Original Article

Antimalarial and Reno-protective Potentials of Combined Stem Bark Extracts of *Khaya grandifoliola* and *Enantia chlorantha* in *Plasmodium* Infected Mice

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ABSTRACT

Background: Malaria is a worldwide threat, which affects millions of people. Although several antimalarial has been reported, they are either not effective or toxic. This study evaluated the antimalarial efficacy and safety of stem bark aqueous extracts of *Khaya grandifoliola* (KG) and *Enantia chlorantha* (EC) in *Plasmodium berghie* (NK65S) infected Swiss mice.

Methods: Forty-two animals were grouped into six groups. Group A (control) comprised uninfected animals given sterile placebo. Group B was infected but untreated. Groups C, D, E, and F were infected and treated with 50 mg/kg artemisinin-based combination therapy (ACT), 400 mg/kg body weight of KG, EC, and the combined extracts (200 mg/ml body weight each) respectively.

Results: Alkaloids, terpenoids, glycosides, phenolics, flavonoids, tannin and saponins were all present in the two extracts. The percentage parasitemia in the treated groups C, D, E, and F was significantly (P<0.05) reduced from 44%, 24%, 35%, and 31% to 17%, 8%, 9% and 8% respectively. The level of creatinine C (1.33±0.08 g/d), D (2.07±0.15 g/d), E (2.17±0.24 g/d) and F (1.20±0.0.12 g/d) increased significantly (P<0.05) compared to group A (0.73±0.08 g/d). There was no significant difference in the urea level, potassium and sodium concentrations among all the groups.

Conclusion: The efficacy and renal safety of oral administration of aqueous stem bark extract of KG and EC were confirmed. Therefore, the extracts could be used as alternatives to standard drugs in the management of malaria.

Keywords: Combination Therapy, *Enantia chlorantha*, *Khaya grandifoliola,* Malaria, Reno Protective Effects.

INTRODUCTION

Malaria is one of the most prevalent, devastating parasitic diseases affecting the whole universe. Since several decades, about 300-500 million clinical incidence plus 1.5-2.7 million casualties related to malaria are recorded worldwide [1]. Malaria is seriously spread in more than 91 nations, apparently in continents like Asia, Latin, America and especially in Africa. The cause of majority of human mortality is malaria and mostly with cold, rigour, fever and profusely sweating as accompanying symptoms. When a child is diagnosed with malaria but necessary therapeutic interventions are not observed, it may lead to brain damage. The case in Nigeria is not any better and particularly holoendemic i.e. there is an all-year-round transmission with greater intensity in the wet season than dry season. The most important problem is that Plasmodia parasites are resistant to most widely available and affordable drugs like fansidar and chloroquine [2]. In addition, mosquitoes control to prevent spread of the disease is made uneasy due to their resistance to drugs.

Plasmodium berghie has been used in studying the activity of potential antimalarial in mice and rats [3]. Because of the high similarity in genetic composition of mice and human, *P. berghie* is used for experimental analysis of malarial disease. As *P. berghie* is transferred from one host to others by *Anopheles* mosquitoes, it can also be transmitted to humans. The *Plasmodium* problem starts from the liver. This happens immediately after injection into the blood by *Anopheles* mosquitoes. After some

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days of infection, the next stage of parasites development is invasion of the erythrocytes.

The multiplication causes anemia and damage essential organs in the body. *P. berghie* infection also affects the brain and can cause cerebral complications in laboratory mice. Four species of *Plasmodium*, namely *P. ovale*, *P. malariae*, *P. vivax* as well as *P. falciparum* are known to infect man but *P. falciparum* causes serious cerebral malaria problems. However, due to all these challenging features of malaria, several ant-malarial therapeutic agents have been developed over the years. Nevertheless, the parasites showed significant level of resistance against these agents. Besides resistance, organ toxicity is another major issue consistent with the use of conventional anti-malarial agents. These problems are further compounded by the fact that only 10% of global research and development resources are directed at diseases including malaria [4]. All these factors have therefore necessitated urgent need to search for more affordable, relatively safe, and highly potent new lead drugs and viable alternative medicines for the management and treatment of malaria.

