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MODULATIVE EFFECTS OF ETHANOL LEAF-EXTRACT OF *STACHYTARPHETA CAYENNENSIS* ON LIPID PROFILE AND OXIDATIVE STRESS MARKERS OF *PLASMODIUM BERGHEI*-INFECTED MICE

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ABSRACT

Effect of ethanol leaf-extract of *Stachytarpheta cayennensis* on lipid profile and some oxidative stress markers of *Plasmodium berghei*-infected mice was investigated using a total of 42 albino mice. The mice were randomly assigned into seven experimental groups of A-G with six mice in each group. Mice in groups A, B, C, D, E, and G were all infected with *Plasmodium berghei,* intraperitoneally with the exception of those in group F. Mice in groups A, B, C and D were treated with graded doses of 200, 400, 800 and 1600 mg/Kg body weight of the ethanol leaf-extract of *Stachytarpheta cayennensis* respectively. The group E (Standard control) mice were treated with 5 mg/Kg body weight of standard drug (Lonart). Mice in group G served as negative control (without any treatment) while mice in group F (Normal control) were administered with normal saline only. All the mice were allowed access to water and feed *ad libitum*. Evaluation of the parameters was done using standard methods. Treatment of the infected mice with the ethanol leaf-extract of *Stachytarpheta cayennensis* reduced parasite count significantly ($p<0.05$) in a dose-dependent manner. Infection of mice with *Plasmodium berghei* caused a significant increase ($p<0.05$) in the levels of low density lipoprotein cholesterol (LDL-c), triacylglycerides (TAG), and malondialdehyde (MDA) while activities of catalase (CAT), superoxide dismutase (SOD) and levels total cholesterol and high density lipoprotein cholesterol (HDL-c) significantly decreased ($p<0.05$) when compared with the normal control. However, treatment of *P.berghei*-infected mice with the ethanol leaf-extract of *Stachytarpheta cayennensis* at the doses of 200, 400, 800 and 1600 mg/Kg of the mice showed a significant dose-dependent reversal ($p<0.05$) in the trends of these markers to a level similar to the level observed in the standard control group, especially the highest dose of 1600 mg/Kg body weight of the mice. This study provides scientific evidence on the antimalarial potentials of *Stachytarpheta cayennensis* and its anti-oxidant potentials.

Keywords: *S. cayennensis,* Lipid profile, Oxidative stress, Malaria and Anti-oxidants

INTRODUCTION

Malaria is a mosquito-borne disease of humans caused by eukaryotic protists of the genus Plasmodium. It is transmitted from one human to another by a bite of an infected female anopheles mosquito. It is widespread in tropical and sub-tropical regions, including much of Sub-Sahara Africa, Asia and the Americas (Clark and Cowden, 2003). Plasmodium species are generally host and vector specific in that each species will only infect a limited range of hosts and vectors. According to the latest World Health Organization (WHO) report, there were 229 million cases of malaria in 2019 compared to 228 million cases in 2018 of which the estimated number of malaria deaths stood at 409 000 in 2019, compared with 411 000 deaths in 2018 (WHO, 2020). WHO African Region continues to carry a disproportionately high share of the global malaria burden. In

2019, the region was home to 94% of all malaria cases and deaths. In 2019,

six countries accounted for approximately half of all malaria deaths worldwide: Nigeria (23%), the Democratic Republic of the Congo (11%), United Republic of Tanzania (5%), Burkina Faso (4%), Mozambique (4%) and Niger (4% each) (WHO, 2020). Children under 5 years of age are the most vulnerable group affected by malaria; in 2019 they accounted for 67% (274 000) of all malaria deaths worldwide. In sub-Saharan Africa, the disease caused an estimated range of 537-907,000 deaths in 2010, many among children under 5 years of age (WHO, 2014). Nigeria suffers the Africa's greatest malaria burden with approximately 30 % of the total malaria burden in Africa, with 97 % of the total population at the risk of infection (Kar, Kumar, Sing, Carlton and Nanda, 2014). The rapid means of movement of large number of people from non-malaria endemic areas to endemic areas may seriously affect them when they return home (WHO, 2014).

