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Luffa cylindrica (Linn. M. J. Roem) Reduces Oxidative Stress *In Vivo* in *Plasmodium berghei*-Infected Albino Mice

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Abstract

Background Malaria is endemic in sub-Saharan Africa, and oxidative stress has been implicated in malaria disease. *Luffa cylindrica* is an ethnomedicinal plant used to treat various diseases, including malaria. The oxidative stress-reducing potential of *L. cylindrica* in malaria-disease state of *Plasmodium berghei* NK-65 parasite-infected mice was carried out *in vivo*.

Materials and Methods Mice were infected with *P. berghei* NK-65, and the effect of administration of methanolic leaves extract (100, 200, and 400 mg/kg b.w) of *L. cylindrica* on percentage parasitemia in blood smear, antioxidant enzymes (catalase CAT, superoxide dismutase SOD, glutathione-s-transferase GST), non-enzymatic antioxidant (reduced glutathione GSH) and malondialdehyde concentration in tissues (plasma, liver, kidneys, and spleen) of mice was investigated and compared to chloroquine and artesunate as reference antimalarial drugs. Phytochemical constituents of the extract were determined by standard methods.

Results Saponins, tannins, terpenes, phenolics, flavonoids, alkaloids, and glycosides were the phytochemical constituents identified in the extract. The extract at three doses (100, 200, and 400 mg/kg b.w.) investigated caused a significant reduction ($p < 0.05$) of parasite growth with over 90% reduction in parasitemia level in mice infected with the parasite. The extract also ameliorated oxidative stress in mice by significantly ($p < 0.05$) increasing the activities of CAT, SOD, and GST in the studied tissues of mice. The level of malondialdehyde, a marker of oxidative stress in mice, was also significantly ($p < 0.05$) reduced by the extract. The results were comparable with chloroquine- and artesunate-treated groups.

Conclusion The study concludes that *L. cylindrica* is an effective therapy for treating malaria and for the management of its oxidative stress-related complications due to its antioxidant properties.

Keywords
- malaria
- *Plasmodium berghei*
- *Luffa cylindrica*
- oxidative stress
- antioxidant enzymes system

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Introduction

Malaria remains a life-threatening disease of global concern as mortality from the disease keeps surging in malaria-endemic countries, particularly in sub-Saharan Africa. The World Health Organization (WHO) reported an estimated 241 million cases of malaria with 627,000 deaths globally in 2020 compared to 227 million malaria cases and 558,000 death cases in 2019.[1] Sub-Saharan Africa is the region among other malaria-endemic countries with the heaviest burden of malaria, accounting for 95% (about 229 million) of all malaria cases and 96% (602,000) of all deaths in 2020. Nigeria accounted for 31.9% of the total death cases, with 80% of deaths occurring in children under the age of 5 years.[1] Malaria is mainly caused by the parasitic protozoan called *Plasmodium*.[2] This parasite is transmitted to humans from the bite of a female anopheles mosquito, a vector of the parasite that bites mainly between dusk and dawn.[3] The parasite invades the human erythrocytes to complete its life cycle and later results in the clinical manifestation of malaria. Oxidative stress, which is a physiological process induced by free radicals, has also been implicated in the pathogenesis of malaria.[4] Reactive oxygen species (ROS) are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($O2^-$), per hydroxyl radical ($HO2^-$), hydroxyl radicals (OH^-), free radical nitric oxide (NO^-) as well as non-free radicals hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$).[5] These oxidants are generated in the mitochondria and peroxisomes from normal intracellular metabolism and as well from a variety of cytosolic enzyme systems. Oxidative stress can damage proteins, cells, tissues, and DNA. Though the role of oxidative stress in malaria is still unclear, some authors suggested that during malaria infection, a primary event that occurs is increased production of reactive oxygen species as part of the host defense to abate the parasite.[6] It has also been reported that the generation of free radicals (reactive oxygen and reactive nitrogen species) associated with oxidative stress plays a significant role in the development of systemic complications caused by malaria.[7]

