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Jiotsa Tchoutezo Boris
Higher Institute of Health Sciences, Université des Montagnes, Cameroon

Gounoue Kamkumo Raceline
Faculty of Science, University of Yaoundé I, Cameroon

Tsakem Nangap Jaurès Marius
Faculty of Science, University of Yaoundé I, Cameroon

Ngueguim Tsofack Florence
Faculty of Science, University of Yaoundé I, Cameroon

Djouwoug Noussi Clarice
Faculty of Science, University of Yaoundé I, Cameroon

Ndelo Josephat
Faculté de Pharmacie, Université libre de Kinshasa, République Démocratique du Congo

Tekam Jean Michel
Higher Institute of Health Sciences, Université des Montagnes, Cameroon

Kouamouo Jonas
Higher Institute of Health Sciences, Université des Montagnes, Cameroon

Dimo Théophile
Faculty of Science, University of Yaoundé I, Cameroon

Corresponding Author:
Gounoue Kamkumo Raceline
Faculty of Science, University of Yaoundé I, Cameroon

Anti-malarial, anti-inflammatory and antioxidative activities of the aqueous extract of *Picralima nitida* (STAPF) TH. &H. Durand fruits (Apocynaceae) in rats

Jiotsa Tchoutezo Boris, Gounoue Kamkumo Raceline, Tsakem Nangap Jaurès Marius, Ngueguim Tsofack Florence, Djouwoug Noussi Clarice, Ndelo Josephat, Tekam Jean Michel, Kouamouo Jonas and Dimo Théophile

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Abstract
Malaria is a life-threatening disease generating secretion of cytokines and inflammatory mediators that trigger inflammatory reactions and oxidative stress often leading to severe forms of the disease. The study investigates the anti-malarial, anti-inflammatory and antioxidative effects of the aqueous extract of *Picralima nitida* fresh fruits in *Plasmodium berghei*-induced malaria in rat. Malaria was induced by intraperitoneal inoculation of 500 µl of $1x10^6$ *Plasmodium berghei*-parasitized erythrocytes. Parasitaemia was assessed using microscopic examination of blood smear Giemsa stained. Infected animals were treated with gradual dose of the extract of *Picralima nitida* fruits, distilled water (10 ml/kg) and chloroquine (10 mg/kg) by single daily administration for 5 days. Haematological, immunological and tissue biochemical and analyses were performed. The treatment with the extract significantly inhibited parasite development, regulated the pro-inflammatory cytokines and improved anti-oxidant status. It also alleviates fever, anaemia, liver and kidney function failure in treated rats. These results express the anti-malarial, anti-inflammatory and antioxidant properties of *Picralima nitida* and justify its traditional used for health care.

Keywords: *Picralima nitida*, inflammatory cytokines, antioxidant properties, *Plasmodium berghei*, rats

1. Introduction
Malaria is a life-threatening disease in population living in tropical and subtropical zone worldwide. The disease affects much more people in developing countries among which the sub-Saharan Africa remains the most affected zone. The WHO numbered about 219 million of malaria cases worldwide, with global death estimated at 435 000, predominantly in young children in sub-Saharan Africa [1]. Malaria stands out among the systemic infectious diseases of humans, presenting with multiple manifestations including intense fever, headache, and muscular pain and sometimes accompanied by potentially fatal symptoms such as severe anaemia, coagulation disturbances, microcirculation impairment that contribute to liver and renal failure and could induce major cerebral pathology [2, 3]. These symptoms are caused by parasitized red blood cells (RBCs) adhering to the endothelium surface, obstructing blood flow and causing regional oxygen deprivation and haemorrhage [4]. Furthermore, during the formation of plasmodium, the cytoadherence of infected red blood cells induces the buildup of parasite components that stimulate the production of reactive oxygen species (ROS) [5]. It has also been shown that heme produced by hemoglobin degradation is a pro-oxidant agent that stimulates the development of reactive oxygen species [6], which are responsible for several harmful effects in the body [7]. Although wide range of drugs exist against malaria and its complications, the development of resistance of plasmodium to current conventional drugs constitutes an obstacle for the treatment of this disease. Therefore, there is an urgent need to strengthen and expand research of novel molecules with benefit effects. In most rural malaria endemic zones, populations use medicinal plant for their health care. *Picralima nitida*

(Apocynaceae) is a small wild tropical tree that antiparasitic activities have been demonstrated [8–10].
This study explores the anti-inflammatory and anti-oxidative activity of the aqueous fresh fruits extract of *Picralima nitida* in malaria situation induced by *Plasmodium berghei* in rat.

2. Material and methods

2.1. Procedure of extraction of the plant material

The fruits of *Picralima nitida* were harvested at Makenene (Region of Centre, Cameroon). The authentication was done at the Cameroon National Herbarium thanks to a botanist staff, Mr Nana Victor, in comparison with an existing specimen deposited number 23183/SRF Cam. Entire fruits was crushed and, a thousand grams of ground material were boiled into 2L of distilled water for 15 min, then cooled at room temperature, filtered and lyophilized giving a crude extract that was stored at -20°C until used.

