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Changes in ocular oxidative indices in *Plasmodium berghei* infected mice treated with aqueous leaf extract of *Nauclea latifolia*

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ABSTRACT

Malaria is associated with increased production of free radicals whose activities can be reduced by antioxidants. The present study investigated effect of the aqueous extract of *Nauclea latifolia* on some antioxidant systems in the eye. Albino mice of mixed sexes, eight weeks old, weighing 12g-25g and divided into 6 groups of 5 mice per group were used for the experiment. Mice were inoculated intraperitoneally with 0.1ml parasitized blood suspension and parasitaemia was assessed by thin blood films stained with Geimsa stain. Aqueous leaf extract of *Nauclea latifolia* was orally administered at different doses (200 mg/kg body weight and 300mg/kg bogy weight daily) to both normal and malaria infected mice for a period of 4 days. Ocular reduced glutathione, superoxide dismutase (SOD), catalase (CAT), and Malondialdehyde (MDA) levels were estimated. Significant ($p<0.05$) reduction in ocular reduced glutathione, decreased activities of ocular SOD and CAT, and increase in MDA levels were observed in parasitized control when compared with normal control mice. However, the oral administration of *Nauclea latifolia* significantly ($p<0.05$) increased ocular SOD, CAT activities with a significant decrease ($p<0.05$) in MDA content of parasitized mice. Non-enzymatic antioxidant (GSH) activity was also enhanced in a dose dependent manner of leaf extract administration. These results suggest that aqueous extract of *Nauclea latifolia* may contribute to the protection of malaria infected mice against oxidative damage by improving antioxidant status in a dose dependent manner.

KEY WORDS: CATALASE (CAT), MALARIA, *NAUCLEA LATIFOLIA*, SUPEROXIDE DISMUTASE (SOD), REDUCE GLUTATHIONE (GSH).

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INTRODUCTION

The consumption of plant is not just for its nutritive value but also for its medicinal effects. A number of plants possess medicinal properties and have been exploited in the management and treatment of diseases. In modern times, a plant part is incorporated into preparation used by non-orthodox practitioners and a number of orthodox medicinal components (Mordi *et al.*, 2013). It has also been established that a number of drugs used as orthodox medicines are derived from plant materials. However, most of these medicines are expensive and have a number of side effects; hence the use of medicinal plants is becoming more popular (Egbung *et al.*, 2013).

Nauclea latifolia (family: Rubiaceae) commonly known as pin cushion tree is used profusely by traditional medicine practitioners. The leaves are also used for the treatment of malaria (Akubue and Mittal, 1982; Benoit-Vical *et al.*, 1998; Abbah et al., 2009) and are active against *Plasmodium falciparum* (Traore *et al.*, 2000; Gidado *et al.*, 2004). It has hypotensive effect and also has shown to decreased the heart rate dose dependently (Gidado *et al.*, 2004; Akpanabiantu *et al.*, 2005; Nworgu et al., 2008).

Disease conditions which are accompanied by changes in the composition of blood and hence the ocular humour could give rise to an elevation or depression of the intraocular pressure (Orban *et al.*, 1966). Malaria is a mosquito-borne infectious disease of humans and other animals caused by protozoa of the genus *Plasmodium* (WHO, 2011). At present, 40% of the world population lives in malaria endemic regions (Snow, 2005). In 2001 about 700,000 lives of children below the age of five were lost to malaria in Africa. The disease is responsible for many medical complications like low birth weights in infants and increase in sudden abortion and stillbirth in pregnant women (Joda *et al.*, 2005). Malaria is responsible for about 66% of all clinic visits in Nigeria. It causes the death of an estimated 250,000 children under the age of five every year (Udobre *et al.*, 2013). Previous studies have shown that malaria infection decreases the levels of some antioxidant enzymes (Egbung *et al.*, 2013) possibly because of the production of free radical. The formation of reactive oxygen species (ROS) by malarial parasites if not checked by the host cyto-protective enzymes and antioxidants could lead to oxidative damage (George *et al.*, 2012).

Oxidative stress has been implicated to cause increased intraocular pressure by triggering trabecular meshwork degeneration and thus contributing to alterations in the aqueous outflow pathway. Previous studies have shown that oxidative damage was significantly increased in trabecular meshwork cells of glaucoma patients and this is also true for the formation of cataract (Zmen *et al.*, 1997; Gupta *et al.*, 2012). Increased retina lipid oxidation was detected in rats with elevated intraocular pressure (Ko *et al.*, 2005; Akpanabiantu *et al.*, 2005).

