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Nauclea latifolia aqueous leaf extract eliminates hepatic and cerebral *Plasmodium berghei* parasite in experimental mice

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ABSTRACT

Objective: To assess the effects of hot water leaf extract of *Nauclea latifolia* (*N. latifolia*) on antioxidant status, lipid peroxidation values and parasite levels in hepatic and brain tissue of experimental mice (BALB/c) infected with *Plasmodium berghei* (*P. berghei*) malaria.

Methods: Forty nine mice were divided into seven groups ($n = 7$) and used for the study. Group A (control) were given 0.2 mL/kg phosphate buffer saline; Group B mice were infected with *P. berghei* and treated with phosphate buffer saline. Groups C and D mice were also infected but treated with 200 and 300 mg/kg body weight of leaf extract respectively. Groups E and F mice were not infected, but received 200 and 300 mg/kg of leaf extract respectively. Group G mice were infected and treated with chloroquine (5 mg/kg). Liver and brain tissues of mice were prepared for both biochemical assay and microscopic examination.

Results: Results showed that *P. berghei* malaria infection induced oxidative stress in both liver and brain tissues as evidenced by the significant ($P < 0.05$) decrease in antioxidants: superoxide dismutase, reduced glutathione and catalase. These reductions perhaps caused compromise in membrane integrity as indicated by the significant increase in lipid peroxidation product malondialdehyde. Malaria parasites were also identified in these tissues. However, *N. latifolia* treatment eliminated the parasites in tissues and protected them from oxidative damage even better than chloroquine treatment did, whose anti-malarial potency also cleared tissue parasites. The measurement of protection by *N. latifolia* against damage was strengthened by the insignificant micro structural alterations.

Conclusions: The bioactive phytochemical(s) in *N. latifolia* should be structured and the mechanism(s) of its antimalarial tendency should be further investigated.

1. Introduction

Malaria remains a devastating global health problem and a major health burden especially for the developing countries [1,2]. Malaria is known as the world's most important tropical infectious disease in humans, which infects about 300–500 million people worldwide and is accountable for 1–3 million deaths annually [3,4].

Malaria infection decreases the levels of antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD)] and reduced glutathione (GSH) system as well as cholesterol and other lipids like triacylglycerol. The degree of severity of malaria is directly proportional to the level of lipid peroxidation [5], an indication of membrane damage prior to oxidative stress. In the brain, nervous system gets involved predominantly in *Plasmodium falciparum* malaria causing increased cytoadherence and resetting of red cells by sequestration of parasitized red blood cells in the cerebral microvasculature that enhances vessel occlusion, hypoxia, endothelial activation and blood–brain–barrier dysfunction [6].

Nauclea latifolia (*N. latifolia*) (family: Rubiaceae) locally nicknamed chloroquine leaf is an evergreen multi-stemmed

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medicinal plant. Also known as ‘Pin cushion tree’ and ‘Bishop’s head’, it is a struggling shrub or small tree native to tropical Africa and Asia [7]. All parts of the plant, the leaves, stem, stem bark and roots are a rich source of monoterpene indole alkaloids. In most parts of the African countries including Nigeria, the plant’s stem, bark, root and leaves are used in treatment of malarial infection [8]. The effect of *N. latifolia* such as lowering blood pressure, antidiabetic, anticonvulsant, antipyretic, analgesic, anxiolytic and sedative properties have been reported [7,9–11]. Nevertheless, the ability of *N. latifolia* leaf extract to eliminate both hepatic and cerebral malarial parasite and reduce oxidative damage to tissues in *Plasmodium berghei* (*P. berghei*) infected mice is yet to be experimentally documented. Hence, the present study was conducted to evaluate the effects of hot water leaf extract of *N. latifolia* on antioxidant status, lipid peroxidation values and parasite levels in hepatic and brain tissue of experimental mice (BALB/c) infected with *P. berghei* malaria.

