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Alterations of Lipid Profile in *Plasmodium berghei*-infected Mice Treated with Ethanol Root Extract of *Sphenocentrum jollyanum*

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ABSTRACT

Effect of ethanol root-extract of *Sphenocentrum jollyanum* on lipid profile in *Plasmodium berghei*-infected mice was carried out using spectrophotometric methods. Thirty six albino mice were randomly assigned into six experimental groups (A, B, C, D, E and F) with six mice in each. Mice in group A (Normal control) were administered normal saline; mice in groups B-F were infected with *Plasmodium berghei* intraperitoneally; mice in group B (Standard control) were treated with 5 mg/Kg body weight of standard drug; mice in group C (Positive control) were left without treatment while mice in groups D, E and F were treated with graded doses of 200, 400 and 800 mg/Kg body weight of the 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 standard drug and plant extract for the period of ten days. Infection of mice with *Plasmodium berghei* caused a significant ($P<0.05$) increase in the levels of low density lipoprotein cholesterol (LDL-C), triacylglycerides (TAG) and significant ($P<0.05$) decrease in the levels of total cholesterol and high density lipoprotein cholesterol (HDL-C). However, treatment of *P. berghei*-infected mice with the ethanol root extract of *Sphenocentrum jollyanum* at the doses of 200, 400 and 800 mg/Kg body weight of the mice showed a significant dose-dependent ($P<0.05$) reversal in the trends of these parameters to levels comparable to those observed among the standard control group, especially the dose of 800 mg/Kg body weight. This study indicates that ethanol root-extract of *Sphenocentrum jollyanum* could be used in the management of cardiovascular diseases in mice infected with *Plasmodium berghei*.

Keywords: Lipid profile, *Plasmodium berghei,* Alterations, Mice and *Sphenocentrum Jollyanum*

INTRODUCTION

The rapidly growing malaria parasite requires large amounts of lipids for increase in surface area and volume of its internal membranes, certain serum lipid

fractions may favour the onset and/or severity of malaria infection. Very little is known about mechanism involved in lipid changes related to malaria. Hypercholesterolemia and hypertriglyceridemia was observed in both uncomplicated and complicated malaria[1,2,3]. Whereas [4] have shown no correlation between severity of malaria attacks and extent of HDL – cholesterol decrease. Human serum HDL is necessary for *P. falciparum* in in-vitro culture. Cholesterol is synthesized in the liver which happens to be the major site of *plasmodium* infection and this raises some questions whether there is any relationship between the cholesterol synthesis by the liver and the *plasmodium* infection of the liver. Although, the parasite has ways that enables it to thrive and multiply using nutrients from the host, they still cannot synthesize majority of their own lipids and cholesterol in vivo. In view of this, one would have to expect that the serum lipid levels to be low compared with the uninfected group. Liver ensures homeostasis of lipid and lipoprotein metabolism, hepatocellular damage often associated with severe and acute *P. falciparum* infections impairs these processes, leading to alterations in plasma lipid and lipoprotein patterns [5,6]. The alterations in serum lipid profile of malarious subjects could be attributable to the level of haemolysis in malaria, which is proportional to severity of infection [7]. Since the erythrocyte membranes are predominantly lipid in composition, the liberation of membrane lipids following sustained haemolysis accounted for the observed alterations in the serum lipid profile of patients presenting this disease [8]. Furthermore, parasitized parenchyma and Kupffer cells compromise lipid metabolism engendering distortions in lipoprotein particles synthesized by the liver with associated alterations in plasma lipid profile [9]. Hyperlipidemia, a hallmark of malarial infection which may results in depletion of natural antioxidants and facilitates the production of reactive oxygen species (ROS) which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA and exert cytotoxic effects on cellular components [10]. Thus increased ROS and impaired antioxidant defense contributes for initiation and progression of micro and macro vascular complications in malaria [11, 12]. Over the years, researchers have developed varieties of antimalaria drugs such as chloroquine, plaquenil, malarone, coartem, doxycyclinem, lonart, mefloquine and aralen used in the treatment of malaria infection, but due to the high cost or expensive nature of these pharmaceutical, and the resistance developed by most of the parasites towards these drugs have lead so many countries in the world including Nigeria, to resort to herbal remedies in the management of malaria.

