

# Free Radical Scavenging Property of *Picralima nitida* Seed Extract on Malaria-Induced Albino Mice

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**Abstract:** Antioxidants help to mop up free radicals that serious damage to the body system and hence are referred to as free radical scavengers. The main objective of this study was to assess the antioxidant property of ethanol seed extract of *Picralima nitida* in malaria-induce mice. The activities of the antioxidant enzymes myeloperoxidase (MPO), superoxide dismutase (SOD), thioredoxin reductase (TrxR) were assayed; malondialdehyde (MDA) and nitrite levels were determined. The levels of nitrite and MDA and the SOD activity of the drug-treated groups of mice were significantly ( $p < 0.05$ ) lower on days 3 and 5 post treatment compared to the group induced with malaria but not treated (positive control). The MPO activity of the drug-treated groups of mice was significantly ( $p < 0.05$ ) higher on day 3 post treatment while its activity in mice treated with 40 and 80 mg/kg b.w. of the extract were significantly ( $p < 0.05$ ) lower on day 5 post treatment compared to the positive control. In the group treated with 80 mg/kg b.w. of the extract, the TrxR activity was significantly ( $p < 0.05$ ) lower on days 3 and 5 post treatment compared to the positive control. *Picralima nitida* seed extract was found to possess good antioxidant properties.

**Keywords:** Antioxidants, Free Radicals, SOD, MDA, TrxR

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## 1. Introduction

Antioxidants are broadly classified into two divisions, hydrophilic and lipophilic antioxidants. Water-soluble antioxidants react with oxidants in the cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet [1]. Antioxidants such as glutathione and ubiquinone are mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few micro-organisms and these compounds can be virulent factors [2] in these micro-organisms. These antioxidants normally interact with one another and with other metabolites and enzyme systems in a synergistic way [3]. The amount of protection by any antioxidant depends on its concentration, its relative reactivity towards a particular reactive oxygen species and the status of the antioxidants with which it interacts. Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from

catalyzing the production of free radicals in the cell.

Oxidation reactions do produce free radicals which can start chain reactions [4] that normally cause damage or death to the cell. Antioxidants which terminate these chain reactions by removing free radicals include thiols, ascorbic acid or polyphenols, glutathione, vitamin A, vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases [5]. Insufficient levels of the antioxidants or inhibition of the antioxidant enzymes, cause oxidative stress [6]. Antioxidants are widely used in dietary supplements though have not been suggested so helpful in dealing with oxidative stress and excess of them may be harmful [7]. Antioxidants also have other industrial uses, such as food preservatives, cosmetics and to prevent rubber and gasoline degradation [8].

Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species [9]. Consequently, organisms contain a complex network of

antioxidant metabolites and enzymes that collaborate to prevent damage to cellular components such as DNA, proteins and lipids [1]. Generally, antioxidants systems either prevent these reactive species from being formed or remove them before they can damage vital components of the cell [4] [9]. However, reactive oxygen species also have useful cellular roles, such as redox signaling. Thus, the function of antioxidant systems is not to remove oxidants entirely, but to keep them at an optimum level [10]. The reactive oxygen species produced in cells include hydrogen peroxide, hypochlorous acid, and free radicals such as hydroxyl radical and superoxide anion, peroxyxynitrite and peroxyxynitrous acid [11]. The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. It is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction [12]. These oxidants damage cells by starting up chemical chain reactions such as lipid peroxidation, oxidizing DNA or proteins. Damage to DNA can cause mutation while damage to proteins causes enzyme inhibition, denaturation and degradation [13, 11].

Superoxide anion is produced as a by-product of several steps in the electron transport chain [14]. Peroxide is produced from the oxidation of reduced flavoproteins, such as complex I of electron transport chain. In plants (algae and cyanobacteria), reactive oxygen species are also produced during photosynthesis [15], especially under high light intensity [16]. This effect is offset by large amounts of iodide and selenium in algae and cyanobacteria [17]. Superoxide dismutases (SODs) are a class of closely related enzymes that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide [18]. They are an important antioxidant defense in nearly all cells exposed to oxygen except *Lactobacillus plantarum* and related *lactobacilli* [19]. Superoxide dismutase plays a critical role in reducing the oxidative stress implicated in atherosclerosis and other life-threatening diseases, reducing internal inflammation and lessening pain associated with conditions such as arthritis [20].