Plants have been considered as sources of medicinal agents for the treatment of various diseases including malaria. In some countries with high records of malarial incidence in the tropics such as Nigeria, the common practice is to use synthetic drugs in combination with herbal preparations. The health personnel in the society have recognized the importance of herbal medicine.

Reports on the efficacy and safety of the use of ethnomedicinal plants for the treatment of several diseases have been in the literature for years in both developed and developing countries [5]. In Africa, particularly West Africa, new drugs are often beyond the reach of the poor. Hence, up to 80% of the population uses medicinal plants as remedy against infections and diseases [6]. Medicinal plant contain potentially useful chemical that serves as basis for the manufacturing of modern medicines, the evaluation of the toxic action of plant extract is indispensable in order to consider treatment safe, it enables the definition of the intrinsic toxicity of the plant and the effect of acute overdose.

Khaya grandifoliola and Enantia chlorantha are two of such popular herbs used in the treatment of malaria. The use of aqueous extract of *K. grandifoliola* for the management of malaria by traditional medical practitioners is common in West Africa, especially Nigeria. The efficacy, chemical compositions and toxicological studies of this plant as antimalarial has been previously reported [7]. In addition, the anti-inflammatory, the effects on

hematological parameters and bone minerals in experimental rats have been elucidated [8]. The stem bark of *E. chlorantha* has wide spectrum antimicrobial activity, antimalarial and antipyretic properties [9].

In view of the foregoing and couple with no previous reports on the synergistic effects of *K. grandifoliola* and *E. chlorantha* against malaria parasites, the present study was conceptualized. Hence, we evaluated the anti-malarial potential and the effects of the separate and combined administration of aqueous stem bark extracts of *K. grandifoliola* and *E. chlorantha* on the renal function parameters of *P. berghie*-infected experimental Swiss mice. The phytochemicals pf the plants were also colorimetrically quantified.

MATERIALS AND METHODS

Chemicals and Reagents

Throughout this research work, unless otherwise stated, all chemicals and reagents used were of analytical grades.

Plant Materials, Authentication, and Extraction

Stem-bark of *E. chlorantha* and *K. grandifoliola* plant material were purchased from local herbal sellers at Emir's Market, Ilorin, Kwara State, Nigeria. The authentication of the plants was made in the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The voucher number, UILH/002/1013 for *E. chlorantha* and UILH/003/1066 for *K. grandifoliola,* were deposited at the Herbarium. Each plant was air-dried in a room at 27 °C for 7 d. Thereafter, the dried stem bark of each plant was cut into pieces with the aid of a sterile knife and then pulverized with a local mortar and pestle after it sieved to remove the large and unwanted particles, leaving the powdery form of the pulverized bark that was later kept in airtight containers. Pulverization was carried out separately for each of the plant stem bark. Exactly 300 gr of each was extracted in distilled water (3 L) with continuous agitation on a laboratory orbital shaker for 48 h. The resulting solution in each case was filtered (Whatman No. 1 filter paper) and subsequently concentrated to dryness using a vacuum oven (60 °C). The crude extract obtained in each case was separately stored in an airtight container and kept refrigerated (4 °C) prior to use.

Preparation of Working Solution of the Drug Sample

Artemisinin-based combination therapy (ACT) was purchased at Fiolu Pharmaceuticals

30

Ilorin, Kwara State, Nigeria. The drug was powdered by grinding with mortar and pestle. One hundred grams of the powdered drug was weighed and dissolved in 200 ml of distilled water to give 0.5 g/ml.

