

Effect of Ethanol Root-Extract of *Sphenocentrum jollyanum* on Liver function 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 Liver function Indices in *Plasmodium berghei*-infected mice. Liver function Indices were determined using standard methods. A total of 36 albino mice were used for the study. They were acclimatized for one week and then randomly assigned into 6 groups of A-F with 6 mice in each group. Mice in group A (Normal control) were given normal saline only. Mice in groups B-F were infected with *Plasmodium berghei* intra-peritoneally. Mice in group B (Standard control) were subsequently treated with standard drug (Lonart®-DS) at a dose of 5 mg/kg body weight of the mice; mice in group C (Positive control) were left without treatment while mice in group D, E and F (treated groups) were treated with graded doses (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 *ad libitum*. Oral route was used for the administration of the Lonart® and the extract. The treatment lasted for ten (10) days. The result showed that infection of mice with *P. berghei* resulted to significant ($P < 0.05$) decrease in the body weight of the mice relative to normal control. Infection of the mice with *P. berghei* also caused significant ($P < 0.05$) increase in the activities of ALT, AST and ALP and level of bilirubin while levels of albumin and total protein decreased significantly ($P < 0.05$) relative to the normal control. However, treatment of the infected mice with graded dose of the extract (200, 400 and 800 mg/Kg) caused a reversal in the pattern of this parameters in a dose dependent manner during the treatment period to a level comparable to the level observed among the standard control group. This study indicates that ethanol root extract of *Sphenocentrum jollyanum* contain principles that may be useful in the management of liver disorders elicited by *Plasmodium berghei* infection.

Keywords: *Plasmodium berghei*, infected mice, *Sphenocentrum jollyanum*, Albumin, Total bilirubin and Total protein.

INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan (a group of single-celled microorganisms) belonging to the *Plasmodium*

species [1]. Malaria parasites belong to the genus *Plasmodium* (phylum Apicomplexa). *Plasmodium berghei* is a protozoan parasite that causes malaria in certain rodents. Due to its ability to infect rodents and relative ease of genetic engineering, *P. berghei* is a popular model organism for the study of human malaria [2]. Like all malaria parasites of mammals, including the four human malaria parasites, *P. berghei* is transmitted by Anopheles mosquitoes and it infects the liver after being injected into the blood stream by a bite of an infected female mosquito. The multiplication of the parasite in the blood causes the pathology such as anaemia and damage of essential organs of the host such as lungs, liver and spleen. Malaria in Nigeria is holoendemic i.e. there is an intense all-year round transmission with greater intensity in the wet season than dry season.

The cells of the liver, which are known as hepatocytes carry out many biochemical activities. Some of these biochemical activities carried include excretion of bile, carbohydrate metabolism, protein metabolism, synthesis of blood clotting factors, storage of iron and some vitamins, detoxification and lipid metabolism [3]. It is therefore very obvious that any disease condition or adverse physiological conditions, which affect the hepatocytes, will cause concerted and tremendous metabolic derangement. In such conditions also, there will be an increase in the serum activities of the mitochondrial-bound liver enzymes since hepatocytic damage causes their release into the serum. It is therefore very pertinent to ascertain the effect of any ingestible food or drug on the serum activities of the liver enzymes so as to ensure the hepato-protectiveness of such food or drug. This can be achieved through liver function tests, which include estimation of plasma protein, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), albumin and bilirubin [3].

The World Health Organisation has estimated that perhaps 80 % of 6 billion people on earth rely upon traditional medicine for their primary health care needs, and a major part of this therapy involves the use of plant extracts or their active principles. Traditionally, some plants species are known to have anti-malaria and hepato-protective properties, with the folkloric use of some of these plants species in the treatment of malaria and diabetes has been proven experimentally [4].