Hypochlorous acid is produced by myeloperoxidase from the reaction between hydrogen peroxide and chloride ion; this potent oxidant contributes to the antimicrobial activity of phagocytes [21]. However, it has been proven that either chronic or prolonged production of hypochlorous acid by the myeloperoxidase-hydrogen peroxide-chloride ion system contributes to tissue damage and the initiation and propagation of vascular diseases [22]. Furthermore, low-density lipoproteins oxidized by this system accumulate in macrophages and exerts pro-inflammatory effects on monocytes and endothelial cells [23, 24]. Another crucial enzyme among the antioxidant enzyme system is thioredoxin reductase; a member of the nucleotide pyridine disulfide oxidoreductase family, which includes glutathione reductase, alkyl hydroperoxide reductase and lipoamide dehydrogenase [25]. Thioredoxin reductase catalyses the disulfide reduction of oxidized thioredoxin using NADPH via the FAD molecule and the redox-active cysteine residues [26]. There are two

main divisions of thioredoxin reductase: the high molecular weight (HMW TrxRs) and low molecular weight (LMW TrxRs) thioredoxin reductase enzymes (27).

The SOD activity of mice infected with *Plasmodium berghei* and treated with graded doses of *Aframomum sceptrum* was significantly enhanced compared to the mice infected but not treated [28]. Seymour *et al.* [29] presented an evidence in support of MPO as a major arm of oxidative killing by neutrophils. In their work, they proposed that the essential contribution of MPO to normal innate host defense is manifest only when exposure to pathogens overwhelms the capacity of other host defense mechanisms. In animals infected with *Schistosoma mansoni*, treatment with artesunate caused significant decrease in expression of *Schistosoma* thioredoxin reductase and cytochrome c peroxidase compared to treatment with praziquantel [30]. Taofit *et al.* [31] conducted a research on the toxicopathological evaluation of *Picralima nitida* seed aqueous extract in Wistar rats and found out that the extract exhibited mild and selected toxicity on the liver.

*Picralima nitida* has been a drug of choice in the treatment of illnesses in traditional medicine practice in some parts of Africa such as Ghana, Nigeria and others. In Ghana, the plant is commonly known as *Akuamma*. Various parts of the plant have been employed in the treatment of diseases such as diarrhea, hypertension, gastrointestinal problems, and malaria among other diseases [32]. In an experiment conducted by Adumanya *et al.* [33], it was observed that the leaves of *P. nitida* when administered to adults confirmed positive with *Plasmodium falciparum*, reduced drastically the level of parasitaemia in the patients. Acute and subchronic toxicities of aqueous seed extract of *P. nitida* showed that the drug is very unlikely to be toxic on chronic administration [34]. This work was aimed at assessing the antioxidant properties of *Picralima nitida* seed extract using some biochemical studies.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Animals

The experimental animals used for this study were white albino mice of either sex weighing 20-34 g. The mice were between 3-4 months old and were obtained from the Animal Unit of Faculty of Biological Sciences, University of Nigeria, Nsukka.

#### 2.1.2. Collection and Identification of the Seeds

Seeds of *Picralima nitida* were collected from Isuofia, Aguata Local Government Area of Anambra State and were authenticated by Mr. Ozioko A. of the Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka.

#### 2.1.3. Chemicals/Reagents

All the chemicals used in this study were of analytical grade and products of May and Baker, England; BDH,

England and Merck, Darmstadt, Germany. The reagents used for all the assays were commercial kits and products of Randox, QCA, USA and Biosystem Reagents and Instruments, Spain.

## 2.2. Methods

### 2.2.1. Extraction

Seeds of *Picralima nitida* plant were harvested and then dried under room temperature (29-35°C) for three weeks, after which they were pulverized into powdered form with a Crestor high speed milling machine. The powdered seed (1 kg) was then macerated in 5 volume (w/v) absolute ethanol and left to stand for 48 hours. Afterwards, the extract was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature of 45°C to avoid denaturation of the active ingredients. The concentrated extract was stored in the refrigerator throughout the period of the experiment.

### 2.2.2. Procurement of Malarial Parasite

Malarial parasite (*Plasmodium berghei*) was obtained from malaria infected mice at Veterinary Medicine Department of University of Nigeria, Nsukka. Ten drops of the parasitized blood obtained with the aid of a capillary tube through the ocular region of the mouse was diluted with 1 ml of normal saline. Thereafter, 0.2 ml of the diluted parasitized blood was used to infect three mice that served as hosts from which subsequent ones were infected.

### 2.2.3. Experimental Design

A total of 180 albino mice of either sex weighing 20-34 g were housed in separate cages, acclimatized for one week and then divided into six groups of thirty mice each as follows:

*Group 1:* Normal mice treated with the vehicle, 3% tween 80 (Normal Control)

*Group 2:* Mice inoculated with malaria parasite and treated with 3% tween 80 (Positive Control)

*Group 3:* Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract

*Group 4:* Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract

*Group 5:* Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract

*Group 6:* Mice inoculated with malaria parasite and treated with the standard drug, artesunate (Standard Control)

The route of administration was via oral route with the aid of an oral intubation tube and the extract was given in 3% tween 80. Treatment lasted for 5 days during which analyses were carried out on days 3 and 5.

