

Antipyretic and haematomodulatory activities of ethanolic extract from *Salacia nitida* root bark in *Plasmodium berghei*-malaria infected mice

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Abstract

Introduction: In southern part of Nigeria, root bark of *Salacia nitida* is use traditionally in the treatment of malaria. Therefore, this study is designed to evaluate the antipyretic and haematomodulatory activities of ethanolic extract from *Salacia nitida* root bark in *Plasmodium berghei*-malaria infected mice.

Materials and methods: Fresh roots of *S. nitida* were collected in February, 2016. Thirty *P. berghei*-infected mice, divided into five groups containing six mice each were used for the study against another six uninfected control mice. 280, 430 and 580 mg/kg of extract were given to infected mice in groups B, C and D, 4mg/kg of artesunate given to group E mice, while group A and F mice were given 4ml/kg of physiological saline daily for five days. Body temperatures and levels of haematological parameters were evaluated.

Results: Results obtained showed significant increase ($P < 0.05$) in haemoglobin concentration, erythrocyte, leukocyte and platelet counts, and decrease in absolute neutrophil and lymphocyte counts. Body temperatures of experimental mice also decreased significantly ($P < 0.05$).

Conclusion: Therefore, this study suggests that ethanolic extract from *S. nitida* root bark is effective in treatment of malaria.

Keywords: Antipyretic, Haematological parameters, Mice, *Plasmodium berghei*, *Salacia nitida*

Introduction

Over 212 million cases of malaria occur every year with about 429,000 deaths, most of who are children from Africa (1). *P. falciparum* is the main cause of most malaria incidences and malaria-related dead (2). Once the parasites are introduced into its victim, they migrate to the liver; invade the liver cells, and grow into merozoites that are released into the blood stream of the victim. Plasmodia invasion of the erythrocytes led to lyses of infected

erythrocytes along with waste substances such as remains of red cell membranes, haemozoin pigments, and glycosyl phosphatyl inositol (GPI), a key parasite toxin (3, 4), which are released into the blood. These products, particularly the GPI, activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators such as tumor necrosis factor (TNF), interferon- γ , interleukin-1 (IL-1), IL-6, IL-8,

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lymphotoxin, as well as superoxide and nitric oxide (NO). These lead to manifestations of malaria (5, 6) with headache, sweat, fever and rigors, chills, nausea and vomiting, diarrhea, tiredness, muscles and joints ache, thrombocytopenia, etc, appearing with haemolyses of the red blood cells that are linked to cytokines released in response to these parasites, and red cell membrane products (7, 8). Also, the plasmodial DNA is highly inflammatory and can induce cytokinemia and fever. This is done by inducing cox-2-upregulating prostaglandins that led to the induction of fever (9, 10). Haemozoin has also been known to induce apoptosis in developing erythroid cells in the bone marrow, thereby causing anaemia (11, 12). In most *P. falciparum* malaria, there may be severe life-threatening conditions that eventually cause dysfunctions of important organs like the liver, kidneys, etc., including cachexia (13). Haematological abnormalities that are caused by malaria episode include changes in haemoglobin concentrations, leukocyte counts and differential leukocytes, platelet counts, and disseminated intravascular coagulation (14 - 17). Anemia is a common disorder that accompanies malaria infections (7, 18), which can cause confusion, restlessness, renal haemorrhages, coma and cardiac failure in very severe cases. This may be caused by haemolysis of the red blood cells by the malaria *Plasmodium* parasites (19).

Salacia nitida is a member of the Celastraceae family, and is traditionally used for treatments of malaria in southern Nigeria. Root bark of *S. nitida* has been reported to contain alkaloids, tannins, sapogenin, flavonoids, phenol, phytate, and anthocyanin which are bioactive phytochemicals (20). Also, the antimalarial activity of ethanolic extract from root bark of *S. nitida* has been reported (21). This study is necessary because scientific reports on the antipyretic and haematomodulatory activities of ethanolic

extract from root bark of *S. nitida* in *Plasmodium berghei*-malaria infected mice is not found in any literature.

Materials and methods

Chemicals and reagents: Chemicals and reagents used are of analytical grade.

Collection and preparation of plant materials: *Salacia nitida* was collected in February 2016, from a farmland in Nyogor-Beerri, Khana local government area of Rivers state, Nigeria, and the plant was identified by Dr. N. L. Edwin-Wosu of the department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria. The voucher number is UPHV-1033, and the plant sample is deposited in the University herbarium. Plant roots were dug from the ground with spade and carried to the laboratory in the department of Biochemistry, University of Port Harcourt, washed in clean water and air dried for about an hour. Barks were removed from the root and reduced to smaller bits with machete onto a clean leather material spread on the floor. The root barks were again air dried under shade for one week. The dried root barks were pulverized with hand grinding machine (corona-16D).