2. Materials and methods

2.1. Harvesting of leaf extract

N. latifolia fresh leaves were harvested from Abraka community in Delta State of Nigeria. The plant was identified at the Nigerian Institute of Forestry Research, Ibadan, Oyo State and classified as *N. latifolia*, belonging to the Rubiaceae family.

2.2. Preparation of leaf extract

The aqueous extract of the leaf (*N. latifolia*) was obtained by boiling 50 g of the fresh leaf in 1000 mL of water for about 10 min under standard atmospheric pressure (760 mmHg). It was allowed to cool before administered to the experimental animals.

2.3. Experimental animals

Forty-nine adults albino male (BALB/c) mice (sixteen weeks old) weighing between 15 and 25 g were obtained from the Animal House, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The mice were fed with growers’ marsh feed (a product of Top-Feeds, Sapele, Delta State, Nigeria) and given water *ad libidum*. The animals were housed in cages constructed of stainless steel and plastic under control condition of 12 h:12 h light:dark cycle. The animals used in this study were maintained in accordance with the guidelines as stated in the guide for the care and use of laboratory animals [12].

2.4. Inoculation of experimental animals

One *P. berghei* parasitized mouse was obtained from the Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. About 0.1 mL of infected blood was collected from the parasitized mouse and diluted with 0.9 mL phosphate buffer (pH 7.2). Twenty-eight mice were inoculated intraperitoneally with 0.1 mL parasitized suspension.

2.5. Animal experimental groups

The 49 mice (21 normal mice and 28 surviving parasitized mice) were divided into 7 groups with 7 mice each as follows:

Group A: non-parasitized mice + 0.2 mL/kg body weight of phosphate buffered saline (PBS); Group B: parasitized mice + 0.2 mL/kg body weight of PBS; Group C: parasitized mice + 200 mg/kg body weight of *N. latifolia*; Group D: Parasitized mice + 300 mg/kg body weight of *N. latifolia*; Group E: non-parasitized mice + 200 mg/kg body weight of *N. latifolia*; Group F: non-parasitized mice + 300 mg/kg body weight of *N. latifolia*; Group G: parasitized mice + 5 mg/kg body weight of chloroquine.

The *N. latifolia* leaf extract and chloroquine were administered for five days by oral gavage.

2.6. Animal sacrifice and collection of specimen

On the 6th day, mice were fasted overnight and sacrificed under anaesthesia (chloroform soaked in cotton wool), and tissues (brain and liver) were excised, refrigerated until needed for biochemical estimation.

2.7. Preparation of tissue homogenate

One gram of frozen tissues (brain or liver) was homogenized in 9 mL of cold normal saline and centrifuged at 3500 r/min for 20 min. The supernatant obtained was decanted and used for the biochemical assay.

2.8. Analysis of specimens

The brain and liver malondialdehyde (MDA) levels were determined using the method of Buege and Aust [13]. Reduced GSH was assayed using the method of Ellman [14]. The antioxidant enzymes SOD and CAT were estimated using the methods of Misra and Fridovich [15], and Kaplan and Groves [16], respectively.

2.9. The histopathological analysis

Liver and brain tissue samples were immediately collected and fixed in 10% buffered formaldehyde solution for a period of at least 24 h before histopathological study. Samples were then embedded in paraffin wax and 5 µm thick sections were prepared with a microtome. These thin sections were stained with haematoxylin and eosin, mounted on glass slides with Canada balsam (Sigma, USA) and observed for pathological changes under a binocular microscope.

2.10. Statistical analysis

The data obtained were subjected to One-way analysis of variance (ANOVA) and Duncan’s multiple range test. Values were considered statistically different at 5% probability level. All statistical analyses were performed using SPSS version 16.

3. Results

The results obtained are presented in Tables 1 and 2 and Figures 1–14. Table 1 shows the changes in brain lipid peroxidation marker MDA and antioxidants like reduced GSH, SOD and CAT induced by *P. berghei* malarial infection and the effect of *N. latifolia*.