Sphenocentrum jollyanum (family Menispermaceae) popularly known as Aduro kokoo (red medicine) and Okramankote (dog's penis) in the Akan language of Ghana is a small erect sparsely branched shrub which grows up to 1.5 meter in height [5]. Different part of the plant has been used extensively for the treatment of various ailments in West Africa Sub-

region. Extracts from the root have been used for the relief of constipation, medicine for treatment of sickle cell disease, rheumatism, aphrodisiac and other inflammatory conditions [5 and 6]. Decoction prepared from fruits, together with the fruits of *Piper guineense* and lime juice, is used for relief of cough. The plant is reported to possess exceptional wound healing properties. Some scientific research has been done on this plant in relation to its antiviral and anti-inflammatory activities, antiviral and antiangiogenic property Nia *et al.*, (2004), [7] and also Raji *et al.*, (2006), [8], have shown that methanolic extract of the root of *Sphenocentrum jollyanum* increased the testosterone levels in dose dependant manner and also reduced the spermatozoa count in mortality and viability in albino rats. In view of the aforementioned medicinal properties of *Sphenocentrum jollyanum*, the present study therefore evaluates the liver function potential of ethanol root extracts of *Sphenocentrum jollyanum* in *Plasmodium berghei*-infected mice.

Aim and Objectives

This study was designed to evaluate the effect of ethanol root-extract of *Sphenocentrum jollyanum* on liver function indices in *Plasmodium berghei*-infected mice

MATERIALS AND METHODS

Plants Collection

Fresh roots of *Sphenocentrum jollyanum* were collected from Aghara-ozza 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°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 [9].

Collection of Blood from Animals

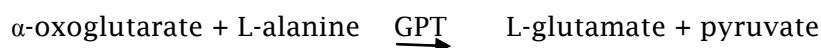
The mice were starved for 12 hours after ten days of administering the mice with the plant extract and Lonart®. The blood samples were collected from the eyes of the mice via ocular puncture using capillary tubes.

Determination of Liver Function Parameters

Determination of Alanine Aminotransferase (ALT) Activity

The activity of ALT was assayed according to the method of [10].

Principle: α -oxoglutarate react with L-alanine to yield L-glutamate and pyruvate and ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.



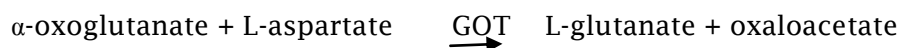
Procedure: Two test tubes were prepared, reagent blank and sample test tubes. Exactly 0.1 ml of the sample which is the serum was added to the sample test tube. Then 0.5 ml of solution R₁ were added to both blank and sample test tubes followed by addition of 0.1 ml of distilled water to the reagent blank only. The tubes were mixed and incubated for exactly 30 minutes at the temperature of 37°C. After that, 0.5 ml of solution R₂ was added to the two test tubes. The test tubes were mixed and allowed to stand for exactly 20 minutes at 20-25°C. Exactly 0.5ml of 0.4N sodium hydroxide was finally added to the test tubes. The tubes were mixed again and the absorbance of the sample (A_{sample}) against the reagent blank was read at the wavelength of 546 nm after 5 minutes.

Calculation: The activity of ALT in the serum was obtained from the standard table

Determination of Aspartate Aminotransferase (AST) Activity

AST activity was assayed according to the method described by [10].

Principle: This was based on the reaction between α -oxoglutarate and L-aspartate to yield L-glutamate and oxaloacetate catalyzed by GOT. AST was then measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.



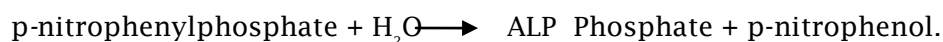
Procedure: Two tests tubes were arranged, the sample and reagent blank test tubes. To the sample test tube, exactly 0.1 ml of the sample (serum) was added followed by addition of 0.5 ml of the reagent1 to the two test tubes. After that, 0.1ml of distilled water was added to the reagent blank test tube. The test tubes were mixed properly and incubated for exactly 30 minutes at 37°C. After the incubation, 0.5 ml of reagent 2 was added to the two test tubes, mixed and allowed to stand for exactly 20 minutes at 20-25°C. Finally, 5.0 ml of 0.4N sodium hydroxide was added to each of the test tubes, mixed and after 5 minutes, the absorbance of sample (A_{sample}) was read against the reagent blank at the wavelength of 546 nm.

Calculation: AST activity was obtained from the standard table.

