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Effect of Ethanol Root-Extract of *Sphenocentrum jollyanum* on $CD4^+$ count and some Oxidative Stress Indices in *Plasmodium berghei*-infected mice

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ABSTRACT

This study was designed to determine the effect of ethanol root extract of *Sphenocentrum jollyanum* on $CD4^+$ count and some oxidative stress parameters in *Plasmodium berghei*-infected mice. The parameters were determined using standard methods. A total of thirty six albino mice were used for the study. The mice were acclimatized for seven days, then randomly assigned into six experimental groups of A, B, C, D, E and F with six (6) mice in each group. Mice in group A were given normal saline only and regarded as normal control. Mice in groups B to F were infected with *Plasmodium berghei* via intra-peritoneal cavity. Mice in group B (Positive control) were treated with 5 mg/Kg body weight of standard drug (Lonart®-DS) while mice in group C (Negative control) were left without treatment. Group D, E and F (treated groups) were treated with graded doses of 200, 400 and 800 mg/Kg body weight of ethanol root extract of *Sphenocentrum jollyanum,* respectively. All the mice were allowed free access to water and feed without restriction. Oral route was used for the administration of the Lonart® and the extract. The treatment lasted for ten days. The result showed that infection of the mice with *P. berghei* revealed a significant ($P<0.05$) low activities of catalase, superoxide dismutase and level of cluster of differentiation 4 while level of malondialdehyde was significantly ($P<0.05$) higher relative to the normal control. Treatment of the infected mice with the stated doses of *S. jollyanum* extract and standard drug (Lonart®) significantly ($P<0.05$) reversed the trend of these parameters in a dose dependent manner to a level similar to that of the normal control. The effect of the extract, especially, the highest dose of 800 mg/Kg body weight was comparable to that of the standard drug as there were no significant ($P>0.05$) difference between these groups. This study indicates that ethanol root extract of *Sphenocentrum jollyanum* contain principles that may be useful in the management of oxidative stress elicited by *Plasmodium berghei* infection.

Key Words: Catalase, Superoxide Dismutase, MDA, *Plasmodium berghei-infected mice, Sphenocentrum jollyanum* and $CD4^+$ Count.

INTRODUCTION

Before the advent of orthodox medicine in the treatment of ailments which include malaria, the Traditional African Society had devised various means of combating such ailments. One of the major ailments that are of concern in the world today is malaria. Malaria is one of humanity's worst diseases and is frequently referred

to as the disease of the poor [1]. Malaria kills more than five million people worldwide [2]. The majority of victims who die are children under the age of five and pregnant women. These children die because they are not protected and are not treated quickly enough to prevent their death [1]. Malaria which is transmitted to humans by mosquito vectors of the *Anopheles* species. In children, malaria presents as a febrile illness with other key symptoms such as vomiting, loss of appetite, restlessness and in severe forms convulsions. Even when it is not fatal, malaria produces considerable impact on the health of the Nigerian children, mostly during their first five years, increasing the susceptibility to other infections and hampering their development [3]. Malaria is now a global crisis, with one - fifth of the world's population at risk and with more than 233 million clinical cases of malaria in 2000, 225 million in 2009 and 244 million in 2000 [4]. The disease continues to spread due to a combination of factors, which include weak health systems, social, cultural and behavioral practices relating to treatment and prevention of malaria, large population movements, and climatic changes among others [4]. Following the high level of threat malaria poses to humanity, with increased level of mortality and morbidity rate as index of its threat, it has become very necessary to look further for an alternative measure for malaria prevention, control and treatment [5]. Conventional drugs used in the treatment of malaria infections are sometimes inadequate and sometimes can have some serious adverse effect [7]. The search for alternative drugs for the treatment of diseases has been on over the years. The anti-malarial potentials of compounds derived from plants have already been established with examples such as quinine, obtained from *cinchoma* species and *Artemisia* obtained from *artemesia species* [7]. The ethnomedicinal use of plants and plant products for the treatment of malaria has been identified as a promising source of wholesome therapy [6]. *Sphenocentrum jollyanum* have been used traditionally for the treatment of malaria.