The biochemical values (Table 1) showed that *P. berghei* malarial infection (Group B) significantly ($P < 0.05$) reduced

Table 1

Changes in brain peroxidation product (MDA) and antioxidants (SOD, GSH, and CAT) in *P. berghei* parasitized and non-parasitized mice.

Groups	MDA (IU/g wet tissue)	GSH (IU/g wet tissue)	SOD (IU/g wet tissue)	CAT (IU/g wet tissue)
A	0.26 ± 0.06 ^a	2.22 ± 0.48 ^a	3.26 ± 0.72 ^a	50.66 ± 5.32 ^a
B	0.33 ± 0.08 ^b	1.24 ± 0.52 ^b	21.17 ± 3.24 ^b	37.12 ± 3.83 ^b
C	0.22 ± 0.09 ^c	2.32 ± 0.42 ^a	16.52 ± 3.46 ^c	39.33 ± 4.01 ^b
D	0.22 ± 0.04 ^c	2.54 ± 0.42 ^a	15.95 ± 3.12 ^c	42.85 ± 4.75 ^c
E	0.26 ± 0.07 ^a	2.36 ± 0.35 ^a	2.54 ± 0.83 ^a	46.38 ± 3.55 ^c
F	0.27 ± 0.05 ^a	2.62 ± 0.52 ^a	4.24 ± 1.02 ^d	53.09 ± 6.36 ^a
G	0.27 ± 0.06 ^a	1.64 ± 0.25 ^c	5.88 ± 1.22 ^e	26.03 ± 3.11 ^d

Values are presented as mean ± SD. Means not bearing same superscript alphabet in a given vertical column differs significantly at $P < 0.05$.

Table 2

Changes in liver MDA, GSH, SOD and CAT activities in both parasitized and non-parasitized mice treated with PBS, *N. latifolia* and chloroquine.

Groups	MDA (IU/g wet tissue)	GSH (IU/g wet tissue)	SOD (IU/g wet tissue)	CAT (IU/g wet tissue)
A	0.24 ± 0.02 ^a	2.02 ± 0.22 ^a	11.56 ± 2.56 ^a	18.92 ± 3.77 ^a
B	0.53 ± 0.02 ^b	1.59 ± 0.19 ^b	27.54 ± 3.57 ^b	13.92 ± 3.92 ^b
C	0.48 ± 0.03 ^c	2.52 ± 0.34 ^c	22.24 ± 4.18 ^c	16.42 ± 4.22 ^c
D	0.48 ± 0.03 ^c	2.65 ± 0.24 ^c	14.85 ± 3.61 ^d	16.04 ± 3.96 ^c
E	0.39 ± 0.02 ^d	1.84 ± 0.17 ^d	13.42 ± 3.11 ^d	19.84 ± 4.11 ^d
F	0.40 ± 0.03 ^d	1.89 ± 0.22 ^d	12.58 ± 2.96 ^d	21.93 ± 4.26 ^c
G	0.42 ± 0.03 ^c	2.38 ± 0.30 ^c	18.58 ± 3.29 ^c	17.09 ± 3.92 ^f

Values are presented as mean ± SD. Means not bearing same superscript alphabet in a given vertical column differs significantly at $P < 0.05$.

brain GSH content and CAT activity but significantly increased ($P < 0.05$) SOD value when compared with the non-parasitized (Group A) control mice. These changes produced significant amount of lipid peroxidation product (MDA), an indication of enhanced membrane lipid damage among the parasitized mice. The two doses of *N. latifolia* (Groups C and D) had increased GSH levels and CAT activity, but reduced SOD when compared with the values for the placebo (PBS) treated parasitized group (Group B), resulting in reduced ($P < 0.05$) lipid peroxidation product. When compared with 200 and 300 mg/kg body weight *N. latifolia* treatment (Groups

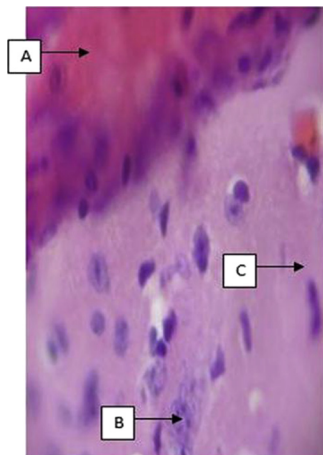


Figure 1. Brain tissue of normal rat (control).
A: Cerebral blood vessel; B: Nucleus of neural cell bodies; C: Axon fibrils (H&E, × 40).

