Augmentation of antioxidant system: Contribution to antimalarial activity of *Clerodendrum violaceum* leaf extract

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ABSTRACT

Reactive oxygen species are known to mediate various pathological conditions associated with malaria. In this study, the antioxidant potential of *Clerodendrum violaceum* leaf extracts, an indigenous antimalarial remedy, was evaluated. Total phenol, flavonoid, selenium, vitamins C and E contents of *Clerodendrum violaceum* leaf extracts were determined. The free radical scavenging activities of the extracts against DPPH, superoxide anion and hydrogen peroxide coupled with their reducing power were also evaluated *in vitro*. Moreover, responses of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) in a rodent malaria model to a 4-day administration of *Clerodendrum violaceum* leaf extracts were also evaluated. The methanolic extract was found to contain the highest amounts of antioxidant compounds/element and also demonstrated the highest free radical scavenging activity *in vitro*. The results showed a significant decrease (*p* < 0.05) in SOD and CAT activities with a concurrent significant (*p* < 0.05) increase in GPx and GR activities in both erythrocytes and liver of untreated *Plasmodium berghei* NK65-infected animals compared to the uninfected animals. The extracts were able to significantly increase (*p* < 0.05) SOD and CAT activities and significantly reduce (*p* < 0.05) GPx and GR activities in both the liver and erythrocytes compared to those observed in the untreated infected animals. The results suggest the augmentation of the antioxidant system as one of the possible mechanisms by which *Clerodendrum violaceum* extract ameliorates secondary effects of malaria infection, alongside its antiplasmodial effect in subjects.

Keywords *Clerodendrum*, antioxidant, *Plasmodium*, oxidative stress

INTRODUCTION

Malaria is still the most prevalent and deadly parasitic disease in the world, infecting between 300 and 500 million people every year and causing about a million deaths annually, mostly among young children in Sub-Saharan Africa (McCombie, 2002). Malaria induces oxidative stress through an increased production of ROS within and outside the parasitized erythrocyte. Oxidative stress is the result of a disturbance in the balance of naturally generated oxidants and antioxidants. This can be caused by an increase in the production of reactive oxygen species (ROS) and/or decrease in the activity of antioxidant systems (Postma et al., 1996). Biologically, free radicals are known to exert some physiological functions in the body such as biosynthesis, detoxification and microorganism clearance (Braganza et al., 1995). Therefore, the antioxidant defense is supposed to maintain a normal homeostatic balance between free radical production and clearance. The defense against free radical in the body comprise a complex antioxidant system including vitamins A, E, C, glutathione and enzymatic antioxidants such as glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) (Halliwell and Gutteridge, 1990).

The role of antioxidants in malaria patients is currently receiving attention; antioxidants have been found to influence host cellular and immunological functions (Spallhoiz et al., 1990, Terahima et al., 2002). There is also increasing evidence that natural antioxidants, especially those contained in some spices, herbs and medicinal plants, may be useful in preventing the deleterious consequences of oxidative stress (Noda et al., 1997). Coincidentally, due to the rapid emergence of widespread drug resistance to most currently available antimalarial drugs (Tona et al., 2001) and the high cost of the same, most people in rural and urban areas in Africa still rely on medicinal plants; about 80% of the population of Countries in Africa use traditional medicine to help meet some of their primary health care needs (WHO, 2001). One of such plants used in traditional herbal treatment of malaria is *Clerodendrum violaceum*. *Clerodendrum violaceum* is commonly called *Clerodendrum* in English and “Ewe isedun” in Yoruba (Nigeria); a decoction of the leaves is used for the treatment of fever/malaria. Our previous studies have authenticated the acclaimed antimalarial activity of its leaf extract in folk medicine (Balogun et al., 2010). However, there is paucity of information on the possible amelioration of secondary ROS-mediated pathological conditions associated with malaria. The present study was therefore set out to evaluate the antioxidant constituents of *Clerodendrum violaceum* leaf extract and its effect on the enzymic antioxidant...
Antioxidant activity of Clerodendrum violaceum leaf extract

### Table 1. Selenium and vitamins C and E concentrations in Clerodendrum violaceum leaf extracts

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Hexane extract</th>
<th>Ethyl acetate extract</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mg/100g)</td>
<td>4.76 ± 0.23</td>
<td>20.85 ± 0.17</td>
<td>29.79 ± 0.18</td>
</tr>
<tr>
<td>Vitamin E (µg/100g)</td>
<td>9.22 ± 0.19</td>
<td>0.09 ± 0.02</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>Selenium (µg/Kg)</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 3 replicates.

