Beni-Suef University Journal of Basic and Applied Sciences xxx (2017) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

Beni-Suef University
Journal of Basic and Applied Sciences

journal homepage: www.elsevier.com/locate/bjbas

Full Length Article

Impact of generic antimalarial or *Phyllanthus amarus* and vitamin co-administration on antioxidant status of experimental mice infested with *Plasmodium berghei*

Matthew Obaineh Ojezele [a,*], Emuesiri Goodies Moke [a], Innocent Onyesom [b]

[a] Department of Pharmacology and Therapeutics, Delta State University, Abraka, Delta State, Nigeria
[b] Department of Medical Biochemistry, Delta State University, Abraka, Delta State, Nigeria

ARTICLE INFO

Article history:
Received 15 December 2016
Received in revised form 24 March 2017
Accepted 21 April 2017
Available online xxxx

Keywords:
Malaria
Vitamins
ROS
Phyllanthrus amarus
Liver
Brain

ABSTRACT

Oxidative stress is a key factor in malaria pathogenesis, particularly, malaria induced anaemia and pathological changes in some organs in the body. This research aimed at investigating the effect of *Phyllanthus amarus* seed extract (PASE), chloroquine (CLQ), and artesunate (ATS) used alone and co-administered with vitamin A, B, C, or E on oxidative stress in *Plasmodium berghei*-infected mice. A total of eighty (80) adult albino male mice infected with *P. berghei* (NK 65 strain) were randomly allotted to 16 treatment groups, mice in another group (17th group) were uninfected; n = 5. Of the treatment groups, fifteen were administered sole generic antimalarials/PASE and combined vitamins A, B, C or E orally for 5 days. Biochemical assay for glutathione peroxidase (GPx), and glutathione reductase (GR) were carried out on the serum. Assay for glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CA), and malondialdehyde (MDA) levels were carried out on homogenised liver and brain. Treatment with PASE, CLQ and ATS combined with vitamin A, B, or E respectively, demonstrated significant increase in the serum levels of GPx and GR concentration. Treatment with PASE/ATS alone and in combination with vitamin A, B, C, and E significantly increased the liver GPX, SOD, and CA levels with significant decrease in liver MDA levels. Treatment with PASE/CLQ alone and in combination with vitamin A, B, C, and E significantly increased the brain GPX, GR and SOD levels with significant decrease in brain MDA levels. PASE showed enhanced antioxidant capacity in plasmodiasis solely or combined with vitamins A, B and E. Furthermore the co-administration of generic agent like artesunate with vitamins A, B, and E enhanced the antimalarial activity and treatment outcome as shown by the antioxidant effects. However, co-administration with vitamin C may be counter-productive.

1. Introduction

Malarial infection persists as a foremost overwhelming global health problem. Plasmodiasis results in over 500 thousand deaths annually, with more than 90% of such deaths reported in the African region (WHO, 2015). The disease burden and economic cost is enormous, particularly in African. The malarial infection is caused by the *Plasmodium* protozoan parasite of which various species have been identified. The most hazardous and deadly of the *Plasmodium*, is the *P. falciparum*. Several antimalarial drugs such as chloroquine, arteminsin, and pyrimethamine have been deployed for the therapy of this infection but resistance to treatment, is a rising major concern globally.

Oxidative stress is a condition in which cellular antioxidant defences are inadequate to completely inactivate the reactive oxygen species (ROS) produced (e.g. superoxide radical, hydroxyl radical, hydrogen peroxide). It leads to damage of the cell components (including the DNA) thus, disrupting basic cellular function. In response to increased oxidative stress, biological antioxidant protective mechanisms have been developed. Antioxidant protection is an in-built physiological mechanism in opposition to harm caused by free radicals. These endogenous antioxidant compounds typically prevent or reduce the oxidation of biomolecules. They include glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx), as well as proteins such as Heat Shock Proteins (HSPS). Deficiency in endogenous antioxidant protection mechanism or an upsurge in ROS generation over the scavenging rate of endogenous antioxidants, renders macromolecules major targets for oxidative

* Corresponding author.
E-mail address: matlar2002@gmail.com (M.O. Ojezele).

http://dx.doi.org/10.1016/j.bjbas.2017.04.008
2314-8535/

modifications (Percario et al., 2012). These oxidative modifications can be injurious and deadly as cell homeostasis and viability can be greatly compromised.

Plasmodiasis has been reported to initiate generation of ROS. The degree of free radical production is sometimes worsened by drug intervention. Report by Erel et al. (2001) revealed that there was a reduced number of platelets and antioxidants activities (superoxide dismutase-SOD, and glutathione peroxidase-GPx), with an upsurge in lipid peroxidation of platelets when malondialdehyde (MDA) level was measured. Thus, the oxidative stress of platelets will generate thrombocytopenia through loss of membrane flexibility and increased brittleness, thereby, resulting in dysfunction of thrombocytes. Generation of hydroxyl (–OH) radicals in the hepatocyte is a probable reason for the generation of free radicals-induced apoptosis in plasmodiasis. Some antimalarials specifically quinolones like amodiaquine and chloroquine, and artemisin and its derivatives may produce free radicals, while exerting their *anti*-malarial effect by the production of ROS which can cause oxidative stress. This may aggravate the ROS/RNS burden and worsen the deteriorating host's health condition.