Although treatment of malaria with conventional anti-malarial drugs has been giving appreciable results, WHO Report (2014), stated that the continued use of oral anti-malarial based monotherapies is a major contributing factor to the development of the resistance to anti-malarial drugs and its derivatives. Also artemisinin and chloroquine resistant *Plasmodium falciparum* has spread to most malarial areas and emerging resistance to artemisinin has become a problem in some parts of Southeast Asia (WHO, 2013). According to Nayyar, Breman, Newton and Herrington, (2012), there is evidence that *Plasmodium falciparium* was becoming resistant to the frontline treatment of malaria in Africa, and on the borders of Thailand and Myanmar.

Due to the high cost or expensive nature of these pharmaceuticals (anti malarial drugs) and the resistance developed by most of the parasites towards these drugs, many countries in the world including Nigeria is resorting to herbal remedies (natural product) in the management of malaria (Ekpono, Aja and Ugwu-Okechukwu, 2018).

Stachytarpheta cayennensis is a weedy and sometime perennial herbaceous flowering plant from the verbenaceae family. They are known by many English common names such as blue snake weed, cayenne vervain, Brazillian tea, cayenne protein weed, and local names such as Ogwu-ugwa, umon, panle by the Ibo, Efik and Yoruba tribes of Nigeria respectively. The use of the herb (leaf) of S*tachytarpheta cayennensis* plant has been shown to offer exciting alternative remedy for malaria treatment and control (REF). In many places, including Nigeria, the

plant has become a casual weed, a garden thug, a crop pest with effects on the ecosystem (Fonseca, 2006). *Stachytarpheta cayennensis* extracts is used in parts of Southern Nigeria, Latin America, India and Peru for treatment of malaria (Kvist *et al.*, 2006). The boiled juice or tea made from the leaves or the whole plant is taken to relieve fever and other symptoms. A tea of the leaves is also taken to help control diabetes in Peru and other areas and for treatment of inflammation, pain, hepatic and renal disorders, helminthiasis, constipation, hypertension and stress (Eliakim-Ikechukwu, Obiri and Igiri, 2013). Studies on the antimalarial effect of *Stachytarpheta cayennensis* leaves on mice infected with *Plasmodium berghei* and also its effect on lipid profile and some oxidative stress markers became necessary, to establish the best effective and efficacious alternative therapy for malaria treatment and control and also aid in better treatment of malaria and its associated biochemical alterations. Hence, this research was designed to determine the effect of ethanol leaf-extract of *Stachytarpheta cayennensis* on lipid profile (total cholesterol, triacyglycerides, HDL-c and LDL-c) and some oxidative stress indices (catalase, SOD and MDA) of *Plasmodium berghei*-infected mice.

Materials and Methods

Biological Materials

Stachytarpheta cayennensis leaves were sourced from a farmland in Ekwe-agbaja, Izzi L.G.A., Ebonyi State. The identification was carried out by a Professor O. O. Onyekwelu from Biological science Dept., EBSU, Abakaliki. Albino mice were sourced from Animal Unit, University of Nigeria, Nsukka, Enugu, Nigeria. They were housed in a well ventilated cage in the Animal House of the Biochemistry Department, Ebonyi State University, Abakaliki. They were allowed to acclimatizated for one week with access to water and food *ad libitum*.

Preparation of Plant Extracts

Fresh leaves of *Stachytarpheta cayennensis* were air-dried at room temperature for 2-3 weeks and thereafter homogenized using a manual blender. A known quantity (425.10g) of the dried and homogenized sample of *Stachytarpheta cayennensis* was macerated in 500 ml of 96% ethanol for 48 hours and filtered with a 2 mm sieve cloth. It was allowed to evaporate to dryness at room temperature. The concentrated *S. cayennensis* was stored in the refrigerator for subsequent studies.

Experimental Design

Forty-two (42) albino mice were randomly assigned into seven (7) experimental groups of six mice in each groups: A, B, C, D, E, F and G. Group A, B, C and D (treated groups) were infected with the parasite and respectively treated with 200, 400, 800 and 1600 mg/Kg body weight through oral intubation route. Group E mice (standard control) were infected with the parasite and treated with standard drug at 5 mg/Kg b.w. Group F mice (normal control) were administered normal saline and feed only while Group G (negative control) was infected without treatment.

