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Effect of *Buchholzia coriacea* Methanol Extract on Haematological Indices and Liver Function Parameters in *Plasmodium berghei*-Infected Mice

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Abstract: Background *Buchholzia coriacea* (Copparidaceae) is an evergreen shrub widely distributed in tropical Africa. In southeast Nigeria, the plant seed extract is used in folk medicine for the treatment of malaria and many other ailments. Alterations in haematological indices and liver function parameters are some of the complications of plasmodium infection which causes malaria. Previous studies demonstrated the antiplasmodial activity of *Buchholzia coriacea* seeds. The present research was designed to evaluate the role of *Buchholzia coriacea* seed extract in amelioration of altered haematological indices and liver function parameters in malaria parasite infected mice. Methods the effect of ethanol seed extract of *Buchholzia coriacea* on percentage parasitaemia, haematological indices and liver function parameters were investigated in malaria-induced mice. A total of thirty six mice (Both infected (Test) and non- infected (Control) consisting of six groups were used in the study. Groups 1-3 were treated with 100, 200 and 300 mg/kg body weight of ethanol seed extract of *Buchholzia coriacea* respectively; group 4 (Standard control) was treated with 28mg/kg body weight of artemeter/lumefatrine; group 5 (Positive control) and 6 (Negative control). Results: the results showed that percentage parasitaemia increased significantly ($p< 0.05$) in group 5 (Positive control) when compared to other groups. The activities of serum alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) as well as the levels of total serum bilirubin were significantly ($p<0.05$) higher in parasitized control mice compared with all other groups. Parasitized mice receiving 100 and 300 mg/kg body weight of *B. coriacea* as well as the standard reference drug have no significant difference ($p >0.05$) in serum AST, ALT, ALP activities as well as bilirubin concentration when compared with the negative control group. However, infection induced significant ($p <0.05$) decreases in packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cell count (RBC) of the positive control group when compared to parasitized- treated groups while the white blood cell count decreased significantly ($p <0.05$) in parasitized positive control group when compared to non- parasitized (Negative control group). Conclusion the results show that the seed extract of *B. coriacea* significantly reduced the parasitaemia in infected mice and restored altered haematological indices in malaria parasite infected mice. Also, treatment with the extract was able to ameliorate altered liver function parameters of malaria parasite- infected mice.

Key words: *Buchholzia coriacea* · *Plasmodium berghei* · Malaria · Haematological Indices Liver Function Parameters

INTRODUCTION

Resistance of Plasmodium falciparum to commonly used antimalarial drugs is increasing in Nigeria as in other parts of Africa [1]. This has resulted in resurgence in transmission and an increase in adverse outcomes due to therapy failure. More recently, artemisinin isolated from the Chinese plant *Artemisia annua*, has been used successfully against chloroquine-resistant *P. falciparum* strains [2]. Pure products have been isolated from some of these plants amongst which are those whose antimalarial activities are comparable to or more active than chloroquine on sensitive and resistant strains of *P. falciparum* [3]. Hence, the need to source antimalarial drugs from plants such as *Buchholzia coriacea*.

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Buchholzia coriacea is widely used in Nigeria by traditional medical practitioners for the treatment of various disorders including cough, urinary tract infections, bronchitis, fever, diabetes, inflammation and malaria [4,5]. The antiplasmodial activity of the seed of the plant has been demonstrated [6]. Previous studies also demonstrated the hypoglycemic activity [7] antibacterial and antifungal activities [8] as well as anti-inflammatory activity of the seed extract [9,10].

The plant seed extract also blocked histamine-induced contractile responses in isolated guinea-pig ileum [11].

There are five species of Plasmodium which can infect and be transmitted by humans. They include *P. falciparum*, *P. vivax*, *P. ovale*, *P. Malariae* and *P. knowlesi*. The vast majority of deaths are caused by *P. falciparum* and *P. vivax [*12]. Malaria is the world's most important parasitic disease especially when *Plasmodium falciparum* is the causative agent [13]. Malaria is endemic in about 100 developing countries and kills an estimated 1.2 million people each year in Africa [14]. In Nigeria and Sub-Saharan Africa, one in five children will die before they are five and 75% of those deaths are attributed to malaria [15]. Despite the need, no effective vaccine exists, although efforts to develop one are ongoing [16]. The present study is a scientific approach to re-establish the traditional uses of the plant seed as antimalarial drug and to evaluate the effect of the plant extract on haematological indices and liver function parameter in *Plasmodium berghei*-infected mice.

