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# Ameliorating effects of ethanol extract of root bark of *Salacia nitida* on blood electrolyte and renal perturbations in *Plasmodium berghei*-infected mice

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Malaria, a disease of public health concern is a known cause of kidney failure, and dependence on herbal medicines for its treatment is increasing due to the high cost of drugs. So this study is designed to evaluate the ameliorating effect of ethanol extract from *Salacia nitida* root bark on electrolyte and renal perturbations in *Plasmodium berghei*-infected mice. Thirty malaria-infected mice divided into five groups of six mice each and another group of six uninfected mice were used for the study. 280, 430, and 580 mg/kg of extract were given to infected mice in groups B, C, and D, 4 mg/kg of artesunate given to group E mice, and 4 ml/kg of physiological saline given to group A and uninfected group F mice for five days. Serum $Na^+$, $K^+$, $HCO_3^-$, $Cl^-$, TB, urea, creatinine, BUN concentrations, and BUN/creatinine ratio were determined using standard methods. Results showed significant increases ($p < 0.05$) in $Na^+$, $K^+$, and $HCO_3^-$ and decreases in $Cl^-$, TB, urea, creatinine, BUN, and BUN/creatinine ratio in the infected treated mice in groups B - E. This study showed that ethanol extract of *S. nitida* root bark is efficient in the treatment of renal disorders and blood electrolyte perturbations.

**Keywords:** Blood electrolytes. Blood urea nitrogen. Malaria. Renal perturbation. Root bark extract. *Salacia nitida*.

## INTRODUCTION

Traditional medicines are commonly used to prevent and cure malaria and other diseases by people of low socioeconomic status in Africa. Malaria, one of the major causes of morbidity and mortality in Africa is caused by *Plasmodium* species. Different types of synthetic medicines are commonly in use for preventing and curing malaria but they posed problems of adverse effects, high cost, and resistance problems. These have increased the dependence on medicinal plants for the treatment of malaria. Also, the use of medicinal plants for the treatment of malaria is gaining more recognition due to the uneven distribution of health workers between rural and urban areas, and the lack of health facilities in the rural areas. Most malaria infections are associated with life-threatening conditions like electrolyte imbalances, liver, and kidney failures (Carter, Mendis, 2002).

Electrolyte disturbances are the common clinical manifestations of severe infectious diseases including malaria. Malaria is associated with abnormalities of fluid, electrolytes (especially sodium, Na and potassium, K), and acid-base balance in most cases. $Na^+$ regulates the normal distribution of water and osmotic pressure in various body fluids. On the other hand, $K^+$ is very crucial in the maintenance of blood pH and water levels in the body. It is used to correct acidosis in malaria infections (Maitland *et al*., 2005). Both $Na^+$ and $K^+$ are important regulators of nerve impulses in the membrane of neurons and muscle fibers. Electrolyte abnormalities seem to be frequent in malaria-associated acute kidney injury (Barsoum, 2000). Hyponatremia is a common feature in both malaria infections and kidney diseases (Barsoum,

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2000), as a result of depletion in blood volume, renal loss due to increased vascular permeability, acute renal tubular failure, or renal tubular acidosis (Pohl, Wheeler, Murray, 2013). Disruption in the normal bicarbonate levels is a feature of kidney disorder (Stoppler, Shiel, 2015). Both chronic and acute renal failures have been reported in cases of malaria infection (Barsoum, Sitprija, 1996). The mechanism responsible for renal failure in malaria infection might be due to a reduction in renal blood flow resulting from intravascular rosettes and parasite cytoadherence that may impede microcirculation, hypovolemia, the release of chemical mediators that increase vascular permeability, renal tubular cell membrane derangements due to ischemia-perfusion syndrome caused by the malaria parasite, intravascular coagulation, increased plasma viscosity due to malaria parasite, or inflammation due to increased vascular permeability. Other factors of kidney injury in malaria infection are hemolysis, and hepatic dysfunction with jaundice, resulting in hyperbilirubinemia, cast nephropathy, and acute kidney disease (Barsoum, Sitprija, 1996; Barsoum, 2000; Mehta *et al*., 2001).