Preparation of ethanolic extract: Extraction was done with Soxhlet extractor; using 280g of powdered material from the root barks and 100ml of ethanol at 80°C with a water bath, for about 18 hours. Extract obtained was concentrated to dryness for a week, with water bath at 80°C and stored in an air-tight container kept in a refrigerator regulated at 4°C until required for use.

Experimental animals: A total of 36 healthy 6-8 weeks old albino mice of mixed sexes, weighing between 19g - 35g which were selected out of eighty (80) albino mice procured from department of pharmacology, college of medicine, University of Port Harcourt, were used for the study. The 80 mice were screened for malaria using the rapid diagnostic test

strips (Access Bio Inc, NJ, USA). Physical appearances and feeding behaviour of the mice were used to ascertain their health status. Only healthy mice were used for the study. They 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 and cleans water *ad libitum* for two weeks. The study was conducted according to the United States National Institute of Health "Principle of Laboratory animals care" (22) and guidelines on the use of laboratory animals of the University of Port Harcourt. Approval for the use of animals in the study was granted by the department of biochemistry animal ethic committee of the University of Port Harcourt, with ethical clearance number UPH/BCH/AEC/2016/015.

Acute toxicity test: Acute toxicity test was done using 24 mice according to Lorke (23). Tests were done in two phases with twelve mice, divided into three groups of four mice per group in each phase. The mice were monitored for 24 hours, for signs of toxicity. Geometric mean of the least dose that killed mice and the highest dose that did not kill mice was taken as the LD₅₀ and multiplied by a factor 0.7 to get doses used for treatments (24).

The parasite and inoculation of mice: Parasite used for the study was *Plasmodium berghei* (NK-65) procured from Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria. They were contained in five (5) donor mice, and carried to the World Bank-Assisted Malaria and Phytomedicines Research Laboratory, University of Port Harcourt, for maintenance. Before inoculation, levels of parasitaemia in donor mice were determined by cutting the tip of the tail of donor mice with sterile pairs of scissors and blood extruded into a small beaker containing 0.5ml normal saline. A drop of

diluted infected blood from the donor mice was placed on the rapid diagnostic malaria test strips (Access Bio Inc, NJ, USA) and colour intensity used to ascertain the level of parasitaemia. All the donor mice with higher levels of parasitaemia were sacrificed by cervical dislocations and blood collected by cardiac puncture into a 150ml beaker, using sterile disposable syringe and needle to avoid any variability in parasitaemia. 0.2ml of parasitized blood from donor mice was added to 9.8ml of normal saline.

Treatment of malaria infected mice with ethanolic extract from *S nitida* root bark and collection of blood for haematological analysis:

The levels of parasitaemia in the experimental mice were tested with malaria test strip as described above on day 1 before infection with the malaria parasites. *P. berghei*-malaria infected mice were treated by oral administration of ethanolic extract from root bark of *S nitida* using the modified method of Ryley and Peters (25). Thirty (30) healthy mice were infected with 0.2ml of the infected diluted blood containing 1×10^7 *P. berghei* infected erythrocytes obtained from donor mice, intraperitoneally on day one, and were randomly divided into five (5) groups labeled A, B, C, D, and E containing six (6) infected mice each, while another six (6) normal mice were placed in group F to serve as positive control. The levels of parasitaemia in the experimental mice were tested again with malaria test strip as described above 72 hours later before treatment with the ethanolic extract from *S. nitida* root bark. All the mice were treated with ethanolic extract from *S. nitida* root bark as described below.

Group A, six *P. berghei*-infected mice given 4ml/kg body weight of physiological saline daily (Negative control group, NC); Group B, six *P. berghei*-infected mice given 280mg/kg body weight/day of ethanolic extract from root bark of *S. nitida*; Group C, six *P. berghei*-infected mice given 430mg/kg body weight/day of

ethanolic extract from root bark of *S. nitida*; Group D, six *P. berghei*-infected mice given 580mg/kg body weight/day of ethanolic extract from root bark of *S. nitida*; Group E, six *P. berghei*-infected mice given 4mg/kg body weight/day of artesunate (Positive control, PC);

Group F, six healthy mice given 4ml/kg body weight/day of physiological saline (Reference control, RC).

All the *P. berghei*-infected mice in groups A through E were orally given 0.3ml of extract and drug once daily, by 8.00am for five consecutive days, using stomach cannula. The mice were also allowed free access to food (grower's marsh) and clean water *ad libitum*. At the end of the treatment period, levels of parasitaemia in the experimental mice were tested with malaria test strip as described above, and all the mice were killed by cervical dislocations and blood collected by cutting the jugular veins with sterilized lancet. Blood were pooled into labeled dry sample bottles containing EDTA for haematological assays.