In contrast, some have argued that malaria infection induces the generation of hydroxyl radicals ($OH^•$) in the liver, which is probably the main reason for the induction of oxidative stress and apoptosis.[8] In malaria infection, the parasite uses the hemoglobin molecule of the host as a source of amino acids and nutrients by breaking down this molecule in the host to release heme, a toxic compound due to its ability to destabilize and lyse membranes, and also inhibit the activity of several enzymes. The iron primarily bound to hemoglobin is in the ferrous state (Fe^{2+}); however, the release of the heme results in ferrous iron (Fe^{2+}) being oxidized to the ferric state (Fe^{3+}). Electrons liberated by this oxidation of iron promote the formation of reactive oxygen species (ROS), causing changes in host erythrocytes and endothelial cells and facilitating the penetration of the parasite into tissues such as the brain and liver.[9] Nitric oxide (NO) is one of the free radicals that appear to be involved in oxidative stress-induced malaria disease[10] though its involvement is controversial. However, there are claims that cerebral malaria is one of the consequences of the high production of NO to promote the death of the parasite.[11] At the same time, some think that cerebral malaria results from the low bioavailability of NO.[12]

Additionally, malaria infection also induces the generation of hydroxyl radicals ($OH^•$). Ataman *et al.*[13,] in their study, reported that erythrocytes infected with *Plasmodium falciparum* produced hydroxyl ($OH^•$) and hydrogen peroxide (H_2O_2) twice as much compared to normal erythrocytes. *Luffa cylindrica*, commonly called sponge gourd, belongs to *the Cucurbitaceae* family and is one of the primary herbs used in Nigerian folk medicine to treat malaria.[14,15] The leaves of the plant are usually prepared in decoction and consumed to treat malaria.[16] Various parts of the plant are also used in other African folk's medicine to treat ailments ranging from fever, jaundice, tumors, leprosy, wounds, bleeding from bowels or bladders, and also to alleviate pain and inflammation.[14–16]

Additionally, the pharmacological activities of *L. cylindrica* are well documented in the literature.[17–19] Our previous studies reported the *in vitro* antioxidant activity[20] and antiplasmodial activities[21] of different extracts of *L. cylindrica* leaf. Thus, in this study, we investigated the ameliorative potential of methanolic leaf extracts of *L. cylindrica* in oxidative stress-related malaria.

Materials and Methods

Materials

Fresh leaves of *L. cylindrica* were collected from Zulle farm in Suleja, Niger State of Nigeria, and authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, with a voucher specimen number NIPRD/H/6650 deposited. Thirty (30) healthy albino mice of both sexes (24.0 ± 2.0 g) were obtained from the Animal House of the Department of Pharmacology at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The mice were housed in clean metabolic cages placed in well-ventilated house conditions (temperature 23 ± 1°C; photoperiod: 12 h natural light and 12 h dark: humidity: 45–50%) and acclimatized for 7 days before the commencement of the experiments. They were also allowed free access to food (Platinum Feed Mills Company, Nigeria) and tap water free of contaminants.

Plasmodium berghei (Chloroquine-sensitive NK-65) was obtained from the Institute for Advanced Malaria Research and Training (IMRAT), College of Medicine, University of Ibadan, Nigeria. Chloroquine diphosphate and artesunate were products of May and Baker, Nigeria Plc. and Sigma Chemical Company, St. Louis, Mo, USA, respectively, while assay kits for superoxide dismutase, catalase, reduced glutathione, and glutathione-S-transferase were the products of Randox Laboratories Ltd. (Co. Antrim, UK). All other reagents were obtained commercially and were of analytical grade.

Methods

For preparation of methanolic leaf extract, the leaves of *L. cylindrica* were air-dried and pulverized with a mechanical

blender (Mazda Mill, MT 4100, Japan). The methanolic extract was prepared according to an earlier report of[28]. The filtrate obtained was concentrated in a rotary evaporator (RE-300B model, China) at 60 to 65°C and was dried to a constant weight on a water bath to give a yield of 28% (w/w). The extract was reconstituted into doses of 100, 200, and 400 mg/kg body weight (b.w.). Standard methods of previously reported studies[22–24] were adopted to screen the methanolic leaves extract of *L. cylindrica* for secondary metabolites. *Plasmodium berghei* (NK-65 strain), a chloroquine-sensitive strain of malaria parasite, was used to infect a donor mouse by intraperitoneal injection of 0.2 mL of infected blood containing approximately 1×10^7 *P. berghei* parasitized erythrocytes. The percentage parasitemia of the donor mouse was determined using a hemocytometer.