### MATERIALS AND METHODS

#### Chemicals and reagents

Chloroquine diphosphate salt was obtained from Sigma Chemical Company, St. Louis, Mo, USA. n-Hexane, ethyl acetate and methanol were obtained from Eagle Scientific Limited, Nottingham. Assay kits for Superoxide dismutase, Glutathione peroxidase and Glutathione reductase were obtained from Randox Laboratories Ltd. (Co. Antrim, U.K.). All other reagents used were of analytical grade and prepared in all glass distilled water.

#### Animals

One hundred and forty four adult Swiss albino mice with an average weight of 20 ± 2 g were obtained from the animal breeding unit of the Department of Biochemistry, University of Jos, Plateau State, Nigeria. The mice were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water ad libitum. The research adhered to the Principles of Laboratory Animal Care (NIH publication #85 – 23, revised in 1985).

#### Parasites

*Plasmodium berghei* NK65 (chloroquine-sensitive strain) was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Oyo state, Nigeria.

#### Plant materials

Fresh leaves of *Clerodendrum violaceum* were collected in Oyo town, Oyo State, Nigeria, in August, 2010 and were botanically authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state, Nigeria. A specimen with voucher number FHI 108879 was deposited.

#### Ethical approval

Ethical clearance for the study was obtained from the Departmental postgraduate committee.

### Determination of antioxidant contents

The total phenolic contents of the extracts were determined by using Folin-Ciocalteu reagent (Nabavi et al., 2008). Total flavonoid contents were determined according to colorimetric method of Chang et al. (2002). Vitamins C and E were assayed by the method described by AOAC (2005).

### Table 2. Total phenol and flavonoid concentrations in Clerodendrum violaceum leaf extracts

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Hexane extract</th>
<th>Ethyl acetate extract</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenol (%)</td>
<td>0.07 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>2.30 ± 0.09</td>
</tr>
<tr>
<td>Total flavonoid (%)</td>
<td>0.10 ± 0.02</td>
<td>0.12 ± 0.04</td>
<td>2.96 ± 0.33</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 3 replicates.

### In vitro antioxidant assays

The free radical scavenging capacity of the extracts were analysed using the DPPH scavenging assay according to the methods of Shimada et al. (1992). The reducing power of the extracts was quantified by the method described by Yen et al. (1995). Hydrogen peroxide scavenging activities of the extracts were evaluated according to the method of Zhao et al. (2006). Moreover, their superoxide anion scavenging activities were assayed as per Yaping et al. (2003).

### In vivo antioxidant studies

Extract administration

The effects of *Clerodendrum violaceum* leaf extracts on activities of selected antioxidant enzymes were evaluated by using the 4-day suppressive test described by Peters (1965) against *Plasmodium berghei* NK65-infection in mice as a model. One hundred and thirty six mice were inoculated intraperitoneally with 0.2 ml of inoculum containing 1 x 10⁷ parasitized red blood cells from the same donor mouse. The remaining eight mice were not inoculated. The inoculated mice were randomly divided into seventeen groups of eight mice each and treated as follows: Groups 1 - 5 were administered 31.25, 62.5, 125, 250 and 500 mg/kg body weight of the n-hexane extract respectively; groups 6 - 10 were administered 31.25, 62.5, 125, 250 and 500 mg/kg body weight of the ethyl acetate extract respectively; and groups 11-15 were administered 31.25, 62.5, 125, 250, and 500 mg/kg body weight of the methanolic extract respectively. Group 16 was treated with 5 mg/kg body weight of chloroquine while animals in group 17 served as infected but untreated control and were administered an equal volume of 5% DMSO (the vehicle in which the extracts were dissolved). The uninfected animals (group 18) were also administered an equal volume of 5% DMSO, thus serving as the uninfected control. Extracts/drug
were administered orally to mice three hours after parasite inoculation and were also administered once daily for three more consecutive days.