Determination of Total Phenol

The Folin–Ciocalteu method [10] was adopted in determining the amount of phenol content of each aqueous stem bark extract. Shortly, 300 µL of extract was dispensed into test tube (in triplicates). To this, 1.5 ml of Folin–Ciocalteu reagent, previously diluted 10 times with distilled water was added. Then, 1.2 ml of solution of Na_2CO_3 (7.5% w/v) was added. After thorough shaking, the mixture was allowed to stand at room temperature for 30 min. Thereafter, a spectrophotometrically measurement of the absorbance was done at 765 nm wavelength. The blank experiment was carried out in the same ways by replacing extracts with distilled water. Total phenol content was expressed as garlic acid equivalent (GAE) in mg/gr material.

Determination of Total Flavonoid

To determine the total flavonoid content of each of the plant extract, a standard reported method was followed [11]. Into a clean test tube, 0.5 ml of each aqueous stem bark extract was pipetted. To this was addition of 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After thorough shaking and allowed to stand at room temperature for 30 min the absorbance of the content of each test tube was determined with spectrophotometer at a wavelength of 514 nm. Total flavonoid content was expressed as quercetin equivalent (QE) in mg/g material.

Determination of Total Tannin

A previously described method was adopted in the determination of tannins [12]. Briefly, to a 0.1 mL extract of the plant, 7.5 mL of distilled water was added. In addition, 0.5 mL of Folin-ciocalteu phenol reagent and 1 mL of 35% sodium carbonate solution were added. The resulting mixture was diluted 10 times with distilled water. Then, after thoroughly shaken and allowed to stand at room temperature of 27 °C for 30 min, the absorbance was spectrophotometrically determined at 725 nm wavelength. A blank test was conducted by replacing extract with distilled water. Total tannin content was expressed as mg tannic acid equivalent/g (mg TAE/g) sample.

Determination of Total Saponins

Determination of the total saponins was carried out using an adapted method [13]. An aliquot (0.25 ml) of each aqueous stem bark extract was dispensed into separate test tubes. Afterwards, 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 were added to each. The reaction mixture in each tube was heated in a water bath at 60 °C for 10 min. Then, the tubes were cooled in ice for 4 min and allowed to cool to ambient temperature. Subsequently, the absorbance was measured in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per gr of the sample.

Determination of total Alkaloid

A previously described method was adopted in determining the total alkaloids in the plant extracts [14]. A mixture of 1 ml of each extract and 1 ml of 0.025 M $FeCl_3$ was made. To this mixture, there was addition of 0.5 M HCl and 1 ml of 0.05 M of 1.10-phenanthroline in ethanol. The mixture was incubated at 70 °C for 30 min. A red-colored complex confirming the presence of alkaloid was formed, and the absorbance was read at a wavelength of 510 nm. Alkaloid contents were calculated and expressed as quinine equivalent in mg/g of sample dry weight.

Determination of Glycoside

The total cardiac glycoside in each aqueous stem bark extract was determined [14]. The extract was first purified using lead acetate and Na_2HPO_4 solutions. Using a pipette, 5 ml of the purified extract was dispensed into a test tube, to which 5 ml of freshly prepared Baljet's reagent (95 ml aqueous picric acid and 5 ml 10 % $NaOHa_{aq}$) was added. The reaction mixture was shaken and allowed to stand for one hour for color development, after which the absorbance was read at 495 nm against a reagent blank. The total cardiac glycoside content was expressed as mg digitoxin equivalent per g sample.

Determination of Total Terpenoids

The vanillin-H_2SO_4 method [15] was followed to determine the total terpenoids content of each aqueous stem bark extract. To 5 ml aliquot of each extract, 2.5 ml of 2% vanilin-H_2SO_4 reagent was added, in a pre-cooled test tube. The reaction mixture was thoroughly agitated with further cooling in an ice bath. The color was developed by incubating the tubes in water bath at 60 °C for 20 min. Thereafter, the tubes were cooled at 25 °C for 5 min and the absorbance was read at 608 nm within 20 min, against a reagent blank. The total terpenoids content was expressed as mg linalool equivalent per g sample.