Thirty (30) albino mice of both sexes were completely randomized into six groups, each consisting of five mice, and were treated as follows: a) control group: (i.e., not infected mice) but received 5% DMSO (dimethyl sulfoxide), b) untreated group: consisted of mice that were infected but left untreated. C-E) treated group(s): consisted of infected mice but administered 100, 200, and 400 mg/kg b.w. of methanolic leaf extract of *L. cylindrica*, respectively. Artesunate group: consisted of infected mice but administered 1.5 mg/kg b.w. artesunate (reference drug 1).

Chloroquine group: consisted of infected mice but administered 5 mg/kg b.w. chloroquine (reference drug 2).

To establish malaria infection, mice in each respective group were intraperitoneally injected with 0.2 mL of infected blood containing approximately 1×10^7 *P. berghei* parasitized red blood cells taken from the donor mouse on the first day (Day 1) of the experiment. The mice were left untreated for 72 hours for infection to be established. After that, thin blood films were collected from the tail of each infected mouse, stained with Giemsa, and examined with a microscope at $\times 100$ of oil immersion to confirm infection. Parasitemia of at least 1000 cells was counted from the blood film, and parasitemia level was determined by employing the formula of Fidock et al[25] below:

% parasitemia = number of parasitized RBCs/total number of RBCs (infected + non-infected) × 100%

Following the infection of rats with malaria, the extract and reference drugs were administered orally to the mice 72 hours (Day 3) post inoculation at the same time for 4 consecutive days using an oropharyngeal cannula. Blood smears were retaken to determine the level of parasitemia, and the percentage chemo-suppression of parasite infection in the treated mice that received different doses of the extract and reference drugs were calculated for each treatment day (Day 4–Day 7) using the formula:

$$\textbf{\% Chemo-suppression} \text{ of parasite} = \frac{\text{Parasitemia in the untreated group} - \text{Parasitemia in treated group} \times 100}{\text{Parasitemia in the untreated group}}$$

The animals were further sacrificed for biochemical analysis.

Animals in each treatment group were anesthetized with diethyl ether on the last day of the experiment. Blood was collected by cardiac puncture into clean, dry EDTA anticoagulant sample bottles, and centrifuged using Uniscope Laboratory Centrifuge (Model SM8) B, Surgrifriend Medicals, Essex, England at (350 $\times g$) for 15 minutes. The supernatant was immediately transferred into clean sample bottles using a Pasteur pipette to obtain the plasma. The animals' liver, kidney, and spleen were removed and homogenized in ice-cold 0.25 M sucrose solution (1:10 w/v). The homogenates were stored and frozen overnight to preserve the activity of the enzymes. Further appropriate dilutions of homogenates (liver, kidney, spleen, and plasma) were used to analyze biochemical parameters. Reduced GSH concentration and lipid peroxidation were determined by previously described procedures[26,27]. The methods described by[28–30] were adopted to assay SOD, CAT, and GST in the selected tissues.

Statistical Analysis

Each data represents the mean of five replicates ± SEM. Data were subjected to statistical analysis using one-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT). Statistical differences with $p < 0.05$ between group means were considered significant.

Results

Phytochemical constituents and chemo-suppressive activity of the extract

The methanolic leaf extract of *L. cylindrica* contained flavonoids, phenolics, terpenes, tannins, saponins, alkaloids, and glycosides as the phytochemical constituents (►**Table 1**). The extract demonstrated a chemo-suppressive activity of parasite in *Plasmodium berghei* NK-65 infected mice at doses of 100, 200, and 400 mg/kg b.w. administered. Each group was administered different doses of the extract, and early suppression of parasitemia was evident from day 5 post-inoculation. The chemo-suppressive activity for the three doses of the extract was quite similar to the chloroquine-treated group, with over 90% suppression from day five post-inoculation. The chemo-suppressive activity of this extract persisted throughout the experiment (►**Table 2**).