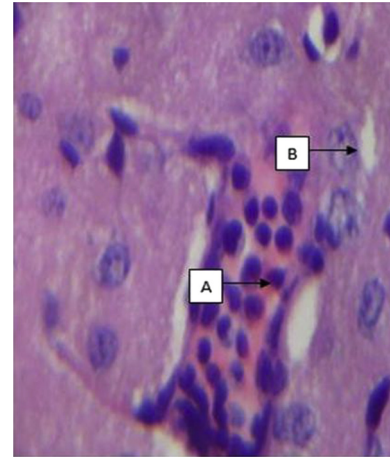


Figure 2. Brain tissue of mice inoculated with *P. berghei* for 5 d.
A: Mild vascular congestion and clogging with parasites; B: Mild tissue separation (H&E, × 40).

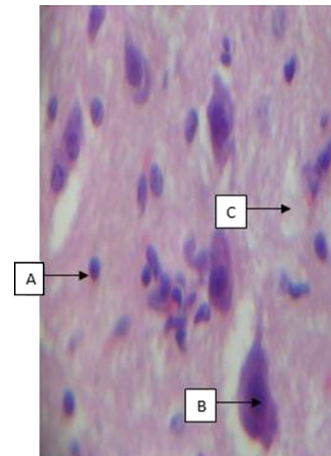


Figure 3. Brain tissue of mice inoculated with *P. berghei* and treated with 200 mg/kg *N. latifolia* for 5 d.
A: Mild infiltrates of lymphocytes; B: Microglial hyperplasia; C: Mild tissue separation (H&E, × 40).

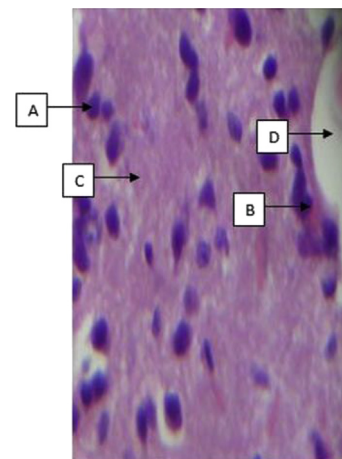


Figure 4. Brain tissue of mice inoculated with *P. berghei* and treated with 300 mg/kg *N. latifolia* for 5 d.
A: Mild infiltrates of lymphocytes; B: Microglial hyperplasia; C: Mild tissue separation; D: Mild vascular dilatation (H&E, × 40).

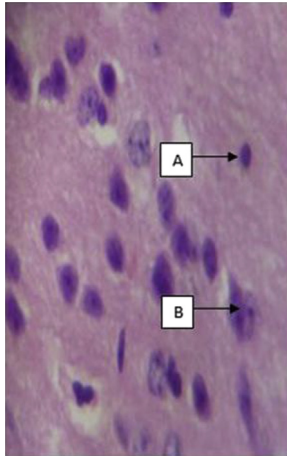


Figure 5. Brain tissue of mice treated with 200 mg/kg *N. latifolia* for 5 d. A: Mild infiltrates of lymphocytes; B: Microglia hyperplasia (H&E, × 40).

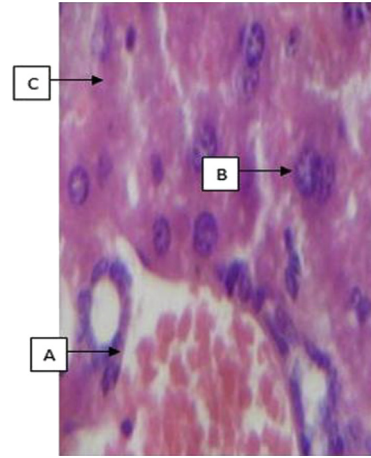


Figure 8. Liver tissue of normal mice (control). A: Portal triad; B: Hepatocytes; C: Separated by sinusoids (H&E, × 40).