Determinations of body temperatures of experimental mice: Body temperatures of mice were measured and recorded using clinical thermometer on the 1st day, on day 3 before treatments commenced, and at the end of study period. Percentage change in body temperatures of mice was determined using the formula:

% change in body temperature = $T_f - T_i / T_f \times 100$. Where T_f = final temperature of mice; and T_i = initial temperature of mice.

Evaluations of haematological indices in *P. berghei*-infected mice given ethanolic extract from root bark of *S. nitida*: Evaluation of haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), total white blood cell (WBC), platelets and white blood cell differentials were evaluated using standard recommended methods (26, 27).

Data analysis

Results were expressed as mean values \pm standard error of means (SEM). The data

obtained were statistical analyzed by the use of one-way analysis of variance (ANOVA), using the SPSS software version 22 statistical package. Multiple comparisons were done using the Scheffe's post hoc test to compare differences between results. Results were considered significant at 95 % confidence level ($P < 0.05$).

Results

The results for the levels of malaria parasites in the experimental mice are shown in Table 1. The results showed that levels of malaria parasites kept increasing in the malaria infected untreated mice in group A (NC) from day1 through 8, while there were reductions in those of the malaria infected treated mice in groups B through E that were given ethanolic extract from root bark of *S. nitida* and artesunate on day 3 to 8. Results for normal mice in group F (RC) showed no trace of malaria parasites, which were the results obtained for all the experimental mice on day 1 (Table 1).

Table 1. Effect of ethanolic extract from *S. nitida* root bark on malaria parasites in experimental mice groups (n = 6).

Group /dose (mg/kg bw/day)	Levels of Malaria		
	Day 1	Day 3	Day 8
A (4)	-	+++	++++
B(280)	-	+++	++
C (430)	-	+++	+
D (580)	-	+++	+
E (4)	-	+++	+
F (4)	-	-	-

++++ = very high malaria parasitaemia; +++ high malaria parasitaemia; ++ = low malaria parasitaemia; + = very low malaria parasitaemia; - = no malaria parasites

The results for percentage change in body temperatures of experimental mice are presented in Table 2. There was significance increase ($P < 0.05$) in percentage change in the body temperatures on day 1, from 0% for mice in all the groups to 3.82% for group A, 3.69% for group B, 4.23% for group C,

2.94% for group D, 4.35% for group E and -1.93% for group F on day 3. From Table 2 below, it is noticed that oral administration of different doses of ethanolic extract from *S. nitida* root bark and artesunate to *P. berghei*-malaria infected mice significantly decrease ($P < 0.05$) percentage change in the body

temperatures on day 8 to -2.53% for group B, -3.37% for group C, -4.11% for group D and -3.73% for group E mice respectively, compare to those of mice in group A (negative control) with 2.83% increase and group F (reference control) with -0.62% decrease in percentage change in the body temperatures on same day.

Table 2. Effect of oral administration of ethanolic extract from *S. nitida* root bark on percentage change in body temperatures of *P. berghei*-malaria infected mice groups (n = 6).

Groups (dose in mg/kg bw/day)	Day 1		Day 3		Day 8	
	Temperature (°C)	Changed temperature	Temperature (°C)	Changed temperature	Temperature (°C)	Changed temperature
A(4)	37.28±0.25	0(0)	38.77±0.00	1.48(3.82)	39.90±0.15	1.13(2.83)
B(280)	37.05±0.16	0(0)	38.47±0.23	1.42(3.69)*	37.52±0.22	-0.95(-2.53)
C (430)	36.70±0.24	0(0)	38.32±0.29	1.62(4.23)*	37.07±0.14	-1.25(-3.37) *
D(580)	37.33±0.25	0(0)	38.47±0.21	1.13(2.94)*	36.95±0.24	-1.52(-4.11)*
E(4)	36.72±0.15	0(0)	38.38±0.32	1.67(4.35)*	37.00±0.24	-1.38(-3.73)*
F(4)	37.40±0.14	0(0)	37.28±0.25	-0.72(-1.93)	37.05±0.13	-0.23(-0.62)

Data are shown as mean ± SEM, * = significant at $P < 0.05$.