MATERIAL AND METHODS

Collection and Identification of Plant Material: Fresh seeds of *Buchholzia coriacea* were bought from Ogige market in Nsukka L.G.A of Enugu State, Nigeria. The seeds were identified by Mr. Alfred Ozioko, a taxonomist at Bioresource Development and Conservation Programme (BDCP) Center, Nsukka.

Chemicals: All chemicals used were of analytical grade and products of May and Baker, England, BDH, England, Riedel-de Haem, Germany, Damststadt, England. Assay kits for liver function tests were obtained from Randox Labouratories Ltd., UK. All other reagents used were of analytical grade and prepared in all glass-distilled water.

Animals: The experimental animals used for this study were white albino mice of either sex weighing 18-30g. The mice were 3-4 months old and were purchased from the animal breeding centre of the Department of Veterinary Medicine, University of Nigeria Nsukka. The mice were housed in metal cages and acclimatized for 7 days under standard laboratory conditions with a 12 hour light/dark cycle and maintained on a regular vital feed and water

Methods

Extraction of Plant Material: The seeds were shade-dried for one week, after which the seeds were pulverized into coarse form with a Crestor high speed milling machine. The coarse form (500g) was then macerated in 2.5 litres absolute ethanol. This was left to stand for 24 hours. After that the extract was filtered through muslin cloth and plug of glass wool in a glass column. The filtrate was concentrated and evaporated to dryness using rotary evaporator at temperature of 40^0C, to avoid denaturation of active constituents. The extract was weighed and recorded and stored in the refrigerator for further analysis.

Experimental Design: After the confirmation of parasitemia, the infected (Parasitized) and non-infected (Normal) were divided into 6 groups of 6 mice per group and treated as follows:

GROUP 1: Parasitized mice treated with 100mg/kg body weight of *Buchholzia coriacea* ethanol seed extract.

GROUP 2: Parasitized mice treated with 200mg/kg body weight of *Buchholzia coriacea* ethanol seed extract.

GROUP 3: Parasitized mice treated with 200mg/kg body weight of *Buchholzia coriacea* ethanol seed extract.

GROUP 4: Not parasitized mice (Standard control) and treated with 28 mg/kg body weight of artemeter/lumenfantrine (Standard drug).

GROUP 5: Parasitized and not treated (Positive control).

GROUP 6: Not parasitized and not treated (Negative control).

The administration of the extract was carried out using intra-gastric tube. Before the treatments, the mice in Groups 1-5 were inoculated with malaria parasite and 3 days after that, analyses were carried out to determine the baseline parameters in all the groups, then, two days later, treatment began. The treatment lasted for 5 days and analyses were done on day 3, day 5 and 28^{th} day of post treatment.

Determination Of Parasitemia: The determination of malaria parasitemia (Mp^+) was carried out according to the method of Dacie and Lewis [17]. This was assessed by their blood film made by collecting blood from the cut-tip of the tail and examined in oil immersion under a microscope.

Blood Sample Collection for Clinical Chemistry Determinations: Blood sample for clinical chemistry determinations was collected from the retro-bulbar plexus of the medial canthus of the eye of mice. A microhematocrit tube was carefully inserted into the medial canthus of the eye to puncture the retro-bulbar plexus and thus enable outflow of about 2ml of blood into a clean glass test tube. The blood sample was kept at room temperature for 30 minutes to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3000 revolutions per minute for ten minutes using a table centrifuge, to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was then carefully aspirated with syringe and needle and stored in a clean sample bottle for the clinical chemistry determinations.

Determination of Alanine Aminotransferase (Alt) Activity: Alanine aminotransferase activity was determined by the Reitman-frankel colorimetric method for *in vitro* determination [18].

Determination of Serum Alkaline Phosphatase Activity: Serum alkaline phosphatase activity was determined using Phenolphthalein monophosphate method of Klien *et al*. [19] and Babson *et al*. [20].