Sphenocentrum jollyanum is one of a well known medicinal plant belonging to the family *Mensipermacea*. It is an erect, small, evergreen, dioecious shrub up to 1.5 m tall, sparingly branched; roots bright yellow; stem thinly short-hairy when young, later glabrous; bark grey [8]. It is called "Ezeogwu" in Igbo, "Akerejupon" in Yoruba, "Aduro kokoo" or "Okramankote" in the Akan language in Ghana [9]. Extracts from *Sphenocentrum jollyanum* roots have been used in the treatment of constipation, rheumatism and inflammatory disorders [10]. The

fruits are used as an anti fatigue snack [11]. The plant has been reported for its use against chronic coughs, worms and other inflammatory conditions as well as tumors [12]. The plant is traditionally used as remedy for feverish conditions as well and as an aphrodisiac [13 and 14]. Though the plant is used locally for the treatment of malaria, not much has been reported in the scientific media about it. This study was aimed at determining effect of root extract of *Sphenocentrum jollyanum* on $CD4^+$ count and some oxidative stress parameters in *Plasmodium berghei*-infected mice.

MATERIALS AND METHODS

Plants Collection

Fresh roots of *Sphenocentrum jollyanum* were collected from Aghara-oza Village in Izzi Local Government Area of Ebonyi State and were identified by Prof. S. S. Onyekwelu in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. Part of the identified plant was kept in Applied Biology Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria, for reference purposes.

Preparation of Extract

The fresh plant material was washed and dried at a room temperature (20-25^{o}C) for the periods of six weeks and ground to coarse form using electrical blending machine sterilized with ethanol. The coarse form was macerated in 99 % ethanol. The solution was allowed to stand for 3 days after which, the extract was filtered using 0.25 mm sieve cloth. The resulting extract was concentrated via evaporation by allowing it to stand overnight. The concentrated extract of *Sphenocentrum jollyanum* root was aerated under the fan for 48 hours and then used for the study.

Experimental Animals

The experimental animals that were used in this study were albino mice purchased from the Animal Unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. They were acclimatized for one week before the commencement of the experiment in the Animal House of the Biochemistry Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Experimental Design

A total of thirty-six (36) albino mice were used for this study. The mice were randomly assigned into six (6) groups of A, B, C, D, E and F with six (6) mice in each group. Group A mice (Normal control mice) were administered with normal saline and feed; group B (Standard control mice) was infected with *Plasmodium berghei* and were treated with 5 mg/Kg body weight of the standard drug (Lonart); group C mice (Positive control

mice) were infected with *Plasmodium berghei* without treatment; groups D, E and F were infected with *Plasmodium berghei* and treated with graded doses of 200, 400 and 800 mg/Kg body weight of the extract of *Sphenocentrum jollyanum* root respectively. Administration of the plant extract of *Sphenocentrum jollyanum* was by oral intubation.

Induction of Parasitaemia

Malaria parasite (*Plasmodium berghei*) was collected from malaria infected-mice at the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. Exactly 10 drops of parasitized blood were collected with the aid of a capillary tube through the ocular region of the mice, and diluted with 1 ml of normal saline. Exactly 0.2 ml of the diluted parasitized blood was used to infect all the mice that were used for the study. This was done according to the method described by [15].

Collection of Blood from Animals

The mice were starved for 12 hours after ten days of administering the mice with the plant extract and Lonart®. The blood samples were collected from the eyes of the mice via ocular puncture using capillary tubes.

Determination of Oxidative Stress Parameters

Determination of Catalase (CAT) Activity

Catalase (CAT) activity was assayed according to the method described by [16].

Principle: The principle was based on ultraviolet absorption of hydrogen peroxide which was measured at 240 nm. On the decomposition of hydrogen peroxide (H_2O_2) with catalase, the absorption decreased with time and from this reduction, catalase activity was measured.

Procedure: Exactly 2.5 ml of phosphate buffer and 2 ml of hydrogen peroxide (H_2O_2) were added to the test tube. After that, 0.5 ml of the sample was also added to the test tube. To 1 ml portion of the reaction mixture, 2 ml of dichromate acetic acid reagent was added. Absorbance was read at 240 nm against the bank at a minute interval.