There is increase in the resistance of *P. falciparum* to antimalarial drugs (Udobre *et al.*, 2013). This development has compromised the efficacy of the available antimalarial drugs such as chloroquine, Fansidar and even artemisinin (Udobre *et al.*, 2013). Records have shown that there are no drugs that can offer protection against malaria in all the regions of the world (WHO, 2011). Therefore, this research on *Nauclea latifolia* herein attempts to contribute to the research efforts towards identifying new potential treatments for malaria as well as investigating the antioxidant capacity of the aqueous leaf extract of *Nauclea latifolia* against *Plasmodium berghei* patho-biochemical changes in the ocular tissue of mice.

MATERIAL AND METHODS

COLLECTION OF *NAUCLEA LATIFOLIA*

Nauclea latifolia was harvested behind Sudoz Nigeria limited (Fuel Filling Station) in neighbourhood of Abraka, in Ethiope East Local Government Area of Delta State, Nigeria. The leaf was identified to the species level at the Forest Research Institute of Nigeria, Ibadan, Nigeria (FRIN), where a copy of the leaf was deposited.

PREPARATION OF EXTRACT

The aqueous extract of *Nauclea latifolia* was carried out as describe by Mordi *et al.*, (2013). Fifty grams (50g) of the leaves was weighed, sliced into pieces and then boiled in 1000 ml of distilled water and boiled for 30 minutes, and then allowed to cool and filtered. Preparation was done in accordance to the local consumption within the Ethiope East Local Government Area of Delta State, Nigeria.

EXPERIMENTAL ANIMALS AND CARE

Thirty albino mice of mixed sexes weighing between 12-26grams, 8-12 weeks old were purchased from the Nigeria Institute of Medical Research (NIMR), Yaba-Lagos, Nigeria, and used for this study. Fifteen of the albino mice were non-parasitized while the other fifteen *Plasmodium berghei* Infected mice were carried out at the Nigeria Institute of Medical Research (NIMR), Yaba-Lagos, Nigeria. The animals were fed on growers mash obtained from Top-Feeds, Sapele, Delta State, and were given water *ad libitum*. The animals were housed in transparent polypropylene cages lined with wood chip bedding under controlled conditions of 12 hours light/

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12 hours dark cycle. The animals used in this study were maintained in accordance with the guidelines approved by the National institute of Health (NRC, 1985).

INOCULATION OF ANIMALS

The mice were infected with parasites (*P. berghei*) by obtaining parasitized blood from the cut-tip of the tail of an infected blood (3 to 4 drops) and diluted in 0.9 ml phosphate buffer (pH 7.4). The mice were inoculated intraperitoneally with One hundred microlitre (100µl or 0.1ml) parasitized suspension. Parasitaemia was assessed by thin blood film made by collecting blood from the cut tip of the tail and this was stained with Geimsa stain. Inoculation was carried out in the Biochemistry Laboratory of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos.

EXPERIMENTAL DESIGN

After the confirmation of parasitaemia, the mice infected (parasitized) and non infected (normal) were divided into six groups of 5 mice per group treated as follows:

Group A: Normal control
Group B: Parasitized control
Group C: Parasitized mice + *N. latifolia* (200 mg/kg b. wt)
Group D: Parasitized mice + *N. latifolia* (300 mg/kg b. wt)
Group E: Normal mice + *N. latifolia* (200 mg/kg b. wt) and
Group F: Normal mice + *N. latifolia* (300 mg/kg b. wt)

The administration of the extract was carried out using an automated micropipette (oral cannula) for a period of four days. On the fifth day mice were made to starve overnight, sacrificed by partial decapitation and the blood and eyes were collected for various biochemical estimations.

PREPARATION OF EYE-TISSUE HOMOGENATE

The wet eye tissue was homogenized in 0.5 ml of freshly prepared normal saline and then centrifuged at 3000rpm for 10 minutes. The supernatant obtained was used for this experiment. This was done on ice to avoid denaturation of biological content.

