TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Prophylactic activity of ethanolic leaf extract from *Hyptis suaveolens* (bush mint) in *Plasmodium berghei* infected Swiss albino mice

Vitus C. Ezuluebo#, Victoria A. Pam, Akwashiki Ombugadu, Ndubisi R. Uzoigwe

Department of Zoology, Faculty of Science, Federal University of Lafia, P.M.B. 146, Lafia, Nasarawa State, NIGERIA.
#Corresponding author. E-Mail: vitusezuluebo@gmail.com; Tel: +2347063043013.

ABSTRACT The development of novel drugs is one of the necessities required to help halt the burden caused by malaria parasite. Thus, the in vivo study on the prophylactic activities of the ethanolic leaf extract of *Hyptis suaveolens* in *Plasmodium berghei* infected Swiss albino mice was investigated. Oral toxicity of the plant extract was first established before the experiment. Also, the mean change in haematological parameters and body weight were determined. The prophylactic test result showed a dose-dependent efficacy of the plant extract on the parasitemia. The group treated with ACT had the least parasitemia while the negative control recorded the highest parasitemia. Thus, the mean parasitemia in relation to the various treatments varied significantly ($P < 0.05$). However, there was no significant difference ($P > 0.05$) in the mean change in Hb, RBC, PCV and mean change in body weight respectively. In conclusion, the result obtained suggests that the ethanolic leaf extract of *H. suaveolens* possesses a dose-dependent prophylactic antiplasmodial activity. This supports the traditional usage of *H. suaveolens* for the treatment of malaria.

KEYWORDS: *Hyptis suaveolens*, ethanolic leaf extract, antiplasmodial activities, *Plasmodium berghei,* haematological parameters

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INTRODUCTION

Malaria is a life-threatening disease caused by *Plasmodium* parasites which remains endemic in the tropics, currently 85 countries and territories are at risk of its transmission (WHO, 2022). World health organization estimated about 241 million cases of individuals infected with malaria parasite and 627,000 malaria associated deaths in 2020. Several African regions carries high global burden, having 95% malaria cases and 96% of malaria related deaths (WHO, 2020).

The major challenge to the control of the spread of the disease is attributed to the development of resistance against the conventional drugs by the parasite (Bhattacharjee & Shivaprakash, 2016). In Southeast Asia and Africa there is a growing concern that the parasites are developing resistance against artemisinin which is currently the most effective drug against the parasites (Duru, 2016).

Plants of medicinal importance have been used by different cultures as source of effective treatment/cure with novel mode of action (Muregi*et al.,* 2007; Ntie-Kang *et al.,* 2014). In Nigeria *Hyptis suaveolens* is used traditionally by various cultures for treatment of malaria (Odugbemi *et al.,* 2007). Thus, the prophylactic activity of *Hyptis suaveolens* crude extract was evaluated in Swiss Albino mice infected with *Plasmodium berghei* in vivo.

MATERIALS AND METHODS

Collection of Experimental Plant

The plant, *Hyptis suaveolens* used for this study was collected from the Permanent Site of Federal University of Lafia, Nasarawa State, Nigeria. After which it was authenticated by Dr. T. P. Terna from the Department of Plant Science and Biotechnology, Federal University of Lafia.

Plant Preparation for Extraction

The plant extraction was carried out using the method described by Harborne (1998) with some modifications. The *H. suaveolens* leaves were rinsed with water to remove dirt and was then spread out on a clean surface to allow air-dry under shade at room temperature.

Mortar and pestle were used to pound the leaves and then sieved using 0.9mm mesh size net to obtain a fine powder. The ratio of plant to solvent was 1:10 (w/v). During maceration, 100 grams of the powdered leaves were exhaustively macerated in 1 liters of 70% ethanol for a period of 72 hours and the solution was agitated repeatedly at intervals and then filtered using Whatman No. 1 filter paper.

The 70% ethanolic leaf extract of *Hyptis suaveolens* obtained was evaporated to dryness using water bath. The yield was then stored in a refrigerator until it was needed for the test.

Ethical Permit

The Ethical permit with Project Identification Code (PIC) – FUL/FS/ZLY/2020/001 was obtained from the Ethical Committee of the Department of Zoology, Faculty of Sciences, Federal University of Lafia, Nasarawa State.

Experimental Animals

The Albino mice used (15–25g) of both sexes were purchased from National Veterinary Research Institute (NVRI) Vom, Plateau State. They were housed in the Department of Zoology, Federal University of Lafia in standard cages (length 290mm, breadth 220mm and height 140mm) and maintained with standard pelleted diet and water for a period of 7 days to enable them acclimatize before carrying out the bioassay.

Acute Oral Toxicity Test

Lorke (1983) acute toxicity test method was used to determine the LD_{50} for the plant material. Two test phases were used to determine the LD_{50}.

In phase 1, nine Swiss Albino mice were used. The mice were randomly divided into three groups, with each of the group having three mice. Each was then administered with 10, 100 and 1000 mg/kg of the ethanolic leaf extract of *Hyptis suaveolens*. The mice were then placed under observation for a period of 24 hours to monitor change in behavior and mortality.