Induction of Parasitaemia

Plasmodium beigheri-infected mice were procured from the National Institute of Medical Research, Yaba Lagos. One mililitre of the parasitized blood was obtained with the aid of a capillary tube through the ocular region of the mice and diluted with 10 ml of normal saline. Exactly 0.2 ml of the diluted parasitized blood was used to infect all the mice according to the method of described by Ekpono *et al.,* 2018. The mice were observed for three days and confirmed infected before treatment with the extract via oral intubation route.

Blood Sample Collection

Blood samples were collected on the 7th day of treatment with the extract as the mice were sacrificed. The blood was collected through peritoneal puncture under chloroform anesthesia and stored in sample bottles in a refrigerator prior to the analysis.

Determination of Percentage Parasitaemia Count

The determination of percentage parasitaemia (Mp^{+}) was carried out according to the method of Dacie and Lewis (2000).

Determination of Biochemical Markers

Catalase (CAT) activity was assayed according to the method described by Aebi (1983) SOD was estimated according to the method described by Fridovich and Mc-Cord (1969). MDA was determined by spectrophotometric method as described by Wallin, Rosengren,

Shertzer, and Camejo, (1993). Triglycerides and total cholesterol concentration was determined according to method of Allain, Poon, Richmond and Fu, (1976). High density lipoprotein cholesterol (HDL-c) concentration was determined by centrifugation method as described by Albers, Warmick, and Cheng, (1978). The equation method of *Friedewald, Levy, and Fredrickson,* (1972) was used in the determination of LDL-c concentrations of the samples.

Statistical Analysis

These analyses were estimated using computer software known as Statistical Package for Social Sciences (SPSS), version18. Data are shown as Mean ± Standard Deviation (n=6). Mean values with different alphabets are significantly different at $p<0.05$.

Results

Percentage Parasitemia in *Plasmodium berghei* infected Mice Treated with Ethanol Leaf Extract of *Stachytarpheta cayennensis.*

The treatment of *Plasmodium berghei*-infected mice with ethanol leaf extract of *Stachytarpheta cayennensis* at the doses of 200, 400, 800 and 1600 mg/Kg body weight of the mice showed a significant ($p<0.05$) dose-dependent decrease in percentage Parasitaemia count as shown in Figure 1.

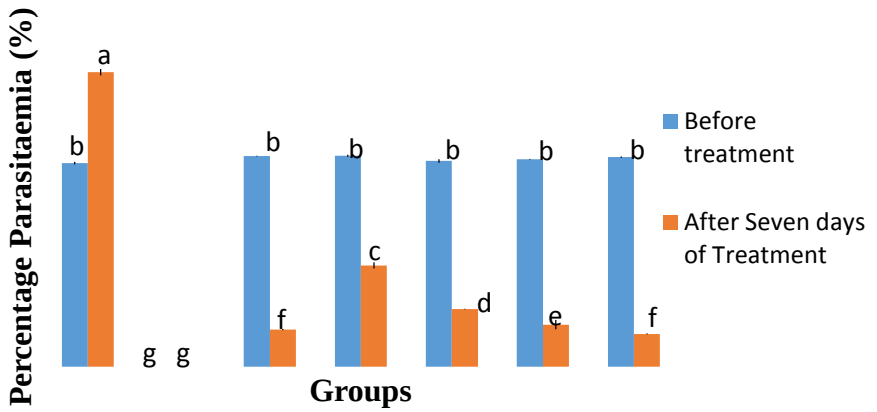

Figure 1: Parasite count in Mice Treated with Ethanol Extract of *Stachytarpheta cayennensis* Leaves.

Effect of Ethanol Leaf-extract of *Stachytarpheta cayennensis* on Oxidative Stress Parameters of *Plasmodium berghei* infected Mice

The result showed that activities of catalase and superoxide dismutase 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. However, treatment of the infected mice with graded doses of *Stachytarpheta cayennensis* ethanol leaf-extract and standard drug significantly ($p<0.05$) reversed the effect in dose-dependent manner to a level similar to that of the normal control as shown in Figures 2, 3 and 4. The effect of the extract, especially the highest dose of 1600 mg/Kg body weight was similar to that of the standard drug.