In Africa, people depend on medicinal plants including *Salacia nitida* for treatments of malaria. *Salacia nitida* L. Benth is a green herbaceous tree of the family Celastraceae. It is found mostly in the southern part of Nigeria. The decoctions of root bark of *S. nitida*, are orally taken for treatments of malaria. The identification and quantification of phytochemical constituents of *S. nitida* root bark using a gas chromatography-flame ionization detector, (GC-FID) has been reported to contain spartein (0.0058 μg/g), epicatechin (24.6059 μg/g), phytate (0.4325 μg/g), anthocyanin (1.2840 μg/g), tannins (20.5901 μg/g), phenol (2.4127 μg/g), lunamarine (14.1437 μg/g), sapogenin (1.7417 μg/g), ribalinidine (7.8886 μg/g), catechin (126.6694 μg/g), rutin (74.9289 μg/g), and kaempferol (62.6072 μg/g) (Nwiloh, Uwakwe, Akaninwor, 2016), which are phytochemicals with pharmacological activities (Robak, Glyglewski, 1988; Lin, Hsu, Lin, 2001; Seeram *et al*., 2002; Lee *et al*., 2003; Han, Shen, Lou, 2007; Oomah, Blanchard, Balasubramanian, 2008; Paolillo, Carratelli, Rizzo, 2011; Marella *et al*., 2013). A preclinical study with the root bark of *S. nitida* has suggested a good level (88.45%) of antimalarial activity (Nwiloh, Akaninwor, Uwakwe, 2017). Hence, this study is designed to evaluate the ameliorating effect of ethanol extract from the root bark of *S. nitida* on blood electrolytes and renal perturbations in induced *P. berghei* malaria-infected mice.

## MATERIAL AND METHODS

### Chemicals and reagents

The reagent kits used were of analytical grade. Bicarbonate, sodium, bilirubin, and urea assay kits were provided by Randox Laboratories Ltd (USA), while creatinine, chloride, and potassium reagent kits were supplied by Teco Diagnostics (USA). The antimalarial drug used was artesunate tablet, 50 mg (Artesunat ®), supplied by Mekophar chemical pharmaceutical Joint-Stock Company, Vietnam.

### Collection of plant materials and extraction

*Salacia nitida* plants were collected in February 2016, from Diidi farm at Nyogor-Beeri, Khana local government area of Rivers State, Nigeria. It was identified and authenticated by Dr. N. L. Edwin-Wosu of the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State, Nigeria, with voucher number UPHV-1033, and sample deposited at the University herbarium. The plant's roots were removed from the ground with a spade and carried to the Department of Biochemistry laboratory at the University of Port Harcourt, washed with clean water, and air-dried under shade for one hour. The barks were removed from the roots and reduced to smaller bits with a machete, onto a clean leather material. The root barks were again air-dried under shade for seven days, and the dried root barks were pulverized using a hand grinding machine (Corona-16D). Extraction was done with Soxhlet extractor using 250 g of powdered root bark material and 500 ml of ethanol (80%) with a heating mantle regulated at 80 $^{o}$C. Extracts obtained were concentrated to dryness with a rotary evaporator (Heidolph 4000, Schwabach, Germany), and the dry extract obtained was stored in a refrigerator at 4$^{o}$C until required for use.

**Experimental animals**

The study was conducted with a total of 36 healthy 12-14 weeks old albino mice of mixed sexes, weighing between 27-38g, selected out of eighty (80) albino mice procured from the Department of Pharmacology, College of Medicine, University of Port Harcourt. The 80 mice were screened for the presence of malaria using rapid diagnostic test strips (Access Bio Inc, NJ, USA). The health statuses of the experimental mice were ascertained by their physical appearances and feeding behaviors. The 36 healthy mice were housed in plastic cages and maintained under standard environmental conditions of humidity, ordinary temperature, and 12 hours light/12 hours darkness cycle, with free access to animal feed (Grower's marsh) and clean water *ad libitum* for two weeks. This study was conducted according to the United States National Institute of Health guides on the care and use of laboratory animals (NIH, 1985), and the ethical rules of the University of Port Harcourt on the use and care of laboratory animals (UPH/BCH/AEC/2016/025B).

**Acute toxicity test**

The acute toxicity test was conducted according to a modified method of Lorke (1983) using 24 mice. Tests were done in two phases with twelve mice divided into three groups of four mice per group in each phase. Doses of 50, 150, and 300 mg/100 g of the ethanol extract were orally given to healthy mice in groups I, II, and III using a stomach cannula in the first phase. The mice were monitored for 24 hours for signs of toxicity. With no sign of toxicity observed, groups IV, V, and VI mice were orally given 400, 700, and 1000 mg/100 g of ethanol extract in the second phase. They were monitored for another 24 hours for signs of toxicity. The square root of the least dose that killed at least one mouse and the highest dose that did not kill any mice was taken as the $LD_{50.}$