Sample collection and preparation
Four animals in each group were sacrificed 24 h after the last extract/drug administration, using slight ether anaesthesia and were dissected. Blood was collected by cardiac puncture into clean, dry sample tubes containing EDTA and centrifuged at 2000 rpm for 5 min to remove plasma. The red blood cells were then washed by re-suspending in 0.9% NaCl and centrifuging for 5 min at 2000 rpm after each wash; they were lysed by re-suspending in cold distilled water and stored frozen until required. The liver of each animal was also quickly removed, cleansed of superficial connective tissue and blood. They were then homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were stored frozen overnight to ensure maximum release of enzymes (Ngaha et al., 1989). On day 8 post-inoculation, the remaining animals were also sacrificed and the same samples were collected.

Biochemical assays
SOD activities in the erythrocytes and liver were assayed by the method of Marklund and Marklund (1974); those of CAT by the method of Aebi (1987). GPx activities in whole blood and liver were assayed by the method of Paglia and Valentine (1967) while GR activities in the erythrocytes and liver were carried out by the method described by Goldberg and Spooner (1983). Protein concentrations in the samples were determined using the Biuret method (Gornall et al., 1949).

Statistical analysis
The group means ± Standard Deviation (SD) for each parameter was calculated and significant differences were determined by Analysis of Variance (ANOVA) and Duncan’s Multiple Range Test at 95% confidence level using SPSS-PC programme packages. (Version 16.0, SPSS Inc, Chicago)

RESULTS
Antioxidant constituents of extracts
The results revealed that the methanolic extract of Clerodendrum violaceum leaf had the highest concentrations of vitamins C and E, selenium, phenols and flavonoids compared to the other two extracts (Tables 1 and 2). Clerodendrum violaceum leaf had the highest concentrations of vitamins C and E, selenium, phenols and flavonoids compared to the other two extracts (Tables 1 and 2).

In vitro antioxidant activities
All the extracts exhibited antioxidant activities in vitro but their capabilities differed for the different indicators considered. For the DPPH radical scavenging activity, there was an increase in scavenging activity with increase in concentration for all extracts; however, the methanolic extract had the highest scavenging activity (88.75%), which compared favourably with that of Butylated HydroxyToluene (BHT) (89.38%), the standard (Fig. 1). Similar results were obtained for the hydrogen peroxide scavenging assay. There was an increase in scavenging activity with increase in concentration for all the extracts. The maximum scavenging activities were 66.4%, 68.0%, 72.0% and 80.8% for hexane extract, ethyl acetate extract, methanolic extract and BHT respectively (Fig. 2). Results for the superoxide radical scavenging activity also showed an increase in activity with increase in concentration for all the extracts, with the methanolic extract comparing favourably well with BHT (Fig. 3). The extracts, except the hexane extract, exhibited increased reducing power with increase in concentration. The methanolic extract had the highest increase, comparing favourably well with the standard (BHT). The hexane extract maintained a plateau at all concentrations (Fig. 4). On the overall, the methanolic extract had the lowest EC50 for the free radical scavenging activities (Table 3).

Effects on Antioxidant enzymes in vivo
The results obtained showed that on day 4 post-inoculation, all the extracts at various doses significantly increased (p < 0.05) superoxide dismutase activities in the erythrocytes and liver of infected animals compared to untreated infected control, except at the dose of 31.25 mg/kg body weight for hexane extract in the erythrocytes (Figs. 5 and 6). Likewise, chloroquine significantly increased (p < 0.05) the superoxide dismutase activity in the erythrocytes and liver of infected animals compared to untreated infected control. The methanolic extract caused the highest percentage increase at all doses, followed by ethyl acetate extract and then hexane extract. On day 8 post-inoculation, the same trend was observed. All the extracts at all doses administered significantly increased (p < 0.05) superoxide dismutase activities in the erythrocytes and liver of infected animals compared to the infected control (Figs. 5 and 6). Also, treatment with chloroquine significantly increased (p < 0.05) the superoxide dismutase activities in the erythrocytes and liver of infected animals compared to untreated infected control, restoring it to the range of the uninfected control in the erythrocyte (p < 0.05).