2.2 Animals

The study was performed on female albinos Wistar rats (two months old; ≈120 g), reared in animal house of the University of Yaoundé I. They were maintained at room temperature, fed and watered *ad libitum*. The experiments were conducted with respect of the Institutional Ethical Committee, by adopting all procedures recommended by the European Union on the protection of animals used for scientific proposes (CEE Council 86/609; Ref N° FWA-IRD 0001954).

2.3 Parasite

The parasite strain used for the study was *Plasmodium berghei* (codified NK-65, ATCC) graciously gifted by Bei Resources (USA).

2.4. Assessment of the antimalarial effect of the plant extract in rat

2.4.1. Procedure of the malaria induction

The parasite strain (*Plasmodium berghei* NK-65) was maintained by continuous blood transfer of parasites from parasitized donor rat to recipient healthy rat. To achieve the test, parasitized rats with parasite count of at less 30% was used. The curative test (Rane's test) [11] was used to perform antiplasmodial activity of the plant. Briefly, blood from *Plasmodium berghei*-parasitized rat was diluted with sodium chloride (0.9%) resulting to 500 µL of inoculum containing $1x10^6$ parasitized erythrocytes as described by Djouhoug *et al*. [12]. The inoculum was then, intraperitoneally inoculated to rat and eighty hours later, *Plasmodium berghei*-parasitized rats were randomly divided and treated with a gradual doses of the aqueous extract of *P. nitida* fruits of 100, 200 and 400 $mg.kg^{-1}$ for the tests groups, chloroquine sulphate (Sigma Aldrich, Germany) at 10 $mg.kg^{-1}$ for the chloroquine control group while distilled water (10 mL/kg) was administered to malaria control. During the five days of treatment, some parameters such as body temperature was recorded using digital thermometer and the parasiteamia that was monitored using 10% Giemsa-stained on thin smears film. Parasitaemia was done by numbering parasite per 100 erythrocytes in three random fields under light microscope (100x objective) with immersion oil. Finally, the percentage of inhibition of the parasite development (%I) of each tested dose of the extract was determined by the formula: % I= [(parasitaemia of malaria control – parasitaemia of the given extract dose)/parasitaemia of malaria control] x100.
The experimental animals were anaesthetized with ketamine (30 mg.kg-1) and diazepam (10 mg.kg-1), and then sacrificed. Blood sample was collected into EDTA tubes for haematology and into dry tubes that, after clotting, were centrifuged at 1500 g for 15 min at 4°C. Serum collected was used to perform some biochemical analysis. Some organs as spleen, kidney and liver were extracted. After weighing, a section of each organ (0.2% w/w) served to perform the oxidative stress assessment.

2.4.2 Assessment of body temperature

Body temperature was taken 2 hours before parasite inoculation of each animals and then daily recorded for the 5 days of treatment (d3 to d8). The rectal temperature was obtained using digital thermometer.

2.4.3 Serum biochemical analysis

Some biochemical parameters analysed were consisted to alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities; creatinine, total bilirubin and uric acid levels; Il-6, IL-1β and TNF-α concentrations.

2.4.4 Assay for evaluation of some parameters of oxidative stress

The portion (0.2 g) sectioned from each organ was homogenized in 2 mL of Tris-HCl 50 mmol buffer, pH 7.4 as described by Djouwoug *et al*. [12]. The homogenate obtained was used for assessment of reduced glutathione (GSH), malondialdehyde (MDA) and nitrites (NO) concentrations, catalase and superoxide dismutase (SOD) activities.

2.4.5 Hematological analysis

Some blood parameters evaluated consisted of the mean cell volume (MCV), haemoglobin (HB) level, haematocrit (Hct) rate, the numbering of erythrocytes (RBC), leucocytes (WBC), platelets (PLA) and the percentage of leucocytes species (lymphocytes (LYM), monocytes (MON), and granulocytes (GRA)). The automated analyser (Sysmex XP-300, Germany) was used for the haematological test.

2.5 Data analysis

The mean ± SEM was used to express the data. Graph pad Prism 8.01 software was used to conduct statistical analyses of the data, which included an analysis of variance (ANOVA) and a Turkey post-test. P value <0.05 was used to determine whether a difference was significant.

3. Results

3.1. Effect of the *Picralima nitida* extract on the body temperature

Plasmodium berghei infection significantly increased ($P< 0.001$) the body temperature of untreated animals by 5.66% related to the normal (Figure 1). The aqueous extract of *Picralima nitida* fresh fruits (400 $mg.kg^{-1}$) significantly decreased the body temperature by 2.01% ($p< 0.01$) and 2.76% ($p< 0.001$) at the days 7 and 8 post-inoculation, respectively, regard to the malaria control. Significant drop of the body temperature ($p< 0.001$) was observed with the treatment chloroquine (10 $mg.kg^{-1}$) from the day 4 to the end of experiment with regard to the malaria control.

