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Assessment of Toxic Effects on Biochemical and Haematological Parameters and Antimalarial and Immunostimulatory Activities of Polyphenol-Rich Fractions of Leaves from *Nauclea latifolia* (Rubiaceae)

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Abstract: *Nauclea latifolia* has been used in the management of malaria in the Burkina and despite its application in ethnomedicine, there is dearth of scientific evidence to justify the acclaimed prophylactic antimalarial usage of the plant. The aim of this study was designed to determine the antimalaria, immunostimulatory activities and toxicological effects of the polyphenol-rich fractions of leaves from *Nauclea latifolia* in animal model. The *in vivo* antimalaria was conducted on *P. berghei* infected albino mice by following 4-day test and Curative tests. The effect of oral administration of the polyphenol-rich fractions on biochemical and haematological parameters did not show any significant effect ($P < 0.05$).The polyphenol-rich fractions of leaves from *Nauclea latifolia* contains antimalaria and immunostimulant substances which help to reduce parasitaemia and the results of the biochemical and hematological parameters show that the polyphenol-rich fractions is non-toxic. Further studies needed to identify and characterize the potent molecules that suppress the malaria parasite for new drug therapies in view of growing resistance to malaria.

Keywords: *Nauclea latifolia*, toxic effects**,** antimalarial and immunostimulatory activities**,** bioactive fraction.

INTRODUCTION

Traditional medicine is widely used for prevention, diagnosis and treatment of physical and mental illness. World population of 80% people depends on herbal medicine for their treatment for the reason that of the high cost and adverse effects of accessible synthetic drugs.

Among these diseases, malaria has an important field. Malaria is a major public-health problem in the world; transmission primarily being common in tropical and subtropical regions [1]. It is an opportunistic disease that benefits from the diminished immune system of the patient. It causes illness by the ability of the causative parasite to invade the red blood cells [2] and the liver where they multiply. Malaria is a serious and often fatal disease caused by plasmodium parasite that is transfected when mosquito feeds on blood meal from humans and it is remains one of the major killer diseases of the world. In extreme infections, up to 80% of the red blood cells can be parasitized and destroyed [3]. This massive cell destruction is known to lead to severe ;anaemia and clogging of the blood circulation of vital organs particularly the brain and eventually death. Despite the fact that malaria is a deadly disease, illness and death from malaria can usually be prevented [4]. In spite of several efforts to combat malaria through chemotherapy, vaccines or chemoprevention, the disease remain unabated simply because of the distribution, widespread and survival of the mosquitoes that carry the parasite and multidrug resistance of the parasite to different drugs designed to cure the disease. In Africa, poverty, strict adherence to treatment regimen to avoid resistance and exposure to mosquito are the major challenges faced in the eradication of the disease from the continent. Several approaches had been developed in the treatment of the disease. One optimistic source for new affordable treatment against malaria lies in the use of traditional herbal remedies. In

effect, multi-drug resistant strains of the parasite to antimalarial drugs proved to be a challenging problem in malaria control in most parts of the world [1]. These recurring problems render development and promotion of phytomedicines as alternative solution to malaria control [5]. Medicinal plants have been playing a vital role in the treatment of malaria for centuries [6] and have always been considered to be a possible alternative and rich source of new drugs. Today, herbal products are being used worldwide in a variety of healthcare settings, and as home remedies [7]. Over 1200 plant species from 160 families are used to treat malaria and fever in endemic countries [8]. In Burkina Faso, the use of traditional medicinal plant is required as an alternative drug to cure malaria. So, *Nauclea latifolia* (syn. *Sarcocephalus latifolius*, Rubiaceae) is a good candidate for malaria treatment and possess immunostimulatory properties [9]. In effect, it is well known that new immunostimulants compounds coming from the plants could help the body to fight against multiple infections [10].