Determination of Aspartate Aminotransferase (Ast) Activity: Aspartate aminotransferase (AST) was determined by the Reitman-Frankel colorimetric method for *in-vitro* determination [18].

Determination of Serum Albumin Concentration: Serum albumin concentration was determined by the method of Anderson [21].

Determination of Total Serum Bilirubin: Total serum bilirubin was determined by Jendrassik-Grof method for the *in vitro* determination [22].

Statistical Analysis: The results were expressed as mean±SD and test of statistical significance was carried out using one-way analysis of variance (ANOVA). The means were separated using Duncan multiple Test. Differences were considered significant when $p < 0.05$. The statistical packaged used was the statistical package for social sciences (SPSS), version 17.

RESULTS

The results in Figure 1 show the effect of different doses of the extract on percentage of parasitemia. On day 3 after inoculation, there was no significant difference in the mean percentage of parasitemia of mice in group 5 (Positive control) ($p>0.05$) compared to groups 1-4. On day 3 of treatment, the mean percentage parasitemia of mice in group 1 decreased significantly ($p<0.05$) when compared to those of mice in groups 2, 3, 5,while the mean percentage parasitemia of mice in group 4 decreased significantly ($p<0.05$) when compared to that of mice in group 5 (Positive control).On day 5 of treatment, there was no significant change ($p>0.05$) in the the mean value for percentage parasitemia of mice in group 4 when compared to the mean values for mice in groups 1-3, while the mean percentage parasitemia of mice in group 5 (Positive control) increased significantly ($P<0.05$) when compared to those of other groups. On day 28 of post treatment, there was no significant change ($p>0.05$) in the mean value for percentage parasitemia of mice in groups 1-3 when compared to that of mice in group 4 (Standard control), while mean values for percentage parasitemia of mice in group 5 (Positive control) increased significantly ($P <0.05$) when compared to other groups.

Figure 2 shows the effect of ethanol seed extract of *Buchholzia coriacea* on white blood cell count. The WBC (Baseline) count obtained 3 days after inoculation for mice in groups 1-4 were not significantly different ($p>0.05$) when compared to the value obtained in groups 5(Positive control). On day 5, mean values for groups 1-5 mice were not significantly ($p>0.05$) different from that of group 6. On day 28 post- treatment, there were significant decreases ($p<0.05$) in WBC count in groups 1-4 when compared to group 5 and group 6 respectively, while there was no significant difference between the WBC values in groups 5 and 6.

Figure 3 shows the mean RBC in groups 1-6. Baseline values obtained 3 days after inoculation in groups 1-5 (Positive control) showed no significant difference ($p>0.05$) compared to the value in group 6 (Negative control) mice. Day 5 of treatment showed significant decreases ($p<0.05$) in RBC count of groups 1-5 (Positive control) mice compared to the mean value for RBC count of mice in group 6 (Negative control).

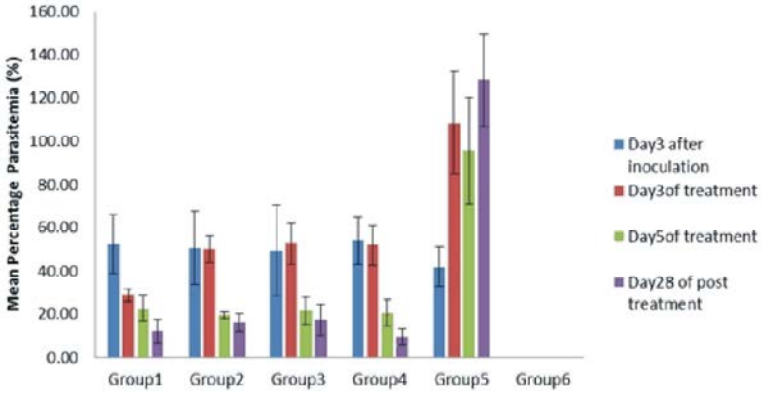

Fig. 1: The effect of ethanol seed extract of Buchholzia coriacea on Percentage Parasitemia in mice

Group1=100mg/kg b.w of *Buchholzia coriacea* Group 2=200mg/kg b.w of *Buchholzia coriacea*
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group4=28mg/kg b.w. Artemeter/lumenfantrine
Group5=Positive control Group 6=Negative control