### 2.2.4. Determination of Myeloperoxidase Activity

Myeloperoxidase (MPO) activity was assayed using the method of Bos *et al.* [35]. An aliquot, 150 µl of serum was added to a mixture containing 0.0 mmol H<sub>2</sub>O<sub>2</sub>, 10 mmol KH<sub>2</sub>PO<sub>4</sub> (pH 6.0) and 0.4 mmol O-dianisidine dihydrochloride. The reaction was performed at 37°C and

absorbance was measured at 460 nm every 15 sec for 4 mins, and the linear portions of the tracing was used for analysis.

### 2.2.5. Determination of Superoxide Dismutase (SOD)

#### Activity

The activity of this enzyme was assayed using the method of Fridovich [36]. An amount, 0.1 ml of serum was added to the tubes containing 0.75 ml of ethanol and 0.15 ml of chloroform under chilled condition and centrifuged. To 0.5 ml of supernatant, 0.5 ml of 0.6 mM EDTA solution and 1.0 ml of 0.1 M carbonate-bicarbonate buffer (pH 10.2) were added. The reaction was initiated by the addition of 0.5 ml of 1.8 mM epinephrine and the increase in absorbance at 30 second interval for 3 minutes was measured. Readings were taken at 480 nm.

### 2.2.6. Determination of Thioredoxin Reductase (TrxR)

#### Activity

Thioredoxin reductase activity was assayed using the method of Holmgren and Bjornstedt [37]. The assay mixture (1 ml) contained 0.2 M Na<sup>+</sup>-K<sup>+</sup>-phosphate buffer (pH 7.6), 1 mM EDTA, 0.25 Mm NADPH, and 1 mM DTNB. After initiating the reaction with NADPH, the increase in absorbance at 412 nm was monitored for 3 min at 25°C.

### 2.2.7. Assay of Lipid Peroxidation Product (MDA)

The lipid peroxidation product (MDA) was determined using the method of Wallin *et al.* [38]. To 0.10 ml of serum in test tube, 0.45 ml of normal saline was added and mixed thoroughly. Thereafter, 0.5 ml of 25% trichloroacetic acid (TCA) (w/v) and 0.5 ml of 1% thiobarbituric acid was added to the mixture. The blank tube contained the same volume of reagents but 0.10 ml of distilled water instead of serum. The mixture in the separate tubes was heated in water bath at 95°C for 40 minutes. The content was allowed to cool before reading the absorbance of the clear supernatant against reagent blank at the wavelength of 532 nm and 600 nm. Thiobarbituric acid reacting substances (TBARS) were quantified as lipid peroxidation product by referring to a standard curve of malondialdehyde (MDA) concentration (i.e. equivalent generated by acid hydrolysis of 1, 1, 3, 3-tetraethoxypropane (TEP) prepared by serial dilution of a stock solution).

### 2.2.8. Determination of Nitrite Concentration

Nitric oxide generation and release was determined using the method of Sanai *et al.* [39]. For this assay, 0.9 ml of distilled water was added to 0.1 ml of the sample. An amount, 0.5 ml of sulphanilamide solution was added to the mixture and allowed to stand for 2 minutes. A little amount, 0.1 ml of N-(1-naphthyl)- ethylenediamine dihydrochloride solution was added and the absorbance was measured at 543 nm.

### 2.2.9. Statistical Analysis

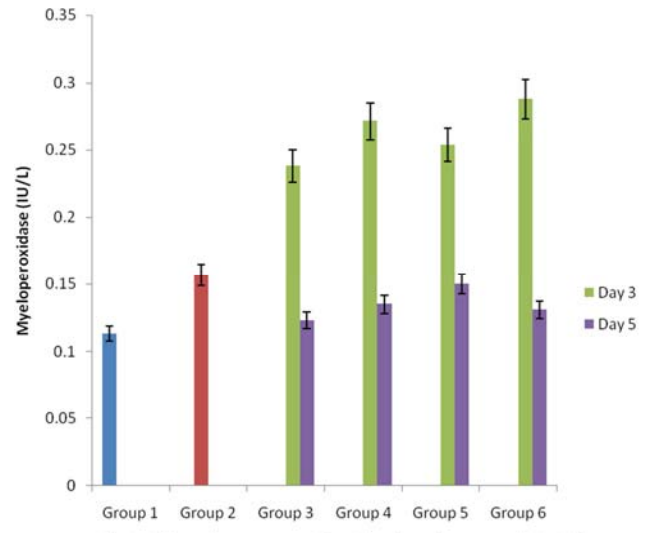
The data results were subjected to one-way ANOVA with repeated measures, t-test and correlation analyses. Significant differences were observed at  $p < 0.01$  and  $p < 0.05$ . The results were expressed as mean  $\pm$  standard error of mean (S. E. M.). This analysis was carried out using Statistical

Package for Social Sciences (SPSS) version 18.