On day 4 post-inoculation, all the extracts at various doses significantly reduced (p < 0.05) the glutathione peroxidase activities in the blood and liver of infected animals compared to untreated infected control, except at the dose of 31.25 mg/kg body weight for hexane and ethyl acetate extracts (Figs. 7 and 8). Likewise, chloroquine significantly reduced (p < 0.05) the glutathione peroxidase activity in the blood and liver of infected animals compared to untreated infected control.

Table 3. In vitro antioxidant activities of Clerodendrum violaceum leaf extracts

<table>
<thead>
<tr>
<th>Fractions/BHT</th>
<th>EC50 (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH scavenging activity</td>
</tr>
<tr>
<td>Hexane extract</td>
<td>0.15</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>0.12</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>0.09</td>
</tr>
<tr>
<td>BHT</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Antioxidant activity of *Clerodendrum violaceum* leaf extract

Fig. 4. Reducing power of *Clerodendrum violaceum* leaf extracts. Values are means of 3 replicates.

restoring it to the range of the uninfected control ($p > 0.05$). Amidst the extracts, the methanolic extract caused the highest percentage reduction at all doses followed by ethyl acetate extract, then hexane extract (Figs. 7 and 8). The same trend was observed on day 8 post-inoculation except that only 31.25 mg/kg body weight of the hexane extract did not significantly reduce ($p > 0.05$) glutathione peroxidase activity in the liver of infected animals compared to the untreated infected control (Figs. 7 and 8). The results also revealed that on day 4 post-inoculation all the extracts at various doses significantly reduced ($p < 0.05$) the glutathione reductase activities in the erythrocytes and liver of infected animals compared to untreated infected control, except at the dose of 31.25 mg/kg body weight for hexane and ethyl acetate extracts in the liver (Figs. 9 and 10). Likewise, chloroquine significantly reduced ($p < 0.05$) the glutathione reductase activity in the erythrocytes and liver of infected animals compared to untreated infected control, restoring it to the range of the uninfected control ($p > 0.05$). On day 8 post-inoculation, the same trend was observed as that of day 4 post-inoculation in the erythrocytes except in the groups treated with 31.25 mg/kg body weight of hexane and ethyl acetate extracts and the group treated with 62.5 mg/kg body weight of the hexane extract. The activities of the enzyme in the liver also showed the same trend as that of day 4 post-inoculation except in the groups treated with 31.25 mg/kg body weight of hexane and ethyl acetate extracts (Figs. 9 and 10). On day 4 post-inoculation, the activities of catalase in the erythrocytes and liver of infected animals were significantly increased ($p < 0.05$) by all the extracts at various doses compared to untreated infected control, except at the doses of 31.25 and 62.5 mg/kg body weight for hexane extracts in the erythrocytes (Figs 11 and 12). Chloroquine significantly increased ($p < 0.05$) the catalase activity in the erythrocytes and liver of infected animals compared to untreated infected control ($p > 0.05$). The methanolic extract caused the highest percentage increase at all doses followed by ethyl acetate then hexane extract (Figs 11 and 12).

On day 8 post-inoculation, the activities of catalase in the erythrocytes and liver of infected animals were significantly increased ($p < 0.05$) by all the extracts at all doses compared to untreated infected control (Figs 11 and 12). Also, chloroquine significantly increased ($p < 0.05$) catalase activity in the erythrocytes and liver of infected animals compared to untreated infected control.

DISCUSSION

Oxidative stress results from an imbalance between the generation of reactive oxygen species (ROS) and endogenous antioxidant systems (Chanda and Dave, 2009; Gyamfi et al., 1999). ROS are known to play a prominent role in the pathophysiology of malaria. A potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical-mediated tissue damage associated with malaria.

Fig. 5. Superoxide dismutase activities in the erythrocytes of experimental animals on (a) day 4 post-inoculation and (b) day 8 post-inoculation. Values are means ± SD of 4 determinations. Values with different alphabets for each extract for each day are significantly different ($p < 0.05$).

Fig. 6. Superoxide dismutase activities in the liver of experimental animals on (a) day 4 post-inoculation and (b) day 8 post-inoculation. Values are means ± SD of 4 determinations. Values with different alphabets for each extract for each day are significantly different ($p < 0.05$).
Antioxidant activity of *Clerodendrum violaceum* leaf extract

From the results obtained in the in vitro studies, all the extracts exhibited DPPH, \( \text{H}_2\text{O}_2 \), and superoxide ion scavenging activities as well as the reducing power on ferric ion, with the methanolic extract having consistent higher activity and comparing favourably with BHT used in the assays (Figs. 1 - 4). This may be due to the presence of phenolic compounds and flavonoids and the appreciable quantities of vitamins C, E, and selenium in the extracts.