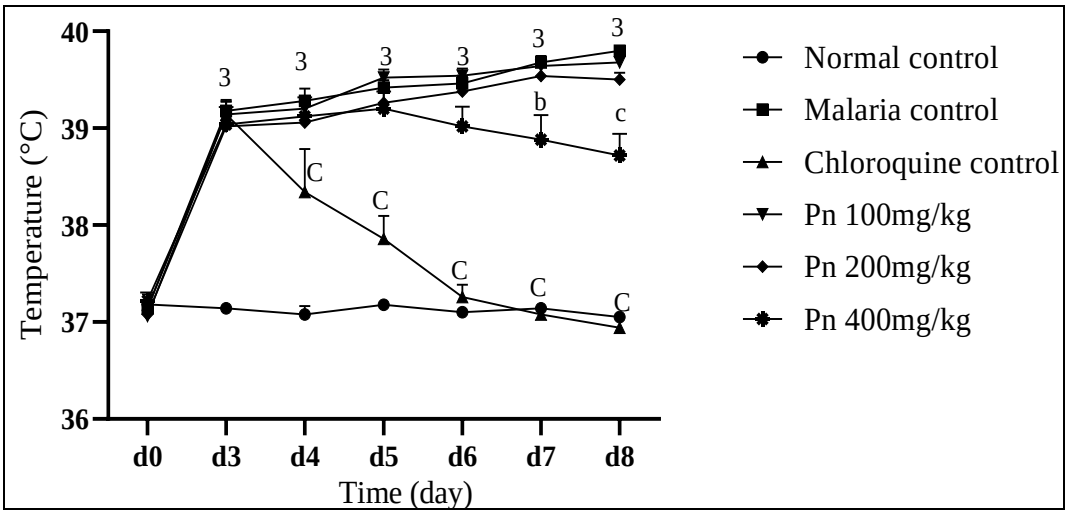


Fig 1: Effect of aqueous extract of *Picralima nitida* fruits on the body temperature

Points represent mean ± SEM, n = 5; the degree of significance when compared to normal control was indicated as ^{3}p 0.001; the level of significance when it came to malaria control was expressed as $^{b}P<$ 0.01, and $^{c}P<$0.001. Malaria control represents the infected rats that were given distilled water (10 mg.kg^{-1}); normal control was rats healthy given distilled water; chloroquine control was malaria-infected rats treated with chloroquine-sulphate (10 mg.kg^{-1}); PN = Malaria-infected rats treated with the plant extract at doses of 100 mg.kg^{-1} (PN 100 mg.kg^{-1}), 200 mg.kg^{-1} (PN 200 mg.kg^{-1}) and 400 mg.kg^{-1} (PN 400 mg.kg^{-1}) (PN 400 mg.kg^{-1})

3.2. Effect on the extract on the parasitaemia

Plasmodium berghei inoculation to rats led to a parasite count of 4.86% ± 0.12 after 3 days, which gradually increased rising up to 37% at the day 8 in untreated group (malaria control) (Fig. 2). The administration of *P. nitida* extract significantly reduced ($p<$ 0.01) with dose-dependent effect the parasite load of infected animals related to the malaria control. The plant extract (100, 200 and 400 mg.kg^{-1}) inhibited parasite growth by 67.67% 76.98% and 85.82% respectively, with regard to the malaria control. The chloroquine sulphate administration induced significant ($p<$ 0.01) complete clearance of parasite (Figure 2).

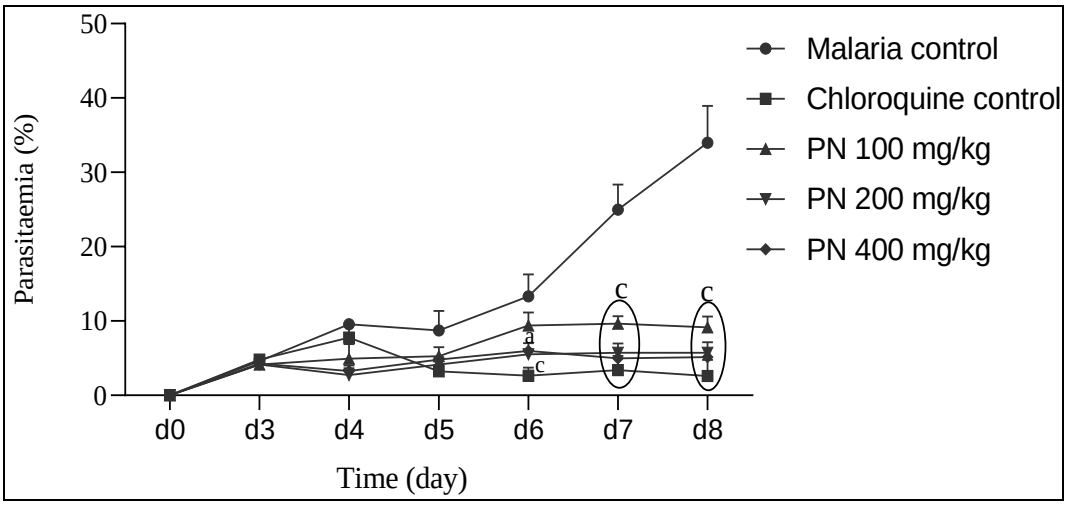


Fig 2: Effect of aqueous extract of *Picralima nitida* fruits on the parasitaemia