Table 3 showed the positive effects of ethanolic extract from root bark of *S. nitida* on haemoglobin (Hb) levels in *P. berghei*-malaria infected mice in groups B to E. There are significant increased ($P < 0.05$) in mean haemoglobin (Hb) levels in *P. berghei*-malaria infected mice in groups B to 9.38±0.16 g/dl, C to 10.35±0.11g/dl, D to 11.27±0.18 g/dl that were given 280, 430, and 580 mg/kg body weight/day of ethanolic extract from root bark of *S. nitida*, and group E that were given 4 mg/kg body weight/day of artesunate to 12.12±0.05 g/dl, compared to those of infected untreated mice in group A (NC) with mean Hb level of 7.75±0.29 g/dl. No significant changes ($P > 0.05$) were observed in levels of Hb in malaria-infected treated mice in group D and group E respectively. It is clearly shown that the packed cell volume (PCV) of malaria infected untreated mice (group A) was decreased significantly ($P < 0.05$) by *P. berghei*-malaria infections to 22.17±0.60% as compared to normal mice in reference control (group F) with PCV value of 40.50±1.06%. Treatments of malaria infected mice with ethanolic extract from root bark of *S. nitida* at difference doses

and artesunate, significantly raised ($P < 0.05$) the PCV levels. The mean PCV for the group B mice given 280 mg/kg body weight/day of extract is 29.33±2.04%, the group C mice given 430 mg/kg body weight/day of extract is 32.00±0.58%, and that for the group D mice given 580mg/kg body weight/day is 35.33±0.33%, while that of mice in group E (PC) given 4 mg/kg body weight/day of artesunate is 36.50±0.22% compared to mean PCV of mice in group A (NC) given 4 ml/kg body weight of physiological saline (Table 3). From Table 3, no significant change ($P > 0.05$) is observed in levels of PCV in groups D and E mice.

There is significant decrease ($P < 0.05$) in level of red blood cells (RBC) in the infected untreated mice in group A with mean RBC value of 3.17±0.16 ($\times 10^{12}/l$) compared to normal mice in group F (RC) with RBC value of 5.68±0.09 ($\times 10^{12}/l$). From Table 3 it is seen that treatments of malaria infected mice with the ethanolic extract and artesunate significantly increase ($P < 0.05$) the levels of RBCs to 3.75±0.03($\times 10^{12}/l$) for group B, 4.30±1.11($\times 10^{12}/l$) for group C, 4.77±0.93($\times 10^{12}/l$) for group D, and

5.13±0.24(x10¹²/l) for group E mice respectively. The results showed no significant change ($P > 0.05$) in RBCs of malaria infected mice in group D treated

with 580 mg/kg body weight/day of the ethanolic extract and those of group E given 4 mg/kg body weight/day of artesunate (5.68±0.09 (x10¹²/l)).

Table 3. Results of effects of ethanolic extract from root bark of *S. nitida* on some haematocological parameters of P.berghei-infected mice groups (n = 6).

Groups (dose)	Hb (g/dL)	PCV (%)	RBC (x10 ¹² /L)	Parameters			
				Platelets (x10 ⁹ /L)	WBC (x10 ⁹ /L)	ANC (x10 ⁹ /L)	ALC (Lymphs/L)
A(4)	7.75±0.29	22.17±0.60	3.17±0.16	185.00 ±4.28	4.67±0.08	2.69±2.55	3665.95±1.11
B(280)	9.38±0.16*	29.33±2.04*	3.75±0.03*	216.67±2.55*	4.32±1.02*	2.26±1.59*	3247.34±0.25*
C (430)	10.35±0.11*	32.00±0.58*	4.30±1.11*	236.67±1.67*	4.17±2.01*	1.93±0.99*	3057.86±0.81*
D(580)	11.25±0.18*	35.33±0.33*	4.77±0.93*	265.00±0.86*	4.03±0.11*	1.54±1.12*	2889.11±2.23*
E (4)	12.12±0.05*	36.50±0.22*	5.13±0.24*	286.67±3.01*	3.93±0.07*	1.21±1.02*	2744.32±0.32*
F(4)	12.82±0.29*	40.50±1.06*	5.68±0.09*	311.67±0.10*	3.78±0.08*	0.96±0.21*	2457.00±1.99*

Data are shown as mean ± SEM, * = significant at $P < 0.05$.

Table 3 also showed significant decrease ($P < 0.05$) in the level of platelet counts in the infected untreated mice in group A with mean value of 185.00±4.28 (x10⁹/l) compared to normal mice in group F (RC) with platelet counts of 311.67±0.10 (x10⁹/l). It is noticed that treatments of malaria infected mice with the ethanolic extract and artesunate increase the levels of platelet counts significantly ($P < 0.05$) to 216.67±2.55(x10⁹/l) for group B, 236.67±1.67(x10⁹/l) for group C, 265.00±0.86(x10⁹/l) for group D, and 286.67±3.01(x10⁹/l) for group E mice respectively. There is no significant change ($P > 0.05$) in platelet counts of malaria infected mice in groups D and E. From Table 3, it is seen that there is significant increase ($P < 0.05$) in level of total white blood cells (WBC) in the malaria infected untreated mice in group A to 4.67±0.08 (x10⁹/L), compared to normal mice in group F (RC) with 3.78±0.08 (x10⁹/l). But treatments of malaria with different doses of ethanolic extract from root bark of *S. nitida* and artesunate significantly decrease ($P < 0.05$) WBC levels in groups B, C, D and E mice when compared to malaria infected untreated mice in group A (NC). There is no significant difference ($P > 0.05$) observed in levels of WBC in malaria-infected treated mice in group D and group E.