Table 1 Phytochemical constituents of methanolic leaf extract of *Luffa cylindrica*

Constituents	Inference
Saponins	+
Tannins	+
Terpenes	+
Phenolics	+
Flavonoids	+
Alkaloids	+
Cardiac Glycosides	+

+Present.

Table 2 Chemo-suppressive activity of methanolic extract of *L. cylindrica* on *Plasmodium berghei*-infected mice post inoculation

Groups	Percentage chemo-suppression			
	Day 4	Day 5	Day 6	Day 7
Untreated	–	–	–	–
Artesunate (1.5 mg/kg b.w)	68.16	43.86	97.76	98.68
Chloroquine (5 mg/kg b.w)	16.48	43.86	97.76	97.35
100 mg/kg b.w extract	41.34	96.42	84.63	97.35
200 mg/kg b.w extract	53.63	90.79	84.63	97.35
400 mg/kg b.w extract	46.55	93.60	97.76	98.82

Antioxidant Enzyme Study

►**Figures 1–5** depict the activity of antioxidant enzymes (CAT, SOD, and GST) in albino mice administered methanolic leaf extract of *L. cylindrica* at 100, 200, and 400 mg/kg b.w. post inoculation with NK-65 *P. berghei*. The antioxidant enzyme status of the treated albino mice was compared with that of the infected untreated mice.

The administration of reference antimalarial drugs (artesunate and chloroquine) and the methanolic leaf extract at 100, 200, and 400 mg/kg b.w. resulted in a significant increase ($p < 0.05$) in the activity of superoxide dismutase in the plasma, liver, and spleen of mice when compared with the infected untreated mice. However, this significant increase was noticed in the kidneys, only at 100 and 400 mg/kg b.w. (►**Fig. 1**).

The results showed that the administration of methanolic leaf extract of *L. cylindrica* at 100, 200, and 400 mg/kg b.w. significantly ($p < 0.05$) increased the catalase activity in the plasma, liver, kidneys, and spleen of mice when compared to infected untreated mice and in a comparable (similar) manner with chloroquine and artesunate-treated groups (►**Fig. 2**).

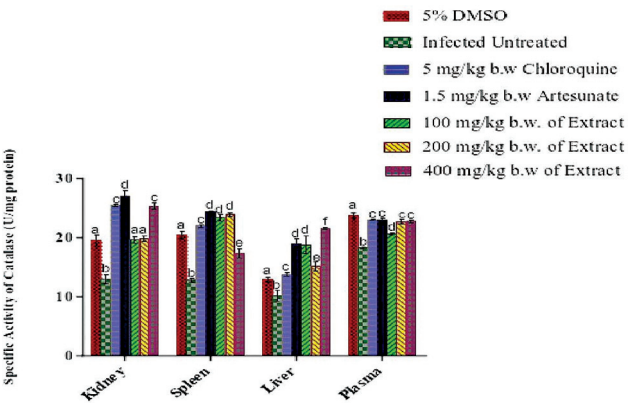

Fig. 2 Effect of administration of methanolic leaf extract of *L. cylindrica* on catalase activity in selected tissues of albino mice day 4 post inoculations Values are means of five replicates ± SEM. Bars with different alphabets are significantly different ($p < 0.05$).

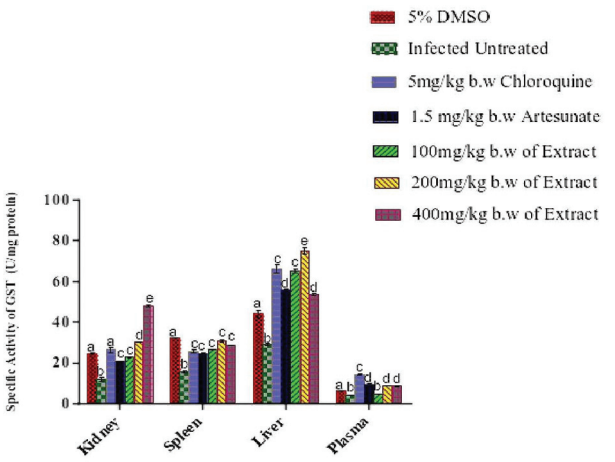

Fig. 3 Effect of administration of methanolic leaves extract of *L. cylindrica* on glutathione-S- transferase activity in selected tissues of albino mice day 4 post inoculations Values are means of five replicates ± SEM. Bars with different alphabets are significantly different ($p < 0.05$).