C and D), chloroquine reduced GSH, SOD and CAT values, and increased MDA level. GSH and CAT may have better protective ability than SOD does against malaria-induced membrane lipid peroxidation and oxidative brain damage.

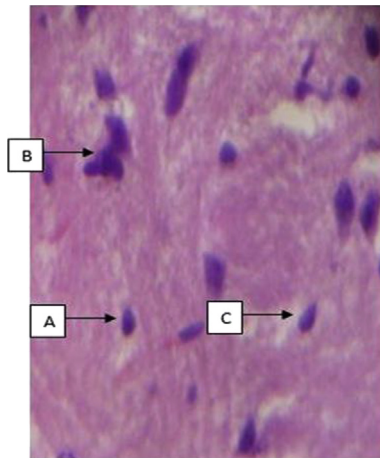


Figure 6. Brain tissue of mice treated with 300 mg/kg *N. latifolia* for 5 d. A: Mild infiltrates of lymphocytes; B: Microglia hyperplasia; C: Mild tissue separation (H&E, × 40).

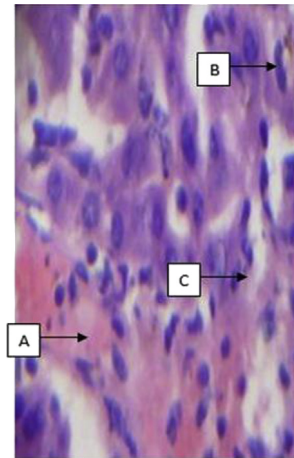


Figure 9. Liver tissue of mice inoculated with *P. berghei* for 5 d. A: Mild portal congestion and clogging with parasites; B: Mild hyperplasia of haemosiderin laden kupffer cells; C: Mild periportal infiltrates of inflammatory cells (H&E, × 40).

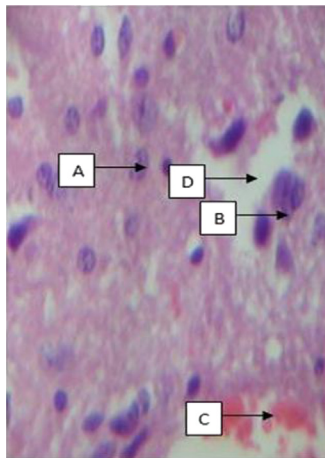


Figure 7. Brain tissue of mice inoculated with *P. berghei* and treated with standard doses of chloroquine for 5 d. A: Mild infiltrates of lymphocytes; B: Microglia hyperplasia; C: Mild vascular congestion and dilatation; D: Mild tissue separation (H&E, × 40).

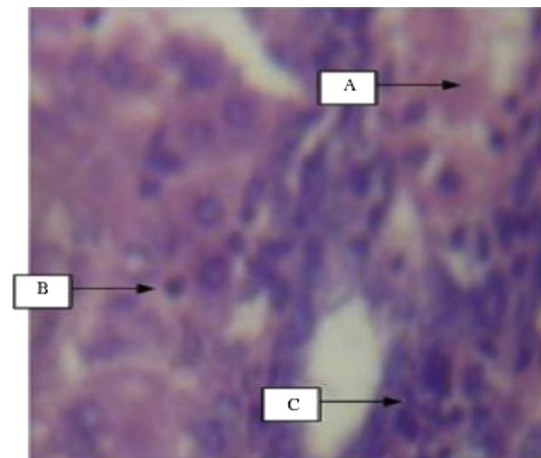


Figure 10. Liver tissue of mice inoculated with *P. berghei* and treated with 200 mg/kg *N. latifolia* for 5 d. A: Mild portal congestion; B: Moderated hyperplasia of haemosiderin laden kupffer cells; C: Mild periportal infiltrates of inflammatory cells (H&E, × 40).