Experimental Animals

Fifty-six Swiss mice of both sexes with an average weight of 14 ± 4 gr were purchased from the Animal Holding Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The animals were allowed free access to food (dry pellets) and portable water *ad libitum*. All the animals were acclimatized to laboratory conditions for two weeks before commencement of the experiment.

The study was performed following approval by the Departmental Independent Ethical Committee on the Use and Care of Laboratory Animals of the Kwara State University, Malete, Nigeria and in accordance with the guidelines of National Research Council Guide for the Care and Use of Laboratory Animals (NRC, 2006).

Malaria Parasite and Inoculation

The rodent malaria parasite, *P. berghie* (strain NK65) was obtained from a donor (infected) mouse at the Institute of Advanced Medical Research and Training (IMRAT), University of Ibadan, Ibadan. *P. berghie* infected red blood cells were obtained from the tail vein of infected mouse by cutting the tip of its tail with sterile scissors. The blood collected was then diluted with phosphate buffered saline (PBS) so that every 0.2 ml that were subsequently injected contained approximately 10^7 infected red cells (>40% parasitemia) per kg of body weight.

The parasite preparation containing *P. berghie* was injected intraperitoneally into 40 healthy Swiss mice using a sterile syringe as described [16, 17]. After three days (72 h), test was carried out to determine the percentage of the parasitemia according to Ryley and Peter [18]. This was done by collecting each mouse's blood on separate slides. The blood was obtained by cutting the tip of each mouse-tail with sterile scissors and massaging it gently to draw blood. After the thin blood smears were made on the slides in duplicate, Leishman staining technique was conducted on the smears according to the method described [19]. Each slide was then examined under x100 magnification oil emersion objectives of a light microscope to check for the presence of the parasite. The estimation of percentage parasitemia in each animal was carried out using the expression previously described [20].

% parasitemia= (number of parasites /number of WBCs counted)x100 (1)

% infected RBCs= (number of infected RBCs/total number of RBCs counted) x 100 (2)

Animal Grouping and Treatment

The experimental animals were divided into five groups (A-E) of 8 animals per group.

Group A (Control): Uninfected animals are given sterile placebo (distilled water).
Group B: (Control) infected but untreated animals
Group C: Infected and treated with 50 mg/ml of ACT/kg b.w. [21]
Group D: Infected and treated with 400 mg/ml of KG/kg b.w. [22]
Group E: Infected and treated with 400 mg/ml of EC/kg b.w. [23]
Group F: Infected and treated with combined (200 mg/ml each) of (KG+EC) /kg b.w.

All the experimental animals had ad libitum access to feed and water throughout the period of treatment. Treatments with the drug and extracts were given orally once daily using a cannula for 4 d starting from day-3 of inoculation (immediately after the confirmation of percentage parasitemia in the animals at day-3). This test was done to check the efficacy and efficiency of the single and combined aqueous stem bark extract of *K. grandifoliola* and *E. chlorantha* compared to that of the Artemisinin-based Combination Therapy in each of the infected and treated groups. The blood sample of each mouse was collected as previously described under "Malaria Parasite and Inoculation". All the animals were also subjected to thorough gross necropsy during this period. Behavioral changes, depression, salivation, sedation ailment and mortality signs were pertinently monitored and observed. The protocols used in this research are in accordance with the guidelines of National Research for the care and use of laboratory animals and principles of Good Laboratory Procedure [24].

Collection of Blood and Serum Preparation

After the completion of the treatment administration, all the experimental mice were sacrificed under chloroform anaesthetization. The neck area was cleared of fur to expose the jugular vein, which was sharply cut with a sterile razor for blood collection. After the blood was allowed to clot, it was then centrifuged at 1500 rpm for 10 min. The serum was carefully aspirated with a pipette into sample bottles for kidney function test.