Each point represents mean ± SEM, n = 5; $^{a}P<$ 0.01, $^{c}P<$ 0.001 were used to express the level of significance as compared to malaria control; Malaria control = Malaria-infected rats are given distilled water (10 mg.kg^{-1}). Chloroquine control = Malaria-infected rats that were given chloroquine-sulphate; Normal control = healthy animals getting distilled water (10 mg.kg^{-1}) PN = malarious rats given plant extract at the respective doses of 100 mg.kg^{-1} (PN 100 mg.kg^{-1}), 200 mg.kg^{-1} (PN 200 mg.kg^{-1}) and 400 mg.kg^{-1} (PN 400 mg.kg^{-1})

3.3. Effect of the extract on pro inflammatory cytokines

Malaria infection caused significant raising ($p<$ 0.001) in the serum concentration of TNF-α, IL-1β and IL-6 in untreated rats (malaria control) versus to those of the healthy rat (Figure 3). The plant extract absorption resulted after 5 days to significant drop ($p<$ 0.001) of the IL-1β level by 39.71% and 58.40%, the IL-6 concentration of 52.38% ($p<$ 0.001) and 15.41% ($p<$ 0.01) at the doses of 200 and 400 mg.kg^{-1}, respectively with regard to the malaria control. The TNF-α concentration also decreased ($p<$ 0.001) by 74.08%, 58.23% and 67.16% at the respective doses of 100, 200 and 400 mg.kg^{-1} related to the untreated malaria group.

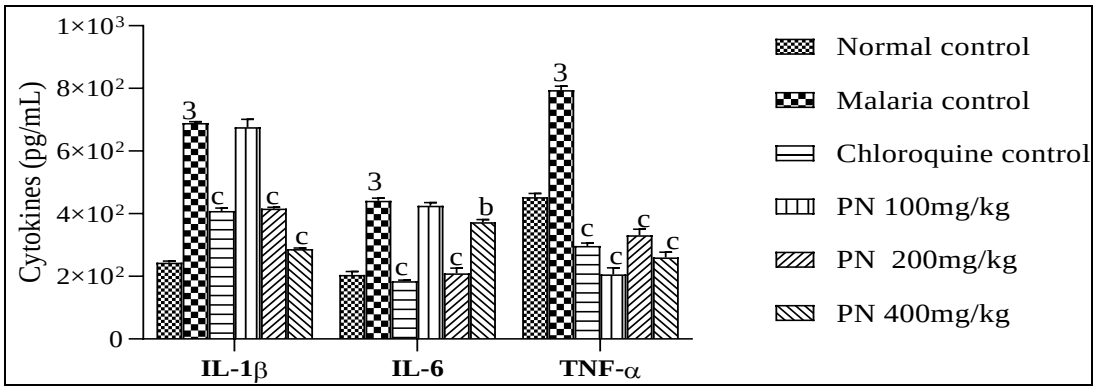


Fig 3: Effect of the extract on some pro-inflammatory cytokines in infected rats

Bars reflect mean ± SEM. n=5. The level of significance as compared to normal control was $^{3}p< 0.001$; when it came to malaria control, the level of significance was $^{b}P< 0.01$, $^{c}P< 0.001$. Malaria control = malaria-infected rats given distilled water (10 mg.kg^{-1}); Normal control = healthy rats given distilled water; Chloroquine control = malaria-infected rats given chloroquine-sulphate (10 mg.kg^{-1}); PN (100, 200 and 400 mg.kg^{-1}) = malaria-infected rats given the plant extract at the respective doses of 100, 200 and 400 mg.kg^{-1}. TNF-α = Tumor necrosis factor α, IL-1β = Interleukin1β and IL-6 = Interleukin 6

3.4. Effect of the plant extract on some oxidative stress parameters

The effects of *P. nitida* fruits extract on some oxidative stress parameters are presented in the Figure 4, where it was noticed that the malaria infection provoked significant increase of the MDA level ($p< 0.01$) in the spleen, kidneys and liver (Fig. 4A); the nitrites rate rise up ($p< 0.001$) in the liver (Fig. 4B) of infected animals whereas the level of GSH (Fig. 4C) and SOD activity (Fig.4D) significantly decreased ($p< 0.01$) in these organs by comparing to the healthy animal batch. With regard to the normal group, the catalase activity (Fig. 4E) dropped significantly ($p< 0.001$) in the spleen of malarious animal.