Sphenocentrum jollyanum is one of a well known medicinal plant belonging to the family Mensipermacea. It is called “Ezeogwu” in Igbo, “Akerejupon” in Yoruba, “Aduro kokoo” or “Okramankote” in the Akan language in Ghana [13]. *Sphenocentrum jollyanum* is widely used for medicinal purposes. The plant, mainly the bark, is used as an emetic and purgative, especially when poisoning is suspected [14]. The root is used as an aphrodisiac tonic for men. The sap from

chewing sticks made from the root is believed to relieve stomach-ache and constipation, and to boost appetite and sexual drive [14]. Pounded roots are taken to treat high blood pressure [15]. The boiled or pulped roots are given in draught or enema against epileptic fits [14]. In Nigeria a decoction of the root is applied to dress tropical ulcers. A decoction of the leafy twigs is used as a wash to stop bleeding of wounds, sores and cuts. Powdered bark can also be used to cover the wounds [13]. The fruit is edible and is taken against fatigue [13]. Extracts from *Sphenocentrum jollyanum* roots have been used in the treatment of constipation, rheumatism and inflammatory disorders [16]. The fruits are used as an anti fatigue snack [17]. The plant has been reported for its use against chronic coughs, worms and other inflammatory conditions as well as tumors [15]. The plant is traditionally used as remedy for feverish conditions as well and as an aphrodisiac [13, 15].

Plate 1: *Sphenocentrum jollyanum* Leaf

Plate 2: *Sphenocentrum jollyanum* Root (Mag. x2)

AIM AND OBJECTIVE

This study was designed to evaluate the alterations in lipid profile in *plasmodium berghei*-infected mice treated with ethanol root extract of *sphenocentrum jollyanum*

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents used were of analytical quality. The chemicals were sourced from May and Baker, England; BDH, England and Merck, Darmstadt, Germany. The reagents used were commercial kits and products of Randox, QCA, USA and Biosystem Reagents and Instruments, Spain.

Biological Materials

Albino mice and *Sphenocentrum jollyanum* roots were the biological materials used for this study. 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. C. 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. 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 experiment in the Animal House of the Biochemistry Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Methods

Preparation of Ethanol Root Extract of *Sphenocentrum jollyanum*

The fresh plant material was washed and dried at a room temperature (20-25^{o}C) for the periods of four to six weeks and ground to coarse form using electrical blending machine sterilized with ethanol. The coarse form was macerated in 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 then used for the study.

Experimental Design for Sub-acute Studies

A total of thirty-six (36) albino mice were used for this study. The mice were randomly assigned into six (6) experimental groups of A, B, C, D, E and F with six (6) mice in each group. Group A (Normal control mice) were administered with normal saline and feed, group B (Positive control mice) were infected with *Plasmodium berghei* and were treated with 5 mg/Kg body weight of the standard drug (Lonart®), group C (Negative control mice) were infected with *Plasmodium berghei* without treatment while 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 route via 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 [18].

Determination of Percentage Parasitaemia Count

The determination of percentage parasitaemia (Mp^{+}) was carried out according to the method of [19].

Determination of Lipid Profile
Determination of Total Cholesterol and Triacylglycerides Concentrations

These were determined by the method as described by [20].

Determination of High-Density Lipoproteins (HDL)–Cholesterol Concentration

High density lipoprotein cholesterol (HDL-C) concentration was determined by centrifugation method as described by [21].

Determination of Low Density Lipoprotein-Cholesterol Concentration

The equation method of [22], was used in the determination of LDL- cholesterol concentrations of the samples.

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

The treatment of *Plasmodium berghei*-infected mice with ethanol root extract of *Sphenocentrum jollyanum* at the doses of 200, 400 and 800 mg/Kg body weight of the mice showed a significant ($P<0.05$) time and dose-dependent decrease in percentage parasitemia count as shown in Figure 1.