Nauclea latifolia (syn. *Sarcocephalus latifolius*, Rubiaceae), commonly called the African pincushion tree, is a plant widely used in folk medicine in different regions of Africa for treating a variety of illnesses, including malaria, epilepsy and pain. *N. latifolia* has not only drawn the interest of traditional healers but also of phytochemists, who have identified a range of bioactive indole alkaloids in its tissue [9]. It is widely distributed in the low land rain forest zones and frequently found in villages. It is found in many villages in western Burkina Faso. It is a plant which has been used in traditional medicine in Burkina Faso to treat health problem, phytochemical profile shows it contains many biologically active substances that include alkaloids, polyphenol compounds, saponin etc. [9]. In Burkina Faso, the plant is used for the treatment of several diseases as jaundice, malaria, infant gastroenteritis, dysentery etc [11]. The plant is also used as a tonic and fever medicine, chewing stick, toothaches, dental cares, septic mouth and malaria, diarrhea and dysentery [9]. These leaves are used for the treatment of yellow fever and malaria while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhea [9]. This research, therefore, is aimed at providing information on the possible anti-malaria and immunostimulatory of Polyphenol-rich Fractions of leaves from *Nauclea latifolia* (syn. *Sarcocephalus latifolius*, Rubiaceae) in animal model.

MATERIALS AND METHODS

Plants material

The vegetable materials (Fresh leaves) of *Nauclea latifolia* (syn. *Sarcocephalus latifolius*, Rubiaceae) were collected in August 2016 in Dedougou, 230 Km West of Ouagadougou, capital of Burkina Faso. This plant was botanically identified by Dr. Traoré Lassina from the plants Biology Department of the University of Koudougou.

Animals Handling

Swiss NMRI mice (20–30 g) and Wistar albino rats (130- 180 g) of both sexes were used for all tests. All animals were housed in cages under controlled conditions of 12 h light/and 12 h without light and 25°C.They received pellets of food enriched with 20% protein and water ad libitum. They were deprived of food for 15 h (but with access to drinking water) and weighed before the experiments. *In vivo* studies were carried out in accordance with guidelines for care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals [12].

Polyphenols extraction

The harvested plant materials fresh (Fresh leaves) were dried in the laboratory at room temperature (20-25°C), afterwards samples were ground to pass a sieve of 0.3 mm. Polyphenols were extracted with aqueous acetone (80%, v/v). The extract was then washed with hexane to remove chlorophyll and other low molecular weight compound. Acetone was evaporated and the extract was lyophilized and stored at 22°C prior to biological tests. For the tests, lyophilized sample was dissolved with 10% DMSO in water at the desired concentration [9].

In vivo antimalarial potential

Animals

Swiss albino mice (20–30 g) of both sexes and Chloroquine sensitive *Plasmodium berghei* were used in this experiment. They were maintained under standard conditions (12hrs light and 12 hrs dark) and have access to mice chow and clean water.

Study design

Fraction administration of drugs and fraction were administered orally using orogastric tube.

Evaluation of Schizontocidal activity in early infection (4-day test)

The schizontocidal activity of polyphenol-rich fractions of leaves from *Nauclea latifolia* was evaluated using the method described by[13]. Swiss albino mice of both sexes were used in this experiment. The animals were divided into six groups of 6 mice of both sexe each. Shortly after inoculation of each mouse with l x 10^6 *P. berghei* they were administered with 100, 200 and 400 mg/kg/b.W/day dose of polyphenol-rich fractions. Chloroquine 5 mg/kg/day (both dissolved in normal saline) and an equivalent volume of distilled water (negative control) for 4 consecutive days (days 0 to 3) percentage parasitaemia was determined using standard laboratory procedures described by [13]. The groups are as indicated below:

Group 1: Uninfected and untreated (normal animals)
Group 2: Infected and untreated (Negative Control)

Group 3: Infected and treated immediately with 100 mg/kg/B.W/day with polyphenol-rich fractions
Group 4: Infected and treated immediately with 200 mg/kg/B.W/day with polyphenol-rich fractions
Group 5: Infected and treated immediately with 400 mg/kg/B.W/day with polyphenol-rich fractions
Group 6: Infected and treated with 5 mg/kg/b.W/day with chloroquine (positive control)

Evaluation of Schizontocidal activity in established infection (Curative Test)