**Inoculation of mice with *Plasmodium berghei***

The parasite used for the study was *Plasmodium berghei* (NK-65) obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria. They were contained in five donor mice and carried to the World Bank-Assisted Malaria and Phytomedicines Research Laboratory, University of Port Harcourt, for maintenance. Before inoculation, levels of parasitemia in donor mice were determined by cutting their tail tips with sterile pair of scissors and blood extruded into a small beaker containing 0.5 ml normal saline. A drop of diluted infected blood from the donor mice was placed on a rapid diagnostic malaria test strip (Access Bio Inc, NJ, USA) and color intensity was used to confirm the level of malaria. All donor mice with higher malaria levels were sacrificed by cervical dislocations and blood was collected by cardiac puncture into a 50 ml beaker using a sterile disposable syringe and needle to avoid any variability in parasitemia. A 0.2 ml of parasitized blood from donor mice was added to 9.8 ml of normal saline. Thirty (30) healthy mice were infected with 0.2 ml each of the infected diluted blood containing $1x10^7$ *P. berghei*-infected erythrocytes injected into the mice intraperitoneally on day one, and randomly divided into five (5) groups labeled A, B, C, D, and E containing six (6) infected mice each. Another six (6) uninfected mice were placed in Group F to serve as the reference control. Standard methods were used to determine the dosages and volume of ethanol extract and drug (ART) used for treatments (OECD, 2000; Oghenesuvwe, Ekene, Lotana, 2014; WHO, 2015).

**Treatment of *Plasmodium berghei*-infected mice with ethanol extract of *S. nitida* root bark**

Seventy-two hours after infections, all the mice in groups A to E were treated with the ethanolic extract of *S. nitida* root bark as follows:

Group A contained six (6) *P. berghei*-infected mice that were given 4 ml/kg body weight of physiological saline daily (negative control group, NC),
Group B contained six (6) *P. berghei*-infected mice that were given 280 mg/kg body weight/day of ethanol extract from the root bark of *S. nitida*,
Group C contained six (6) *P. berghei*-infected mice that were given 430 mg/kg body weight/day of ethanol extract from the root bark of *S. nitida*,

Group D contained six (6) *P. berghei*-infected mice that were given 580 mg/kg body weight/day of ethanol extract from the root bark of *S. nitida*,
Group E contained six (6) *P. berghei*-infected mice that were given 4 mg/kg body weight/day of artesunate (positive control, PC),
Group F contained six (6) uninfected mice that were given 4 ml/kg body weight/day of physiological saline (reference control, RC).

All the *P. berghei*-infected mice in groups B to E were orally given 0.3 ml of the extract and drug, while those in groups A and F were given physiological saline. Treatments were done once daily by intragastric administration of extract and drug in the morning time for five consecutive days with oral metal gavage. The mice were also allowed free access to food (grower's marsh) and clean water *ad libitum*.

#### Analyses of renal biomarkers and blood electrolytes

At the end of the treatment period, all the experimental mice were killed by cervical dislocations, and blood was collected by cutting the jugular veins with a sterilized lancet into dry bottles containing lithium-heparin. The blood sample was centrifuged for 10 minutes at 4000 rpm, and the supernatant was used to evaluate the blood electrolytes. Serum $Na^+$ was determined according to Trinder (1951), $K^+$ was analyzed by turbidimetric spot test (Tubino, de Souza, Hoehr, 2004), $HCO_3^-$ measured using the back titration method (Van Slyke, Spillman, Cullen, 1919), and $Cl^-$ estimated using the colorimetric method (Schoenfeld, Lewellen, 1964). Jendrassik-Grof method was used to analyze serum total bilirubin, TB (Dangerfield, Finlayson, 1953), and serum urea (Ur) determined with the Urease-Berthelot method (Fawcett, Scott, 1960), while creatinine (Cr) was estimated with Jaffe's direct endpoint reaction method (Toora, Rajagopal, 2002). Also, blood urea nitrogen, BUN (mg/dl), and BUN-creatinine ratio (BCR) were determined using mathematical expressions (Crook, 2006; Higgins, 2016).

#### Statistical analysis

Results were expressed as mean values ± standard error of means (SEM). The data obtained were statistically analyzed by the use of one-way analysis of variance (ANOVA), using the SPSS software version 22.0 statistical package. Multiple comparisons were done with Scheffe's post hoc test to compare differences between results. Results were considered significant at a 95 % confidence level ($p < 0.05$).