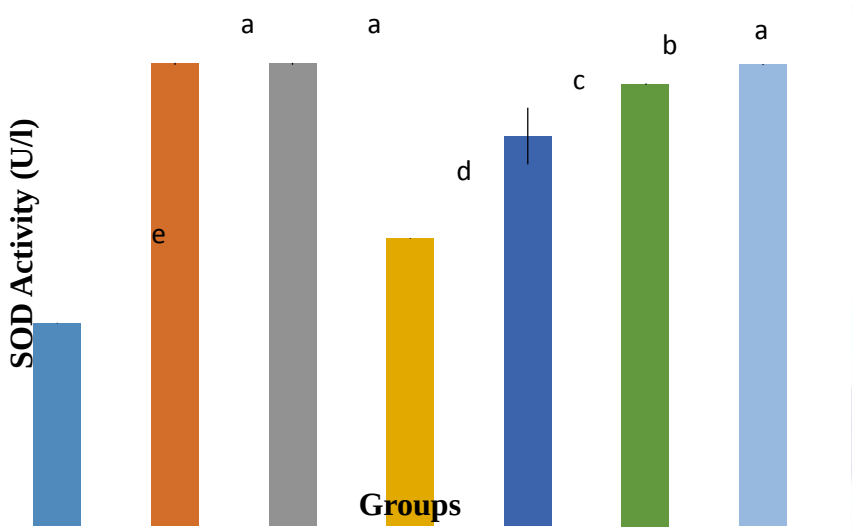

Figure 2: SOD Activity of *Plasmodium berghei*-infected Mice Treated with Ethanol Leaf-extract of *Stachytarpheta cayennensis*.

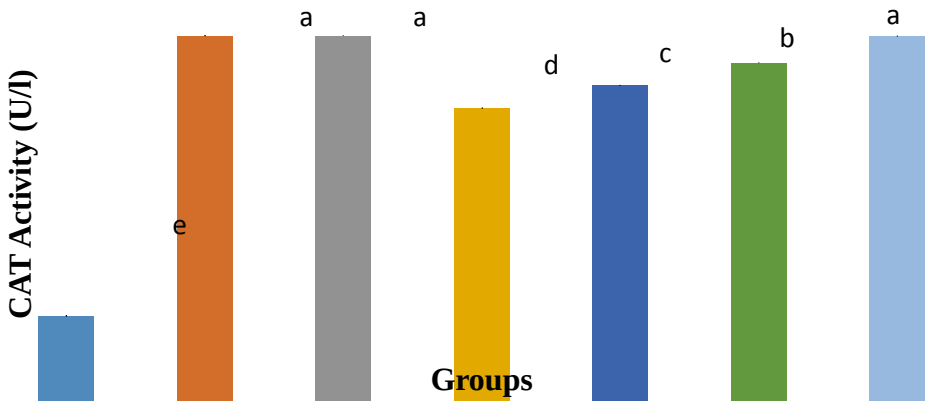

Figure 3: Catalase Activity of *Plasmodium berghei*-infected Mice Treated with Ethanol Leaf-extract of *Stachytarpheta cayennensis*.