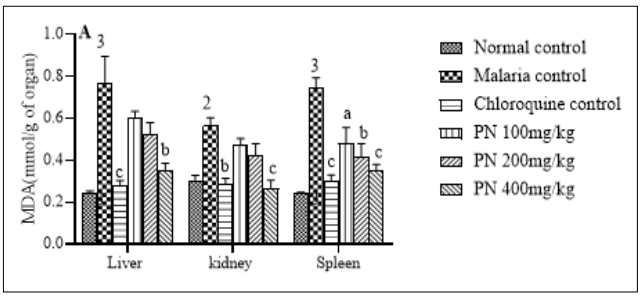
The plant extract (400 mg.kg^{-1}) induced significant decrease in MDA concentration by 54.36% ($p< 0.01$) in the liver and 53.09% ($P< 0.001$) in the kidney, in comparison to the untreated group. Likewise, with regard to the malarious animals, a dose dependant reduction in the MDA level by 35.47% ($p< 0.05$), 44.17% ($p< 0.01$), and 53.27% ($p< 0.001$) at the gradual extract doses of 100, 200 and 400 mg.kg^{-1}. Chloroquine (10 mg.kg^{-1}) significantly reduced the MDA level by 64.01% ($p< 0.001$) in the liver, 49.38% ($p< 0.01$) in the kidney and 59.57% ($p< 0.001$) in the spleen related to the malaria control.

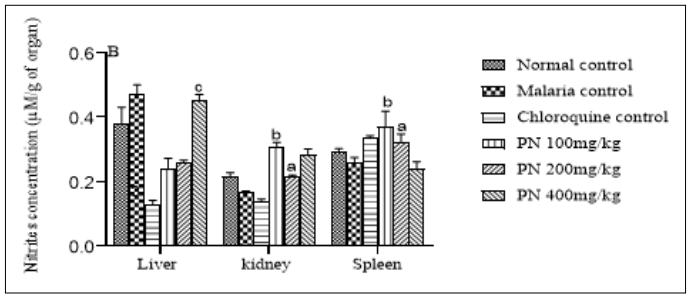
It was observed significantly dropped ($p< 0.001$) of the nitrites concentration in the liver by 48.93% and 44.68% at the extract doses of 100 and 200 mg.kg^{-1} respectively, as compared to the malaria control. By contrary, the extract increased the nitrites rate by 87.50% ($p< 0.001$) and 75.00% ($p< 0.01$) in the kidney at the dose of 100 and 200 mg.kg^{-1} respectively, and by 48.00% ($p< 0.05$) in the spleen at the dose of 100 mg.kg^{-1} related to the malaria control. The chloroquine treatment provoked significant drop ($p< 0.001$) of the nitrites level in the liver.

The treatment with the extract as with the chloroquine (10 mg.kg^{-1}) significantly enhanced ($p< 0.05$) the glutathione level in the liver related to the untreated malaria group.

The activity of SOD significantly increased in the liver of 89.49% ($p < 0.01$) at the plant extract (400 mg.kg^{-1}) compared to the malaria control. In the kidney and spleen, the extract induced significant gain ($p< 0.01$) of SOD level as compared to the malaria group. Significant enhancement in the SOD activity in the liver by 67.80% ($P< 0.05$), in the kidney of 164.51% ($P< 0.001$) and more than three time ($P< 0.001$) in the spleen with the chloroquine with regard to the malaria control.

In the liver, the extract (200 mg.kg^{-1}) significantly raised up the catalase activity by 24.69% ($p< 0.05$) with regard to malaria control. Likewise, by comparing to the malaria control, the plant extract involved significant increase ($p< 0.001$) of catalase activity by 67.88% and 40.87% at the respective doses of 200 and 400 mg.kg^{-1} in the spleen. The catalase activity also increased by 48.90% ($p< 0.001$) in the spleen, with the chloroquine treatment.