The evaluation of the curative potential of polyphenol-rich fractions was done using the methods described by [14]. Albino mice were used in the experiment. Ninety six hours after parasite inoculation of each mouse with 1 x 10^6 *P. berghei*. The animals were divided into five groups of 6 mice each. These mice were treated with 100, 200 and 400 mg/kg/day doses of polyphenol-rich fractions. Chloroquine 5 mg/kg/day (both dissolved in normal saline) and an equivalent volume of distilled water (negative control). The drug or fraction was given once daily to the appropriate group at 8.00 am. The levels of parasitaemia were determined using standard laboratory procedure [13]. The groups are as under listed.

Group 7: Infected and untreated

Group 8: Infected and treated on day 5 with 100 mg/kg/b.W/day with polyphenol-rich fractions for 3 consecutive days.

Group 9: Infected and treated on day 5 with 200 mg/kg/b.W/day with polyphenol-rich fractions for 3 consecutive days.

Group 10: Infected and treated on day 5 with 400 mg/kg/b.W/day with polyphenol-rich fractions for 3 consecutive days.

Group 11: Infected and treated on day 5 with 5 mg/kg/b.w/chloroquine for 3 consecutive days.

Animal weights

After assessment of percentage of parasitemia and percentage of chemo-suppression inhibition**,** the body weights of animals were measured during the period of study.

Blood analysis for antimalarial potential

After measuring the weight of the animals during the study period, blood samples were collected by cardiac puncture in tubes for haematology, and serum biochemistry. The blood samples with heparin for haematology parameters analysis and without anticoagulant were centrifuged at 3000 rpm for 5 min to obtain plasma or serum for biochemical parameters. The serum for biochemical parameters such as aspartate aminotransferase (AST) and alamine aminotransferase (ALT) determined according [15], estimated by [16, 17] methods, creatinine [18], total protein [19], total bilirubin determined according [20]. All these biochemical parameters were measured by Selectra XL Vital Scientific (Elitech Group Company). Haematological analyses were performed on whole blood, using the automatic counter (Mindray Auto hematology Analyser BC- 5500).

***In vivo* immunostimulatory potential [21]**

Animal treatment

Adult albinos wistar rats (130 - 180 g) of both sexes were used. All animals were housed in cages under controlled conditions of 12 h light/and 12 h without light and 25°C.They received pellets of food enriched with 20% protein and water ad libitum. They were deprived of food for 15 h (but with access to drinking water) and weighed before the experiments. The animals were divided into five groups of six animals each one. Groups 1 and 2 were used as controls groups.

Group 1: The rats received 10% DMSO as control by oral way during 21 days.

Group 2: The rats received cyclosporine A as control (5 mg/kg, by oral route) during 07 days (from 1st to 7th day) and then received DMSO 10% (from 8th to 21st day).

Group 3: The rats received fraction (25 mg/kg bw.)

Group 4: The rats received fraction (75 mg/kg bw.).

Group 5: The rats received fraction (100 mg/kg bw.).

The animals of groups 2 to 5 initially received cyclosporine A (5 mg/kg bw oral route) 1st to 7th day in order to lower the immune system. From group 3 to group 5, the animals received various concentrations of polyphenol-rich fractions (25, 75, 100 mg/kg bw) dissolved in 10% DMSO and were managed during 14 days by oral route (8th to 21 st day of treatment). The 21 st day, the animals were deprived of water and food during 15 hours.

Blood analysis for immunostimulatory potential

At the end of 21-days period, the animals were deprived of food for 15 h and blood samples were collected by cardiac puncture in two tubes for hematological and serologic parameters analysis. The blood samples (with heparin and without anticoagulant) were centrifuged at 3000 rpm for 5 min to obtain plasma or serum. Hematological analyses were performed on whole blood, using automatic counter (Mindray Auto hematology Analyser BC-5500) to evaluate following parameters: total white blood cells (TWBC), total lymphocytes, using automatic Counter System (SB FACS) serologic parameters (CD8 and CD4) were determined.