Various studies have shown that a number of natural products including polyphenols, terpenes and various plant extracts exert antioxidant activity (Chanda and Dave, 2009; Zhou and Zheng, 1991). Phenolic compounds are a class of antioxidant agents, the activity of which is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Hasan et al., 2009; Rice-Evans et al., 1997; Shahidi and Wanasundara, 1992). Phenolic compounds have been used to reverse, retard, or delay the onset of some diseases (Farombi and Owoeye, 2011). Flavonoids also have an established antioxidant activity (Cook and Samman, 1996; Farombi et al., 2002). The advantage of the antioxidant property of flavonoids has been revealed in neurotoxic studies which established that flavonoids can traverse the blood brain barrier (Youndim et al., 2003). Vitamin C is the major circulating water soluble antioxidant and acts as a free radical scavenger; it has been shown to take part in the metabolism of folic acid, red blood cell formation and maturation, bone formation and immune response mechanisms (De Tullio, 2010; Rao, 2006). Vitamin E is a lipid soluble vitamin and its main function is to prevent the peroxidation of membrane phospholipids and avoid cell damage through its antioxidant action (Herrera and Barbas, 2001; Padayatty et al., 2003). Selenium protects the cells by inhibiting free oxygen radical production but is best known for its role in glutathione enzyme system (Balakrishnan and Anuradha, 1998; Yalçın et al., 2003). Furthermore, vitamin E is transported by selenoproteins (Allin-Slater and Morris, 1963). Thus, these antioxidant species in the extracts may play an important role in scavenging the ROS produced during Plasmodium species infection either independently or synergistically through mechanisms which may include interference with lipid peroxidation, which has been shown to occur in malaria (Kulkarni et al., 2003) and termination of free radical chain reactions (Sardesai, 1995). Furthermore, the higher activities obtained for the methanolic extract may result from the higher concentrations of these antioxidant species in this extract compared to other extracts. This implies that the methanolic extract of the leaf extract of *C. violaceum* may be better in counteracting the deleterious effects of ROS produced in vivo during Plasmodium species infection and its antioxidant species may complement the endogenous antioxidant systems.

The results obtained for the in vivo antioxidant enzymes showed that treatment with the various extracts was able to increase the activities of SOD in the erythrocytes and liver which were reduced by infection (Figs. 5 - 8). SOD is a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide, thus protecting the cell from superoxide toxicity (Zelko et al., 2002). They are present in almost all aerobic cells and in extracellular fluids (Bannister et al., 1987). The observed decrease in the activities of both erythrocytic and hepatic SOD due to infection may result from the higher concentrations of these antioxidant species in this extract compared to other extracts. This implies that the methanolic extract of the leaf extract of *C. violaceum* may be better in counteracting the deleterious effects of ROS produced in vivo during Plasmodium species infection and its antioxidant species may complement the endogenous antioxidant systems.

The production of ROS species by the immune cells and the synchronized release of \( \text{O}_2^- \) during haemoglobin degradation by the malaria parasites might have contributed to the depletion of SOD possibly by overstretching the induction of its synthesis (Sohail et al., 2007). Furthermore, the antioxidant enzymes degraded by malaria parasites to derive amino acids cannot be replenished by the red blood cells due to lack of machinery for protein synthesis (Das and Nanda, 1999). However, the extracts.

CAT is an enzyme present in the peroxisomes of nearly all aerobic cells; it serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals using either an iron or manganese co-factor (Chelikani et al., 1996; Farombi and Owoeye, 2011). These antioxidant species in the extracts may play an important role in scavenging the ROS produced during Plasmodium species infection either independently or synergistically through mechanisms which may include interference with lipid peroxidation, which has been shown to occur in malaria (Kulkarni et al., 2003) and termination of free radical chain reactions (Sardesai, 1995). Furthermore, the higher activities obtained for the methanolic extract may result from the higher concentrations of these antioxidant species in this extract compared to other extracts. This implies that the methanolic extract of the leaf extract of *C. violaceum* may be better in counteracting the deleterious effects of ROS produced in vivo during Plasmodium species infection and its antioxidant species may complement the endogenous antioxidant systems.