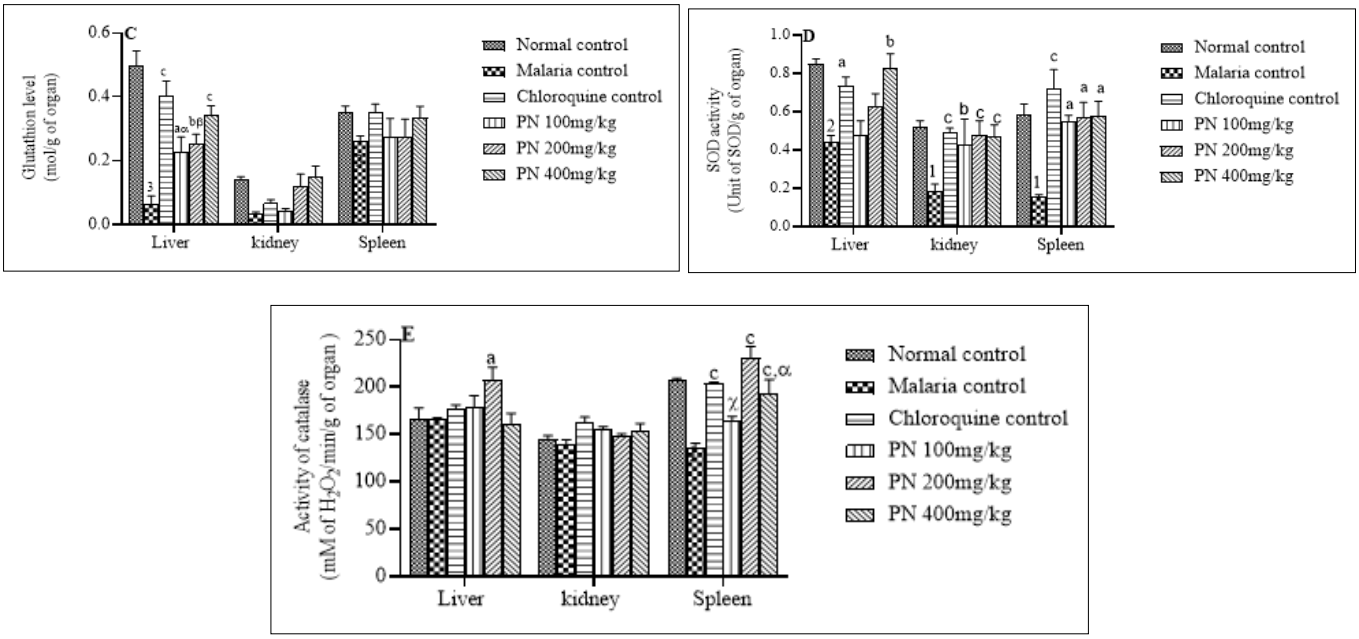


Fig 4: Effects of the extract from *Picralima nitida* fruits on some oxidative stress parameters (A) MDA, (B) nitrites, (C) GSH, (D) catalase and (E) SOD in infected rats

Bars express mean ± SEM, n=5, The degree of significance was stated as $^{1}p< 0.05$, $^{2}p< 0.01$, $^{3}p< 0.001$ when compared to normal control, and as $^{a}p< 0.05$, $^{b}P< 0.01$, $^{c}P< 0.001$. $^{\alpha}p< 0.05$, $^{ß}p< 0.01$ when it came to malaria control. Malaria control = malaria-infected rats given distilled water (10 mg.kg^{-1}); Normal control = healthy animals given distilled water (10 mg.kg^{-1}); Chloroquine control = malaria infected rats given chloroquine-sulphate (10 mg.kg^{-1}); PN = malarious rats given the plant extract at doses of 100 mg.kg^{-1} (PN 100 mg.kg^{-1}), 200 mg.kg^{-1} (PN 200 mg.kg^{-1}) and 400 mg.kg^{-1} (PN 400 mg.kg^{-1}).

3.5. Effect of the extract *Picralima nitida* fruit on some biomarkers of the liver and kidney function

P. berghei-infected rats had a significant rise ($p< 0.001$) in bilirubin concentration, creatinine and urea level and in transaminases activities in comparison to healthy rats (Table 1). At doses of 200 and 400 mg.kg-1, respectively, the extract significantly recovered ALT activities by 32.81% ($p < 0.01$) and 46.39% ($p < 0.001$) and the AST by 18.05% ($p < 0.05$) and 39.81% ($p < 0.001$) at the doses of 200 and 400 mg.kg^{-1} respectively, compared to the untreated malaria control. The treatment with chloroquine (10 mg.kg^{-1}) resulted in substantial reductions in ALT and ALT levels ($p< 0.001$) by 54.80% and 36.57% respectively, with regard to malaria control.

Moreover, the plant extract decreased significantly the total bilirubin concentration of 42.18% ($p< 0.01$) and 80.66% ($p< 0.001$) at the doses of 200 and 400 mg.kg^{-1} respectively, in comparison to malaria-infected rats (Table 1). The reduction in this parameter was of 79.53% ($p< 0.001$) with chloroquine treatment (10 mg.kg^{-1}) as compared to untreated malaria-infected rats.

The decrease in serum urea level was of 25.54% ($p< 0.01$), 45.98% ($p< 0.001$) and 43.06% ($p< 0.001$) in treated animals with the *P. nitida* extract at the doses of 100, 200 and 400 mg.kg^{-1} respectively, comparing to the untreated group. Chloroquine administration induced significant decrease by 59.12% ($p< 0.001$) related to the malaria control.

Table 1: Effect of *Picralima nitida* fruits extract on some parameters of the liver and kidney function.