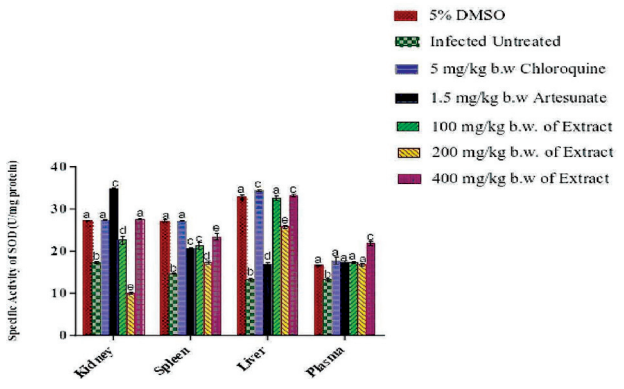

Fig. 1 Effect of administration of methanolic leaf extract of *L. cylindrica* on superoxide dismutase activity in selected tissues of albino mice day four post inoculations Values are means of five replicates ± SEM. Bars with different alphabets are significantly different ($p < 0.05$).

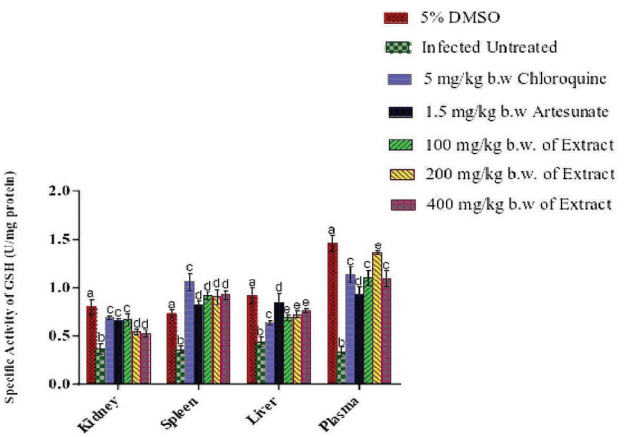

Fig. 4 Effect of administration of methanolic leaf extract of *L. cylindrica* on reduced glutathione concentration in selected tissues of albino mice day four post inoculations Values are means of five replicates ± SEM. Bars with different alphabets are significantly different ($p < 0.05$).

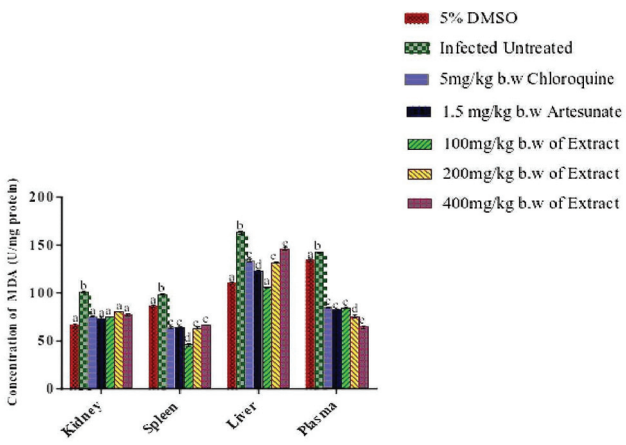

Fig. 5 Effect of administration of methanolic leaves extract of *L. cylindrica* on malondialdehyde concentration in selected tissues of albino mice day four post inoculations Values are means of five replicates ± SEM. Bars with different alphabets are significantly different ($p < 0.05$).

The administration of 100, 200, and 400 mg/kg b.w. of the extract as well as artesunate and chloroquine significantly ($p < 0.05$) increased the glutathione transferase activity in the plasma and organs (liver, kidneys, and spleen) of mice compared with the infected untreated group. This activity observed in the extract treated groups was compared favorably with artesunate- and chloroquine-treated groups. However, mice administered the extract showed a dose-dependent activity of GST in the kidney of albino mice (►**Fig. 3**).