### 3. Results and Discussion

#### 3.1. Results

In figure 1 and table 1, the serum myeloperoxidase (MPO) activity of normal mice treated with 3% tween 80 (normal control) is shown to be  $0.11 \pm 0.00$  IU/L while that of infected mice treated with 3% tween 80 (positive control) was higher with the value  $0.16 \pm 0.00$  IU/L. The serum MPO activities of the mice groups 3, 4 and 5 treated with 20, 40 and 80 mg/kg b.w. of the extract were found to be  $0.24 \pm 0.01$ ,  $0.27 \pm 0.01$  and  $0.25 \pm 0.02$  IU/L respectively on day 3 post treatment. On day 5 post treatment, their MPO activity decreased significantly to  $0.12 \pm 0.01$ ,  $0.14 \pm 0.01$  and  $0.15 \pm 0.01$  IU/L. The treated with 5 mg/kg b.w. of artesunate (standard control) showed an MPO activity of  $0.29 \pm 0.02$  IU/L on day 3 post treatment which further decreased to  $0.13 \pm 0.01$  IU/L on day 5 post treatment. Compared to the positive control, the MPO activity of all the extract-treated groups were significantly ( $p < 0.05$ ) higher on day 3 post treatment while on day 5 post treatment, the enzyme's activity in groups 3 and 4 were significantly ( $p < 0.05$ ) lower. Even when compared to the standard control, the MPO activity of group 3 was significantly ( $p < 0.05$ ) lower and was found to have decreased by 0.05 IU/L on day 3 post treatment while the enzyme's activity of group 5 was significantly ( $p < 0.05$ ) higher and would have increased by 0.02 IU/L on day 5 post treatment.



**Figure 1.** Effect of treatment with ethanol seed extract of *P. nitida* on serum myeloperoxidase activity of malaria-infected mice.

Group 1: Normal mice treated with the vehicle, 3% tween 80 (Normal Control)  
 Group 2: Mice inoculated with malaria parasite and treated with 3% tween 80 (Positive Control)  
 Group 3: Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract  
 Group 4: Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract  
 Group 5: Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract  
 Group 6: Mice inoculated with malaria parasite and treated with the standard drug, artesunate (Standard Control)

**Table 1.** Values for serum myeloperoxidase (MPO), superoxide dismutase (SOD) and thioredoxin reductase (TrxR) activities, malondialdehyde (MDA) and nitrite levels.

Parameters/Post Treatment Days	Mice Groups					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
MPO (IU/L)						
Day 3	0.11 ± 0.00*	0.16 ± 0.00	0.24 ± 0.01*	0.27 ± 0.01*	0.25 ± 0.02*	0.29 ± 0.02*
Day 5	N. D.	N. D.	0.12 ± 0.01*	0.14 ± 0.01*	0.15 ± 0.01	0.13 ± 0.01*
SOD (IU/L)						
Day 3	87.94 ± 1.52	89.84 ± 0.91	67.99 ± 4.66*	74.50 ± 1.70*	78.29 ± 2.78*	74.84 ± 2.84*
Day 5	N. D.	N. D.	55.67 ± 0.56*	47.26 ± 1.96*	52.49 ± 1.35*	50.87 ± 2.74*
TrxR (IU/L)						
Day 3	0.162 ± 0.000*	0.164 ± 0.000	0.156 ± 0.010	0.146 ± 0.000	0.134 ± 0.000*	0.142 ± 0.000*
Day 5	N. D.	N. D.	0.138 ± 0.010*	0.126 ± 0.010*	0.124 ± 0.000*	0.129 ± 0.000*
MDA (mg/dL)						
Day 3	5.42 ± 0.13*	6.05 ± 0.16	2.85 ± 0.05*	2.78 ± 0.01*	2.91 ± 0.17*	2.10 ± 0.05*
Day 5	N. D.	N. D.	3.66 ± 0.18*	3.08 ± 0.20*	3.34 ± 0.08*	3.14 ± 0.03*
Nitrite (mg/dL)						
Day 3	0.048 ± 0.007	0.050 ± 0.008	0.013 ± 0.000*	0.014 ± 0.000*	0.013 ± 0.000*	0.014 ± 0.000*
Day 5	N. D.	N. D.	0.031 ± 0.001*	0.034 ± 0.001*	0.031 ± 0.002*	0.034 ± 0.001*

\* = significant ( $p < 0.05$ ) compared to the positive control

Results are expressed as Mean ± Standard Error of Mean

N. D. = Not Determined

Group 1: Normal mice treated with the vehicle, 3% tween 80 (Normal Control)