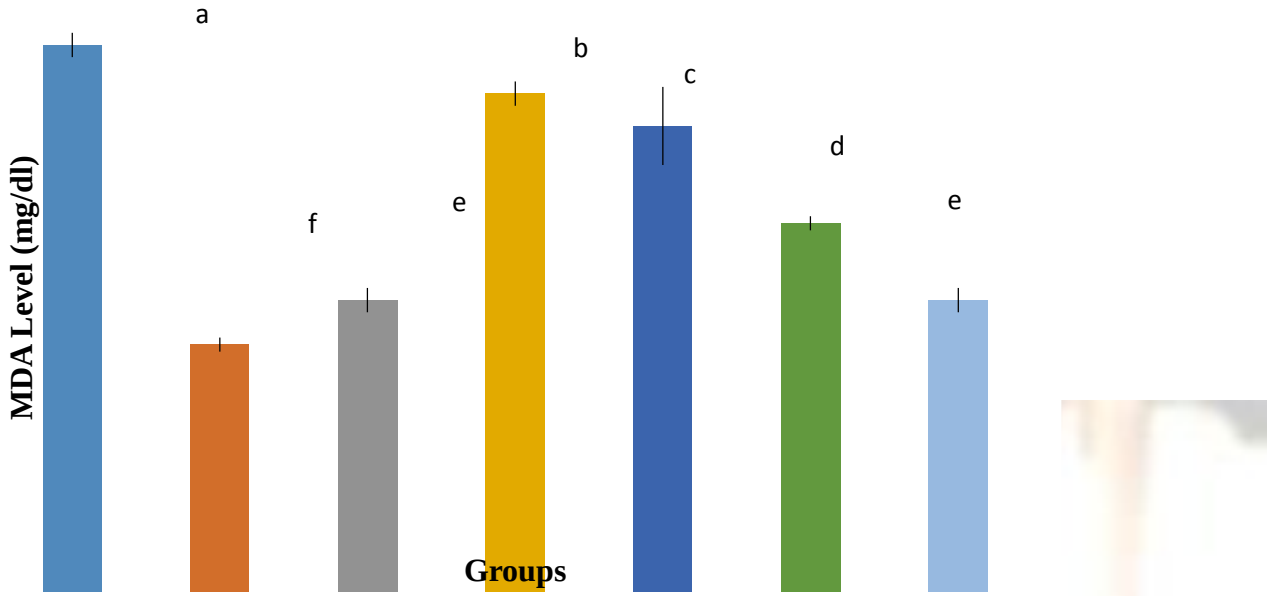

Figure 4: MDA Level of *Plasmodium berghei*-infected Mice Treated with Ethanol Leaf-Extract of *Stachytarpheta cayennensis*.

Effect of Ethanol Leaf-extract of *Stachytarpheta cayennensis* on Lipid Profile of *Plasmo-dium berghei*-infected Mice

Infection of mice with *Plasmodium berghei* caused a significant decrease ($p<0.05$) in levels of high density lipoprotein cholesterol and total cholesterol and significant increases ($p<0.05$) in triacylglycerides and low density lipoprotein cholesterol relative to normal control as shown in Figure 5, 6, 7 and 8. However, treatment of the infected mice with the ethanol leaf-extract of *Stachytarpheta cayennensis* at the doses of 200, 400, 800 and 1600 mg/Kg body weight of the mice showed a significant ($p<0.05$) dose-dependent reversal in the trends of these parameters towards the level found in the normal control. The effect of the extract, especially the highest dose of 1600 mg/Kg body weight was similar to that of the standard control group.

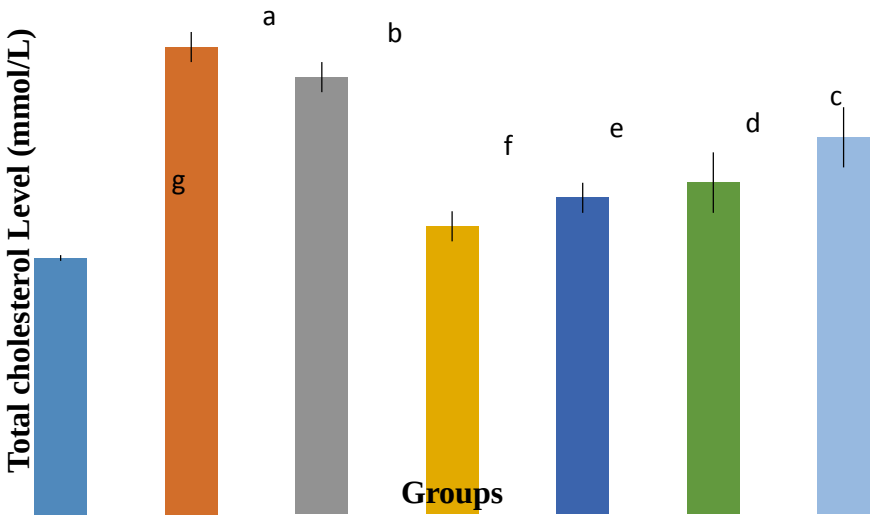

Figure 5: Total Cholesterol Level of *Plasmodium berghei*-infected Mice Treated with Ethanol Leaf-extract of *Stachytarpheta cayennensis*.