Significant increase ($P < 0.05$) in level of absolute neutrophil count (ANC) in

malaria infected untreated mice (group A) to 4.67±0.08 (x10⁹/L) compared to healthy mice in group F (RC) with 3.78±0.08 (x10⁹/l) was recorded. Oral administrations of different doses of the ethanolic extract and artesunate significantly decrease ($P < 0.05$) ANC levels in infected treated mice in groups B to 2.26±1.59 (x10⁹/L), C to 1.93±0.99 (x10⁹/L), D to 1.54±1.12 (x10⁹/L), and E to 1.21±1.02 (x10⁹/L) respectively, compared to mice in group A (NC). No significant difference ($P > 0.05$) was observed in ANC of malaria-infected mice in group D treated with 580 mg/kg body weight/day of the ethanolic extract and those in group E treated with artesunate (4 mg/kg body weight/day).

The results for effect of ethanolic extract from *S. nitida* root bark on absolute lymphocyte counts are presented in Table 3. It is shown that administration of the ethanolic extract and artesunate caused a significant decrease ($P < 0.05$) in absolute lymphocyte counts (ALC) in the malaria infected treated mice in groups B through E to 3247.34±0.25 lymphs/L for B, 3057.86±0.81 lymphs/L for C, 2889.11±2.23 lymphs/L for D, and 2744.32±0.32 lymphs/L for E respectively, compared to malaria infected untreated mice in group A with 3665.95±1.11 lymphs/L. But no significant difference was observed in ALC of malaria-infected mice in group D treated and group E.

Discussion

The results obtained for the treatments of malaria in the *P. berghei*-infected mice with ethanolic extract from *S. nitida* root bark agreed with the work reported by Nwiloh and colleagues (21). Also, the effect of administrations of the extract on body temperatures of the experimental mice are in tandem with that reported by Dikasso and colleagues (28). Fever, which is one of the features of malaria, is induced by exogenous pyrogens like parasites, bacteria, etc, that is transported to the liver where it activate the kuffer cells and other phagocytic cells that produced endogenous pyrogens such as cytokines and other inflammatory mediators such as interleukins (IL), tumor necrosis factor-B (TNF-B), macrophage inflammatory protein, and nitric oxide into the bloodstream and transported to the hypothalamus, where they induce the expression of cyclooxygenase-2 (COX-2) and, up-regulate prostaglandin-2 (PGE-2), the ultimate regulator of temperature (29). PGE2, in turn, inhibits the activity of warm-sensitive neurons, causing heat conservation and production, thereby acting as the proximal mediator of fever (30), by upregulating the thermoregulator center in the hypothalamus of the brain. The set point temperature will remain raised until PGE2 is no longer present, inhibited or suppress.

Blood cells count help in revealing the seriousness of malaria infection and its possibility of causing other problems, like kidney failure, anaemia, etc. Some haematological biomarkers are very useful in the diagnosis and study of malaria. The decreased in haemoglobin (Hb) concentration observed in the group A mice in this study might be due to increased haemolyses of the RBCs by the *P. berghei* parasites (31), which could result to haemolytic anaemia (27, 32). So, the decreased in RBCs level in malaria infected untreated mice recorded in this study might be due to haemolyses of RBCs

by the malaria parasites. Decreased RBCs is an indication of loss of Hb, and decreases in the levels of PCV and Hb are consequences of anaemia (33). In severe malaria, anaemia might arise from deformed erythrocytes, splenic phagocytosis and pooling or both (34), and it is a risk factor of cardiovascular disease (35). Anaemia resulting from a fall in concentrations of Hb due to malaria and other infections could possibly cause a fall in PCV levels. Decreased in platelet counts in the malaria infected mice observed in this work agreed with the works reported by Lee and co-workers (36), Oh and colleagues (37), and that of Taha and colleagues (38). The fall in platelet counts observed in the study might be caused by increased destruction of platelets by malaria parasites (27), sequestration and pooling of the platelets in the spleen, and destruction of circulating platelets initiated by the immune system (16, 39, 40). The results in Table 2 showed increased levels of WBC counts in malaria infected untreated mice. Derangements in WBCs and platelets are features associated with malaria infections (27). Increased concentration of total WBC signifies inflammations of the body cells and elevated number of neutrophil is linked to acute infection, while lymphocytosis is a useful index for the diagnosis of parasitic infections (33).