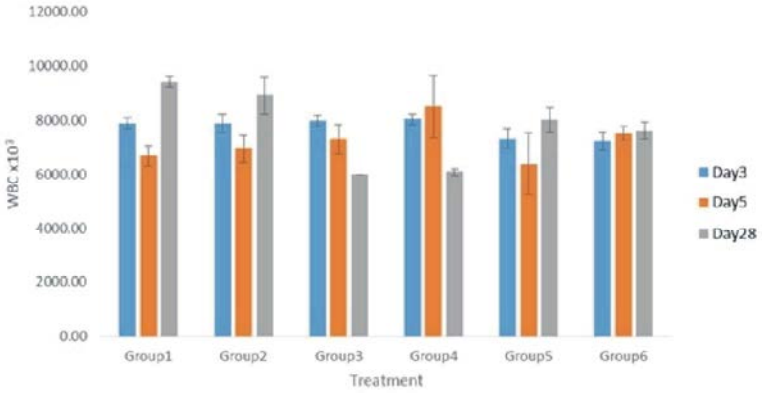

Fig. 2: Effect of seed Extract of *Buchholzia coriacea* on WBC in mice

Group 1= 100mg/kg b.w of *Buchholzia coriacea* Group 4=28mg/kg b.w of artemether/lumefantrine
Group 2= 200mg/kg b.w of *Buchholzia coriacea* Group 5= Positive Control
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group 6=Negative Control

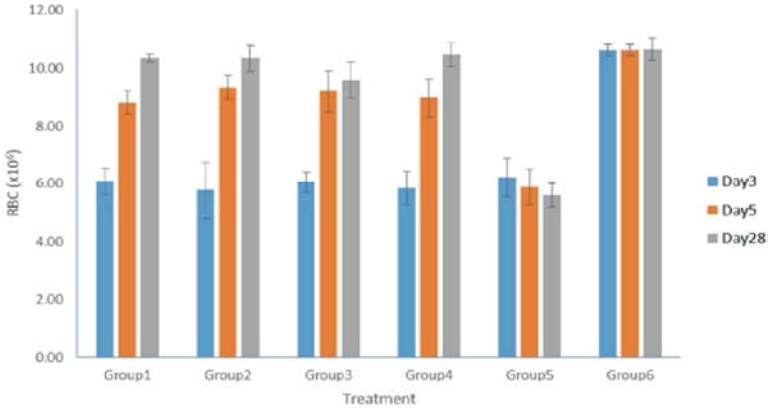

Fig. 3: Effect of seed Extract of *Buchholzia coriacea* on RBC in mice

Group 1= 100mg/kg b.w of *Buchholzia coriacea* Group 2= 200mg/kg b.w of *Buchholzia coriacea*
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group 4=28mg/kg b.w of artemether/lumefantrine
Group 5= Positive Control Group 6=Negative Control

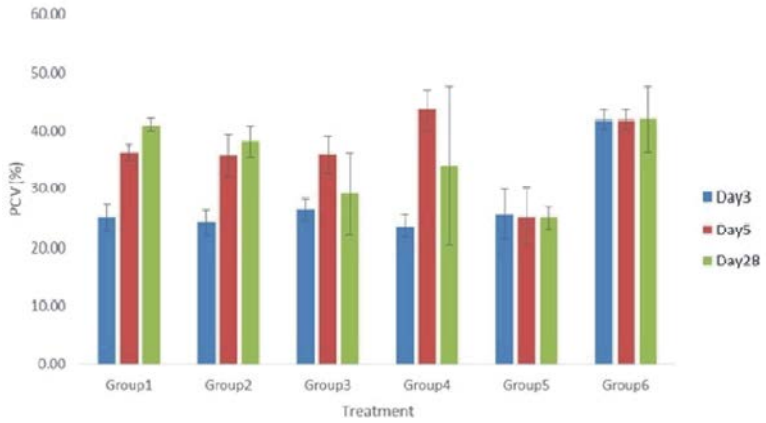

Fig. 4: Effect of seed Extract of *Buchholzia coriacea* on PCV in mice
Group 1= 100mg/kg b.w of *Buchholzia coriacea* Group 2= 200mg/kg b.w of *Buchholzia coriacea*
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group 4=28mg/kg b.w of artemether/lumefantrine
Group 5= Positive Control Group 6=Negative Control