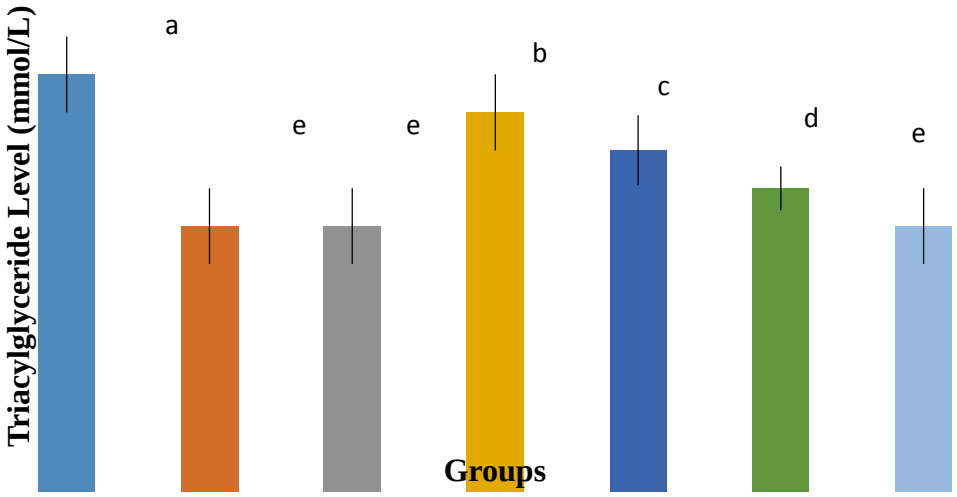

Figure 6: TAG Level of *Plasmodium berghei*-infected Mice Treated with Ethanol Leaf-extract of *Stachytarpheta cayennensis*.

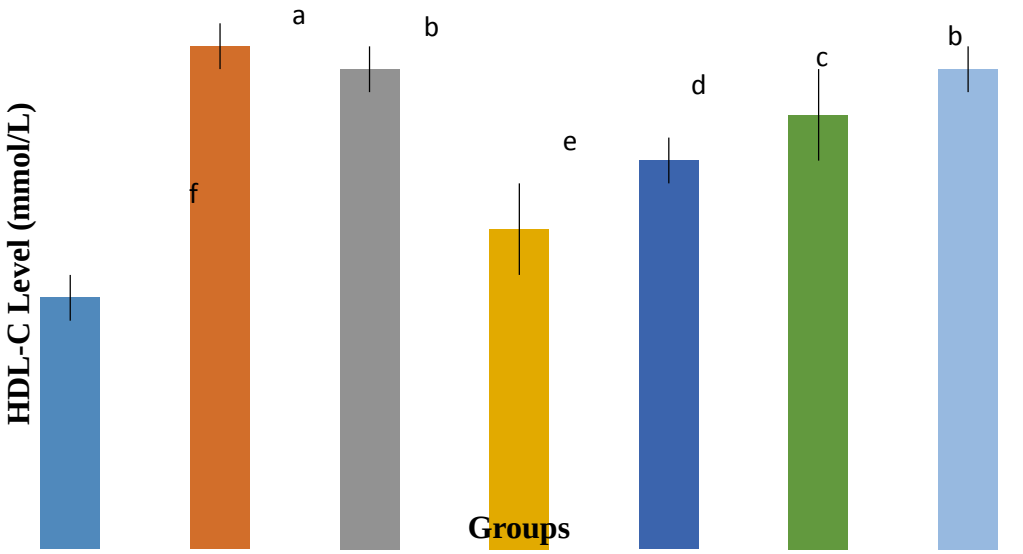

Figure 7: HDL-C Level of *Plasmodium berghei*-infected Mice Treated with Ethanol Leaf-extract of *Stachytarpheta cayennensis*.