## RESULTS AND DISCUSSION

The results of the oral toxicity test showed that the extract was safe with an $LD_{50}$ of 837 mg/100g. Results for the effect of treatments with the ethanol extract from the root bark of *S. nitida* on serum $Na^+$, $K^+$, $HCO_3^-$, and $Cl^-$ are shown in Table I. The results showed significant decreases ($p < 0.05$) in the mean concentrations of $Na^+$ and $HCO_3^-$, and a significant increase ($p < 0.05$) in the mean concentration of $Cl^-$ in the *P. berghei*-infected untreated mice in group A (NC) compared to those in group F (RC). A decrease in the mean concentration of $K^+$ in mice of group A compared to uninfected untreated mice in group F were also observed. It was noticed that treatments of *P. berghei*-infected mice in groups B through E with different graded doses of ethanol extract from *S. nitida* root barks and artesunate significantly increased ($p < 0.05$) the mean concentrations of $Na^+$ and $HCO_3^-$, and moderately increased the mean concentration of $K^+$ compared to those in the reference control mice in group F. Also, treatments of *P. berghei*-infected mice in groups B through E with different graded doses of ethanol extract of *S. nitida* root barks and artesunate significantly decreased ($p < 0.05$) the mean concentration of $Cl^-$ when compared to that of the reference control in group F.

**TABLE I -** Effect of ethanol extract of *S. nitida* root bark on blood electrolytes in *P. berghei*-infected mice (n = 6)

| Parameters | Group/Dosage | | | | | |
|---|---|---|---|---|---|---|
| | A (4ml/kg) | B (280mg/kg) | C (430mg/kg) | D (580mg/kg) | E (4mg/kg) | F (4ml/kg) |
| $Na^+$ (mmol/l) | 44.67 ± 1.20[a] | 82.17 ± 4.77 | 144.83 ± 1.66* | 148.33 ± 0.59* | 145.09 ± 0.82* | 151.50 ±1.84 |
| $K^+$ (mmol/l) | 4.17 ± 0.11 | 4.62 ± 0.10 | 4.92 ± 0.07 | 5.02 ± 0.83 | 5.03 ± 0.12 | 5.15 ± 0.14 |
| $HCO_3^-$ (mmol/l) | 19.17 ± 0.60[a] | 22.67 ± 0.36* | 26.33 ± 0.49* | 28.33 ± 0.42* | 29.50 ± 0.43* | 30.67 ± 0.67 |
| $Cl^-$ (mmol/l) | 119.67 ± 0.67[a] | 115.00 ± 0.36 | 111.33 ± 0.88 | 106.17 ± 3.23* | 104.17 ± 2.23* | 99,83 ±1.56 |

Values = mean ± SEM; *, [a] = values are significant ($p < 0.05$).

Results for serum total bilirubin, creatinine, urea, BUN, and BCR are presented in Table II. It is shown that there were significant increases ($p < 0.05$) in the levels of bilirubin, creatinine, urea, BUN, and BCR in the *P. berghei*-infected untreated mice in group A compared to those in the uninfected untreated mice in the reference control group (group F). Treatments with different doses of the ethanol extract and artesunate significantly ($p < 0.05$) decreased the plasma levels of bilirubin, creatinine, urea, BUN, and BCR in the *P. berghei*-infected treated mice in groups B, C, D, and E compared to those in the infected untreated mice in group A.

**TABLE II -** Effect of ethanol extract of *S. nitida* root bark on some renal biomarkers in *P.berghei*-infected mice (n = 6)

| Parameters | Group/Dosage | | | | | |
|---|---|---|---|---|---|---|
| | A (4ml/kg) | B (280mg/kg) | C (430mg/kg) | D (580mg/kg) | E (4mg/kg) | F (4ml/kg) |
| Bilirubin (µmol/l) | 8.80 ± 0.17[a] | 7.80 ± 2.10* | 7.50 ± 1.16* | 6.96 ± 3.15* | 5.67 ± 1.22* | 5.30 ± 2.10 |
| Creatinine (µmol/l) | 137.33 ± 3.05[a] | 121.17 ± 0.87 | 112.17 ± 1.58 | 101.33 ±1.26 | 99.50 ±1.38* | 92.83 ±1.66 |
| Urea (mmol/l) | 13.30 ± 0.67[a] | 9.17 ± 0.09* | 8.02 ± 0.31* | 6.80 ±0.07* | 5.22 ±0.22* | 4.42 ±0.04 |
| BUN (mg/dl) | 37.25 ±0.33[a] | 25.67 ±0.27* | 22.46 ±0.11* | 19.05 ±0.23* | 14.62 ±1.31* | 12.38 ±1.79 |
| BCR | 24.03[a] | 18.74 | 17.69* | 16.57* | 12.94* | 11.79 |