It is also seen from the results of the study (Table 1) that treatments of rodent malaria with ethanolic extract from root bark of *S. nitida* reduced the elevated temperatures, an indication that the ethanolic extract exhibited antipyretic potential. This activity might be due to the phytochemicals contained in the root bark extract. Compounds rich in flavonoids and quinoline alkaloids have been shown to exhibit antipyretic or hypothermic activities (41). Flavonoids are reported to inhibit cyclooxygenase-2 (COX-2) expression (41-43), and quinoline alkaloids shown to suppress prostaglandin productions (44, 45). The present of these

phytochemicals in ethanolic extract from root bark of *S. nitida* could be implicated as the causes of temperature reductions observed in the malaria infected treated mice in this study. The presence of some pharmacologically bioactive ingredients in the ethanolic extract from root bark of *S. nitida* might be implicated in the antipyretic potential and positive modulation of haematological parameters in malaria infected mice observed in this study. Oral administration of ethanolic extract from *S. nitida* root bark positively modulated the derangements in haematological indices caused by the *P. berghei* malaria parasites, as seen in this study. These might also be due to the present of phytoconstituents with pharmacological activities present in the ethanolic extract under investigation. The observed positive modulatory ability of the ethanolic extract from root bark of *S. nitida* might be due to the presence of phytoconstituents with antioxidant activities present in it. The alkaloids, tannins, flavonoids, anthocyanin, phenol and phytate present in the ethanolic extract have known antioxidant and radical scavenging abilities (46 - 54). Antioxidants prevent RBCs from haemolysis by preventing peroxidation (55). This action might be responsible for the positive modulation of haematological disorder observed in this study.

References

1. Snow, RW, Guerra, CA, Noor, AM, Myint, HY, Hay, SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*. 2005; 434(7030):214-7.
2. World Health Organization, WHO. Malaria control today. WHO, Geneva, Switzerland: Roll Back Malaria Department. 2005; P.1-75.
3. Mackintosh, CL, Beeson, JG, Marsh K. Clinical features and pathogenesis of severe malaria. *Trends Parasitol*. 2004;20(12):597-603.
4. Chakravorty, SJ, Hughes, KR, Craig, AG. Host response to cytoadherence in *Plasmodium falciparum*. *Biochem Soc Trans*. 2008; 36:221-8; doi:10.1042/BST0360221.
5. Ntsaluba, A. Guidelines for the prevention of malaria in South Africa. National Department of Health. 2003; P.1-44. www.fidssa.co.za/content/..., retrieved 20/6/2016.
6. Lamar J, Martschinske R, Tetreault G, Doud C. Pocket guide to malaria prevention and control. Preventive medicine, Navy medical department,

Conclusion

Generally, changes in body temperatures and haematological indices caused by *P. berghei* malaria infections in mice were positively modulated via treatments with ethanolic extract from root bark of *S. nitida* as observed in this study. These might be due to the anti-oxidative and anti-inflammatory effects of phytochemicals present in the ethanolic extract. This study has proven that ethanolic extract from root bark of *S. nitida* is antimalarial and has the potential for influencing the state of anaemia due to malaria in infected mice, hence its use will go a long way in alleviating the usual problems of health-care delivery. Therefore, the use of ethanolic extract from *S. nitida* root bark as antimalarial regimen traditionally does not adversely affect the haematological parameters and is antipyretic.

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Conflict of Interest

No conflict of interest exists.