Infection of mice with *Plasmodium berghei* caused a significant ($P<0.05$) decrease in high density lipoprotein cholesterol and total cholesterol and significant ($P<0.05$) increase in triacylglycerides and low density lipoprotein cholesterol relative to normal control as shown in Figure 2, 3, 4 and 5. However, treatment of the infected mice with the ethanol root extract of *Sphenocentrum jollyanum* at the doses of 200, 400 and 800 mg/Kg body weight of the mice showed a significant ($P<0.05$) dose-dependent reversal in the trend of these parameters towards the level found in 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 thesegroups.

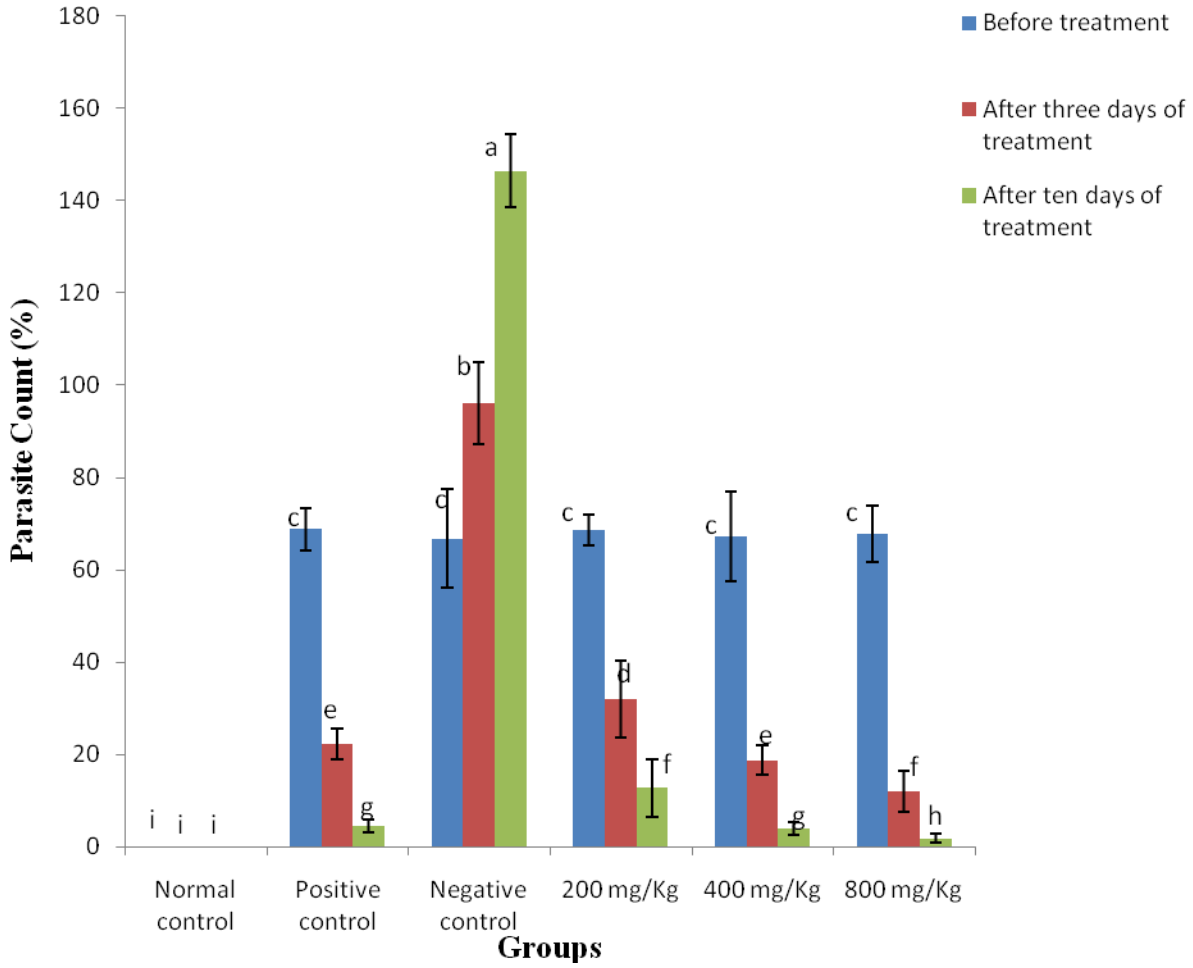

Figure 1: Parasite count in *Plasmodium berghei*-infected Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