Group 2: Mice inoculated with malaria parasite and treated with 3% tween 80 (Positive Control)

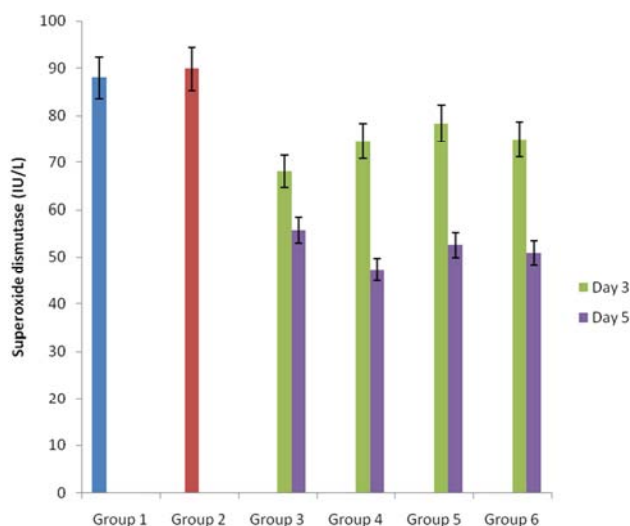
Group 3: Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract

Group 4: Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract

Group 5: Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract

Group 6: Mice inoculated with malaria parasite and treated with the standard drug, artesunate (Standard Control)

Table 1 and figure 2 show the effect of treatment on serum superoxide dismutase (SOD) activity of malaria-infected mice. The serum SOD activity of the normal control was  $87.94 \pm 1.52$  IU/L while the positive control showed higher activity of  $89.84 \pm 0.91$  IU/L. The result of day 3 post treatment assay revealed that the groups of mice treated with the extract showed SOD activity of  $67.99 \pm 4.66$ ,  $74.50 \pm 1.70$  and  $78.29 \pm 2.78$  IU/L respectively. Day 5 post treatment assay result showed decreased activities of  $55.67 \pm 0.56$ ,  $47.26 \pm 1.96$  and  $52.49 \pm 1.35$  IU/L respectively. The standard control showed SOD activity of  $74.83 \pm 2.84$  IU/L on day 3 post treatment which further decreased to  $50.87 \pm 2.74$  IU/L on day 5 post treatment. The SOD of all the extract-treated groups of mice were significantly ( $p < 0.05$ ) lower on days 3 and 5 post treatment compared to the positive control. Even in comparison with the standard control, the SOD activity of group 4 was non-significantly ( $p > 0.05$ ) lower and was found to have decreased by 3.27 IU/L from day 3 to day 5 post treatment.



**Figure 2.** Effect of treatment with ethanol seed extract of *P. nitida* on serum superoxide dismutase activity of malaria-infected mice.

**Group 1:** Normal mice treated with the vehicle, 3% tween 80 (Normal Control)

**Group 2:** Mice inoculated with malaria parasite and treated with 3% tween 80 (Positive Control)

**Group 3:** Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract

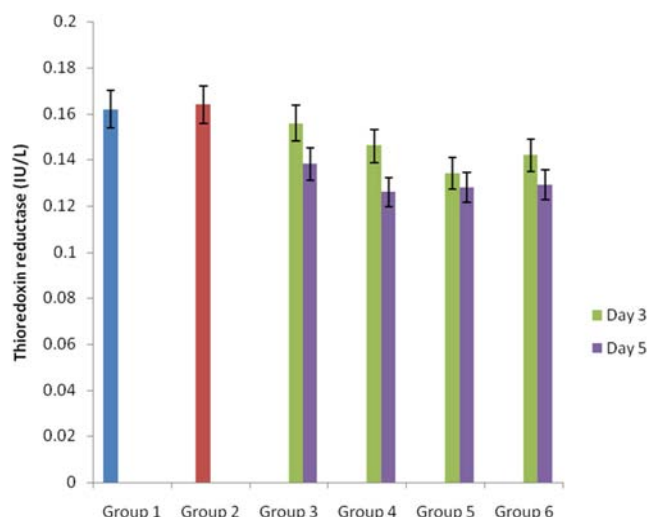
**Group 4:** Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract

**Group 5:** Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract

**Group 6:** Mice inoculated with malaria parasite and treated with the standard drug, artesunate (Standard Control)

The serum thioredoxin reductase (TrxR) activity (table 1, figure 3) of the normal control was  $0.162 \pm 0.000$  IU/L while the positive control showed a higher activity of  $0.164 \pm 0.000$  IU/L. The result of the assay on day 3 post treatment revealed that the extract-treated groups of mice

showed TrxR activity of  $0.156 \pm 0.010$ ,  $0.146 \pm 0.000$  and  $0.134 \pm 0.000$  IU/L respectively which reduced significantly to  $0.138 \pm 0.010$ ,  $0.126 \pm 0.010$  and  $0.124 \pm 0.000$  IU/L on day 5 post treatment. The standard control showed TrxR activity of  $0.142 \pm 0.000$  IU/L on day 3 post treatment which further reduced to  $0.129 \pm 0.000$  IU/L on day 5 post treatment. The TrxR activity of group 5 was significantly ( $p < 0.05$ ) lower on day 3 of post treatment compared to the positive control while the activity of TrxR of all the experimental groups were significantly ( $p < 0.05$ ) lower on day 5 post treatment.



