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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 anti-oxidants: superoxide dismutase, reduced glutathione and catalase. These reductions perhaps caused compromise in membrane integrity as indicated by the significant increase in lipid peroxidation product malondialdhyde. 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