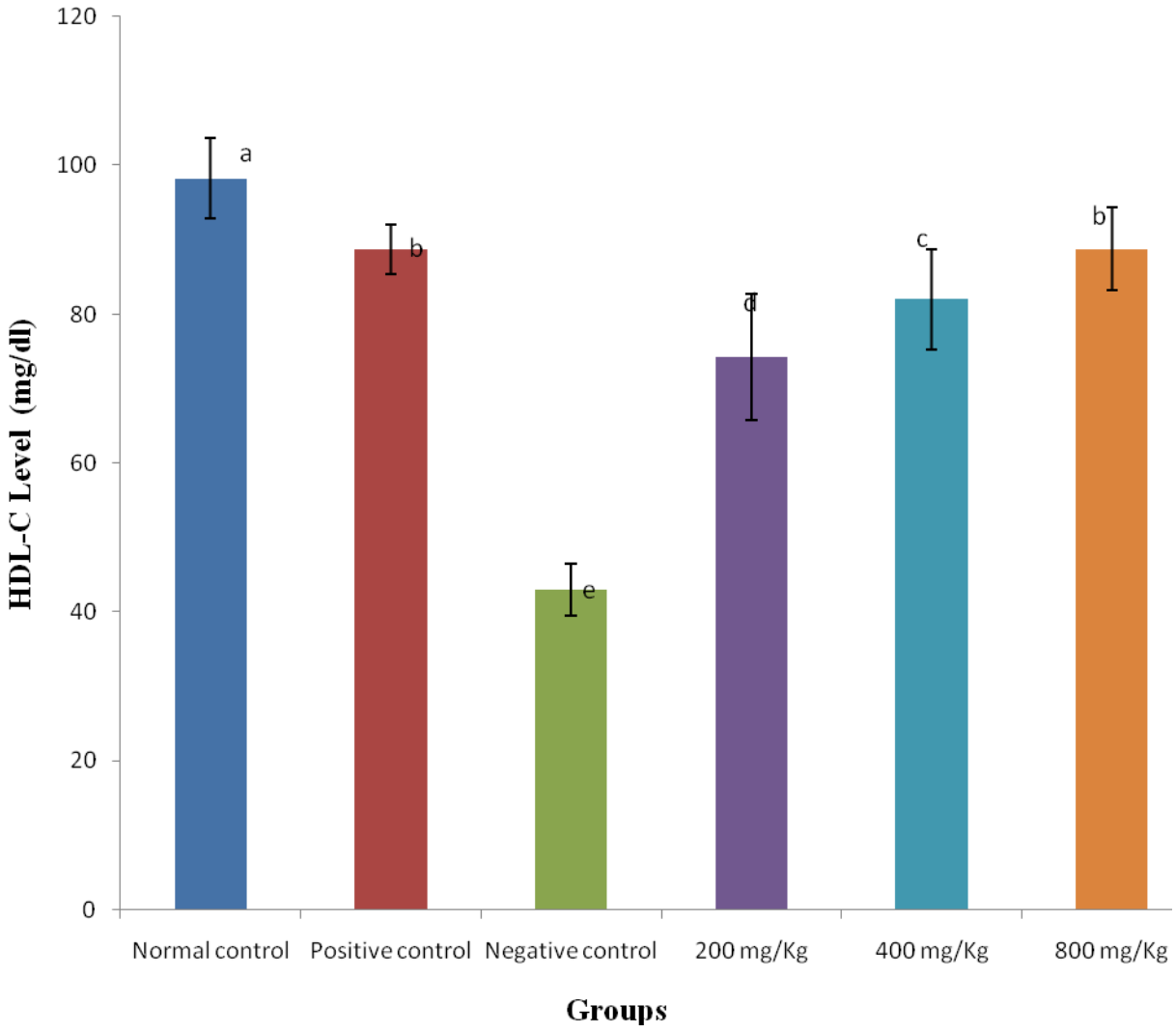

Figure 2: HDL-C Level in *Plasmodium berghei*-infected Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