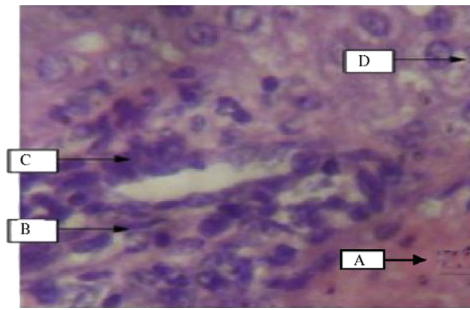


Figure 11. Liver tissue of mice inoculated with *P. berghei* and treated with 300 mg/kg *N. latifolia* for 5 d.
A: Mild portal congestion; B: Moderate hyperplasia of haemosiderin laden kupffer cells; C: Mild periportal infiltrates of inflammatory cells; D: Increased number of hepatocytes (H&E, $\times 40$).

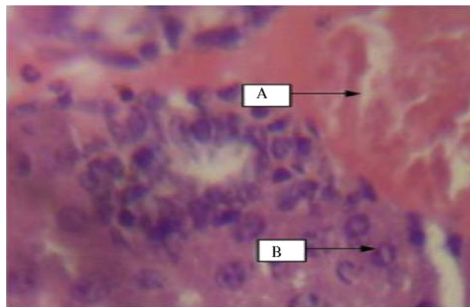


Figure 12. Liver tissue of mice treated with 200 mg/kg *N. latifolia* for 5 d.
A: Mild portal congestion and dilatation; B: Hepatocytes with nuclei containing numerous inconspicuous nucleoli (H&E, $\times 40$).

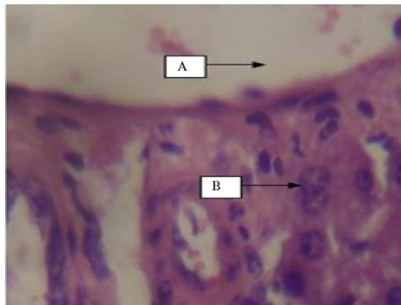


Figure 13. Liver tissue of mice given 300 mg/kg *N. latifolia* for 5 d.
A: Mild portal congestion and dilatation; B: Hepatocytes with nuclei containing numerous inconspicuous nucleoli (H&E, $\times 40$).

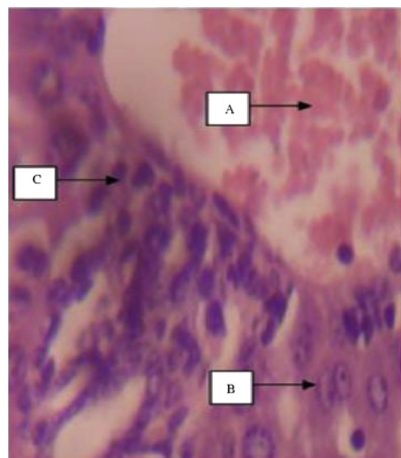


Figure 14. Liver tissue of mice treated with standard doses of chloroquine after inoculation for 5 d.
A: Moderate portal congestion and dilatation portal congestion and dilatation; B: Hepatocytes with nuclei containing numerous inconspicuous nucleoli; C: Mild periportal infiltrates of inflammatory cells (H&E, $\times 40$).

Data (Table 1) also show that *N. latifolia* treatment offers higher defense to oxidative damage in the brain than chloroquine does.

Table 2 contains data on the changes in liver MDA, GSH, SOD and CAT activities induced by chloroquine sensitive *P. berghei* malarial parasite and the effect of treatment with *N. latifolia* leaf extract.

In the liver, the trends in changes of the lipid membrane peroxidation marker MDA and antioxidants (GSH, SOD and CAT) activities were similar to the observation for the brain. *P. berghei* infection reduced both hepatic GSH and CAT activities ($P < 0.05$) when compared with the non-parasitized control group (Group A), and these changes resulted in lipid membrane damage as indicated by the significant increase in MDA value.