Values = mean ± SEM; BUN = Blood urea nitrogen; BCR = BUN-Creatinine ratio; [a], * = significant values ($p$ <0.05)

Perturbations in blood electrolytes and some biochemical indices of renal disorder were noticed in this study. Significant ($p < 0.05$) increase in total bilirubin level in the *P. berghei*-infected mice in group A compared to their healthy counterparts in the reference control (group F) observed in this study might be due to hemolytic jaundice (Mehta *et al*., 2001; Okafor *et al* 2020), which is commonly associated with acute kidney failure due to malaria infections (Crook, 2006; Irwin, Rippe, 2008). The results obtained for $Na^+$ and $K^+$ in *P. berghei*-infected untreated mice in group A are in tandem with the work reported by Jasani and colleagues (Jasani *et al*., 2012). The decrease in plasma bicarbonate concentration recorded in group A mice in this work might be due to increased chloride ions concentration (Baron, Whicher, Lee, 2011). Increased serum chloride ions and hypokalemia have been reported as consequences of distal renal tubular acidosis and raised serum urea levels as a consequence of increased catabolic processes of protein (Chatterjea, Shinde, 2007). Increased serum creatinine and urea are caused by reduced glomerular filtration rate (GFR), and are indications of renal disease (Higgins, 2016). The elevated chloride, urea, and creatinine concentrations recorded in this work in the *P. berghei*-infected untreated mice in group A are indications of a disorder in renal function (Henderson, 2016). A rise in urea and creatinine levels characterizes pre-renal acute kidney injury (Lelevich, Popechits, 2010). The raised serum urea level seen in this work might result from the increase in catabolic processes of protein as a result of malaria infections or fever. The elevated BUN seen in this work is an indication of impaired kidney function or conditions that could reduce renal blood flow (Areekul, 1988), and the elevated BCR obtained is a prognostic indicator of acute kidney injury (Higgins, 2016). The increase in the concentrations of both urea and creatinine might be the underline cause of the increased BCR seen in this work (Higgins, 2016).

The results obtained from this study showed that oral administrations of different graded doses of ethanolic extract from the root bark of *S. nitida* and artesunate to the *P. berghei*-infected treated mice in groups B through E positively modified the biochemical alterations caused by plasmodium malaria infection, thus ameliorating the disorders in electrolytes and renal functions in these mice. Antimalarial activities of ethanolic extract from the root bark of *S. nitida* have been reported (Nwiloh, Akaninwor, Uwakwe, 2017) and could be the cause of the amelioration observed in this study. The extract from *S. nitida* root bark has been reported to contain some phytochemicals with antioxidant, anti-inflammatory, membrane stabilizing, improve blood flow, angiogenic, hemostatic, decrease vascular permeability activities, and ability to repair inflamed membranes(Wilairatana, Looareesuwan, Charoenlarp, 1994; Nwiloh, Uwakwe, Akaninwor, 2016), which could be implicated for the ameliorations of electrolytes and renal perturbations observed in this work by the extract in the *P. berghei*-infected treated mice. Spartein, lunamarine, and ribalinidine (quinolone alkaloids), tannins, phenols, anthocyanin, epicatechin, and phytate which are phtytochemical constituents of *S. nitida* have been shown to possess antimalarial, antioxidant, anti-inflammatory, and metal chelating activities (Robak, Glyglewski, 1988; Lin, Hsu, Lin, 2001; Seeram *et al*., 2002; Lee *et al*., 2003; Han, Shen, Lou, 2007; Oomah, Blanchard, Balasubramanian, 2008; Paolillo, Carratelli, Rizzo, 2011; Marella *et al*., 2013; Nwiloh, Uwakwe, Akaninwor, 2016).

From the study, ethanol extract of *S. nitida* root bark ameliorated the disorders in blood electrolytes and renal functions that resulted from induced malaria infection in mice. Therefore, this study showed that ethanol extract from the root bark of *S. nitida* is very useful in the treatment of renal disorders and the amelioration of blood electrolytes resulting from malaria. These findings corroborated the traditional use of the alcoholic decoctions of *S. nitida* root bark for the treatment of malaria in Nigeria, and could also be a very useful source of novel drugs for treatments and management of malaria, blood electrolytes, and renal disorders.

## CONFLICT OF INTEREST

No conflict of interest exists.

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