Statistical analysis

The data were expressed as Mean±Standard deviation (SD) of six determinations (n=6). Results were analysed by one-way ANOVA followed by Dunnett's *t*-test using Prism 4 software. The level of significance was accepted at $p \leq 0.05$.

RESULTS

Evaluation of Schizontocidal activity in early infection (4-day test)

From day-4 test, obtained results signify that, polyphenol-rich fractions displayed very good activity against Plasmodium berghei *in vivo* in experimental mice. We noticed that during the early infection oral administration of 100, 200 and 400 mg/kg body weight/day concentration of polyphenol-rich fractions caused chemo-suppression of 40.22, 63.54 and 81.63% respectively on day-4 which was significant at $P < 0.05$ when compared to control negative. The standard drug chloroquine (5 mg/ kg b.wt./day) caused 100% chemo-suppression which was highly significant when compared to the polyphenol-rich fractions treated groups (Table 1). The highest concentration of extract (400 mg/kg b.wt./day) shown 81.63% chemo-suppression which is almost like to that of standard drug chloroquine (5 mg/kg b.wt./day). In the 4-day suppressive test, all the doses of the polyphenol-rich fractions showed a preventive effect on weight reduction and normalized the weight in infected mice at all dosages when compared to control negative group (Table 1).

Table-1: Inhibition of percentage and body weight in 4-day suppressive test after administration of polyphenol-rich fractions of leaves from *Nauclea latifolia* against *Plasmodium berghei* infected experimental mice

Test substance	Dose (mg/kg/day)	Inhibition (%)	Weight on day 0 (g)	Weight on day 4 (g)
Polyphenol-rich fractions	100	40.22	28.00±2.52	28.50±1.76
	200	63.54	28.83±1.83	29.00±1.27
	400	81.63	28.83±1.51	29.00±1.27
Vehicle (-)	1ml	-	28.33±1.63	28.67±1.21
Chloroquine (+)	5	100	28.00±2.10	29.00±0.89

Evaluation of Schizontocidal activity in established infection (Curative Test)

From day 5 to 7 in the established infection, oral administration of 100, 200 and 400 mg/kg b.wt./day concentration of polyphenol-rich fractions suppressed parasitemia and was statistically significant at $P < 0.05$ when compared to negative control. The standard drug chloroquine (5 mg/kg b.wt./day) caused 100% chemo-suppression which was highly significant when compared to the treated groups. The highest concentration of polyphenol-rich fractions used (400 mg/kg b.wt./day) showed 90.54 % chemo-suppression which was almost like to that of standard drug chloroquine (5 mg/kg b.wt./day). During the established infection, all the doses of the polyphenol-rich fractions showed a preventive effect on weight reduction and normalized the weight in infected mice at all dosages when compared to control negative group and the increase in body weight was not dose dependent (Table 2).

Table-2: Inhibition of percentage and body weight in Curative Test after administration of polyphenol-rich fractions of leaves from *Nauclea latifolia* against *Plasmodium berghei* infected experimental mice

Test substance	Dose (mg/kg/day)	Inhibition (%)	Weight on day 5 (g)	Weight on day 7 (g)
Polyphenol-rich fractions	100	48.19	28.50±1.76	29.17±0.98
	200	69.71	29.00±1.27	29.33±0.82
	400	90.54	29.00±1.27	29.50±0.84
Vehicle (-)	1ml	-	28.67±1.21	29.50±0.84
Chloroquine (+)	5	100	29.00±0.89	29.67±0.41