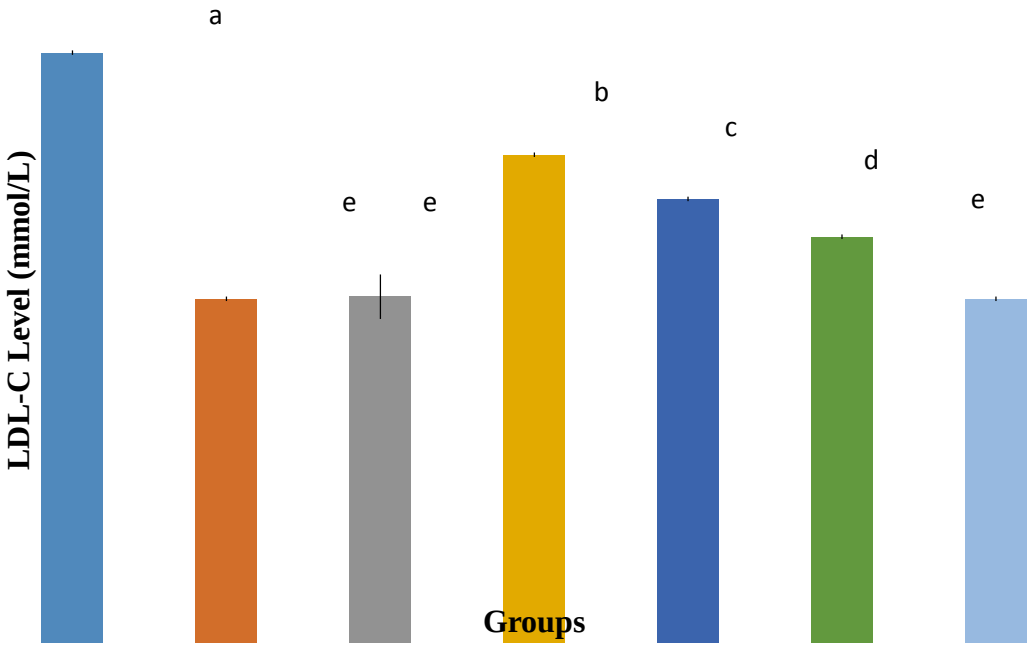

Figure 8: LDL-C Level of *Plasmodium berghei*-infected Mice Treated with Ethanol Leaf-Extract of *Stachytarpheta cayennensis*.

Discussion

Treatment of *Plasmodium berghei*-infected mice with ethanol leaf-extract of *Stachytarpheta cayennensis* and standard drug at the stated doses showed a significant ($p<0.05$) dose-dependent decrease in percentage Parasitaemia count. *Stachytarpheta cayennensis* leaf has been reported to contain alkaloids, (Alice *et al.,* 1991), Ipolamide, beta hydroxyipolamide and verbascoside, (Schapoval *et al.,* 1998), steroids, triterpenes and irridoids (Futuro, 1997). Antiplasmodial screenings of plants have implicated alkaloids, terpenes and flavonoids in this activity (Philipson and Wright, 1991). Although the mechanism of action of this extract has not been elucidated, some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation (Etkin, 1997), or by inhibiting protein synthesis (Kirby *et al.,* 1989), depending on their phytochemical constituents. The extract could have exerted its action through either of the two mechanisms mentioned above or by some other unknown mechanism.

This result correlates with the findings of Ekpono *et al.* (2018), which stated a significant increase ($p<0.05$) in the level of parasitaemia in the parasitized untreated groups and a significant ($p<0.05$) dose and time dependent decrease in the level of parasitaemia in the parasitized groups treated with varying doses of ethanol root extract of *Sphenocentrum jollyanum.* The result also agrees with the report of Uraku *et al.* (2015), who 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 some medicinal plants and standard drug. The reduction in the percentage parasitemia may be due to effect of the extract on the parasites-*plasmodium* level (Odeghe *et al.,* 2012).