BIOCHEMICAL INVESTIGATIONS

The biochemical investigations in ocular humour homogenate samples were carried out with the following

ASSESSMENT OF LIPID PEROXIDATION (MDA)

Lipid peroxidation (MDA) in the ocular humour homogenate was estimated spectrophotometrically by Thiobarbituric acid reacting substance (TBARS) method as described by Varshney and Kale, (1990). The reaction mixture contained 0.4ml of sample mixed with 1.6ml 0.15M Tris KCl buffer, 0.5ml of 30% TCA and 0.5ml of 52mM TBA. The mixture was placed on a water bath for 45min at 80°C, cooled in ice and centrifuged at room temperature for 10mins at 3000rpm. The absorbance of the clear supernatant was measured against the reference blank of distilled water at 532nm in a spectrophotometer.

DETERMINATION OF CATALASE

Ocular humour homogenate was estimated in a UV spectrophotometer at 240nm by monitoring the decomposition of H_2O_2 as described by Aebi, (1984). The reacting mixture (1ml) contained 0.02ml of suitably diluted cytosol in phosphate buffer (50mM, pH 7.0) and 0.1ml of 30nM H_2O_2 in phosphate buffer. The specific activity of catalase was been expressed as moles of H_2O_2 reduce per minute mg protein.

ESTIMATION OF REDUCED GLUTATHIONE (GSH)

The amount of reduced glutathione (GSH) was estimated by a colorimetric method using 5, 5-dithiobis (2-nitrobenzoic acid) as described by Boyne and Ellman, (1972). One milliliter of the sample was treated with 4 ml of metaphosphoric acid precipitating solution (1.67g of glacial metaphosphoric acid, 0.2g EDTA and 30g NaCl dissolved in 100 ml water). After centrifugation at 17000 Xg for 15 minutes, 2 ml of the protein-free supernatant was mixed with 0.2 ml of 0.4M Na_2HPO_4 and 1.0 ml of 5, 5-dithiobis-2-nitrobenzoic acid (DTNB - Ellman's reagent). Absorbance was read at 413 nm within 2 minutes. The GSH concentration was expressed as nmol/mg protein.

SUPEROXIDE DISMUTASE (SOD) ASSAY

Ocular humour homogenate superoxide dismutase was assayed utilizing the technique of Fridovich, (1989). 1ml of sample ocular humour homogenate was diluted in 9ml of distilled water to make a one in ten dilution of ocular humour homogenate. An aliquot of 2.0ml of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, and 0.3ml of the substrate (adrenaline) and 0.2ml of distilled water. The increase in absorbance at 480nm was monitored every 30seconds to 150seconds. A single unit of enzyme is defined as the quantity of SOD required to produce 50% inhibition of auto-oxidation.

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STATISTICAL ANALYSIS

The results were expressed as Means ± Standard Deviation (SD). Level of significance was assessed by one-way ANOVA (Analysis of variance) while the Duncan's multiple –range test was used to test for differences between individual treatments groups using Sigma-stat 2.0 software. P <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

In traditional medicine the leaf of *Nauclea latifolia* has been used in the treatment of malaria, skin rashes, jaundice, fever, oedema, haemolysis, dysentery, hernias, diarrhoea and sexually transmitted diseases (Burkill, 1997). The folkloric use of this plant prompted the need to evaluate the *in vivo* antiplasmodial activity of its aqueous leaf extract to provide some support for its ethnobotanical uses.

Malaria is associated with increased production of free radicals (George *et al*., 2012; Egbung *et al*., 2013). Infections' including malaria activates the immune system of the body causing the release of reactive oxygen species (ROS), which can attack the membrane of cells, compromising their integrity (Kulkarni *et al*., 2003). Result from Table 1 revealed a significant decrease (p<0.05) in ocular GSH, catalase, CAT, superoxide dismutase activities (SOD) as well as a significant increase (p<0.05) in MDA level of the parasitized mice when compared to the non-parasitized and *Nauclea latifolia* treated groups. The depletion of GSH, SOD and CAT activities in the parasitized control group might be as a result of increased utilization of these endogenous antioxidants due to oxidative stress or promotion of ROS.

Biological system protect itself against the damaging effect of activated species by the actions of free radical scavenging, direct quenching and chain termination by enzymes such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) system (Kurata *et al*., 1993). Reduced glutathione (GSH) acts as primary line of defence to cope with the deleterious effect of reactive oxygen species. It is well established that reduced glutathione (GSH), the most important biomolecule protecting against chemically induced cytotoxicity, can participate in the elimination of reactive intermediates by conjugation or of free radicals by direct quenching (Gupta *et al*., 2012).