Calculation

$$\text{Catalase concentration (U/L)} = \frac{0.23 \times \log \text{Absorbance 1/Absorbance 2}}{0.00693}$$

Estimation of Superoxide Dismutase (SOD) Activity

This was estimated according to the method described by [17].

Principle: The superoxide dismutase ability to block the autoxidation of adrelanine was the basis of the SOD assay. Superoxide generated by xanthine oxidase is known to cause the oxidation of

adrelanine to adenochrome. The production of the adenochrome formed per superoxide added increased with elevation of pH and also with increased adrelanine.

Procedure: Exactly 0.2 ml of the sample was introduced into 2.5 ml of 0.05 phosphate buffer. At pH of 7.8, 0.3 ml of newly prepared adrenaline solution was added to the reaction mixture followed by quick mixing by inversion in the cuvette. The increase in absorbance was taken every 30 seconds for 3 minutes at 480 nm against blank. Blank contained 0.3 ml of adrenaline and 2.5 ml buffer. Super Oxide Dismutase (SOD) activity was measured by determining the inhibition of auto oxidant of adrenalins.

Determination of Malondialdehyde (MDA) Level

MDA was determined by spectrophotometric method as described by [18].

Principle: The principle of this test was based on the fact that malondiadehyde (MDA) reacted with thiobarbituric acid to form a red or pink colored complex which in acid solution absorbed maximally at the wavelength of 532 nm.

$$MDA + 2TBA \longrightarrow MDA: TBA\ adult + H_2O$$

Procedure: Exactly 0.1 ml of sample, 0.9 ml of distilled H_2O, 0.5 ml of 25% TCA reagent and 0.5 ml of 1% TBA reagent in 0.3% NaOH were added to a test tube. The test tube was incubated at 95^0C for 40 minutes. After that, the test tube was allowed to cool in water and exactly 0.1 ml of 20% SDS (sodium dodecyl sulphate) was added to the test tube. The absorbance of the sample was read against the blank reagent at 532 and 600 nm.

Calculation: $\%TBARS = \frac{A532-A600}{0.5208\times0.1} \times 100$

Determination of $CD4^+$ Count

This was done using Flow cytometry technique according to the method of [19].

Principle: CD4-PE fluorescence is analysed on a cyflow cytometer with an excitation light source of 488 nm or 532 nm.

Procedure: Exactly 20 µl of well mixed anticoagulated blood was added to a test tube. To this was added 20 µl of CD4 mAb PE mixed gently and incubated for 15 mins at room temperature protected from light. 800 µl of no lyse buffer was added and shaken. The mixture was loaded into the cyflow device and analysed.

Statistical Analysis

The results were expressed as mean and standard deviation (SD) and data was subjected to One-way Analyses of Variance (ANOVA). Significant differences were obtained at $P<0.05$. This analysis

was estimated using computer software known as Statistical Package for Social Sciences (SPSS), version 18.

RESULTS

Effect of Ethanol Root Extract of *Sphenocentrum jollyanum* on some Oxidative Stress Para-meters and $CD4^+$ Count in *Plasmodium berghei* infected Mice

The result showed that activities of catalase, superoxide dismutase and level of cluster of differentiation 4 count were significantly (P<0.05) lower in the *Plasmodium berghei*-infected mice while level of malondialdehyde was significantly (P<0.05) higher relative to the normal control mice. Treatment of the infected mice with graded doses of *S. jollyanum* extract and standard drug (Lonart®) significantly (P<0.05) reversed the effect in a dose dependent manner to a level similar to that of the normal control. The effect of the extract, especially the highest dose of 800 mg/Kg body weight was comparable to that of the standard drug as there was no significant (P>0.05) difference between these groups.