	Normal control	Malaria control	Chloroquine control	PN 100 mg.kg^{-1}	PN 200 mg.kg^{-1}	PN 400 mg.kg^{-1}
AST (U/L)	141.00±6.46	216.00±6.95^{3}	137.00±7.33 ^{c}	195.00±4.17	177.00±3.67 ^{a}	130.00±8.26 ^{c}
ALT (U/L)	38.50±2.52	83.20±5.75^{3}	37.60±3.33^{c}	103.00±2.78	55.90±6.05 ^{c}	44.60±4.56 ^{c}
Bilirubin (mg/dL)	0.02±0.00	0.25±0.03^{3}	0.05±0.00 ^{c}	0.22±0.02	0.14±0.03 ^{a}	0.04±0.02 ^{c}
Creatinine (mg/dL)	0.39±0.01	1.41±0.17^{3}	0.38±0.02 ^{c}	0.35±0.01 ^{c}	0.35±0.00 ^{c}	0.32±0.04 ^{c}
Urea (mg/dL)	9.24±1.17	27.40±1.40^{3}	11.20±0.08 ^{c}	20.40±1.58 ^{b}	14.80±1.21 ^{c}	15.60±1.20 ^{c}

Values are expressed as mean ± SEM. n=5. $^{2}P< 0.01$. $^{3}p< 0.001$: the degree of significance when compared to a normal control $^{a}p<0.05$, $^{b}P< 0.01$, $^{c}P< 0.001$: the degree of significance as regard to malaria control. Malaria control = malaria-infected rats given distilled water (10 mg.kg^{-1}); Normal control = healthy animals given distilled water; Chloroquine control = malaria-infected rats given chloroquine-sulphate (10 mg.kg^{-1}); PN = malaria-infected rats given the plant extract at the doses of 100 mg.kg^{-1} (PN 100 mg.kg^{-1}), 200 mg.kg^{-1} (PN 200 mg.kg^{-1}) and 400 mg.kg^{-1} (PN 400 mg.kg^{-1}).

3.6. Effect of extract on some haematological parameters

Plasmodium infection caused a substantial drop ($p< 0.001$) in hemoglobin (HB) level, red blood cells (RBC) count, hematocrit (Hct) rate, mean packed cells volume (MCV) and platelets count, while white blood cells (WBC) raised ($p< 0.001$) as regard to the normal group after 8 days (Table 2). At doses of 100, 200 and 400 mg.kg^{-1}, the aqueous extract of *P. nitida* fresh fruits increased RBC by 30.04% ($P< 0.01$), 94.61% ($P< 0.001$), and 120.62% ($P< 0.001$), in HB level by 32.65% ($P< 0.05$), 83.67% ($P<0.001$) and 122.44% ($P< 0.001$), respectively, compared to the malaria control. In the malaria-infected rats, the hematocrit rate increased ($p< 0.001$) by 45.02% and 66.49%, respectively, at the extract doses of 200 and 400 mg.kg^{-1} When compared to the malaria untreated animal, WBC count decreased by 64.25% ($P< 0.01$), 72.16% ($P< 0.001$), and 75.41% ($P< 0.001$) after treatment with the

extract at doses of 100, 200 and 400 mg.kg^{-1}, respectively. The extract significantly reduced ($p< 0.01$) monocytes count at doses of 200 and 400 mg.kg-1 with regard to the malaria untreated rat. The plant extract significantly raised ($p< 0.05$) the number of platelets in treated animals compared to the malarious group. In comparison to malaria control, chloroquine (10 mg.kg^{-1}) considerably reduced (p 0.05) haematological disturbance.

Table 2: Effect of aqueous extract of *Picralima nitida* on some haematological parameters in infected rats

	Normal control	Malaria control	Chloroquine control	PN 100 mg.kg^{-1}	PN 200 mg.kg^{-1}	PN 400 mg.kg^{-1}
RBC (10^6/mm3)	7.70 ± 0.28	2.23±0.11^3	6.34±0.43^c	2.90±0.42^b	4.34±0.53^c	4.92±0.27^c
HB (g/dl)	13.20 ± 0.61	3.92±0.25^3	12.30±0.50^c	5.20±0.75^b	7.20±0.77^c	8.72±0.58^c
Hct(%)	46.60 ± 1.79	19.10±1.20^3	42.10±1.41^c	1.04 ±1.91	27.70±1.36^c	31.80±1.71^c
MCV (fl)	80.60 ± 1.21	72.50±4.32^3	75.50±2.33	70.00±2.26	76.80±1.66	74.30±1.12
WBC (10^3/mm3)	5.72 ± 1.34	27.70±2.79^3	4.82±0.23^c	9.90±3.18^b	7.71±1.59^c	6.81±1.56^c
LYM (10^3/mm^3)	4.04 ± 0.64	11.90±0.67^2	3.94±0.19^b	6.15±2.05	2.08±1.77	2.47±0.36^b
MON (10^3/mm^3)	0.45 ± 0.05	12.60±2.34^3	0.43±0.15^c	2.63±1.10	3.79±0.98^b	3.57±0.71^b
GRA (10^3/mm^3)	1.23 ± 0.39	3.24 ± 0.32^3	0.47 ± 0.11^c	1.12±0.23	1.84±0.32^a	0.77±0.04^c
PLA (10^3/mm^3)	659.00 ± 23.75	162.00 ±14.25^3	683.00 ± 99.06^c	472.00 ±74.0^a	435.00 ±58.85^a	534.00 ±42.8^b