Determination of Alkaline Phosphatase (ALP) Activity

ALP activity was assayed by colorimetric method as described by [11].

Principle: The principle of this test was based on the hydrolysis of p-nitrophenylphosphate to yield phosphate and p-nitrophenol catalyzed by ALP.



Procedure: Exactly 0.01 ml and 0.5 ml of the sample and reagent respectively were added into micro cuvette. The mixture was properly mixed and the initial absorbance was read at 405 nm wavelength and timer start simultaneously. The absorbance was read again after 1, 2 and 3 minutes to get the changes in absorbance.

Calculation: ALP (U/L) = 2760 x ΔA nm/minutes

Determination of Total Bilirubin Concentration

Total bilirubin concentration was determined according to the method described by [12].

Principle: The principle was based on colorimetric method whereby total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphanilic acid.

Procedure: Two cuvettes were prepared and arranged, the sample blank and sample cuvette. Exactly 4 drops (200 μl) of reagent 1 was added to the two cuvette followed by addition of 1 drop (50 μl) of the reagent two only to the sample cuvette. After that, 1000 μl and 200 μl of reagent three and sample (serum) respectively were added to the two cuvettes. The cuvette was properly mixed and incubated for 10 minutes at 20-25°C. Finally, 1000 μl of reagent four was added to both sample blank and sample cuvette, mixed and incubated for a further 5-30 minutes at 25°C and the absorbance of the sample against sample blank was read at the wavelength of 578 nm.

Calculation: T. Bilirubin (mg/dl) = 10.8 x A_{TB}

Determination of Albumin Level (ALB)

Albumin concentration was determined according to the method of [13].

Principle: The principle was based on the quantitative binding of albumin to the indicator 3, 3', 5, 5'-tetrabromo-m cresol sulphonephthalein (bromocresol green BCG). The albumin-BCG-complex absorbed maximally at 578 nm and the absorbance was directly proportional to the concentration of albumin in the sample.

Procedure: Test tubes were prepared and designated as reagent blank, standard and sample test tubes. Exactly 0.01 ml of distilled H₂O was added to the reagent blank. Then 0.01 ml of standard was added to standard test tube followed by the addition of 0.01 ml of serum to the sample test tube. Finally, 3.0 ml of BCG reagent was added to the three test tubes. The tubes were mixed properly and incubated for 5 minutes at 20-25°C. The absorbance of the sample (A_{sample}) and that of the standard (A_{standard}) were read against the reagent blank at the wavelength of 630 nm.

Calculation:

$$\text{ALB Conc. (}\frac{\text{g}}{\text{dl}}\text{)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

Determination of Total Protein

This was determined according to the method of [14].

Principle: The principle was based on the formation of a coloured complex when cupric ions in an alkaline medium interact with protein peptide bonds.

Procedure: Test tubes were prepared, reagent blank, standard and sample test tubes. Exactly 0.04 ml each of distilled water, standard and serum were added to reagent blank, standard and sample test tubes respectively. This was followed by addition of 2.0 ml of reagent one to all the test tubes. The mixture was incubated for 30 minutes at 20-25°C and the absorbance of the sample was read at 570 nm wavelength against the reagent blank at a room temperature.

Calculation: $\text{Total Protein Conc. (g/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration}$

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.

RESULT

Effect of Ethanol Root Extract of *Sphenocentrum jollyanum* on the body Weight of *Plasmodium berghei* infected Mice

The result showed that the weight of the infected mice but not treated with either the extract or the standard drug decreased significantly ($P < 0.05$) as the days of the exposure increased. But treatment with the extract and standard drug reversed the effect on the weight which then increased as the days of exposure increased. The effect of the standard drug was higher than that of the extract as shown in Figure 1.