Table-3: Result of Biochemical Analysis

Parameters	Controls, Doses (mg/kg/day) of polyphenol-rich fractions and chloroquine (5 mg/kg/b.w)					
	Normal control	Negative control	100	200	400	Chloroquine
Creatinine (mg/dl)	1.22±0.52^{f}	1.69±1.18^{f}	0.95±0.12^{f}	0.90±0.22^{f}	0.91±0.32^{f}	0.86±0.11^{f}
AST (ul)	80.42±1.58^{a}	91.22±0.52^{a}	71.30±0.32^{a}	76.20±0.54^{a}	79.52±0.11^{a}	70.20±1.58^{a}
ALT (ul)	30.62±0.54^{b}	33.12±0.62^{b}	26.18±0.18^{b}	28.60±0.64^{b}	29.30±0.54^{b}	26.80±0.18^{b}
Total bilirubin (mg/dl)	2.48±0.12^{e}	2.62±0.01^{e}	1.42±0.01^{e}	1.56±0.06^{e}	1.52±0.22^{e}	1.32±1.18^{e}
Total protein (mg/dl)	13.22±0.32^{d}	12.54±0.54^{d}	12.88±1.58^{d}	12.86±0.01^{d}	12.90±0.01^{d}	12.62±0.32^{d}
ALP (ul)	16.34±0.54^{c}	16.20±0.06^{c}	15.60±0.64^{c}	15.80±0.31^{c}	15.92±0.54^{c}	15.20±0.01^{c}

Mean±SEM. Means with same letter in same column are not significantly different.

Blood analysis for antimalarial potential

The Polyphenol-rich fractions did not have any significant effect on RBC, Hb, MCHC, MCH, PCV and MCV while WBC was significantly reduced ($P < 0.05$) in the group treated with 100 mg/kg body weight. The platelet was significantly reduced ($P > 0.05$). The Polyphenol-rich fractions did not have exact any significant effect ($P < 0.05$) on alkaline phosphatase (ALP), total bilirubin, and total protein. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine were significantly decreased ($P < 0.05$). Results are summarized in Table 3 and Table 4.

Table-4: Result of Hematological Analysis

Parameters	Controls, Doses (mg/kg/day) of polyphenol-rich fractions and chloroquine (5 mg/kg/b.w)					
	Normal control	Negative control	100	200	400	Chloroquine
Hb Conc. (g/dl)	13.68±0.01^{e}	12.20±0.58^{e}	11.40±0.54^{e}	11.60±0.01^{e}	11.80±0.54^{e}	12.10±0.01^{e}
PCV (%)	42.24±1.18^{c}	40.10±0.10^{c}	38.94±1.58^{c}	38.60±0.06^{c}	39.22±1.58^{c}	40.30±1.54^{c}
WBC (x10^{12}/µl)	10.24±0.10^{f}	8.22±0.10^{f}	4.86±0.20^{f}	4.70±0.20^{f}	4.30±0.32^{f}	5.10±0.30^{f}
RBC (x10^{12}/L)	4.70±0.54^{g}	3.70±0.20^{g}	4.18±0.10^{f}	4.30±0.54^{f}	4.40±0.58^{f}	4.60±0.54^{f}
MCHC (g/dl)	32.12±0.30^{d}	31.80±0.54^{d}	29.68±0.54^{d}	30.10±0.32^{d}	30.80±0.10^{d}	31.20±0.06^{d}
Platelet (x10^{9}/L)	185.82±0.58^{a}	182.20±1.58^{a}	203.40±1.58^{a}	196.20±1.58^{a}	200.10±1.18^{a}	182.20±1.10^{a}
MCH (Pg)	31.36±0.30^{d}	29.40±0.58^{d}	29.10±0.32^{d}	29.50±0.54^{d}	29.60±0.58^{d}	30.80±0.54^{d}
MCV (*f*l)	96.44±0.20^{b}	95.12±0.54^{b}	94.70±0.10^{b}	95.10±0.32^{b}	94.80±0.54^{b}	96.30±0.20^{b}

Mean±SEM. Means with same letter in same column are not significantly different.

Blood analysis for immunostimulatory potential

Figure 1 shows the effects of polyphenol-rich fractions on the hematologic and serologic parameters of the rats. It is noticeable that the effect of the polyphenol-rich fractions concentrations of 25 mg/kg, 75 mg/kg and 100 mg/kg bw involved a significant decrease of hematologic and serologic parameters ($p<0.01$) compared to the group controls (10% DMSO). But, there a small significant difference ($p<0.01$) between the group control (cyclosporin A) and the test groups (3 to 5) for the effects of polyphenol-rich fractions on the various parameters.