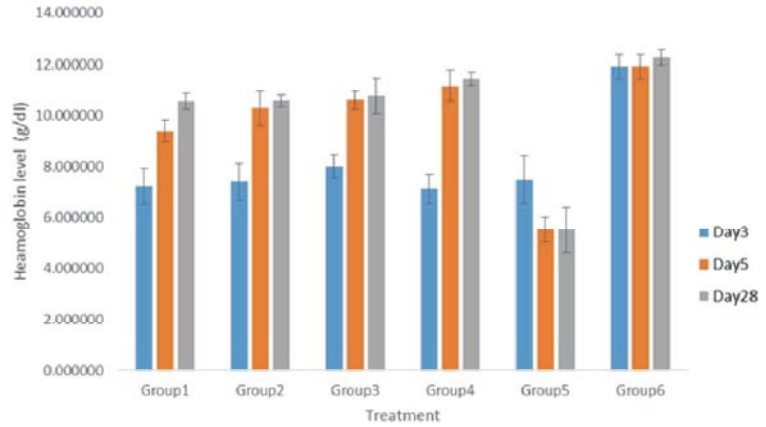

Fig. 5: Effect of seed Extract of *Buchholzia coriacea* on Hemoglobin in mice
Group 1= 100mg/kg b.w of *Buchholzia coriacea* Group 2= 200mg/kg b.w of *Buchholzia coriacea*
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group 4=28mg/kg b.w of artemether/lumefantrine
Group 5= Positive Control Group 6=Negative Control

Day 28 of post treatment showed significant increases ($p<0.05$) in the mean RBC count in groups 1- 4,6 (Negative control) mice when compared to the mean RBC count of group 5 (Positive control) mice. Also, there were significant decreases ($p<0.05$) in RBC count of groups 1-5 (Positive control) mice compared to the mean value for RBC count of mice in group 6 (Negative control).

Figure 4 shows the mean values of PCV of mice in groups 1-6. They were significantly decreased ($p<0.05$) when compared to group 6 (Negative control) after 3 days of inoculation. On day 5 of treatment, mean values for PCV for groups 1- 3 were similar and showed significant increase ($p<0.05$) when compared to group 5 (Positive control). On the other hand, values obtained for group 4 showed significant increase ($p<0.05$) above the value for animals in the groups1-3, 5 (Positive control) and 6. For day 28 post treatment, whereas the mean PCV values for groups 1-4 were not significantly ($p<0.05$) different from that of group 6,mean values for those in groups 1,2 showed significant increases ($p<0.05$) when compared to the value in group 5.

Figure 5 shows the mean values for hemoglobin in groups 1-6. 3days after inoculation, there were significant decreases ($p<0.05$) in the mean values for hemoglobin in other groups when compared to group 6. On day 5 of treatment the mean values for hemoglobin in groups 1-4, 6 significantly increased ($p<0.05$) when compared to group 5 (Positive control). Finally, on day 28 of post

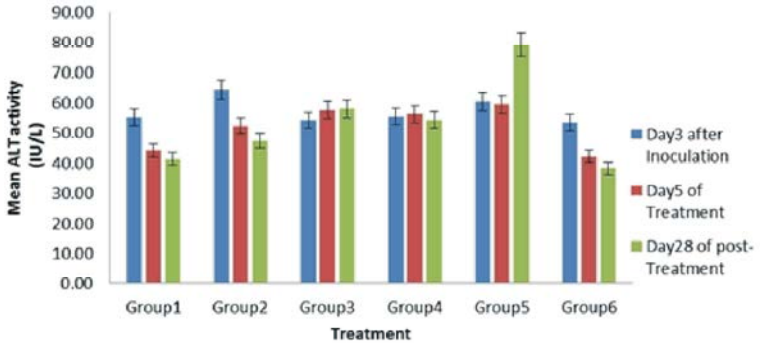

Fig. 6: Effect of ethanol seed extract of *Buchholzia coriacea* on serum ALT activity in mice
Group 1= 100mg/kg b.w of *Buchholzia coriacea* Group2= 200mg/kg b.w of *Buchholzia coriacea*
Group 3= 300mg/kg b.w of *Buchholzia coriacea* Group 4= 28mg/kg b.w.of Artemeter/lumenfantrine
Group 5 = Positive control Group 6 = Negative control