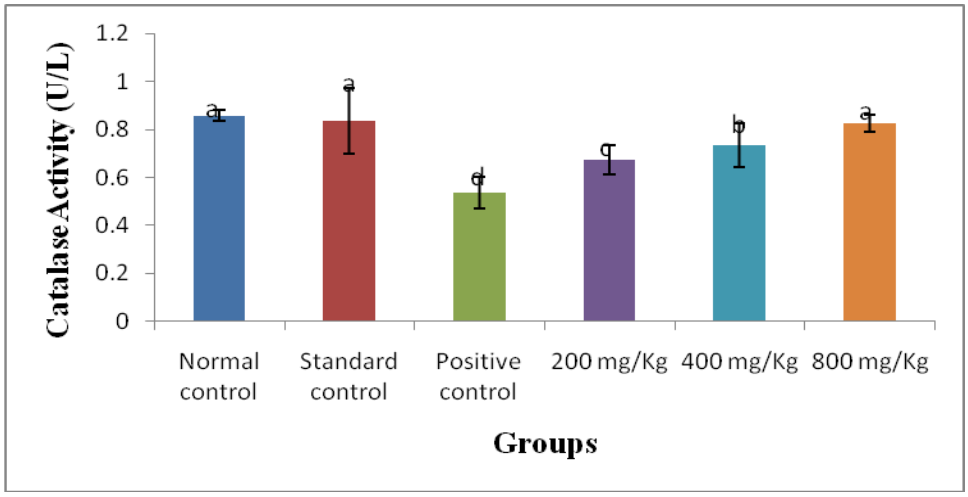

Figure 1: Catalase Activity in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root. Data are shown as Mean ± Standard Deviation (n=6). Mean values with different alphabet showed significant different at (P<0.05).

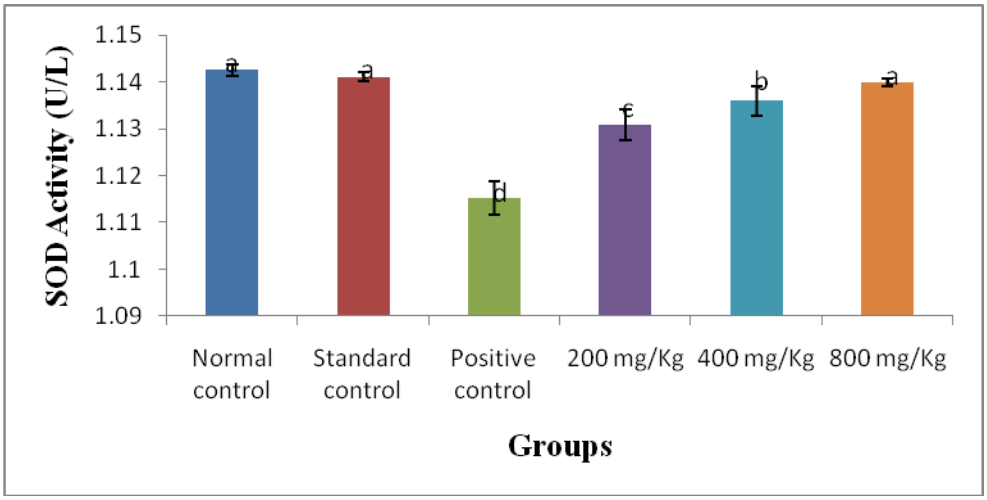

Figure 2: SOD Activity in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

Data are shown as Mean ± Standard Deviation (n=6). Mean values with different alphabet showed significant different at (P<0.05).