- public health center, Navy and Marine corps, Portsmouth, Virginia. 2007; P. 1-112. Retrieved on 15/4/2015 from www.public.navy.mil/surfor/Documents/6250_1.pdf.
7. World Health Organization, WHO. Severe malaria. *Trop Med Int Health*. 2014; 19(suppl.1):7-131.
 8. Clark IA, Budd AC, Alleva LM, Cowden WB. Human malarial disease: a consequence of inflammatory cytokine release. *Malar J*. 2006; 5:85. doi:10.1186/1475-2875-5-85.
 9. Schumann RR. Malarial fever: Hemozoin is involved but toll-free. *PNAS*. 2007;104(6):1743-4.
 10. Parroche P, Lauw FN, Goutagny N, Latz E, Monks BG, Visintin A, et al. Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9. *PNAS*. 2007; 104:1919–1924.
 11. Lamikanra AA, Theron M, Kooij TWA, Roberts DJ. Hemozoin (malarial pigment) directly promotes apoptosis of erythroid precursors. *PLoS One*. 2009; 4(12):e8446. doi:10.1371/journal.pone.0008446.
 12. Awandare GA, Ouma Y, Ouma C, Were T, Otieno R, Keller CC, et al. Role of monocyte-acquired hemozoin in suppression of macrophage migration inhibitory factor in children with severe malarial anemia. *Infect Immun*. 2007; 75(1):201–10.
 13. Dondorp A, Nosten F, Stepniewska K, Day N, White N. Artesunate versus quinine for treatment of severe falciparum malaria: a randomized trial. *Lancet*. 2005;366(9487):717-25.
 14. Wickramasinghe SN, Abdalla SH. Blood and bone marrow changes in malaria. *Baillieres Best Pract Res Clin Haematol*. 2000;13(2):277-99.
 15. Reyburn H, Mbakilwa H, Mwangi R, Mwerinde O, Okomi R, Drakeley C, et al. Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania randomized trial. *BMJ (clinical research ed)*. 2007; 334(7590):403. doi: 10.1136/bmj.39073.496829.AE
 16. Maina RN, Walsh D, Gaddy C, Hongo Waitumbi J, Otieno L, et al. Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya. *Malar J*. 2010; 9(suppl3):S3-S4.
 17. Chandra S, Chandra H. Role of haematological parameters as an indicator of acute malaria infection in Uttarakhand state of India. *Mediterr J Hematol Infect Dis*. 2013; 5(1):e2013009. doi: 10.4084/MJHID.2013.009.
 18. Menendez C, Fleming AF, Alonso PL. Malaria-related anaemia. *Parasitol Today*. 2000; 16:469-76.
 19. Beare NA, Taylor TE, Harding SP, Lewallen S, Molyneux ME. Malaria retinopathy: a newly established diagnostic sign in severe malaria. *Am J Trop Med Hyg*. 2006; 75(5):790-7.
 20. Nwiloh BI, Uwakwe AA, Akaninwor JO. Phytochemical screening and GC-FID analysis of ethanolic extract of root barks of *Salacia nitida* L. Benth. *JMPS*. 2016; 4(6):283-7.
 21. Nwiloh BI, Akaninwor JO, Uwakwe AA. Antimalarial activity of ethanolic extract of root bark of *Salacia nitida* L. Benth in mice infected with *Plasmodium berghei*. *JOCAMR*, 2017; 3(3):1-9.
 22. National Institute of Health (NIH). Guide for the care and use of laboratory animals. NIH Publication, 1986; 83: 823.
 23. Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol*. 1983;54(4):275-87.
 24. Ghosh MN. Fundamentals of experimental pharmacology, scientific book agency, Calcutta. 1984; P. 153-8.
 25. Ryley JF, Peters W. The antimalarial activity of some quinoline esters. *Ann Trop Med Parasitol*. 1970;64(2):209-22.