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status (Mishra et al., 1994). It has been shown that trophozoite infected human red cells produce twice as much hydrogen peroxide and hydroxyl radicals as normal erythrocytes (Sohail et al., 2007). Increased intracellular iron levels from haemolysis can also lead to an increased production of hydrogen peroxide and hydroxyl ions (Vander et al., 1992). The observed significant decrease in the activity of catalase in both the erythrocytes and liver of infected animals may be due to depletion of catalase in combating oxidative stress produced by the infection. Decrease in the enzyme activities may also be due to utilization of erythrocytic proteins by the parasite. Hydrogen peroxide produced by the parasite during digestion of host cell cytosol also appears to be partially handled by host catalase (Becker et al., 2004). However, the extracts, most especially the methanolic extract, were able to ameliorate this reduction in CAT activities to a significant extent in the treated mice, possibly by sparing it because of their own inherent antioxidant composition.

GPx is the key enzyme responsible for the detoxification of cellular $\text{H}_2\text{O}_2$. It exists in two forms: Selenium dependent and Selenium independent. The former both detoxifies $\text{H}_2\text{O}_2$ and converts lipid hydroperoxides to non-toxic alcohols (Dreher et al., 1997); whereas, the latter enzyme is responsible for metabolizing lipid peroxides (Moon et al., 1983). All glutathione peroxidases catalyze the reduction of $\text{H}_2\text{O}_2$ using glutathione as substrate. Both GPx and catalase have the ability to inactivate intracellular $\text{H}_2\text{O}_2$, although GPx is considered the preferential pathway for elimination at low concentrations of $\text{H}_2\text{O}_2$ (Claudio et al., 2008; Jacob and Jandl, 1966). The observed increase in the activity of glutathione peroxidase may be due to an increased synthesis of the enzyme as a compensatory response to increased oxidative stress in both liver and blood of untreated infected animals. This observation agrees with that of Claudio et al. (2008) who reported an increase in the activity of glutathione peroxidase in the blood of patients with malaria. The increased activity may also be due to the fact that Plasmodium species produce glutathione peroxidase in response to reactive oxygen species formed during haemoglobin degradation (Atamna and Ginsburg, 1993).

GR is a flavoprotein that catalyzes the reduction of $\text{GSSG}$ to the GSH which is an important cellular antioxidant (Muller, 2004). It is part of the glutathione enzyme system along with glutathione peroxidases and glutathione-S- transferase (Hayes et al., 2005). The observed significant increase in the activity of glutathione reductase in the untreated infected mice may be for the same reason as that of glutathione peroxidase because these two enzymes are closely linked in recycling glutathione (Postma et al., 1996). The observation that these two enzymes were reduced in a dose-dependent manner in both the blood and liver of animals treated with extracts, suggest an effective clearance of the free radicals generated as a result of the infection by the extracts. Moreover, since the extracts have the capacity of reducing parasitaemia in the infected animals (Balogun et al., unpublished data), the amount of free radicals generated will be reduced which may not necessitate increased synthesis of the enzymes, thus accounting for the reduction in activities of GPx and GR in a dose-dependent manner.

The results obtained in this study suggest that *Clerodendrum violaceum* leaf extracts, in conjunction with its direct parasite clearance activity, may ameliorate the deleterious effects of ROS produced in vivo during malaria infection and its antioxidant species may complement the endogenous antioxidant systems in subjects.

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Fig. 9. Glutathione reductase activities in the erythrocyte of experimental animals on (a) day 4 post-inoculation and (b) day 8 post-inoculation. Values are means ± SD of 4 determinations. Values with different alphabets for each extract for each day are significantly different ($p < 0.05$).

Fig. 10. Glutathione reductase activities in the liver of experimental animals on (a) day 4 post-inoculation and (b) day 8 post-inoculation. Values are means ± SD of 4 determinations. Values with different alphabets for each extract for each day are significantly different ($p < 0.05$).

et al., 2004). CAT and glutathione peroxidase are involved in the elimination of hydrogen peroxide although catalase has been regarded as a major determinant of hepatic antioxidant
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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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