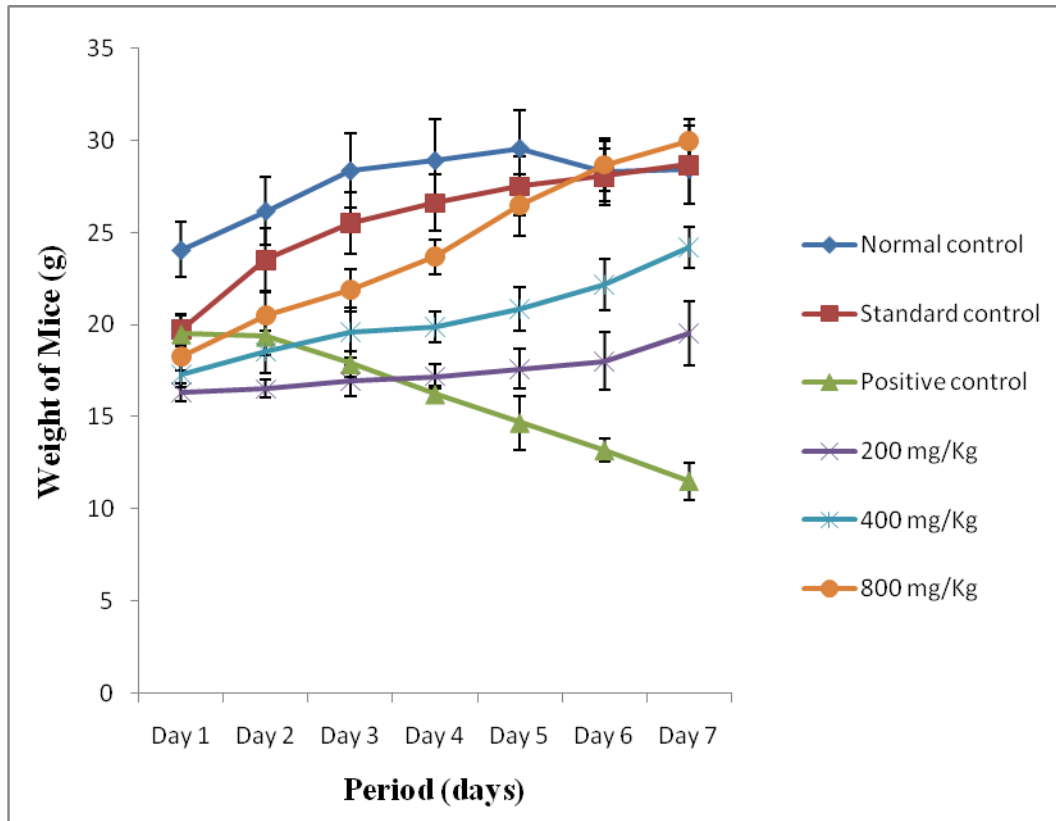


Figure 1: Weight of Mice during the last Seven (7) Days of Treatment with Ethanol Extract of *Sphenocentrum jollyanum* Root.

Data are shown as Mean ± Standard Deviation (n=6).

Effect of Ethanol Root Extract of *Sphenocentrum jollyanum* on Liver Function indices in *Plasmodium berghei*-infected Mice

Infection of the mice with *P. berghei* caused significant ($P < 0.05$) increase in the activities of ALT, AST and ALP and level of bilirubin while levels of albumin and total protein decreased significantly ($P < 0.05$) relative to the normal control. However, treatment of the infected mice with graded dose of the extract (200, 400 and 800 mg/Kg) caused a reversal in the pattern of this parameters in a dose-dependent manner during the treatment period to a level

comparable to the level observed among the standard control group. 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 difference ($P < 0.05$) between the groups.