In vivo Non-enzymic Antioxidant Study

Reduced glutathione concentration in the plasma, kidneys, liver, and spleen of mice administered 100, 200, and 400 mg/kg b.w. of methanolic leaf extract of *L. cylindrica* significantly ($p < 0.05$) increased when compared with infected mice that received no medical attention. The concentration of GSH in all studied tissues of mice that received the extract at the three investigated doses compete favorably with the groups of mice that received artesunate and chloroquine (►**Fig. 4**).

Malondialdehyde concentration increased significantly ($p < 0.05$) in infected untreated mice when compared with the control (animals administered 5% DMSO). Administration of 100, 200, and 400 mg/kg b.w. of the extract resulted in a significant decrease ($p < 0.05$) in malondialdehyde concentration when compared to infected mice with no medical attention and as well compete favorably with the artesunate and chloroquine treated mice (►**Fig. 5**)

Discussion

Our previous study investigated the in vivo antimalarial activity of different extracts of *L. cylindrica* leaf.[31] We established that methanolic leaf extract of *L. cylindrica* demonstrated the best antimalarial efficacy among other extracts of the plant in vivo.[21] The increase in parasitemia level in mice infected with *Plasmodium* and received no medical intervention (Group B) confirmed the establishment of malaria state in the mice recruited for this study. We previously also established that methanolic leaf extract of *L. cylindrica* caused over 90% chemosuppression of malaria parasite in the infected mice at the three doses (100, 200, and 400 mg/kg b.w.)[21], thus substantiating the antimalarial efficacy of the methanolic leaf extract of the plant. Oxidative stress has been implicated in the physiopathology of malaria, and *L. cylindrica* is documented to demonstrate antioxidant activities in vitro,[20] thus the need to investigate the antioxidant potential of this plant in oxidative stress-related malaria. Malondialdehyde is a known marker of oxidative stress. The increased MDA concentration observed in malaria-infected mice with no treatment compared with the control indicates an increased rate of oxidative stress. This, therefore, corroborates previous reports that oxidative stress markers such as MDA in infected humans and animals occur at high levels compared to uninfected ones[4,8,31] and the suggestion that oxidative stress is an important mechanism in parasite infection such as malaria.[10] In addition, the reduction in the activity of the antioxidant enzymes (SOD, CAT, and GST) in infected mice further substantiate claims that oxidative stress play an important key in the physiopathology of malaria. The studies of[31–33] reported a reduction in the activity of these enzymes as well as glutathione peroxidase (GSH-Px) in malaria patients infected by *Plasmodium vivax* and *falciparum*. Similarly, a decrease in the concentration of glutathione concentration as observed in this study was also reported by[34,35] in malaria patients. The reduction in activities of SOD and CAT in the erythrocyte of the experimental mice, suggest a reflection of increased generation of superoxide and hydrogen peroxide radicals due to the erythrocyte infection by the parasite resulting into inactivation of SOD and CAT because both enzymes are responsible for scavenging these radicals respectively. Though the mechanism of oxidative stress in malaria is still controversial, it is also possible that the reduction is a response defense mechanism adopted by the host cell (mice) to abate parasite infection in the erythrocyte. There is a correlation between oxidative stress and inflammatory response during an infection.[36] However, innate immune cells recognize pathogens and respond by strongly triggering inflammatory responses. The innate immune cells engulf these pathogens and attempt to eliminate them by rapidly increasing the production of ROS in their phagosomes in a mechanism called the oxidative or respiratory burst. ROS produced during the oxidative burst are also released extracellularly, contributing to the increase in the oxidative state in infected host.[37] Therefore, it may be noteworthy to say that mice infected with *P. berghei* NK-65 adopt a similar mechanism in the plasma (erythrocytes) to abate the malaria parasite invasion, which eventually led to a decrease in the activity of the antioxidant enzymes and to oxidative stress consequently. Malaria infection has been reported to induce the generation of reactive oxygen species such as hydroxyl radicals ($OH^{\bullet}$) in vital organs, especially the liver, and is the main reason for the induction of apoptosis in organs.[8] The further reduction in the activities of the antioxidant enzymes observed in the liver, brain, kidneys, and spleen is indicative of oxidative stress extension from the erythrocytes to these tissues due to the parasite invasion because the *plasmodium*