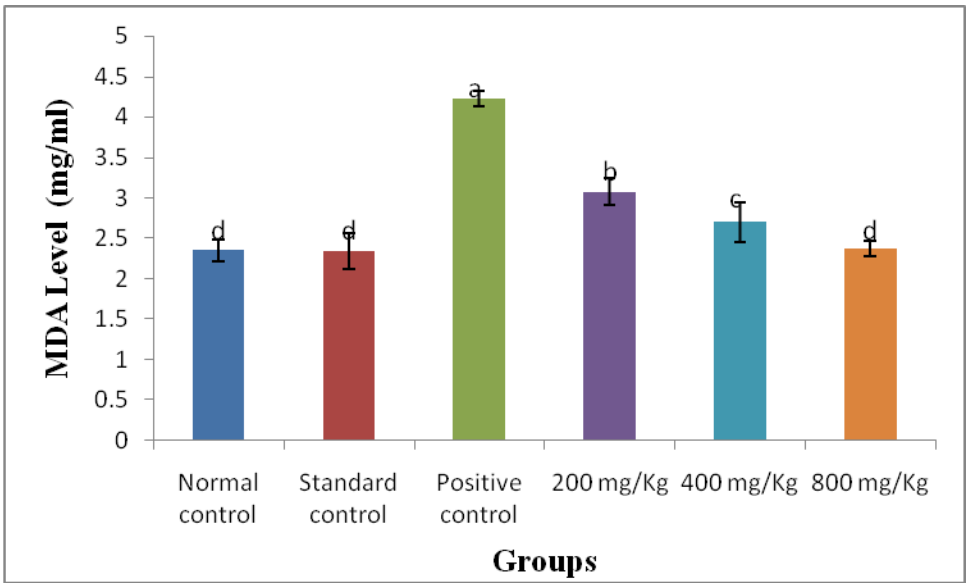

Figure 3: MDA Level in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

Data are shown as Mean ± Standard Deviation (n=6). Mean values with different alphabet showed significant different at (P<0.05).

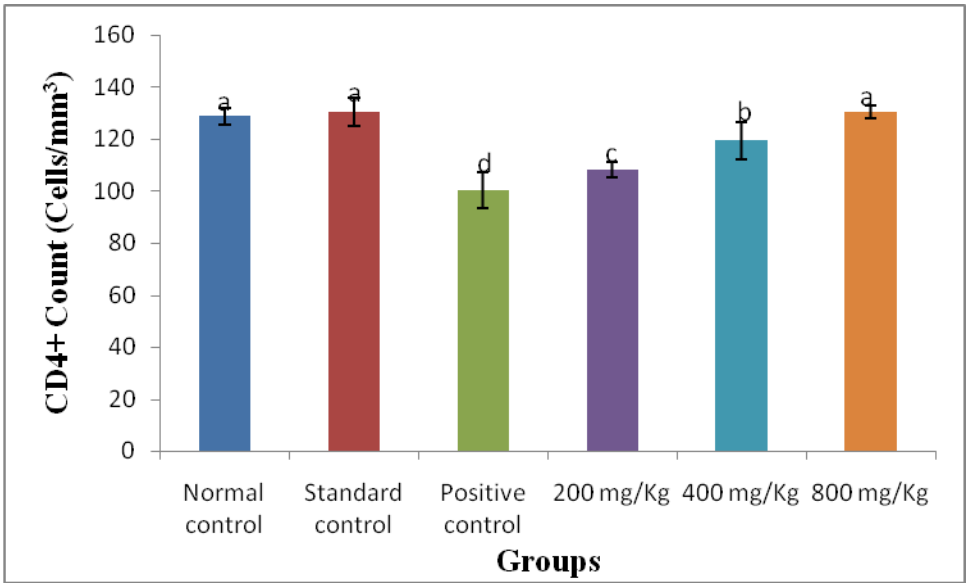

Figure 4: CD4⁺ Count in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

Data are shown as Mean ± Standard Deviation (n=6). Mean values with different alphabet showed significant different at (P<0.05).

DISCUSSION

The threatening of multidrug resistant malaria parasite and the absence of a functional safe and widely available malaria vaccine have stimulated researchers in the direction of development of new antimalarial drugs. In this contest, it is very important to investigate medicinal plant used by traditional practitioners for malaria treatment.

The result showed that catalase and superoxide dismutase activities were significantly (P<0.05) low while malondialdehyde level was significantly (P<0.05) high in the *Plasmodium berghei*-infected mice relative to the normal control mice The results revealed an increase in lipid peroxidation levels and decrease in the activities of superoxide dismutase (SOD) and catalase (CAT) in parasitized untreated mice when compared to treated mice, which suggest an oxidative environment and stress in parasitized mice. This could be because of dynamic changes in multiple body systems which result in increased basal oxygen consumption [20]. Also, this might have occurred as a result of toxic effect of upsurge reactive oxygen species produced by immune system as well as