Phyllanthus amarus (family Euphorbiaceae) is a perennial herb which is widely distributed in tropical and subtropical countries of the world, including Nigeria (Mazumder et al., 2006; Tahseen and Mishra, 2013). *P. amarus* is a common weed found in cultivated and waste lands (Joseph and Raj, 2011). Ethnomedically, different parts of the plant are employed in several medical problems, including diabetes, inflammation, gastro-intestinal disorders, urinary bladder disturbances, ulcers, cancer, microbial infections, amongst others (Rajeshkumar et al., 2002; Khatoon et al., 2004; Saranraj and Sivasakthivelan, 2012; Ushie et al., 2013). The antioxidative activity (Lim and Murtijaya, 2007; Sen and Batra, 2013) and antiplasmodial effect (Ajala et al., 2011) of *P. amarus* have also been reported.

In Nigeria, as is the case in many sub-Saharan nations, the use of herbs alone or combined with conventional antimalarial is a very common practice. Although, administration of antimalarial combination therapy (ACT) is advocated by World Health Organisation, the use of monotherapy is not uncommon. Therapeutic failures, anecdotal support of vitamins efficacy in malaria and the need to improve treatment outcome informed the inclusion of some vitamins in the 'cocktail for malaria therapy'.

This research aimed at investigating the effect of *Phyllanthus amarus* seed extract, chloroquine, and artesunate used alone and co-administered with vitamin A, B, C, or E on oxidative stress in *Plasmodium berghei*-infected mice.

2. Materials and methods

2.1. Animal care

A total of eighty-five (85) adult male Swiss albino mice weighing between 25 and 30 g were obtained from the Animal House, Faculty of Basic Medical Science, Delta State University, Abraka, Delta State, Nigeria. They were acclimatized for fourteen (14) days prior to commencement of the experiment. Guidelines followed in the handling of animals were in accordance with the ethical standards of the Institutional Animals Ethics (IAEC), as adopted by the ethical committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

2.2. Plant sourcing and extract preparation

Fresh plants of *Phyllanthus amarus* were collected from Abraka in Ethiope East Local Government Area of Delta State, Nigeria, in the month of January 2016. The plant was identified and authenticated by Dr. (Mrs.) V. Ilondu of the Department of Botany, Delta State University Abraka, Delta State, Nigeria (Herbarium Number-1013568).

The seeds of the *Phyllanthus amarus* plant were sorted, air dried at room temperature and blended into coarse powder. Fifty grams (50 g) of the powdered sample was soaked in ethanol and refluxed for two hours in a distillatory flask as described by Kabiru et al. (2013). The extract was filtered using Whatman's filter paper. The filtrate was concentrated using rotary evaporator under reduced pressure at a temperature of 40 °C. The crude extract, paste, was stored in a refrigerator prior to use. The extract was prepared by reconstituting one gram of the concentrated crude extract in distilled water (the vehicle).

2.3. Malaria parasite and inoculation

The malaria parasite, *Plasmodium berghei* strain NK65 was obtained from the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos State, Nigeria. The mice were passaged intraperitoneally with 1×10^6 erythrocyte infected by the parasite, *P berghei,* from donor mice (Idowu et al., 2015). The experimental mice were observed and assessed for the level of parasitaemia for four consecutive days after passaging before the commencement of treatment. The level of parasitaemia was assessed by staining thin blood films, obtained from the tail, with Giemsa stain and examined under the microscope (Preeti et al., 2012).

2.4. Experimental design

The mice were randomly selected and grouped into seventeen (17) groups with five mice each. Drug dosage was determined according to the findings of Akira et al. (2006), Tolulope et al. (2011) and Anne et al. (2014).

Group 1 – Uninfected Control (NC)
Group 2 – Infected and untreated Control (IUC)
Group 3 – *P. amarus* Seed Extract (PASE) 300 mg/kg body weight
Group 4 – Chloroquine (CLQ) 20 mg/kg body weight
Group 5 – Artesunate (ATS) 20 mg/kg body weight
Group 6 – *P. amarus* Seed Extract 300 mg/kg + Vitamin A 66.67 IU/kg (PASE + VITA)
Group 7 – *P. amarus* Seed Extract 300 mg/kg + Vitamin B 10 mg/kg (PASE + VITB)
Group 8 – *P. amarus* Seed Extract 300 mg/kg + Vitamin C 0.056 mg/kg (PASE + VITC)
Group 9 – *P. amarus* Seed Extract 300 mg/kg + Vitamin E 28.57 IU/kg (PASE + VITE)
Group 10 – Chloroquine 20 mg/kg + Vitamin A 66.67 IU/kg (CLQ + VITA)
Group 11 – Chloroquine 20 mg/kg + Vitamin B 10 mg/kg (CLQ + VITB)
Group 12 – Chloroquine 20 mg/kg + Vitamin C 0.056 mg/kg (CLQ + VITC)
Group 13 – Chloroquine 20 mg/kg + Vitamin E 28.57 IU/kg (CLQ + VITE)
Group 14 – Artesunate 20 mg/kg + Vitamin A 66.67 IU/kg (ATS + VITA)
Group 15 – Artesunate 20 mg/kg + Vitamin B 10 mg/kg (ATS + VITB)
Group 16 – Artesunate 20 mg/kg + Vitamin C 0.056 mg/kg (ATS + VITC)
Group 17 – Artesunate 20 mg/kg + Vitamin E 28.57 IU/kg (ATS + VITE)

Please cite this article in press as: Ojezele, M.O., et al. Impact of generic antimalarial or *Phyllanthus amarus* and vitamin co-administration on antioxidant status of experimental mice infested with *Plasmodium berghei*. Beni-Suef Univ. J. Basic Appl. Sci. (2017), http://dx.doi.org/10.1016/j.bjbas.2017.04.008