However, *N. latifolia* and chloroquine treatments (Groups C, D and G) increased GSH and CAT activities, but reduced SOD activity when compared with the parasitized group treated with PBS (Group B). The increase in GSH concentration and CAT activity as observed reduced the level of peroxidation (MDA estimation) in both *N. latifolia* and chloroquine treated mice (Groups C, D and G) compared with Group B, but this reduction was significantly higher than that observed in comparison of Groups C, D and G, and the control mice (Group A).

Figures 1–14 show the histopathological microstructure of the brain (Figures 1–7 for Groups A–G) and liver (Figures 8–14 for Groups A–G).

Mice infected with *P. berghei* showed parasites in both liver and brain tissues in addition to significant microstructural alterations indicative of damage. *N. latifolia* and chloroquine treatments eliminated both liver and brain parasites and mitigated the microstructural alteration and damage caused by *P. berghei*.

4. Discussion

Malaria is an endemic parasitic infection in Africa and remains a contributory factor to the morbidity and mortality of many populations in the world with about 3.3 billion people at risk of malaria, and the mortality caused by malaria is currently estimated at about 781 000 people per year [17,18]. Malaria is associated with increased production of free radicals. Biological system protect itself against the damaging effect of activated species by the actions of free radical scavengers and chain terminator antioxidant enzymes such as SOD, CAT, and reduced GSH system [19]. Reduced GSH in conjunction with reduced nicotinamide adenine dinucleotide phosphate protects the cell by destroying hydrogen peroxide and hydroxyl free radicals. Regeneration of GSH from its oxidized form GSH disulfide requires the nicotinamide adenine dinucleotide phosphate produced in the glucose 6-phosphate dehydrogenase reaction [20]. SOD breaks up superoxide into water and hydrogen peroxide. CAT, then breaks up the hydrogen peroxide into water and dioxygen to prevent the hydrogen peroxide from becoming a free radical.

The result of this study showed a higher lipid peroxidation and also an increased level of MDA in the brain and liver of the *P. berghei* parasitized mice (Group B). Administration of 200 and 300 mg/kg body weight of *N. latifolia* leaf extract reduced both brain and liver MDA levels and improved antioxidant (SOD, CAT, and GSH) defense even when compared with the chloroquine treatment. The extract contributes to the protection

of the brain and liver tissues from being compromised by free radicals produced by the *P. berghei* malaria parasite possibly via antioxidant boost; either by contributing to the induction of GSH reductase which enhances the conversion of oxidized GSH to GSH, and consequently increasing the blood and hepatic GSH value in parasitized mice, or donating electrons to the free radicals, thereby reducing them to a less reactive radical, or reducing the parasites. Therefore, aqueous leaf extract of *N. latifolia* could contribute to the protection against oxidative damage in malaria and improve the antioxidant status in a dose dependent manner.

Confirming the biochemical values, results of the histopathological pictures show that inoculation of mice with malarial parasite *P. berghei* caused mild loosening of brain tissues together with mild vascular congestion and clogging of vessels with the parasite (parasitaemia and mild congestion). However, treatment with *N. latifolia* (200 and 300 mg/kg body weight) revealed mild congestion in the vessels, and infiltration of the tissue by lymphocyte and microglia; also, the clogging of the blood vessels with parasite was not observed and tissue loosening was negligible. With chloroquine administration, there was mild vascular congestion and no parasitaemia in Group G. However, the infiltration by lymphocytes and microglia cells was slightly less pronounced than that observed in mice treated with extract. Similar trend was observed for the liver tissue.

P. berghei malarial infection could result in a marked depletion of host cytoprotective enzymes and antioxidants in the brain and liver, thus elevating the levels of lipid peroxidation, which was observed in the form of its product MDA. Treatment of infected mice with *N. latifolia* aqueous leaf extract reduced lipid peroxidation and oxidative stress, by probably improving antioxidant defense.