Values are expressed as mean ± SEM. n=5. The degree of significance when compared to normal control was stated as $^2P< 0.01$. $^3p< 0.001$; when compared to malaria control, the degree of significance was expressed as $^ap<0.05$, $^bP< 0.01$, $^cP< 0.001$. PN = malaria-infected rats given the plant extract at doses of 100 mg.kg^{-1} (PN 100 mg.kg^{-1}), 200 mg.kg^{-1} (PN 200 mg.kg^{-1}) and 400 mg.kg^{-1} (PN 400 mg.kg^{-1}). Malaria control = malaria-infected rats given distilled water (10 mg.kg^{-1}). Chloroquine control = malaria-infected rats given chloroquine-sulphate (10 mg.kg^{-1}); Normal control = healthy animals given distilled water.

4. Discussion

Malaria infection is a multisystem and life-threatening disease. It was demonstrated that fever represents the hallmark, but, clinical findings in malaria remains extremely diverse and may range in severity from mild headache to serious physiological changes leading to death. This study explored the anti-inflammatory and anti-oxidative effect of the aqueous extract of *P. nitida* fresh fruits in malaria condition caused by *Plasmodium berghei* in rat. Malaria infection was obtained by injection of 1 x 10^6 *P. berghei*-parasitized erythrocytes via intraperitoneal route in rats. The infection was expressed by gradual increase of parasitaemia along the experimental days followed by significant hyperthermia in untreated animals. The physiological impairment currently described in malaria infection was accompanied by an increase of some proinflammatory cytokines and nitrites levels as observed in the study. This pathophysiological state results from the synchronous lysis of the erythrocytes after maturation of schizonts that release in the bloodstream of plasmodium antigens such as glycosylphosphatidylinositol (GPI) and oxidizing agent, hemozoin endowed of pyrogenic activity that are capable to stimulate production of some endogenous pyrogens like pro-inflammatory cytokines (IL-1β, TNF-α and IL-6) from polynuclear cells and lymphocytes [13, 14]. These cytokines provoke the synthesis of prostaglandins which disturb the thermoregulation centre causing fever [15]. The plant extract administration to the malarious animals significantly inhibited not only the parasite growth, but, prevented hyperthermia and reduced the proinflammatory cytokines level and leucocytes count, indicating the antiparasitic, antipyretic and anti-inflammatory potential of the plant. These benefit effects may result from some alkaloid compounds contained in the *P. nitida* fruit that act on apicop last in the Plasmodium parasite by interfering with the protein synthesis in the parasite [9, 13, 16–18]. In addition, flavonoids component exhibit antiplasmodial activity either by inhibiting the influx of myoinositol and L-glutamine in parasitized erythrocytes or through inhibition of the biosynthesis of fatty acid (FAS II) in Plasmodium [19, 20]. Moreover, antipyretic activity have been assigned to terpenoids, phenols, flavonoids, alkaloids and saponins which are some phytochemicals compounds identified in the extract of *P. nitida* fruit [21]. These bioactive compounds exert their antipyretic effects by inhibiting synthesis of molecules as lipooxygenase, cyclooxygenase, leukotrienes and prostaglandins contributing to inflammatory reactions [22, 23].

In the present study, plasmodial infection caused anaemia, liver and kidney impairments which are some severe life-threatening complications described in the infection [3]. Dysfunction observed in the kidneys of malarious animals could result from the hemodynamic disturbances and/or immunological reactions. Oxidative stress as demonstrated in malarial infection could also be responsible of these physiological alterations [24]. The formation of free radicals linked with oxidative stress is often the cause of various systemic abnormalities in malaria, demonstrating the complex host-parasite connection. [25]. The generation of free radicals in malaria could be owing to the host's inflammatory response, as observed in this study, or to high levels of free iron, which catalyse Haber-Weiss and Fenton reactions [24]. During malaria, this disturbance causes ischemia and reperfusion syndrome, which is caused by cytoadherence that could result in a diminished capacity of red blood cells to transport oxygen [24, 26–28].

The plant extract administration to the infected animals significantly protected from the damages, indicating the ability of the plant to alleviate physiological alterations. This protective antioxidant effect could assign to some ingredients contained in the plant such as flavonoids which inhibit the production of inflammatory mediators and along with saponins improve the microcirculation into the liver and kidney due to their surfactant properties [29–31], phenolic compounds interfere with the production of reactive oxygen species and play an important activity in neutralisation while tannins act by free radical scavenging properties [32].

5. Conclusion

The pharmacological effect of the aqueous extract of *P. nitida* fresh fruit in *Plasmodium berghei*-induced malaria in rats is demonstrated in this work. The plant extract prevented from anaemia, leucocytosis, liver and kidney failure, decreased the inflammatory cytokine level and improved the antioxidant status of infected animals. The extract also protected from fever and inhibited the parasite growth. These results testify the pharmacological properties of the plant and justify its traditional use for different ailments.

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