutic agents [50]. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are important liver enzymes that serve as key indicators of liver damage [51]. Abnormal elevation of the liver enzymes is usually associated with liver damage or alteration in bile flow. When a liver cell is damaged, these enzymes leak into the blood and thus raise the enzyme level in the blood [52]. Total serum bilirubin may also be used to detect liver damage in addition to ALT, ASP, and AST. Bilirubin is a pigmented compound produced when macrophages degrade heme from aged red blood cells. Globin and iron from hemoglobin are recycled, and the unconjugated bilirubin (water insoluble and indirect reacting) circulates to the liver bound to albumin. Elevated bilirubin is a sign of liver cell damage. When the liver cell is unable to conjugate bilirubin, there is gradual increase in the serum levels of unconjugated bilirubin in the blood and this is a sign that there is liver function impairment [51].

In preclinical toxicity assessments, kidney biochemical changes are particularly liable to occur because of the fact that the kidneys eliminate many drugs and their metabolites. In this study, urea and creatinine assessment were critical as markers of kidney function. Urea and creatinine are usually increased in acute and chronic renal diseases. Diseased kidneys are unable to clear urea and creatinine, and as a result, urea and creatinine tend to accumulate and tend to be at high level in the sera of the blood [52, 53]. After 14 days of treatment with *Cassia nodosa* extract, changes in biochemical parameters between the control and treatment groups up to a dose of 2000 mg/kg were statistically insignificant (Figure 5). This indicates that there were no significant changes in serum levels of both enzyme and nonenzyme parameters (total bilirubin, indirect bilirubin, direct bilirubin, ALP, ALT, AST, urea, and creatinine), verifying the nontoxic nature of *Cassia nodosa* extract at these concentrations. The results are similar to another study where these parameters (total bilirubin, ALP, ALT, and AST) were not significantly elevated in mice fed orally with methanolic seed extract of *Cassia fistula* at single high dose of 5,000 mg/kg [55].

9. Conclusion

The findings in this study showed that stem bark extract of *Cassia nodosa* does possess antiplasmodial, antioxidant, and anti-inflammatory activities. Phytochemicals such as glycosides, coumarins, alkaloids, tannins, and sterols were detected in the plant extract and may be responsible for these activities. Toxicological evaluation of the extract revealed no significant effect on the body weight of the rats used in the study. Additionally, no mortalities or adverse clinical

manifestations were recorded. The biochemical data for the renal and hepatic function tests were similar for both control and treatment groups and indicate that *Cassia nodosa* extracts are safe at the concentrations tested. This study provides valuable scientific credence for the safety and efficacy of *Cassia nodosa* extracts in traditional herbal medicine. Isolation of the active principles responsible for the biological activities will provide useful compounds for potential use in drug discovery programs.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethical Approval

The project proposal and procedures were reviewed and approved by the Institution Ethics Review Board for Animal Use at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

LSB, GA, and MKL conceived the study. All experiments were designed by LSB, MKL, and GA. Plant materials were collected by GA and MKL. MKL carried out the antiplasmodial work. Phytochemical screening, inflammation, toxicity, and antioxidant works were done by GA. Data analysis was by MKL, GA, and LSB. Manuscript was prepared by LSB, MKL, and GA. All authors read and approved the final manuscript.

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