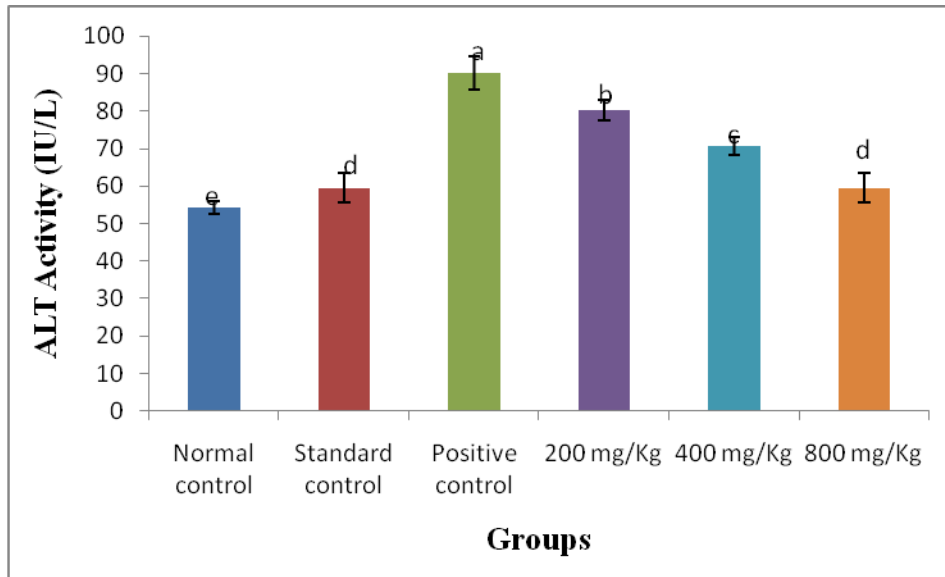


Figure 2: ALT Activity in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

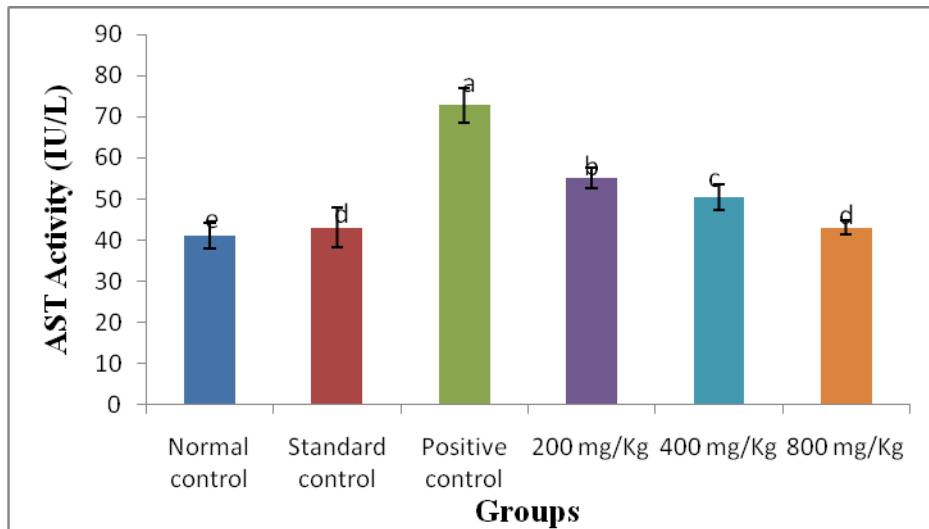


Figure 3: AST Activity in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

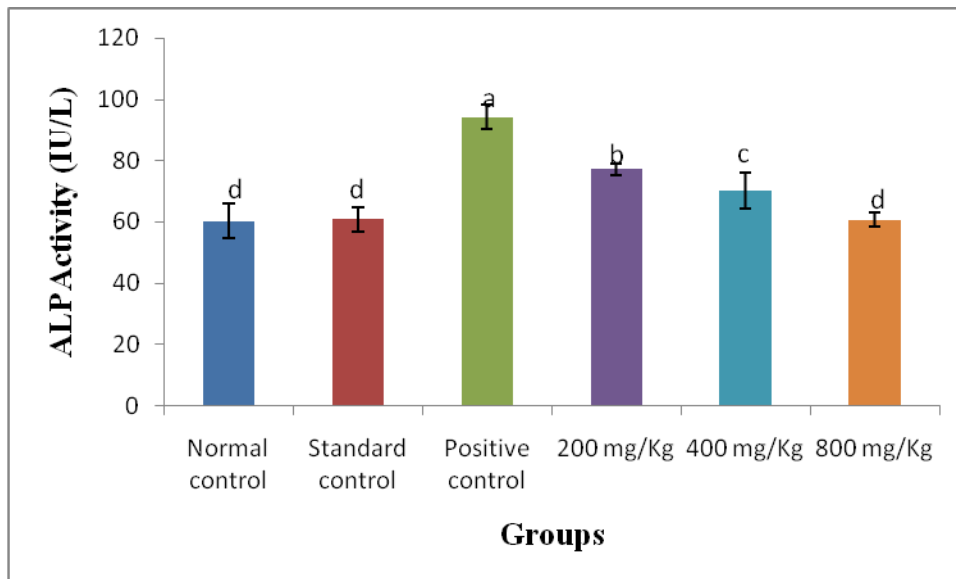


Figure 4: ALP Level in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

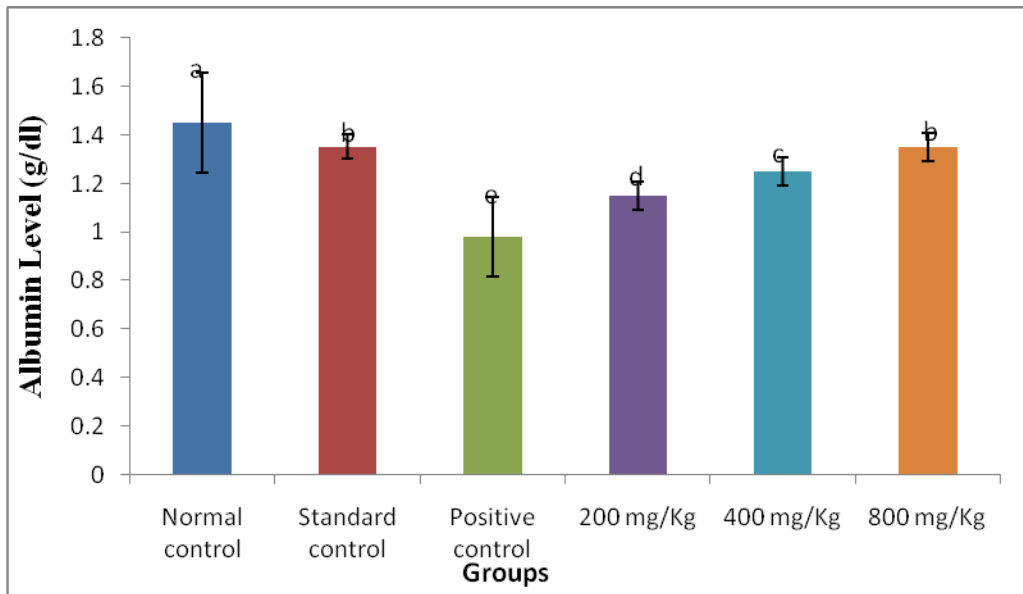


Figure 5: Albumin Level in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

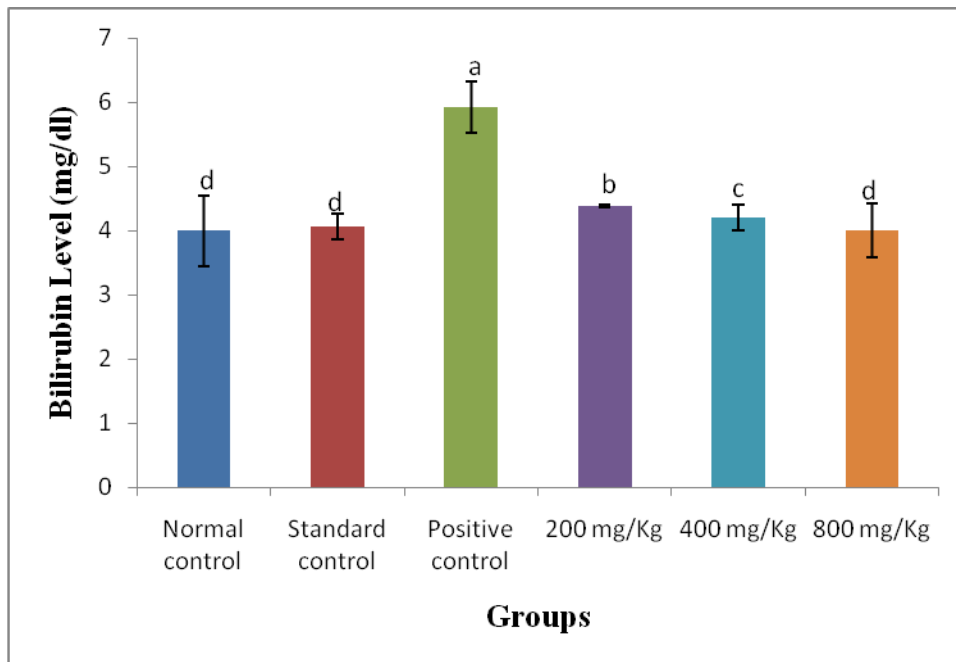


Figure 6: Bilirubin Level in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

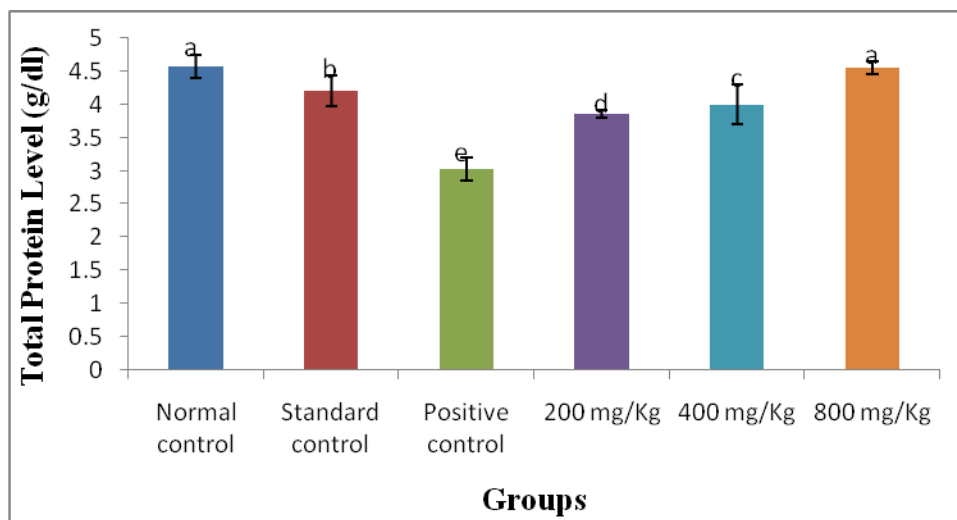


Figure 7: Total Protein Level in Mice Treated with Ethanol Extract of *Sphenocentrum jollyanum* Root.

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

DISCUSSION

The results showed that infection of mice with *Plasmodium berghei* significantly (P<0.05) reduced the body weight relative to the level found in the normal control mice. But treatment of the infected mice with graded doses of *Sphenocentrum jollyanum* root extract significantly (P<0.05) restored the body weight