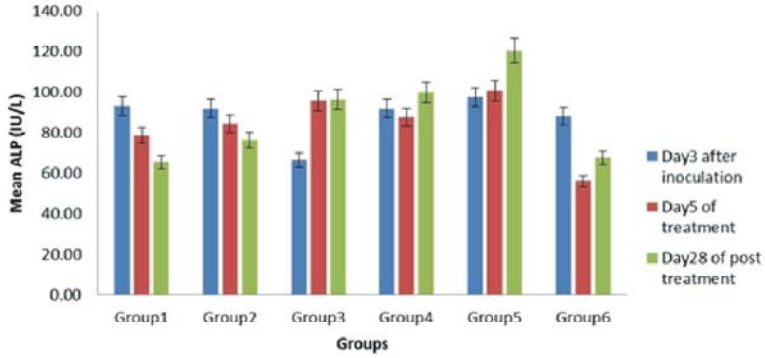

Fig. 7: Effect of ethanol seed extract of *Buchholzia coriacea* on serum ALP activity in mice.
Group 1=100mg/kg b.w of *Buchholzia coriacea* Group 2=200mg/kg b.w of *Buchholzia coriacea*
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group4=28mg/kg b.w.of Artemeter/lumenfantrine
Group5=Positive control Group 6=Negative control

treatment the mean values for hemoglobin in groups 1- 4, 6 (Negative control) significantly increased ($p<0.05$) when compared to group 5 (Positive control).

On day 3 after inoculation, Figure 6 shows that the mean value for serum ALT activity of group 2 increased significantly ($p<0.05$) when compared to group 6 (Negative control), while there were no significant increases ($p>0.05$) in the mean values for groups 1, 3-5 when compared to that of group 6 (Negative control). On day 5 of treatment, the mean values for ALT in groups 2-5 increased significantly ($p<0.05$) when compared to that of group 6 (Negative control), while that of group 1 did not increase significantly ($p>0.05$) when compared to group 6 (Negative control). On day 28 of post-treatment, the mean values for ALT of groups 3-5 increased significantly ($p<0.05$) when compared with that for mice in group 6 (Negative control), while that for groups 1, 2 did not increase significantly ($p>0.05$) when compared to that of group 6 (Negative control).

Figure 7 shows the effect of *B.coriacea* extract on the mean values of ALP in groups 1-6. On day 3 of inoculation, there were no significant difference ($p>0.05$) in the mean values of ALP in groups 1, 2, 4, 5 when compared to group 6 (Negative control). On day 5 of treatment, mean values for ALP in all groups increased significantly when compared to group 6 (Negative control). On day 28 post treatment, mean values for ALP in group 1 was not significantly different ($p>0.05$), from that of group 6 (Negative control). Also, the mean value for group 5 increased significantly ($p<0.05$) when compared to groups 1- 4,6.

Figure 8 shows the mean values of AST in groups 1-6. On day 3 after inoculation, mean values for AST in groups 1- 5 increased significantly ($p<0.05$) when compared to group 6 (Negative control). The mean values of groups 1-4 were not significantly different from ($p>0.05$) from that of group 5 (Positive control). On day 5 of treatment, mean values of all other groups increased

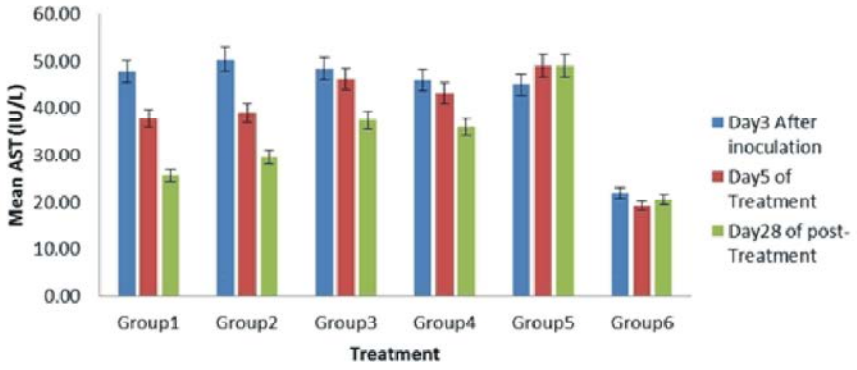

Fig. 8: Effect of ethanol seed extract of *Buchholzia coriacea* on serum AST activity in mice.
Group 1=100mg/kg b.w of *Buchholzia coriacea* Group 2=200mg/kg b.w of *Buchholzia coriacea*
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group 4=28mg/kg b.w.of Artemeter/lumenfantrine
Group5=Positive control Group 6=Negative control