**Figure 3.** Effect of treatment with ethanol seed extract of *P. nitida* on serum thioredoxin reductase activity of malaria-infected mice.

**Group 1:** Normal mice treated with the vehicle, 3% tween 80 (Normal Control)

**Group 2:** Mice inoculated with malaria parasite and treated with 3% tween 80 (Positive Control)

**Group 3:** Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract

**Group 4:** Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract

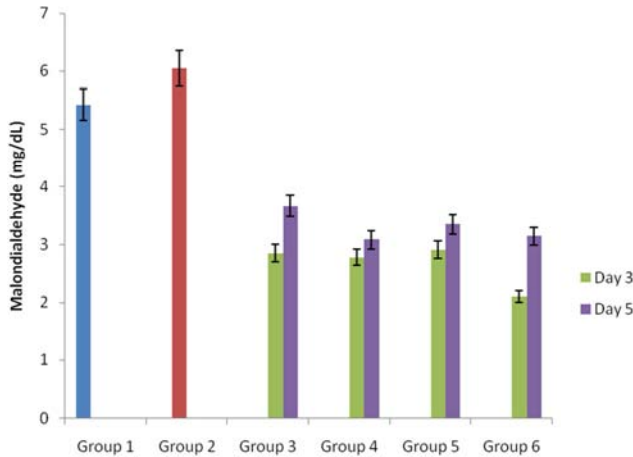
**Group 5:** Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract

**Group 6:** Mice inoculated with malaria parasite and treated with the standard drug, artesunate (Standard Control)

As shown in table 1 and figure 4, the serum malondialdehyde (MDA) concentration of normal mice treated with 3% tween 80 was found to be  $5.42 \pm 0.13$  mg/dl while that of infected mice treated with 3% tween 80 was  $6.05 \pm 0.16$  mg/dl. On day 3 post treatment, the result of the test on MDA concentrations of the mice groups treated with extract were  $2.85 \pm 0.05$ ,  $2.78 \pm 0.01$  and  $2.91 \pm 0.17$  mg/dl respectively. These concentrations further decreased to  $3.66 \pm 0.18$ ,  $3.08 \pm 0.20$  and  $3.34 \pm 0.08$  mg/dl on day 5 post treatment. The standard control showed an MDA concentration of  $2.10 \pm 0.05$  mg/dl on day 3 post treatment which further got elevated to  $3.14 \pm 0.03$  mg/dl on day 5 post treatment. The MDA concentrations of the extract-treated groups of mice on



days 3 and 5 post treatment were significantly ( $p < 0.05$ ) lower compared to the positive control. Also, in comparison with the standard control, the MDA concentration of group 3 was significantly ( $p < 0.05$ ) higher and was found to have increased by 0.23 mg/dl from day 3 to day 5 post treatment.



**Figure 4.** Effect of treatment with ethanol seed extract of *P. nitida* on serum malondialdehyde level of malaria-infected mice.

**Group 1:** Normal mice treated with the vehicle, 3% tween 80 (Normal Control)

**Group 2:** Mice inoculated with malaria parasite and treated with 3% tween 80 (Positive Control)

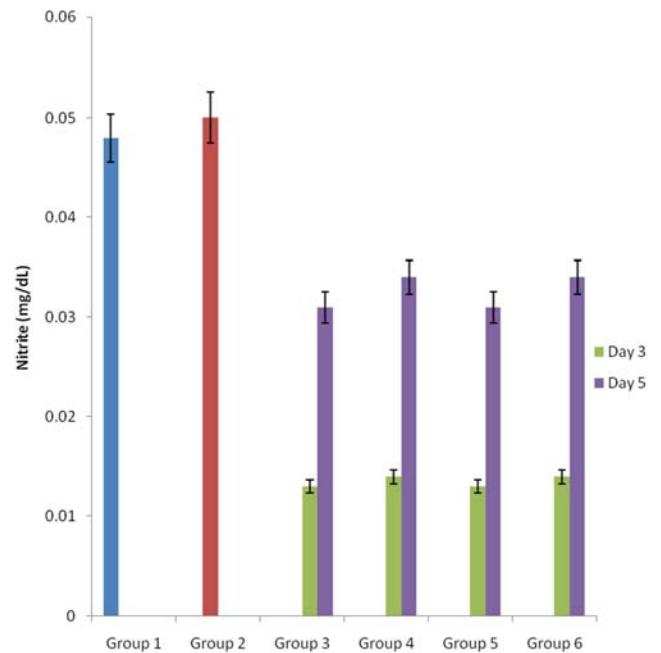
**Group 3:** Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract

**Group 4:** Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract

**Group 5:** Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract

**Group 6:** Mice inoculated with malaria parasite and treated with the standard drug, artesunate (Standard Control)

The results of the effect of the extract on serum nitrite concentration (table 1, figure 5) showed that the normal control produced  $0.048 \pm 0.007$  mg/dl of nitrite whereas the positive control produced  $0.050 \pm 0.008$  mg/dl of nitrite. The test result on day 3 post treatment revealed that the extract-treated groups of mice produced the serum nitrite concentrations of  $0.013 \pm 0.000$ ,  $0.014 \pm 0.000$  and  $0.013 \pm 0.000$  mg/dl respectively. However, on day 5 post treatment, the serum nitrite concentrations of these groups were further elevated to  $0.031 \pm 0.001$ ,  $0.034 \pm 0.001$  and  $0.031 \pm 0.001$  mg/dl. The standard control produced  $0.014 \pm 0.000$  mg/dl of nitrite anion on day 3 post treatment and  $0.034 \pm 0.001$  mg/dl on day 5 post treatment. The nitrite levels of groups 3, 4 and 5 on days 3 and 5 post treatment were significantly ( $p < 0.05$ ) lower compared to the positive control. In comparison with the standard control, the nitrite concentrations of the mice groups 3, 4 and 5 were non-significantly ( $p > 0.05$ ) lower and were found to have decreased by 0.002, 0.000 and 0.002 mg/dl respectively on days 3 and 5 post treatment.



**Figure 5.** Effect of treatment with ethanol seed extract of *P. nitida* on serum nitrite level of malaria-infected mice.

**Group 1:** Normal mice treated with the vehicle, 3% tween 80 (Normal Control)

**Group 2:** Mice inoculated with malaria parasite and treated with 3% tween 80 (Positive Control)

**Group 3:** Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract

**Group 4:** Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract

**Group 5:** Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract

**Group 6:** Mice inoculated with malaria parasite and treated with the standard drug, artesunate (Standard Control)

### 3.2. Discussion

At inflammatory sites, activated neutrophils produce MPO to naturally deal with harmful micro-organisms; hence, increase in the activity of this enzyme is an indication of the formation of cytotoxic hypochlorous acid and tyrosyl radicals. These radicals are able to kill pathogenic organisms during infections [40]. On day 3 post treatment, group 4 (mice treated with 40 mg/kg b.w. of the extract) showed the highest MPO activity, followed by group 5 (mice treated with 80 mg/kg b.w. of the extract) and the least activity was shown by group 3 (mice treated with 20 mg/kg b.w. of the extract). The group of mice treated with 5 mg/kg b.w. of artesunate (the standard control) showed a higher activity of MPO than the extract treated groups on day 3 post treatment but a lower activity than group 4 and group 5 on day 5 post treatment. The evidence showing that the extract treatment caused a non dose-dependent decrease on day 3 post treatment and a dose-dependent decrease on day 5 post treatment revealed that the extract affects the formation of cytotoxic hypochlorous acid and tyrosyl radical. The significant ( $p < 0.05$ ) increase in MPO activity of the treated groups when compared with the positive control on day 3

post treatment could be attributed to the priming period of the treatment. Group 3 had the least MPO activity on day 5 post treatment among the extract treated groups; an indication that the anti-inflammatory activity of this extract is better at lower doses. The time dependent effect of the treatment with this extract on MPO was better on day 5 than day 3 post treatment. The findings of this study on MPO agrees with the observation of Saraf *et al.* [41] who observed that *emblin* isolated from *Embelia robes*, tested against acute lung inflammation was found to significantly reduce MPO activity.

