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• Research Article

Antioxidant defense system induced by cysteine–stabilized peptide fraction of aqueous extract of *Morinda lucida* leaf in selected tissues of *Plasmodium berghei*–infected mice

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ABSTRACT

OBJECTIVE: This study evaluated the responses of some antioxidant parameters in selected tissues of *Plasmodium berghei*-infected mice treated with cysteine-stabilized peptide fraction (CSPF) of aqueous extract of *Morinda lucida* leaf.

METHODS: Fifty-six mice were randomly divided into seven groups. Group A (normal control) was uninfected and received 5% dimethyl sulfoxide (DMSO). Mice in Groups B (negative control), C, D, E and F were inoculated with P. berghei NK65 and were administered with 5% DMSO and 15.63, 31.25, 61.5 and 125 mg/kg body weight of CSPF respectively. Group G animals, were also inoculated with *P. berghei* NK65, and received 20 mg/kg body weight of chloroquine. The administration lasted for three days, after which malondialdehyde (MDA) concentration and various antioxidant parameters in selected tissues of mice were determined on days 4 and 8 post-inoculation.

RESULTS: The results revealed that MDA concentration was significantly increased ($P < 0.05$) in the tissues of the negative control and chloroquine-treated groups. The increased MDA concentration was reduced by CSPF in a dose-dependent manner, which was significant ($P < 0.05$) at higher doses. The activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase and the concentration of reduced glutathione were significantly reduced ($P < 0.05$) in the tissues of the negative control animals compared to the normal controls. This observed reduction in the negative control animals was reverted in a dose-dependent manner in infected animals given CSPF, even to the range of the normal controls at highest dose, as did chloroquine.

CONCLUSION: The results suggest that CSPF of *M. lucida* leaf extract may induce the antioxidant defense system *in vivo* against *Plasmodium* species infection.

Keywords: *Morinda lucida*; peptide extract; *Plasmodium berghei*; antioxidant; oxidative stress

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1 Introduction

Malaria is an infectious disease affecting many people in Africa and other parts of the world. It is a major public issue, especially in Africa, having serious impact on health, economy and social life with about 214 million clinical cases annually, resulting in an estimated number of 438 000 deaths, primarily in children.[1] Different approaches have been employed in the fight against malaria, which include prevention of infection and the use of antimalarial drugs.[2] The major limitation of antimalarial drugs is resistance developed against them by the parasite. For instance, resistance of *Plasmodium falciparum* to chloroquine (CQ) has reduced its effectiveness in the treatment of malaria.[3] The currently effective artemisinin combination therapy is very expensive, especially to rural dwellers, who are the major victims of the disease.

Although conventional medicine is available in major communities of the developing world, herbal medicines are still popular as many Africans rely on them for the treatment of minor ailments.[4] Some medicinal plants have been reported to express cysteine-stabilized peptides in their tissues. These peptides, which were thought to serve as natural protection against pathogens in plants, have been found to have pharmacological potentials, including immunomodulatory, haemolytic, antimicrobial and anticancer activities.[5] It is believed that oxidative stress contributes to secondary complications in malaria pathophysiology[6] and that reactive oxygen species are produced during *Plasmodium* infection, mainly from heme Fe^{2+} released from the digestion of host haemoglobin by the parasite.[7,8] These reactive oxygen species lead to an imbalance in the body's antioxidant system, which may lead to oxidative stress.[9] Thus, plant products, such as plant-derived peptides, which can clear the parasites and mop-up free radicals that are strongly linked to the secondary complications of malaria, will present good candidates for antimalarial drug development. Our preliminary work has established that the cysteine-stabilized peptide fraction of aqueous extract of *Morinda lucida* (Rubiaceae) leaf possesses antimalarial activity. This study was therefore carried out to evaluate its effect on the antioxidant defense system in selected tissues of mice with *Plasmodium berghei*-induced oxidative stress.

2 Materials and methods

2.1 Animals

Fifty-six adult Swiss albino mice, weighing between 20 and 24 g, were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Life Science, University of Ilorin, Nigeria.

2.2 Parasites

A CQ-sensitive strain of *P. berghei* NK65 was obtained from the Institute for Advanced Medical Research and Training, University College Hospital, Ibadan, Nigeria.

2.3 Plant material

M. lucida leaves were collected at the University of Ibadan campus, Ibadan, Oyo State, Nigeria. A sample was identified and authenticated at the Forest Research Institute of Nigeria, Ibadan, Oyo State, Nigeria, where a sample was deposited and assigned a voucher number (FHI: 110187).

2.4 Ethical approval

This study was conducted according to guidelines approved by the University of Ilorin Ethical Review Committee (UERC/ASN/2015/067).

2.5 Extraction and identification of peptides from *M. lucida* leaf

The cysteine-stabilized peptide fraction was obtained according to the method described by Koehbach et al.[10] *M. lucida* leaves were dried under shade and pulverized. The powder (500 g) was percolated in 6 L of dichloromethane-methanol mixture (1:1, v/v) and left for 16 h at room temperature. The mixture was filtered using muslin cloth to remove plant debris. The filtrate was transferred into a separating funnel, where an equal volume of distilled water was added; thereafter, the aqueous layer was collected, concentrated in a rotary evaporator and freeze-dried. A preloaded C_{18} solid phase extraction column was preconditioned with methanol, activated with solvent B (acetonitrile-distilled water mixture; 9:1, v/v) and equilibrated with buffer A (distilled water-trifluoroacetic acid mixture; 100:0.05, v/v). A portion of the concentrated aqueous extract was loaded onto the C_{18} column; peptides and other hydrophilic compounds in the extract were eluted with increasing concentrations (20%, 80% and 100%) of solvent B. The 80% solvent B fraction (hereafter referred to as cysteine-stabilized peptide fraction, CSPF) was concentrated in a rotary evaporator and dried using a lyophilizer (Gallenkhamp, UK), giving a percentage yield of 0.5%, which was stored at –20 °C for further use.

2.6 Animal handling

The animals were housed in well-ventilated plastic cages. They were fed with standard mouse feed (Grand Cereals Ltd., Jos, Plateau State, Nigeria) and clean tap water *ad libitum*. Animals were treated according to the Guide for Care and Use of Laboratory Animals (NIH publication No. 85–93, revised 1985).

2.7 Induction of oxidative stress and administration of extract fraction

P. berghei NK65 was maintained in mice by serial passages of blood from an infected donor mouse to naive recipients. The 4-day suppressive test described by Peters[11] and modified by Carvalho et al.[12] was adopted