Since aqueous leaf extract of *N. latifolia* appears promising, its potency in antiplasmodial and antioxidant activities need to be further exploited.

Conflict of interest

We declare that we have no conflict of interest.

References

- [1] Wang ML, Feng YH, Pang W, Qi ZM, Zhang Y, Guo YJ, et al. Parasite densities modulate susceptibility of mice to cerebral malaria during co-infection with *Schistosoma japonicum* and *Plasmodium berghei*. *Malar J* 2014; **13**: 116.
- [2] Chilengi R, Gitaka J. Is vaccine the magic bullet for malaria elimination? A reality check. *Malar J* 2010; **9**(Suppl 3): S1.
- [3] Pierce SK, Miller LH. World Malaria Day 2009: what malaria knows about the immune system that immunologists still do not. *J Immunol* 2009; **182**: 5171-7.
- [4] Donovan MJ, Messmore AS, Scrafford DA, Sacks DL, Kamhawi S, McDowell MA. Uninfected mosquito bites confer protection against infection with malaria parasites. *Infect Immun* 2007; **75**(5): 2523-30.
- [5] Asagba SO, Eriyamremu GE, George BO, Okoro I. Biochemical indices of severity in human malaria. *J Med Sci* 2010; **10**: 87-92.
- [6] Beare NA, Harding SP, Taylor TE, Lewallen S, Molyneux ME. Perfusion abnormalities in children with cerebral malaria and malarial retinopathy. *J Infect Dis* 2009; **199**: 263-71.
- [7] Gidado A, Ameh DA, Atawodi SE. Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. *Afr J Biotechnol* 2005; **4**(1): 91-3.
- [8] Abdalla SH. Peripheral blood and bone marrow leucocytes in Gambian children with malaria: numerical changes and evaluation of phagocytosis. *Ann Trop Paediatr* 1988; **8**: 250-8.
- [9] Nworgu ZAM, Onwukaeme DN, Afolayan AJ, Ameachina FC, Ayinde BA. Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. *Afr J Pharm Pharmacol* 2008; **2**(2): 37-41.
- [10] Taiwe GS, Bum EN, Talla E, Dimo T, Weiss N, Sidiki N, et al. Antipyretic and antinociceptive effects of *Nauclea latifolia* root decoction and possible mechanisms of action. *Pharm Biol* 2011; **49**(1): 15-25.
- [11] Ngo Bum E, Taiwe GS, Moto FC, Ngoupaye GT, Nkantchoua GC, Pelanken MM, et al. Anticonvulsant, anxiolytic, and sedative properties of the roots of *Nauclea latifolia* Smith in mice. *Epilepsy Behav* 2009; **15**: 434-40.
- [12] National Research Council. *Guide for the care and use of laboratory animals*. 8th ed. Washington: The National Academies Press; 2011.
- [13] Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978; **52**: 302-10.
- [14] Ellman GL. Tissue sulphhydryl groups. *Arch Biochem Biophys* 1959; **82**: 70-7.
- [15] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; **247**: 3170-5.
- [16] Kaplan JH, Groves JN. Liver and blood cell catalase activity of tumor-bearing mice. *Cancer Res* 1972; **32**: 1190-4.
- [17] World Health Organization. World malaria report 2012. Geneva: World Health Organization; 2012 [Online] Available from: http://www.who.int/malaria/publications/world_malaria_report_2012/wmr2012_no_profiles.pdf?ua=1 [Accessed on 3rd November, 2014].
- [18] World Health Organization. World malaria report 2010. Geneva: World Health Organization; 2010 [Online] Available from: http://www.who.int/malaria/world_malaria_report_2010/worldmaliareport2010.pdf [Accessed on 21st September, 2013].
- [19] Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nat Protoc* 2010; **5**: 51-6.
- [20] Chayen J, Howat DW, Bitensky L. Cellular biochemistry of glucose 6-phosphate and 6-phosphogluconate dehydrogenase activities. *Cell Biochem Funct* 1986; **4**: 249-53.