The reactive oxygen species, superoxide ion, is normally dismutated into oxygen and hydrogen peroxide by superoxide dismutases [42] in living systems. Group 5 produced the highest SOD activity; followed by group 4 and the least activity was showed by group 3 on day 3 of post treatment. However, on day 5 post treatment, group 3 produced the highest SOD activity, followed by group 5 and the least activity by group 4. The standard control showed lower SOD activity than groups 4 and 5 on day 3 post treatment while on day 5 of post treatment, it showed a lower SOD activity than groups 3 and 5. The evidence showing that the extract treatment caused a dose-dependent decrease on day 3 of post treatment and a non dose-dependent decrease on day 5 of post treatment revealed that the extract was able to mop up superoxide ion radical. Since Group 3 showed the least SOD activity on day 3 of post treatment, it shows that at shorter treatment regimen, the dismutation property of this extract is better with lower doses but at longer treatment regimen, intermediate doses are better; hence, Group 4 showed the least SOD activity on day 5 of post treatment. The time dependent effect of the treatment showed better effect on day 5 than day 3 post treatment. In support of the findings of this study, George *et al.* [43] observed a decrease in the activity of SOD when malaria infected mice were treated with aqueous extract of *Aframomum sceptrum*; an indication that *P. nitida* exhibits its antioxidant property by dismutation of superoxide ion radical into oxygen and hydrogen peroxide.

Thioredoxin reductase (TrxR) is an antioxidant enzyme system responsible for transferring electrons from NADPH to thioredoxin which plays a role in the antioxidant capacity of cells [44]. The effect of the treatment with *P. nitida* seed extract on TrxR of mice on day 3 post treatment showed that group 3 had the highest TrxR activity; followed by group 4 and the least activity was shown by group 5. On day 5 post treatment, group 3 showed the highest activity followed by group 5 and the least activity was shown by group 4. The standard control produced a lower TrxR activity than groups 3 and 4 on day 3 post treatment while on day 5 post treatment; it produced a higher TrxR activity than groups 4 and 5. The evidence showing that the extract treatment caused a dose-dependent decrease in TrxR on day 3 post treatment and a non dose-dependent decrease on day 5 of post treatment revealed that the extract plays vital role in reducing the amount of oxidants in biological system, hence a reduction in the rate at which this enzyme transfers

electrons to thioredoxin in its antioxidant role. The time dependent of effect of the treatment showed better effect on day 5 than day 3 post treatment. This observation correlates with that of Shadia *et al.* [45], show asserted that chloroform extract of *Citharexylum quadrangular* leaves showed ameliorative action on the decreased thioredoxin reductase (TrxR) activity due to *S. mansoni* infection.

Degradation of polyunsaturated fatty acid peroxides yields MDA as one of the end products and the measurement of this compound has been used as an indicator of lipid peroxidation [46]. Group 5 produced the highest MDA concentration; followed by group 3 while group 4 produced the least MDA concentration on day 3 post treatment. On day 5 post treatment, group 3 showed the highest amount of MDA, followed by group 5 while group 4 showed the least concentration. The standard control showed a better activity against lipid peroxidation than the extract treated groups on days 3 post treatment while group 4 produced a better activity against lipid peroxidation than the standard control on day 5 post treatment. The evidence showing that the extract treatment caused a non dose-dependent decrease in MDA concentration revealed the extract possesses the potential of maintaining the integrity of cell membrane. The time dependent effect of the treatment showed better effect on day 5 than day 3 of post treatment. In agreement with the finding of this study, Fakeye *et al.* [47] reported that methanol and hydroethanol extracts of the stem bark and leaves of *P. nitida* exhibited significant reduction in MDA level *in vivo* by the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging method.

Nitrite anion ( $\text{NO}_2^-$ ) is an intermediate in the oxidation of ammonium to nitrate [48]. It is the acute marker of nitric oxide formation in biological systems [49]. Nitrite has a deleterious effect on haemoglobin by converting it to methaemoglobin which has lower capacity to bind oxygen. Group 4 produced the highest nitrite concentration while groups 3 and 5 showed the same concentration of nitrite on days 3 and 5 post treatment. The standard control equally produced a decreased nitrite concentration compared with the positive control. Groups 3 and 5 produced better effect than group 4 on days 3 and 5 post treatment. The evidence showing the extract treatment caused a non dose-dependent decrease in nitrite concentration revealed that the extract possesses the ability to clear nitrite (storage form of nitric oxide) thereby reducing the rate of oxidation of haemoglobin content of the red blood cells. The time dependent effect of the treatment showed better effect on day 3 than day 5 post treatment.

Correlation statistics between the antioxidant parameters (MPO, SOD, TrxR and nitrite) showed that the values for nitrite concentration were negatively significant ( $p > 0.01$ ) with the values for MPO activity but positively significant ( $p < 0.05$ ) with values for SOD activity and also positively significant ( $p < 0.01$ ) with TrxR activity while the values for SOD activity were positively significant ( $p < 0.01$ ) with the values for TrxR activity.

## 4. Conclusion

In conclusion, the ethanol seed of extract of *P. nitida* has been proven to enhance the activities of superoxide dismutase, myeloperoxidase and thioredoxin reductase in malaria-infected mice. The levels of malondialdehyde and nitrite in malaria-infected mice were significantly reduced by the same extract; hence, good antioxidant properties which help in mopping up free radicals, due to malaria and other diseases.

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