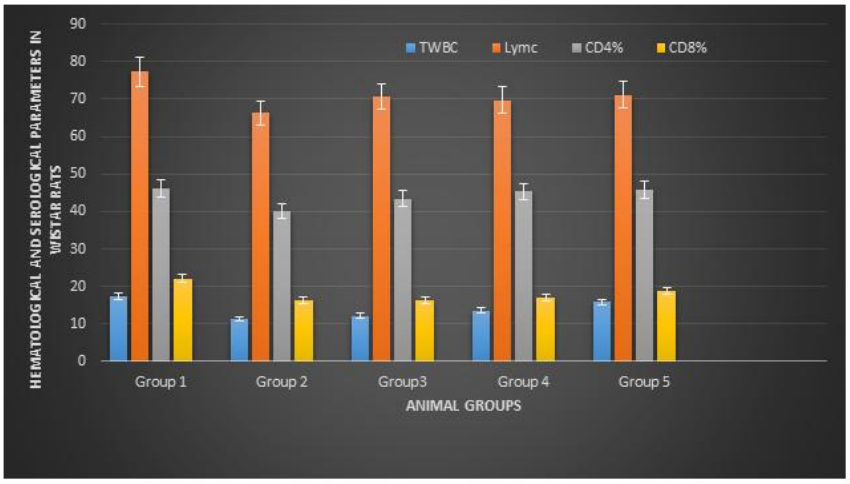

Fig-1: Effects of polyphenol-rich fractions of leaves from *Nauclea latifolia* on hematological and serological parameters in wistar rats.

DISCUSSION

The World Health Organization, has estimated that between the years 2000 and 2020 nearly one billion people will be infected and more than 200 million will develop the disease [22]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. About our study, results from investigation suggest that the polyphenol-rich fractions of leaves from *Nauclea latifolia* has anti-malaria activities to mice when administered. In effect, *In vivo* antimalaria activity can be classified as moderate, good and very good if an plant extract demonstrated the percentage of parasitemia suppression equal to or greater than 50% at a dose of 500, 250 and 100 mg/kg b.wt./day respectively [23]. Based on this classification, we could say effectively that *Nauclea latifolia* has shown good antiplasmodial activity.

Drugs lead to decreased parasitemia and subsequent recovery of symptomatic malaria. They also reduce parasitemia through different ways like reducing parasite nutrient intake, interfering with parasite metabolic pathways like a heame metabolic pathway which is involved in the metabolism of iron [24]. Drugs

also negatively influence the parasite reproduction and growth [25]. The polyphenol-rich fractions reduced the level of parasitemia and increased the mice survival time. Chloroquine had a good chemo-suppression with 100% inhibition. Some studies reported that plant whose phytochemical compounds may have antimalarial activities [26, 27] Hence, various chemical compounds may be present in high concentration in polyphenol-rich fractions of leaves from *Nauclea latifolia* which may be responsible for their high antimalarial activity. These reports are similar to those obtained in this study as polyphenol-rich fractions of leaves from *Nauclea latifolia* contains cardenolides and tannins that are polyphenol compounds [9].

In this study, the results also indicated that the polyphenol-rich fractions of leaves from *Nauclea latifolia* are low poisonous. In effect, changes in body weight could be due to the adverse side effects. For [28,29], weight loss is a simple and sensitive index of toxicity after exposure to toxic substance and this fact was noticed by the low variation between animal weights compared with control group. However, the decrease in body weight observed in the rats treated with the doses of the polyphenol-rich fractions of leaves from *Nauclea latifolia* may be due to low feed intake and utilization [30]. Reported severed growth depression as a consequence of reduce feed intake in rats fed high tannin containing diet. Certainly the tannins would be responsible in this fact; because, according to recent studies, tannins have been known to occur in high concentrations in the polyphenol-rich fractions [9].