Renal Function Tests

Serum Creatinine Determination

Colorimetric method was used in determination of serum creatinine in accordance with an earlier described method [25]. The principle involves that creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

32

Serum Urea Determination

The procedure described previously [26] was adopted for serum urea determination. The principle involves that urea in a serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

Sodium Determination

Sodium in the serum is determined colorimetrically [26]. The principle includes that sodium is precipitated as sodium magnesium uranyl acetate. The excess uranium will then react with ferrocyanide to produce a chromophore. The absorbance of chromophore varies inversely as the concentration of sodium in the test specimen.

Potassium Determination

The amount of potassium is determined using sodium tetraphenyl boron in a specially prepared mixture to produce a colloidal suspension [27]. The turbidity of this solution is proportional to potassium concentration in the range of 2-7 mEq/l and used to determine potassium in sample.

Statistical Analysis

The numerical values were expressed as means ± S.E.M from each experiment. The results were analyzed using analysis of variance (ANOVA) and a statistically significant comparison was taken at $P<0.05$.

RESULTS

Anti-malarial potentials of KG and EC were tested on malarial infected Swiss mice and the results are presented in Fig. 1. In each of the treated animal groups, the percentage parasitemia in the animals before the treatment was higher than after treatment indicating the efficacy of the agents. The percentage parasitemia in each treated group B(44%→17%), C(24%→8%), D(35%→9%) and E(31%→8%) was significantly reduced following treatment with the extracts and the ACT. The extracts-treated groups (Groups C-E) competed favorably well with the ACT-treated animals (Group B) in reducing the percentage of parasitemia in the RBC of the animals. The tentative phytochemicals responsible for this bioactivity were colorimetrically quantified in each extract. In Fig. 2, the results of the quantitative analysis on both plants indicated that total phenolic (26%) was found the most abundant in EC followed by tannins (22%), total saponins (15%), total flavonoids (12%), total alkaloids (10%), terpenoids (10%), with glycosides (5%) being the least in the extract. In KG, as shown in Fig. 3, tannins (26%) was found

the most abundant followed by total phenolic (21%), total saponins (19%), total alkaloids (18%), terpenoids (10%), glycosides [4] with total flavonoids (2%) being the least constituent in the extract.
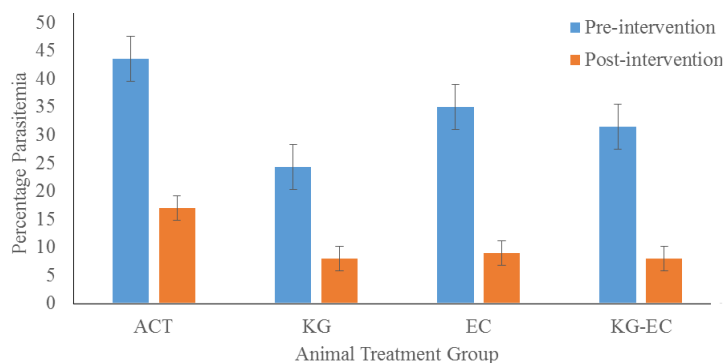

Figure 1. Pre- and Post-intervention Tests on the Effects of stem bark aqueous extracts of *K. grandifoliola* S and *E. chlorantha*. [ACT = Antimalarial-based combination therapy, KG = *Khaya grandifoliola*, EC = *Enantia chlorantha*].
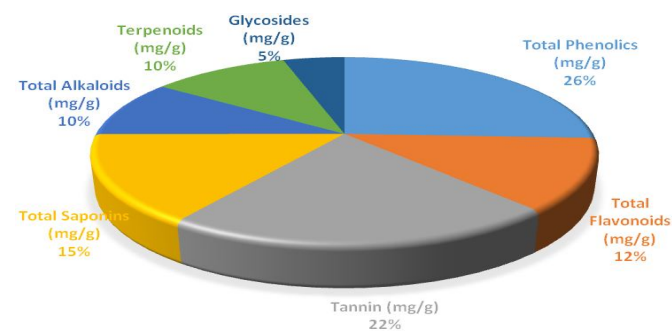