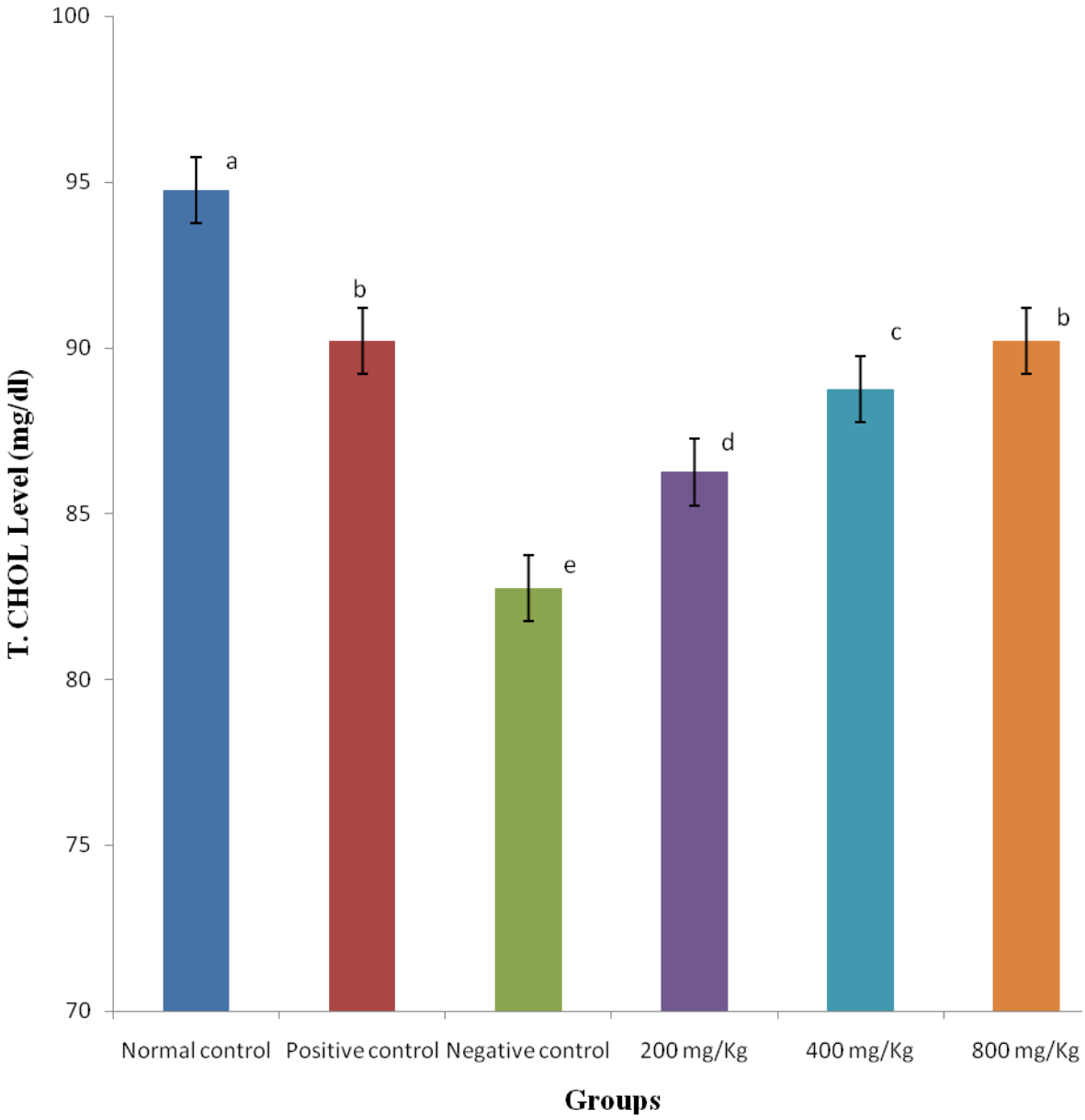

Figure 3: Total Cholesterol Level in *Plasmodium berghei*-infected Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