The concentration of malondialdehyde (MDA), an index of lipid peroxidation was increased in the parasitized mice (Group B), but treatment with *Nauclea latifolia* reduced its formation (Table 1). Free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food products (Chen and Ho, 1997). *Nauclea latifolia* extract might have suppressed lipid peroxide through different chemical mechanisms, including free radical quenching, electron transfer, radical addition, or radical recombination.

The administration of 200mg and 300mg/kg body weight of *Nauclea latifolia* extract as shown in Table 1, improved (increased) SOD, CAT and GSH levels in the ocular tissue when compared with the Plasmodium berghei treated group only. The extract contributes to the protection of the ocular tissue from being compromised by free radicals produced by *Plasmodium berghei* malaria parasite possibly via antioxidant boost, either by contributing to the induction of glutathione reductase which enhances the conversion of GSSG to GSH and consequently increasing ocular GSH value in parasitized mice or donating electrons to the free radicals thereby

TABLE 1: Changes in ocular reduced glutathione, malondialdehyde, superoxide dismutase and catalase activities in both parasitized and non-parasitized mice treated with *Nauclea latifolia*.

Group	Ocular GSH (µmol/g of wet tissue)	Ocular Catalase (µmol/g of wet tissue)	Ocular SOD (µmol/g of wet tissue)	Ocular MDA (µmol/g of wet tissue)
Group A	3.14±0.39*	62.31±5.14	4.10±0.51	2.81±1.98
Group B	0.99±0.05*	49.02±4.72*	2.74±0.76*	6.95±1.77*
Group C	1.68±0.06*	56.76±5.42*	5.75±0.60*	5.30±1.33*
Group D	2.05±0.15	52.42±8.13*	4.23±0.80	3.83±1.47*
Group E	2.63±0.08	63.39±6.11	4.73±0.45	2.58±2.08
Group F	2.88±0.18	62.60±8.36	4.39±0.63	2.75±1.60

Values are expressed as means ± sd with n = 5. The columns with *are significantly different (p<0.05) from other groups.

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reducing them to a less reactive radical or reducing the level of the parasites.

The aqueous extracts of *N. latifolia* have been shown by previous studies to contain bioactive agents such as saponins, polyphenol and flavonoids, alkaloids, anthraquinones, terpenoids, and tannins (Egbung *et al.*, 2013). Some secondary metabolites of plants such as alkaloids and terpenes which are present in the aqueous extract of *N. latifolia* (Abbah *et al.*, 2009) have been associated with anti-plasmodial activity (Christensen and Kharazmi, 2001) and the presence of alkaloids in the aqueous form is an indication of the detoxifying and antihypertensive properties exhibited by the plant extract since alkaloids are known to be effective for these purposes (Trease and Evans, 1989; Cheng, 1997). Also, flavonoids and phenolic compounds, which are widely distributed in plants including *N. latifolia* were considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living system (Block, 1992; Hertog and Feskens, 1993).

Group A: Normal control;
Group B: Parasitized control;
Group C: Parasitized mice + *N. latifolia* (200 mg/kg body wt);
Group D: Parasitized mice + *N. latifolia* (300 mg/kg body wt);
Group E: Normal mice + *N. latifolia* (200 mg/kg body wt)
Group F: Normal mice + *N. latifolia* (300 mg/kg body wt).

The outcome of this present study indicates that malarial infection caused by *P. berghei* release free radical which could lead to oxidative stress in the ocular tissue as well as other part of the organism and that treatment with *N. latifolia* showed positive improvement in the antioxidant status.

CONCLUSION

Plasmodium berghei malarial infection could result in a marked depletion of the host protective cytoprotective enzymes and antioxidants in the ocular tissue thus elevating the levels of lipid peroxidation, which was observed in the form of its product, malondialdehyde (MDA). Treatment of infected mice with *N. latifolia* leaf extract may have likely caused reduction in lipid peroxidation and oxidative stress, but improvement in antioxidant defence mechanism. Thus, since aqueous leaf extract of *N. latifolia* appears promising, the mechanisms by which the extract exerts its anti-plasmodial activities should be subsequently investigated and the principle characterized. Furthermore, we recommended that the biochemical mechanism involving antioxidant enzymes activities should be carried out, as the mode of action of most anti-malarial drugs have also been found to increase free radicals in the body (Kirk, 2001; Abbah *et al.*, 2009; Gupta *et al.*, 2012).

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