synchronized release of O^{2-} during haemoglobin degradation by malaria parasite [21]. However, treatment of the infected mice with graded doses of *Sphenocentrum jollyanum* extract and standard drug (Lonart®) significantly (P<0.05) reversed the trend of these parameters in a dose dependent manner to a level similar to that of the normal control. The effect of the extract, especially, the highest dose of 800 mg/Kg body weight was comparable to that of the standard drug as there were no significant difference (P<0.05) between the groups. This work correlate with that of Agbafor *et al.* (2015), [22], which reported a general significant (P<0.05) decrease in the lipid peroxidation concentrations of the parasitized mice treated with ethanoilc extracts of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citrates* when compared to parasitized untreated mice on the last day and a general significant dose dependent increase (P<0.05) in superoxide dismutase (SOD), catalase and glutathione perioxidase activities as well as reduced glutathione concentrations in the treated mice. This work also agrees with the report of Momoh *et al.*(2014), [23], which reported that ethanolic leaf extract of *Alstonia boonie* significantly increase (P<0.05) the level of CAT, SOD and GSH in the liver homogenate induced with oxidative stress using H_2O_2 while the MDA values reduced significantly with the administration of the extract in Swiss albino mice infected with *Plasmodium berghei* and Olorunnisola and Afolayan (2013), [24], which reported that treatment of parasitized mice with leaf extract of *Sphenocentrum jollyanum* had a significant (P<0.05) reductions in elevated levels of serum, kidney and liver malondialdehyde (MDA) concentrations, but caused a significant (P<0.05) increased in the activities of serum and liver catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) level when compared with parasitized non-treated group (PNT) respectively.

There was general increase in the activities of superoxide dismutase (SOD), catalase (CAT) and decrease in MDA level in the parasitized treated animals when compared to parasitized untreated animals. Decrease in the activities and concentrations of these antioxidants in parasitized untreated mice were an indication of a surplus amount of free radical generation which promotes oxidative stress induced by malaria parasites in *Plasmodium berghei* and the host cell. Similarly, decreased SOD and CAT activities are linked to exhaustion of the enzymes as a result of oxidative stress caused by *Plasmodium berghei* but Restoration of SOD and CAT activities by the ethanol extracts indicated that the

extracts scavenged superoxide radicals. Thus, the reversal effects were their protective actions via a decreased production of *Plasmodium bergheiI*-derived-free-radicals or through the antioxidant activity of the *S. jollyanum* extract.

The result showed that infection of mice with *Plasmodium berghei* significantly ($P<0.05$) reduced the levels of $CD4^+$ count relative to the level found in the normal control mice. However, treatment of the infected mice with the graded doses of extract of *Sphenocentrum jollyanum* significantly ($P<0.05$) restored the $CD4^+$ count in the animals in a dose dependent manner, with the effect of the highest dose of 800 mg/Kg body weight being similar to that observed in the group treated with standard drug (Lonart®) as shown in Figure 4. $CD4^+$ T cells consist of several helper-subtypes which shape immune responses against particular pathogens. During malaria, $CD4^+$ T cell subsets have multiple roles in protection, pathogenesis and also escape from immune responses. $CD4^+$ T cells have been demonstrated to be the major source of both interferon-y (IFN-y) and tumor necrosis factor alpha (TNF-a) during experimental malaria in mice (Muxel *et al.,* 2011), [25], which are implicated in both protection and pathology of this disease. A study which investigated the effects of chronic malaria on MSP1-specific transgenic $CD4^+$ T cells (Stephens and Langhorne, 2010), [26], on mice which were infected with 10^5 *P. chabaudi* showed that one half of the mice treated with Chloroquine on days 30-34 cleared chronic malaria while after 60 days, flow cytometric analysis of transgenic T cells found that approximately 25% of memory $CD4^+$ T cells were lost in untreated mice compared to drug-treated mice which had cleared the infection [26]. The loss of $CD4^+$ T cells during malaria was perhaps first suggested when up to 99% of parasite-specific T cells labeled with the fluorescent dye 5-(and -6)-carboxy-fluorescein succinimidyl ester (CSFE), were found to be deleted only following infection of mice [27]. Subsequent studies also found deletion of T cells specific for the malaria vaccine, $MSPl_{19}$ during malaria infections [28]. However, FOXP3-expressing $CD4^+CD25^+$ regulatory T cells were also shown to correlate with more rapid parasite growth in human malaria infections [29], which may explain why protective cells subside.

CONCLUSION

In conclusion, the results of the study suggest that the ethanol root extract of *Sphenocentrum jollyanum* possesses potent antioxidant activity and immune boosting property. This may partly explain their use in traditional medicine as an antimalarial remedy.

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