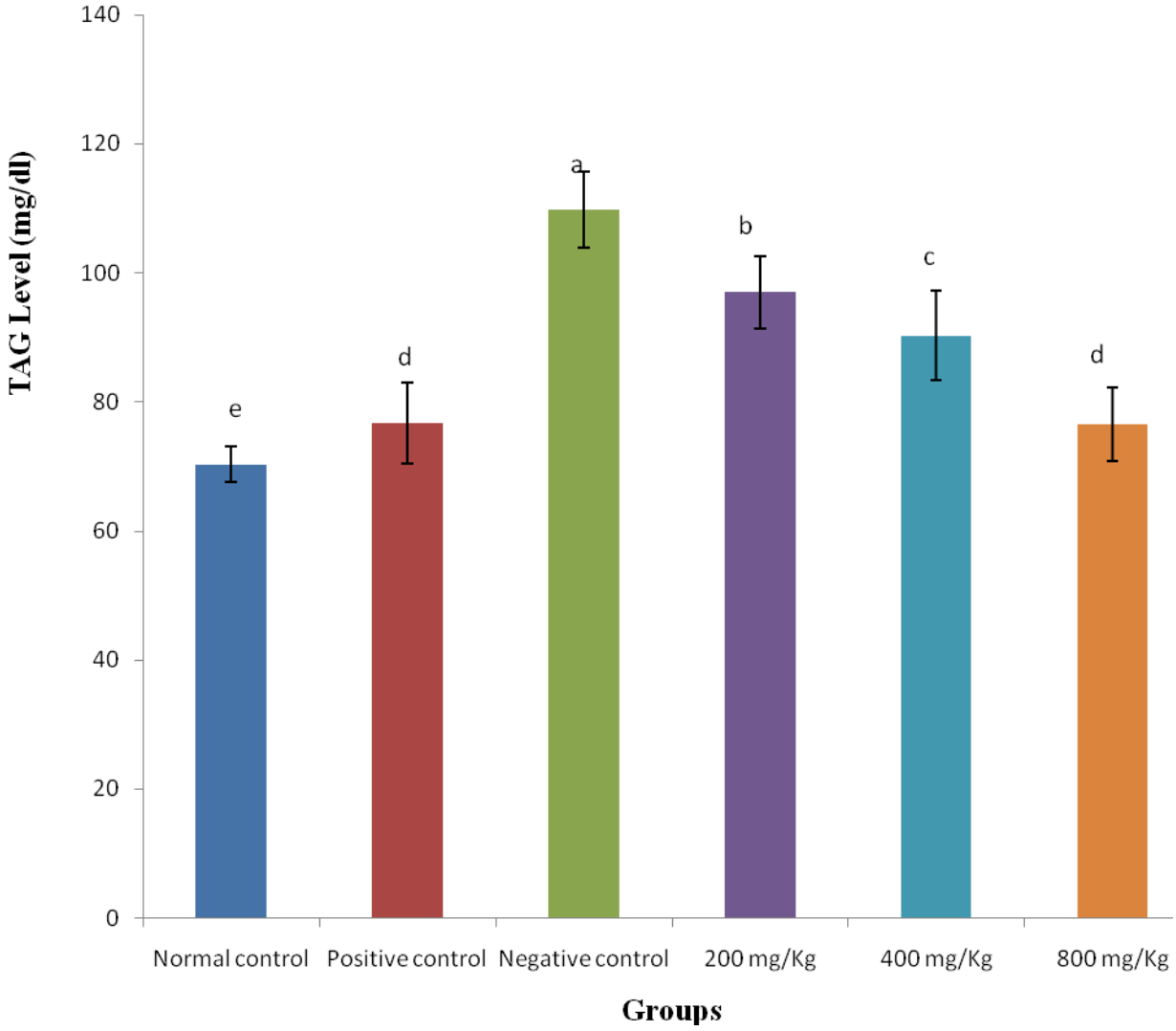

Figure 4: TAG Level in *Plasmodium berghei*-infected Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