in the animals 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. This observation could be attributed to malaria infection. Infection of animals with malaria parasite leads to loss of weight due to loss of appetite or blood quality of the animals (Haruna *et al*, 2013), [15], as well as generation of reactive oxygen species (ROS) by the parasites inside host erythrocytes [16]. The gain in weight of animals treated with root extract of *Sphenocentrum jollyanum*

may be attributed to the presence of some metabolites found in the plant that reduce the level of malaria parasite and thereby increase appetite of the animals.

Infection of the mice with *Plasmodium berghei* also caused significant ($P < 0.05$) increase in ALT, AST and ALP activities relative to the normal control. This result is in consistent with studies which reported that majority of malaria patients showed elevated serum activities of AST, ALT and ALP indicating liver damage [17]. 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 ($P < 0.05$) reduction in activities of these parameters as shown in Figure 2, 3 and 4 respectively in a dose dependent manner during 10 days treatment to a level comparable to the level observed among the standard control group especially, the highest dose of 800 mg/Kg body weight as there was no significant ($P < 0.05$) difference between them. This finding correlates with the following findings; Olorunnisola and Afolayau (2013), [18], which reported that treatment of parasitized mice with leaf extract of *S. jollyanum* had a significant ($P < 0.05$) reductions in elevated levels of AST, ALT, ALP, and GOT when compared with parasitized non-treated group (PNT), Uraku, (2016), [19], which reported a dose dependent significant reductions ($P < 0.05$) in the activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in parasitized mice treated with the extract of *Spilanthes uliginosa*, *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citrates* leaves and Momoh *et al.* (2014), [20], which reported that treatment of *Plasmodium berghei*-infected mice with chloroquine and ethanolic leaf extract of *Alstonia boonie* significantly decreases ($P < 0.05$) plasma liver marker enzymes AST, ALT, ALP and GGT when compared to the untreated group.