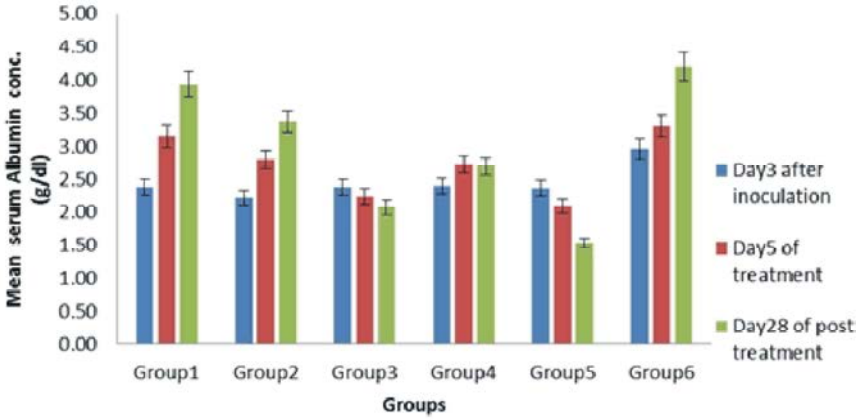

Fig. 9: Effect of ethanol seed extract of *Buchholzia coriacea* on concentration of serum albumin in mice.
Group 1=100mg/kg b.w of *Buchholzia coriacea* Group 2=200mg/kg b.w of *Buchholzia coriacea*
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group4=28mg/kg b.w.of Artemeter/lumenfantrine
Group5=Positive control Group 6=Negative control

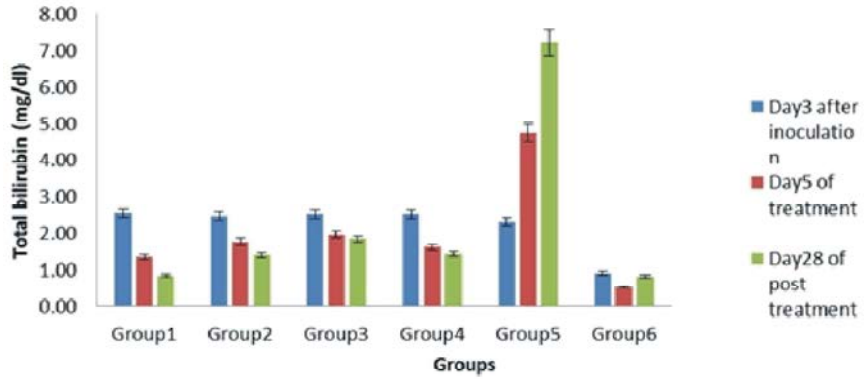

Fig. 10: Effect of ethanol seed extract of *Buchholzia coriacea* on the level of serum total bilirubin in mice.
Group 1=100mg/kg b.w of *Buchholzia coriacea* Group 2=200mg/kg b.w of *Buchholzia coriacea*
Group 3=300mg/kg b.w of *Buchholzia coriacea* Group4=28mg/kg b.w.of Artemeter/lumenfantrine
Group5=Positive control Group 6=Negative control

significantly ($p<0.05$) when compared to group 6 (Negative control). On day 28 of post treatment, the mean values of AST (In groups 1-4,6) decreased significantly ($p<0.05$) when compared to group 5. (Groups 1-4,6). Also, the mean values of all other groups increased significantly ($p<0.05$) when compared to group 6 (Negative control).