parasites can penetrate organs from the erythrocytes.[9] Based on this, our study suggests that the observed decrease in the level of antioxidant enzymes in the studied tissues may further result in tissue damage and severe complications. It is well documented that the oxidation of ROS and RNO in malaria state contributes to the development of complications in malaria such as cerebral malaria and acute kidney injury[11] based on the severity of the invasion of the plasmodium strain, e.g., *P. falciparum* and *vivax* strains that cause malaria in human.[4,38] Antioxidant enzymes such as SOD, CAT, and glutathione enzymes are important in the defense system as they act directly on some free radicals to terminate or detoxify their actions and thus avert cell, tissue, organ, and DNA damage. SOD scavenges the superoxide radicals by converting it to hydrogen peroxide (H_2O_2) and oxygen. The H_2O_2 from the dismutation reaction above can penetrate the biological membrane and decompose into hydroxyl radicals, a more powerful damaging reactive oxygen species. However, catalase is one of the major antioxidant enzymes to combat the dismutation process that generates H_2O_2 by reducing it to water. Also, the GSH molecule is a powerful antioxidant in protecting eukaryotic cells in the host defense against oxidative stress, acting upon several different mechanisms.[35] The increased expression of SOD, CAT, and GST observed in the studied tissues following the administration of methanolic leaves extract of *L. cylindrica* is an indication of the antioxidant property of the extract acting by stimulating their *in situ* production in the affected tissues of the malaria-infected mice, thus restoring normalcy from the assault caused by the free radicals[39] which could avert further complications associated with malaria. Saliu *et al*[20] have demonstrated the antioxidant activity of different fractions of *L. cylindrica* leaves, one of which is the methanolic fraction. Although the administration of the extract at different doses investigated in this study ameliorated the depleted antioxidant enzymes in variable proportions in studied tissues of the infected mice; however, the highest dose (400 mg/kg b.w.) demonstrated better antioxidant activity in ameliorating the SOD activity in all tissues. Plants and compounds with antioxidant activity may ameliorate the progression of malarial infection and probably prevent its sequelae, as experiments carried out in animals showed that antioxidants prevented the development of cerebral malaria. Additionally, studies have shown that the production of glutathione transferase and catalase reduces complications of malaria and the occurrence of severe malaria.[31,33] Though the mechanism at which the extract boosts the antioxidant enzymes is not clear, this development may be facilitated by flavonoidal components of the extract acting either in synergy or additive with other chemical constituents identified in the methanolic leaf extract of *L. cylindrica*. Flavonoids play a significant role in the stabilization of antioxidant enzymes. Flavonoids contain hydroxyl groups and mediate their antioxidant effects by scavenging free radicals.[40] These compounds act as hydrogen-donating antioxidants and can react with lipid peroxyl radicals, resulting in the termination of the generation cycle of new radicals.[40,41] An agent that exhibits high antioxidant capacity promotes a reduction in lipid peroxidation and increases the total antioxidant capacity of the host. These changes are correlated with significant suppression of parasitemia. Our previous study demonstrated the antimalarial activity of the methanolic leaf extracts of *L. cylindrica*[21]. The present study suggests that the antimalarial activity of *L. cylindrica* could be attributed to its antioxidant property because the plant extract ameliorated the antioxidant status of superoxide dismutase, catalase, reduced glutathione, glutathione transferase in malaria-infected mice upon administration and so could avert oxidative stress-related malaria complications.

Conclusion

The study concludes that *L. cylindrica* is an effective therapy for treating malaria and managing its oxidative stress-related complications due to its antioxidant properties.

Authors' Contributions

Saliu OA conducted the laboratory research. Akanji MA supervised the research and reviewed the manuscript. Idowu OA conducted the statistical analysis for the research and also prepared the manuscript.

Sponsorship and Funding

None.

Compliance with Ethical Principles

Ethical approval for the use of experimental animals was issued by the University of Ilorin ethical committee on the use of experimental animals.

Conflict of Interest

None declared.

Acknowledgment

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