This work disagrees with the following findings; Mbaka *et al.* (2010), [21], which reported no significant ($P < 0.05$) changes in alanine amino transferase (ALT) and aspartate amino transferase (AST) in the animals treated with extract of *Sphenocentrum jollyanum* leaves and Mbaka and Owolabi (2011), [22], which reported that the liver function indices of the rats treated with extract of *Sphenocentrum jollyanum* seed showed no significant ($p > 0.05$) difference relative to the control This variation in the results could be as a result of different locations in which the plants were collected or may be attributed to the part or species of the plant used. The difference may also be directly depended to the plant physiological

setup and climatic changes [23]. This variation may equally be attributed to the methodology, experimental model or doses of the extracts used.

An increase in the activities of ALT, AST and ALP in the blood serum of parasitized untreated mice may be as a result of liver injury and altered hepatocyte integrity caused by the *Plasmodium* infection and the consequent release of the enzymes into the blood stream. An activity of ALT rises in diseases is associated with death of hepatocytes like viral hepatitis [10]. AST on the other hands is not found to be specific for liver damages but has been found to be a cardiac marker as it is found in cardiac and skeletal muscles [24]. The serum ALP is related to the function of hepatic cell and increase in serum level of ALP may be due to increased synthesis of the enzymes in presence of increasing biliary pressure [25]. Generally, an increase in this enzyme indicates injury or toxicity to the organ. Several African medicinal plants have been shown to have curative effects [25]. This effect is possibly due to flavonoids, alkaloids and other chemical composition which exert membrane-stabilizing action that protects the liver cells from injury [24].

This result equally showed significant ($P < 0.05$) decrease in albumin and total protein levels and significant ($P < 0.05$) increase in total bilirubin level following *Plasmodium berghei* infection relative with the normal control. The decreased serum total protein and albumin in the parasitized non-treated mice was suggested to be due to cellular response to hyper-parasitemia [26]. A rise in serum bilirubin concentration is an indication of liver damage, parenchyl or biliary [27]. However, treatment of *Plasmodium berghei*-infected mice with graded dose of *Sphenocentrum jollyanum* ethanol root extract, caused a significant ($P < 0.05$) dose dependent reversal on the trend of these parameters in the mice, with 800 mg/Kg body weight exerting effect comparable to that of the standard drug (Lonar®) as shown in Figure 5, 6 and 7. This correlated with observation of Olorunnisola and Afolayan (2013), [18], which showed that leaf extract of *Sphenocentrum jollyanum* administered to malaria-infected mice significantly decreased the total bilirubin concentration. In conformation to the present study too, crude aqueous extract of the fruiting body of medicinal mushroom, *Ganoderma lucidum* has been found to significantly reduce the total bilirubin level in malaria-infected mice [28]. This current work also agrees with the finding of Momoh *et al.* (2014), [20], which reported that total protein and albumin values significantly increased

($P < 0.05$) in *Plasmodium berghei* infected mice treated with ethanolic leaf extract of *Alstonia boonie*.

The evidence showing that extract treatment caused a decrease in total bilirubin concentration and increase in levels of albumin and total protein revealed that this extract enhance the liver function of clearing the blood unconjugated hydrophobic bilirubin by combining it with glucuronic acid making it water soluble conjugated bilirubin using the enzyme UDP-glucuronide transferase.

CONCLUSION

This work support local users in the treatment of malaria due to its curative effect on liver function indices.

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