Figure 9 shows the effect of extract on serum albumin in groups 1- 6. On day 3 after inoculation in mice, there were no significant changes ($p>0.05$) in the mean values of serum albumin in group 6 (Negative control) when compared to other groups. On day 5 of treatment, the mean values for serum albumin of groups 1,2 were not significantly different ($p>0.05$) from that of group 6 (Negative control), while the mean values for groups 3- 5 (Positive control) decreased significantly ($p<0.05$) when compared with that for group 6 (Negative control). On day 28 of post treatment, there was significant increase ($p<0.05$) in the mean value of serum albumin in group 6 when compared to groups 2-5.

Figure 10 shows the effect of the ethanol extract on serum total bilirubin after inoculation in groups 1-6. On day 3 after inoculation, there were significant ($p<0.05$) increases in the mean values of serum total bilirubin in groups 1-5 when compared to that of group 6. On day 5 of post treatment, there was a significant increase in the mean value of total serum bilirubin in group 5 when compared to those of other groups. Also, on day 28 of post treatment, there was a significant increase in the mean value of total serum bilirubin in group 5 when compared to those of other groups. Also, there were significant ($p<0.05$) increases in the mean values for serum total bilirubin in groups 1- 5 when compared to that of group 6.

DISCUSSION

The evaluation of the effect of ethanol seed extract of *Buchholzia coriacea* on percentage parasitemia in groups treated with 100mg/kg, 200mg/kg and 300mg/kg body weight of the extract showed that the extract has antiplasmodial activity when compared to group 5 (Positive control). This is in accordance with the work of Okoli *et al*. [6] which demonstrated that the ethanol seed extract of *Buchholzia coriacea* reduced parasitemia level of malaria parasite -infested mice. This was demonstrated in Fig. 1 which showed a significant decrease ($P<0.05$) in percentage parasitemia of mice in extract- treated groups and the standard control groups.

As shown in Figures 3-5, infection caused significant decreases in PCV, Hb concentration and red blood count when compared to other groups. Packed cell volume and red blood cell concentration are used to assess anaemia, erythrocytosis, haemodilution and haemoconcentration. A decrease in packed cell volume and red blood cell could be as a result of anaemia [17]. Anemia is the major clinical sign and cause of mortality in animals with malaria infection where malaria parasites invade erythrocytes of infected animals, resulting in the destruction of parasitized erythrocytes. In this study, *B. coriacea* seed extract was able to significantly increase the reduced number of erythrocytes as well as the haemoglobin content and packed cell volume. At day 5 and day 28 of treatment, both the PCV and RBC increased significantly ($p<0.05$) in extract treated mice when compared to group 5. This suggests that the ethanol extract of *B. coriacea* contains biologically active agent that encourages proliferation of the cells at the period [23]. Similarly, the value of Hb also increased with the number of days of treatment as shown in Fig. 5. Also, the extract was able to significantly lower the increased number of WBC due to infection (Fig. 2).

A significant increase in the activities of ALT, AST and ALP in the blood of control parasitized mice as compared with all the other groups was observed and this was more pronounced on day 28 post -infection. The observed increase in enzyme activities may be as a result of liver injury and altered hepatocyte integrity caused by the plasmodium infection and consequent release of the enzymes into the blood stream. Alanine aminotransferase (AST) and alkaline phosphatase (ALP) are marker enzymes for liver function and are considered indicators of hepatocellular health [24].The administration of *B. coriacea* tends to normalize these enzymes (ALT, AST and ALP) activities. Treatment with extract in this study has shown that the extract protects hepatocyte integrity of parasitized mice. Also, treatment of parasitized mice with the extract was able to bring about significant increase in the serum albumin levels of parasitized non-treated control when compared to the treated groups (Fig. 9).

There was significant increase ($P<0.05$) in the level of total serum bilirubin of group 5 (Positive control) when compared to other groups, this could be as a result of destruction of red blood cells by the malaria parasite [25]. Treatment restored the alteration in total bilirubin concentration observed in the parasitized non- treated control group of mice (Fig. 10).

CONCLUSION

The results show that the plant extract significantly reduced the parasitaemia in the infected mice and also restored altered hematological and liver function

parameters in malaria parasiteinfested mice. This further support the use of ethanol seed extract of *Buchholzia coriacea* in numerous ethnomedicinal practices to combat malaria. This study also indicates clearly that *Buchholzia coriacea* possesses invaluable but yet to be tapped potentials which, if exploited, will benefit the pharmaceutical industry.

ACKNOWLEDGEMENTS

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