The result of the oxidative stress parameters in *Plasmodium berghei* infected mice showed that activities of catalase and superoxide dismutase were significantly lower ($p<0.05$) in the *Plasmodium berghei*-infected mice while level of malondialdehyde was significantly higher ($p<0.05$) relative to the normal control mice. These suggest an oxidative stress in parasitized mice (Ekpono *et al.,* 2018). This could be because of dynamic changes in multiple body systems which result in increased basal oxygen consumption (Romero *et al.,* 2003). Also, this might have occurred as a result of toxic effect due to upsurge of reactive oxygen species produced by immune system as well as synchronized release of oxygen radicals during haemoglobin degradation by malaria parasite (Erel *et al.,* 1997). However, treatment with graded doses of *Stachytarpheta cayennensis* ethanol leaf-extract and standard drug significantly ($p<0.05$) reversed the effect in dose-dependent manner.

This finding agreed with a report by Ekpono *et al.* (2018), that infection of the mice with *P. berghei* showed a significant decrease ($p<0.05$) in the activities of catalase and superoxide dismutase while level of malondialdehyde was significantly higher ($p<0.05$) relative to the normal control. However, treatment of the infected mice with the stated doses of *S. jollyanum* extract and standard drug significantly ($p<0.05$) reversed the trend of these parameters in a dose dependent manner. Olorunnisola and Afolayan (2013) reported that treatment of parasitized mice with leaf extract of *S. jollyanum* showed significant reductions ($p<0.05$) in elevated levels of serum, kidney and liver malondialdehyde (MDA) concentrations, but caused a significant increased ($p<0.05$) in the activities of serum and liver catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) level when compared with parasitized non-treated group (PNT). These findings equally agrees with the study by Agbafor *et al.* (2015), who reported a general significant decrease ($p<0.05$) 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 peroxidase activities as well as reduced glutathione concentrations in the treated mice.

Increase in the activities and concentrations of these parameters in parasitized untreated mice were an indication of a high amount of free radical generation which promotes oxidative stress induced by malaria parasites of *Plasmodium berghei* and the host cell. Similarly, decreased SOD and CAT activities are linked up to exhaustion of the enzymes as a result of oxidative stress caused by *Plasmodium berghei* (Ekpono *et al.,* 2018). Restoration of SOD and CAT activities by the extracts indicates that the extracts scavenged superoxide radicals. Thus, the reversal effects were their protective actions via a decreased production of *Plasmodium berghei* derived free radicals or through the antioxidant activity of the bioactive agents in the plant extract (Ekpono *et al.,* 2018).

The result of lipid profile showed that infection of mice with *Plasmodium berghei* caused a significant decrease ($p<0.05$) in levels of high density lipoprotein cholesterol and total cholesterol and significant increase ($p<0.05$) in triacylglycerides and low density lipoprotein cholesterol relative to normal control. However, treatment of the infected mice with the ethanol leaf-extract of *Stachytarpheta cayennensis* at the doses of 200, 400, 800 and 1600 mg/Kg body weight of the mice showed a significant ($p<0.05$) dose-dependent reversal in the trends of these parameters towards the level found in the normal control.

The decrease in LDL-c demonstrated the presence of hypolipidaemic agent in the extract. This property has been attributed to the presence of some of the phytochemicals like saponins and alkaloids identified in the crude extract of this plant (Kabiru *et al.*, 2012). 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 (Barnett and O'Gara, 2003; Ogbonnia *et al.,* 2014). The beneficial effect of the extract on lipid profile status accounts for its use in the treatment of diabetes and diabetic complications ((Barnett and O'Gara, 2003). These positive effects on lipid profile status 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 (Achuba, 2005).

This finding agrees with the work of Ekpono *et al.* (2018), who reported that infection of mice with *Plasmodium berghei* caused a significant increase ($p<0.05$) in the levels of low density lipoprotein cholesterol (LDL-C), triacylglycerides (TAG) and significant decrease ($p<0.05$) 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* reversed the trends of these parameters. It also agrees with the report of Mbaka *et al.* (2010) 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.

Conclusion

This study provides scientific evidence on the antimalarial and antioxidant potentials of *Stachytarpheta cayennensis*. It also proves that this plant could be helpful in the management of lipid related disorders.

Recommendations

The individual phytochemical constituents of this plant can be isolated and their biological activities and effects tested on different models in order to bring about the discovery of novel and effective anti-malaria drugs. Other disease conditions may also be induced on experimental model and treated with this extract to obtain its efficacy.

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