26. Dacie JV, Lewis SM. Practical Haematology, 7th ed. England: ELBS with Churchill Livingstone. 1991. P. 37-85.
27. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge University press, Edinburgh, United Kingdom, 2004. P. 296-320.
28. Dikasso D, Mekonnen E, Debella A, Abebe D, Urga K, Mekonnen W, et al. In vivo antimalarial activity of hydro-alcoholic extracts from *Asparagus africanus* L. in mice infected with *Plasmodium berghei*. *Ethiopian J Health Dev.* 2006a; 20(2):112-8.
29. Wolfe LS, Coceani F. The role of prostaglandins in the central nervous system. *Ann Rev Physiol.* 1979; 41:669-684. doi: 10.1146/annurev.ph.41.030179.003321
30. Blatteis CM. Fever as a host defense mechanism. In: Amason, B.G. editor, the brain and host defense. *Neuroimmune biology.* Volume 9. Elsevier, 2010. P. 213-235.
31. Goselle ON, Onwuliri COE, Onwuliri VA. Malaria infection in HIV/AIDS patients and its correlation with packed cell volume (PCV). *J Vector Borne Dis.* 2009; 46(3):205-11.
32. Bakhubaira, S. Hematological parameters in severe complicated *Plasmodium falciparum* malaria among adults in Aden. *Turkish J Hematol.* 2013; 30(4):394-9.
33. Kemal J. Laboratory manual and review on clinical pathology. Foster City, USA. OMICS group ebooks. 2014. Retrieved on 2/7/2016 from www.esciencecentral.org/ebooks.
34. Dondorp AM, Angus BJ, Chotivanich K, Silamut K, Ruangveerayuth R, Hardeman MR, et al. Red blood cell deformability as a predictor of anaemia in severe *falciparum* malaria. *Am J Trop Med Hyg.* 1999;60(5):733-7.
35. Pereira AA, Sarnak MJ. Anaemia as a risk factor for cardiovascular disease. *Kidney Int.*2003; 64(S87):S32-S39.
36. Lee HK, Lim J, Kim M, Lee S, Oh EJ, Lee J, et al. Immunological alterations associated with *plasmodium vivax* malaria in South Korea. *Ann Trop Med Parasitol.* 2001;95(1):31-9.
37. Oh MD, Shin H, Shin D, Kim U, Kim N, Chol MH, et al. Clinical features of vivax malaria. *Am J Trop Med Hyg.* 2001;65(2):143-6.
38. Taha K, El-Dein SD, Idrees M., Makboul G, Baidas G. Haematological changes in malaria: Relation to *Plasmodium* species. *Kuwait Med J.* 2007; 39(3):262-7.
39. Pain A, Ferguson DJ, Kai O, Urban BC, Lowe B, Marsh K, et al. Platelet-mediated clumping of *Plasmodium falciparum*-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. *Proc Natl Acad Sci USA.* 2001;98(4):1805-10.
40. Erhart LM, Yingyuen K, Chuanak N, Buathong N, Laoboonchai A, Miller RS. et al. Hematologic and clinical indices of malaria in a semi-immune population of Western Thailand. *Am J Trop Med Hyg.* 2004; 70(1):8-14.
41. Varma S, Kinoshita JH. Inhibition of lens aldose reductase by flavonoids-their possible role in the prevention of diabetic cataracts. *Biochem Pharmacol.* 1976;25(22):2505-13.
42. Cheng L, Ming-Liang, H, Lars B. Is COX-2 a perpetrator or a protector? Selective COX inhibitors remain controversial. *Acta Pharmacol Sin.* 2005;26(8):926-33.
43. Sharma USH, Umesh KS, Abhishek S, Niranjana S, Puspak JS. Screening of *Terminalia bellirica* fruits extracts for its analgesic and antipyretic activities. *Jordan J Biol Sci.* 2010; 3(3):121-4.
44. Bi Y, Stoy P, Adam L, He B, Krupinski J, Normandin D, Pongrae R, et al. Quinolines as extremely potent and selective PDE₅ inhibitors as

- potential agents for treatment of erectile dysfunction. *Bioorg Med Chem Lett*. 2004;14(6):1577-80.
45. Lunniss CJ, Cooper AWJ, Eldred CD, Kranz M, Lindvall M, Lucas FS, et al. Quinolines as a novel structural class of potent and selective PDE₄ inhibitors optimization for oral administration. *Bioorg Med Chem Lett*. 2009;19(17):5261-5. doi: 10.1016/j.bmcl.2009.04.012.
46. Parmar NS, Parmar S. Anti-ulcer potential of flavonoids. *Indian J Physiol Pharmacol*. 1998;42(3):343-51.
47. Seeram NP, Schutzki R, Chandra A, Nair MG. Characterization, quantification, and bioactivities of anthocyanins in *Cornus* species. *J Agric Food Chem*. 2002;50(9):2519-23.
48. Lee MRF, Harris LJ, Dewhurst RJ, Merry RJ, Scollan ND. The effect of clover silages on long chain fatty acid rumen transformations and digestion in beef steers. *Animal Sci*. 2003; 76(3):491-501. doi:10.1017/S1357729800058719.
49. Okwu DE. Phytochemicals and vitamins content of indigenous species of southeastern Nigeria. *J Sustain Agric Environ*. 2004; 6(1):30-7.
50. Han X, Shen T, Lou H. Dietary polyphenols and their Biological significance. *Int J Mol Sci*. 2007; 8(9):950-88.
51. Buzzini P, Arapitsas P, Goretti M, Branda E, Turchetti B, Pinelli P, et al. Antimicrobial and antiviral activity of hydrolysable tannins. *Mini Rev Med Chem*. 2008;8(12):1179-87.
52. Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L. Condensed and hydrolysable tannins as antioxidants influencing the health. *Mini Rev Med Chem*. 2008;8(5):436-47.
53. Oomah BD, Blanchard C, Balasubramanian P. Phytic acid, phytase, minerals, and antioxidant activity in Canadian dry bean (*Phaseolus vulgaris L.*) cultivars. *J Agric Food Chem*. 2008 56(23):11312-9. doi: 10.1021/jf801661j.
54. Fernandez-Bachiller MI, Perez C, Gonzalez-Munoz GC, Conde S, Lopez MG, Villarroya M, et al. Novel tacrine-8-hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper complexing properties. *J Med Chem*. 2010;53(13):4927-37. doi: 10.1021/jm100329q.
55. Vasudevan, DM, Sreekumari, S. Free radicals and anti-oxidants: textbook of biochemistry for medical students. 5th ed. New Delhi, India: Jaypee Brothers Medical Publishers (p) Ltd; 2007. P. 353.