Figure 2. Quantitative phytochemical composition of the aqueous stem bark extract of *Enantia chlorantha*.
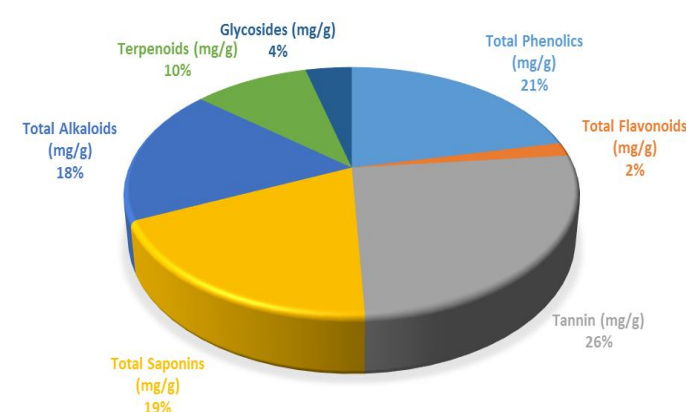

Figure 3. Quantitative phytochemical analysis of the aqueous stem bark extract of *Khaya grandifoliola*.

For the renal function test, the effects of KG and EC on the concentrations of serum creatinine in the experimental animals are presented in Fig. 4. The level of creatinine in groups B(1.33±0.08 g/d), C(2.07±0.15 g/d), D(2.17±0.24 g/d) and

E(1.20±0.0.12 g/d) increased significantly ($P<0.05$) as compared to group A(0.73±0.08 g/d). The control (Group A) was considered to have a normal creatinine level. Administration of ACT and plant extracts resulted in a significant ($P<0.05$) elevation in creatinine. The KG and EC extract treated animals had statistically ($P<0.05$) elevated creatinine level when compared to both the control and ACT treated group. The 50%-50% fortified KG-EC treated group caused no significant changes in the level of creatinine when compared with the ACT treated group but shows significant ($P<0.05$) increase when compared to the control.
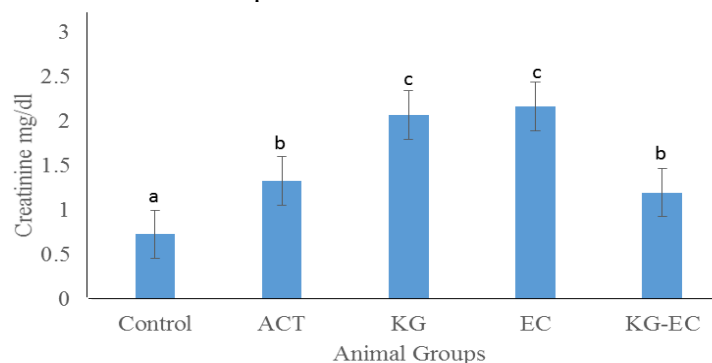

Figure 4. Effect of *Khaya grandifoliola* and *Enantia chlorantha* on serum creatinine concentrations in the experimental Swiss albino mice. Data are expressed as Mean ± S.E.M, [n=5]. Bars with different letters are significantly different. [ACT = Artemisinin-based Combination Therapy, KG = *Khaya grandifoliola*, EC= *Enantia chlorantha*].

The results of the two extracts on the concentrations of blood urea nitrogen (BUN) in the experimental animals are shown in Fig. 5. There was no significant difference in the BUN level among groups A (22.53±2.26), C (22.23±0.27), D (27.30±0.56) and E (23.60±2.55). The BUN level of the control group was considered the normal in the range (8-33 mg/dl) for mice. Administration of ACT resulted in a significant ($P<0.05$) increase in BUN level. However, there was no significant difference between the effect of the separate doses of KG and EC treated animals and that of the control. However, there was a statistically significant increase ($P<0.05$) compared with the ACT treated group. Although for the fortified (50%-50%) KG-EC treated groups, there was no significant difference compared with the control, there was a significant reduction in the BUN level when compared with the ACT treated group.