The various biochemical and haematological parameters investigated are useful indices of evaluating the toxicity of plant extract in animals [31]. For the results of biochemical parameters, we notice a variation between the different doses administered but this variation is low. There is a low significant difference between the control group (10% DMSO) and the other treated assay groups ($p<0.01$). Biochemical evaluation is important, because kidney and liver toxicity has been reported the use of phytotherapeutic products [32]. In the present study, creatinine determination was critical as marker of kidney function [33]. There is not much significant difference in creatinine comparatively to the control group ($p<0.01$). Among the parameters evaluated, AST, ALT and ALP are considered markers of liver function [34]. There is not much differences in AST, ALT and ALP comparatively to the control group ($p<0.01$). The results revealed relationship between these enzymatic markers and liver function. Indeed dose-dependent elevations were observed in serum enzymes in the treated groups. This indicates hepatocellular damage [35, 36]. Reported that the increase in the activity of these enzymes in the plasma is often seen following liver damage and it is attributed to the loss of the enzyme from damaged hepatocytes rather than increased production. Polyhenol-rich fractions treated groups showed significantly lowered lilirubin level. The decrease in bilirubin concentration may be attributed to the depressant effect of the polyhenol-rich fractions.

Assessment of haemotological parameters cannot only be used to determine the extent of deleterious effect of plant extract on the blood of an animal but it can also be used to explain blood relating functions of a plant extract on its products [37]. Analysis of blood parameters is relevant in risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies [38]. The non-significant effect of the polyhenol-rich fractions on the RBC may be an indication that the balance between the rate of production (erythropoiesis) and destruction of the blood corpuscles was not altered. MCHC and MCH relate to individual red blood cells while Hb, RBC and PCV are associated with total population of Red Blood Cells. Therefore, the absence of significant effect of the the polyhenol-rich fractions on RBC, Hb, PCV, MCH and MCHC could mean that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was altered [39]. Platelet activity may play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, antiplatelet agents have been used clinically in patients at risk of myocardial infarction [40]. Polyphenol compound have shown to act at the blood platelet level by preventing platelet activity-related thrombosis [41]. *Nauclea latifolia* has been reported to contain polyphenol compounds as one of its active compounds [42]. The serum creatinine level was decreased significantly suggesting that the the polyhenol-rich fractions was not toxic to the kidney. Creatinine is the major catabolic products of the muscle and is excreted in the kidneys. Creatinine levels are useful as indicators of renal failure [43].

About immunostimulatory activity, it is well know that new immunostimulants compounds coming from the plants could help the body to fight against multiple infections. These immunogenic substances of plants origin could present three advantages namely restoration of immunity in certain case of immunosuppression, minimizing certain side effects of modern drugs and especially the reduction in the cost of therapy [10]. Concerning our study, it is well know that cyclosporine A has an immunosuppressive potential. The increase in hematological and serological parameters of the test groups, compared to the control group 2 (cyclosporine A control) allows to say that the polyphenol-rich fractions of *Nauclea latifolia* (100mg/kg bw; $p<0.01$) has a immunostimulatory effect comparatively to control group (cyclosporine A). These results could be explained certainly by the presence of biologically active antioxidants such as polyphenol compounds contained in the polyphenol-rich fractions

of *Nauclea latifolia* [44]. Because, recent study reported that polyphenol compounds are considered as the major contributors to the antioxidant potential of plants [45], and antioxidant play an important role in controlling oxidative stress and decreasing disease activity [46].

CONCLUSION

We hereby conclude that the polyphenol-rich fractions of *Nauclea latifolia* have antimalaria activity against chloroquine-sensitive *P. berghei* parasites and have an immunostimulatory effect. Moreover, the polyphenol-rich fractions of *Nauclea latifolia* does not exhibited toxicity on the biochemical and haematological parameters. These results lend support to claims of herbalists that decoctions of *Nauclea latifolia* are useful medicines in the treatment of malaria. Hence, more research is needed to identify and characterize the potent molecules that suppress the malaria parasite for new drug therapies in view of growing resistance to malaria. In this fact, the polyphenol-rich fractions of *Nauclea latifolia* could be useful alternatives to antimalarial drug or useful in combination therapy since they are cheaper.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest regarding this publication.

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