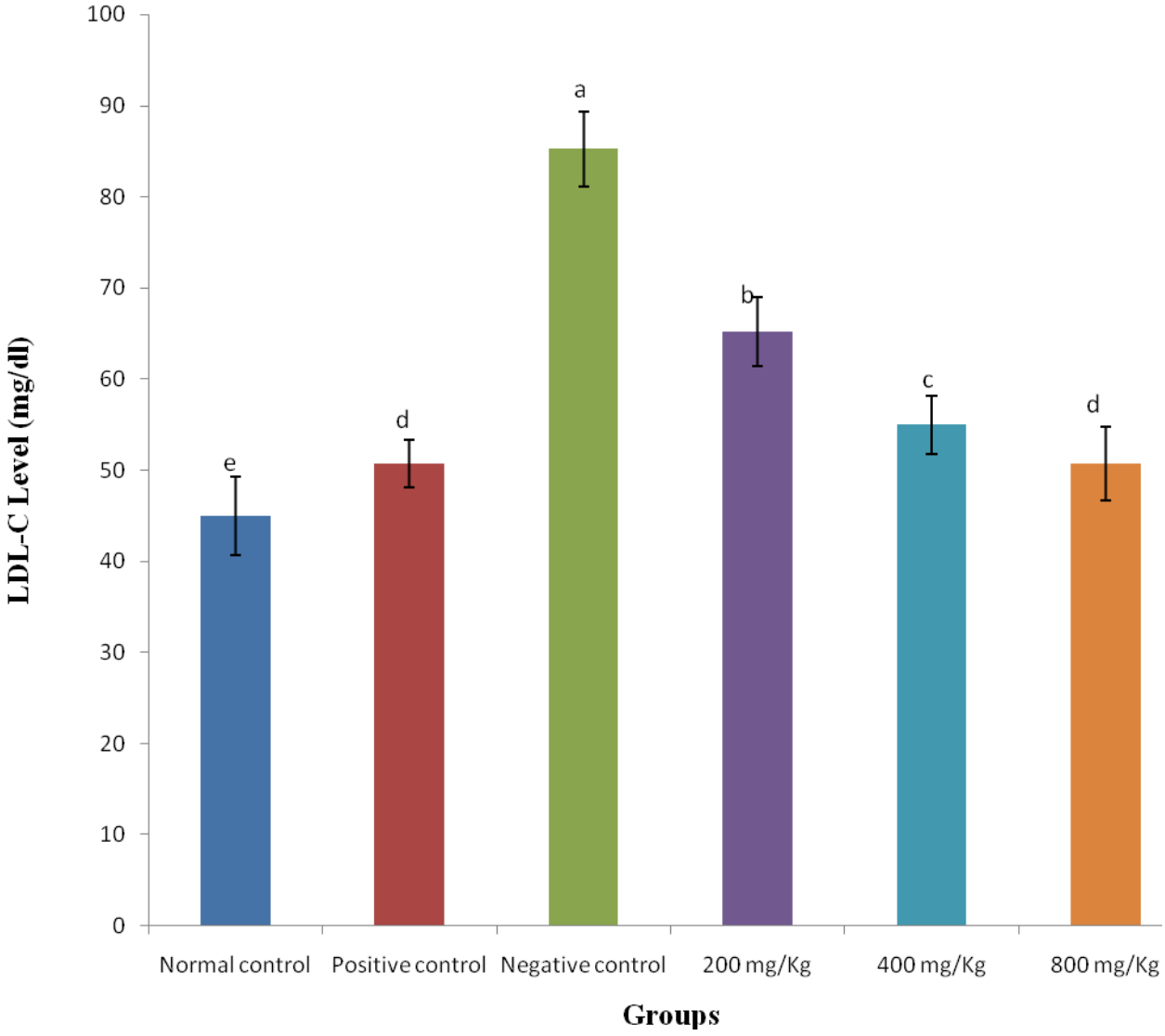

Figure 5: LDL-C Level in *Plasmodium berghei*-infected Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

DISCUSSION

The results showed that infection of mice with *Plasmodium berghei* significantly (P<0.05) showed high percentage parasitemia count relative to the normal control mice. But treatment of the infected mice with graded doses of *Sphenocentrum jollyanum* root extract significantly (P<0.05) reversed the effect

in both time and dose dependent manner, with the effect of the highest dose being similar to that observed in the group treated with standard drug (Lonart®) as shown in Figure 1. The presence of the parasite alone in the blood does not induce disorder, but the response of the host immune system against foreign autogenic organism via free radical generation, activation of phospholipase cascade series and generation of prostaglandins and other haemolytic principles such as free fatty acids does [23]. The reduction in the percentage parasitemia may be due to effect of the extract on the parasites-*plasmodium* level [24]. This result correlates with the findings of [25], which reported that leaf extract of *Sphenocentrum jollyanum* exhibits a significant (P<0.05) dose dependant anti-plasmodial activities. The results also agrees with those of [26], which reported a significant (P<0.05) daily increase in the level of parasitaemia in the parasitized untreated groups and a significant (P<0.05) dose dependent decrease in the level of parasitaemia in the parasitized groups treated with varying doses of the various medicinal plants (*Spilanthes uliginosa, Ocimum basilicum* (Sweet Basil), *Hyptis spicigera* and *Cymbopogon citrates*) and the standard drug.

The result of lipid profile showed that infection of mice with *Plasmodium berghei* significantly (P<0.05) decreased the levels of HDL-C and total cholesterol and increased the levels of LDL-C and triacylglycerides relative to the level found in the normal control mice. However, treatment of the infected mice with ethanol root extract of *Sphenocentrum jollyanum* at varied doses significantly (P<0.05) reversed the effect in the model in a dose-dependent manner with the effect of the highest dose being similar to that observed in the group treated with the standard drug (Lonart®) as shown in Figure 2, 3, 4 and 5. 