The combined therapy of the extracts was also tested on electrolytes concentration in the experimental animals. The effects of KG and EC on the sodium concentration in the experimental

34

animals are presented in Fig. 6. Although, the control group was considered a normal Na^+ concentration, the ACT, and other extracts treated groups indicated no statistically significant difference ($P<0.05$) in the level of sodium compared with the control group.

Potassium concentrations obtained in all groups were significantly different from that of group A. The effects of the separate and combined extracts of KG and EC on the concentrations of potassium (K^+) electrolyte in the experimental animals are presented in Fig. 7. The control group had normal K^+ levels. The ACT treated group (4.86±0.18) showed significant decrease ($P<0.05$) in the level of K^+ when compared with the values of the control group (5.47±0.09). The 100% KG treated group was also statistically reduced ($P<0.05$) compared to the control group. However, there was no significant difference when compared with the ACT treated group. The single dose EC and the fortified KG-EC treated groups were not statistically different ($P<0.05$) from the control and ACT treated group as well.
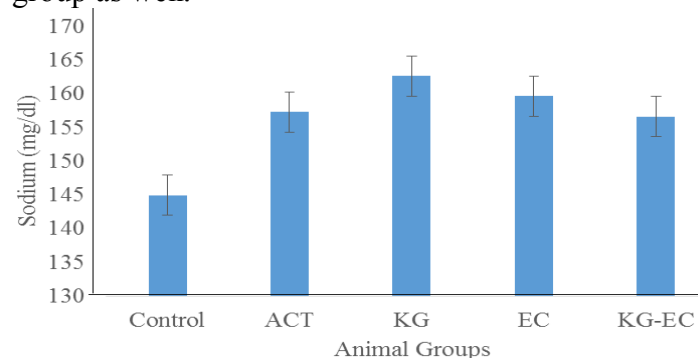

Figure 6. Effect of *Khaya grandifoliola* and *Enantia chlorantha* on sodium electrolytes concentration in the experimental Swiss mice. Results are Mean ±S.E.M, [n=5]. [ACT= Artemisinin-based Combination Therapy, KG= *Khaya grandifoliola*, EC= *Enantia chlorantha*].
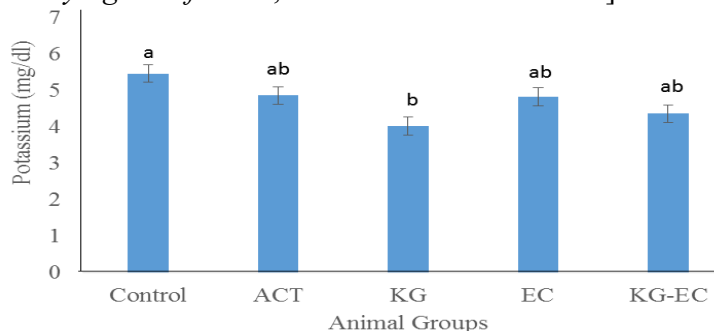

Figure 7. Effect of *Khaya grandifoliola* and *Enantia chlorantha* on potassium electrolyte concentration in experimental Swiss mice. Results are Mean ±S.E.M, [n=5]. Bars with different letters are significantly different. [ACT= Artemisinin-based Combination Therapy, KG= *Khaya grandifoliola*, EC= *Enantia chlorantha*].

DISCUSSION

The efficacy of the extract of the plant as antimalarial agent on *P. berghie*-infected mice has been reported [28]. In the same line, the stem bark of *E. chlorantha* has wide spectrum antimicrobial activity [29] antimalarial and antipyretic properties. The plant was also found to contain phenolic, flavonoids, tannin, alkaloids, terpenoids, glycosides and saponins and these secondary metabolites have been reported to cure several debilitating diseases including malaria [30].