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.

This finding agrees with the work [27], which reported that total cholesterol levels demonstrated dose-dependent decrease while high density lipoprotein cholesterol (HDL-cholesterol) increased with dose in *P. berghei* infected animals treated with extract of *Sphenocentrum jollyanum* leaves. This result also agrees with the report of [26], which reported dose-dependent significant (P<0.05) reductions in the plasma level of total cholesterol, triacylglycerol and low density lipoprotein cholesterol (LDL-C) and dose-dependent significant (P<0.05) increase in high density lipoprotein cholesterol (HDL-C) of the parasitized mice treated with ethanolic extracts of

Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera and *Cymbopogon citratus*. The decrease in total cholesterol and LDL-C demonstrated the presence of hypolipidaemic agent in the extract. The extract equally exhibited marked increase in HDL-cholesterol which is an indication that it has the tendency to minimize cardiovascular risk factors a major contributor of death in diabetes [28, 29]. The beneficial effect of the extract on lipid profile accounts for its use in the treatment of diabetes and diabetic complications. These results obtained from lipid profile in this study could be attributed to the antioxidants content of the plant extracts. A number of studies have established that antioxidant prevent lipid oxidation and oxidative damage, thus impeding the progression of altherosclerosis [30].

CONCLUSION

The result of this study indicates that ethanol root-extract of *Sphenocentrum jollyanum* could be used in the management of cardiovascular diseases in mice infected with *Plasmodium berghei*.

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