KG and EC elicit their antimalarial properties due to presence of important phytochemicals in them. This work also has confirmed the presence of these phytochemicals as presented in the Figs. 2 and 3 for KG and EC respectively. Interestingly, the quantitative phytochemical composition of the aqueous stembark extract of KG and EC revealed the presence of bioactive principles with alkaloids, terpenoids, glycosides, phenolics, flavonoids, tannin and saponins in significant quantities. The effects elicited by these plants may be attributed to their phytochemical constituents. One of the oldest and most important antimalarial drugs, quinine, belong to this class of compounds and are still relevant [31]. The presence of alkaloids could afford the extracts their antimalarial properties. In addition, flavonoids are considered as the most diverse and widespread groups of phenolics with numerous therapeutic potentials. The flavonoid content of both plants as reported in this study perhaps could be another supportive evidence for the displayed attributes by their aqueous stem bark extract in this study. Tannins, saponins, glycosides, terpenes are also very important compounds used for various products including drug for different diseases and as anti-malarial agents [32]. The agents have cytotoxic effect on malarial parasites thereby kill most of them. Thus, the potential of KG and EC as antimalarial is confirmed in this work, as previously been reported [33].

Besides the liver, the kidney is also an important xenobiotic metabolizing organ that is often used to assess the toxicological implication of new therapeutic agents including plant extracts [34]. Hence, the need for the evaluation of the effects of these plants extracts on renal function parameters in this study. The serum creatinine and BUN are considered as nephrotoxicity markers, but serum BUN concentration is often a reliable renal function predictor. Administration of separate dose of KG and EC led to significant increase in the levels of creatinine in the *P. berghie* infected Swiss mice in comparison with control where there was no administration of ACT nor extracts. Whereas, there was no significant difference in the level of creatinine in ACT treated animal group and all the extracts treated groups (groups B to E). This means that the effects of the extracts and the synthetic ACT are not different on the level of creatinine in mice. In addition to their antimalarial efficacy of the extracts, they are also as safe as ACT in term of renal function. This deduction buttresses the previous reports [30]. This is in line with another finding [34] that observed a stabilized level of creatinine and blood urea nitrogen in *P. berghie* infected Swiss mice treated with the extract of selected antimalarial plants.

The results of this work indicated that the levels of sodium in all the treated animal groups were not significantly different from one another. There was no negative effect of both the ACT and the plant extracts on the serum sodium concentration. Electrolyte imbalance or disturbance is most commonly associated with renal failure [35]. In the intracellular and extracellular environment, the most important and electrolytes in terms of volume regulations are sodium and potassium in the living system. Sodium regulates the total amount of water in the body and its transmission across cells play roles critical to body functions while increased adequate level of potassium ions is essential for normal cell function. The movements of these ions are critical in generation of these electrical signals. The sodium pump maintains the intracellular K^+ of 140 mM against the extracellular K^+ concentration of 5 mM. Thus, the safety of these extracts is indicated by the unaltered level of the ion concentration in all the treated animal groups. A consonance was observed in all of the aqueous extracts treated groups in the Na^+ and K^+ electrolytes level, which is consistent with that of both the negative and standard control groups. This is in correlation with other reports [35]. In the same line, although, the level of potassium was slightly reduced in the treated animal groups, the reduction was not significant among all the treated groups. The ACT and the plant extracts possess Renoprotective activities as reported previously [30].

CONCLUSION

The administration of combined aqueous stem bark extract of KG and EC was suggestive of reno-protection on the animal implying that it is safe for treating *Plasmodium* infection as evidenced in the reduction of percentage parasitemia and safety

35

effect on renal function indices of Swiss mice. Thus, the administration of the combined aqueous extract of *K. grandifoliola* and *E. chlorantha* could be used safely as an alternative to standard drugs in the management of malaria.

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37