In phase 2, three mice were used and distributed into three groups with each having one mouse and were administered with higher doses of 1600, 2900 and 5000 mg/kg of ethanolic leaf extract and then observed for 24 hours for change in behavior and mortality.

Test Parasite

The *Plasmoduim berghei* strain used for the study was purchased from the in vivo laboratory of National Institute for Pharmaceutical Research and Development (NIPRID), Federal Capital

Territory, Abuja, Nigeria. The parasites were maintained through serial blood passage, 0.2ml of the diluted blood from the infected mice was passage into a parasite free mice using 1ml syringe every four to five days until the test was carried out.

Inoculation of P. berghei in Mice

The percentage parasitemia and the erythrocytes (RBCs) count of the donor mouse was determined before diluting the blood with normal saline and infecting the mice (Odetola and Basir, 1980). The individual mice were inoculated intraperitoneally with 0.2mL of infected blood using 1ml syringe containing about 1×10^7 *P. berghei* parasitized erythrocytes.

Drug Administration

Artemisinin Combination Therapy (Arthemeter-lumefantrin) tablet was the drug used as positive control, 8mg/kg/day was administered. Distilled water served as negative control and 10mg/kg/day was administered. For the ethanolic leaf extract of *Hyptis suaveolens* 100mg/kg/day, 200mg/kg/day and 400mg/kg/day was administered. The controls and the ethanolic leaf extract of *Hyptis suaveolens* were orally administered using a feeding cannula as described by Okokon *et al.* (2017).

Test for the In Vivo Antiplasmodial Prophylactic Activity of the Extract

The prophylactic activity of the ethanolic leaf extract of *Hyptis suaveolens* was assessed using the method described by Peters (1965). Twenty-five Swiss albino mice used for this study were randomly divided into five groups having five mice each. Group 1-3 were administered 100mg/kg/day, 200mg/kg/day and 400mg/kg/day of the ethanolic leaf extract of *Hyptis suaveolens* respectively. Groups 4 were administered 8mg/kg/day of Artemisinin Combination Therapy (positive control) and Group 5 received 10mg/kg/day of distilled water (negative control). The distilled water, ethanolic leaf extract of *Hyptis suaveolens* and drug were administered for a period of three days i.e. (D_0–D_2) then followed by inoculation activity on the fourth day (D_3) with *P. berghei*. After 72 hours, the parasitemia level was assessed by preparing a thin blood smear.

Determination of Parasitemia in Mice

Blood was collected from the mice after bleeding their tail veins and the blood collected was used to prepare a thin blood smears on a clean grease free microscope slides. The slides containing the blood were allowed to air dry followed by fixing with methanol and stained with 10% Giemsa stain. The Giemsa stain slide was allowed to air dry for 20 minutes and was then rinsed with water. The slides were viewed under the microscope using x100 objective with the aid of oil immersion. Percentage parasitemia for each mouse was determined by counting the number of parasitized red blood cells for at least six different fields and calculated using Equation (1).

$$\text{Percentage Parasitemia} = \frac{\text{No. of infected RBCs}}{\text{Total No. of RBCs}} \; x \; 100\% \quad (1)$$

Determination of the Effect of Ethanolic Leaf Extract of Hyptis suaveolens on the Haematological Parameters of the Swiss Albino Mice

Methods described by Cheesbrough (2004) were used to determine the haematological parameters. The Packed Cell Volume (PCV), Haemoglobin (Hb) and Erythrocyte (RBC) were determined for each mouse before infection and after treatment for the prophylactic test.

Statistical Analysis

Data obtained was recorded in Microsoft excel spreadsheet and analyzed using R Console software (Version 4.0.3). One sample Kolmogorov Smirnoff test was used to test for normality of the data. One-way analysis of variance (ANOVA) was used to compare the mean parasitemia in albino

mice in relation to the concentrations of ethanolic leaf extract of *Hyptis suaveolens* and ACT treatments. Also, mean change in body weight as well as haematological parameters were compared using one-way ANOVA. The output from the ANOVA test that was significant was followed by a post-hoc test using LSD test. Level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Acute Oral Toxicity of Ethanolic Leaf Extract of Hyptis suaveolens (LD_{50}) for the Prophylactic Test

During the Lorke's toxicity test method, in phase one (1) 10, 100 and 1000mg/kg, and phase two (2) 1600, 2900 and 5000mg/kg of the plant extract were administered respectively. After the 24 hours of observation no mortality was recorded in all the groups, although the group that was administered the highest dose of 5000mg/kg portrayed erection of hair, loss of appetite, rigidity and reduction in locomotion. Thus, the LD_{50} of ethanolic leaf extract of *Hyptis suaveolens* is above 5000mg/kg. All the toxicity signs showed are negligible or insignificant toxicity signs (Okokon *et al.*, 2017; Zemicheal and Mekonnen, 2018).

Prophylactic Activity of Ethanolic Leaf Extract of Hyptis suaveolens

Mean Parasitemia in Mice Treated with Ethanolic Leaf Extract of Hyptis suaveolens and ACT

The result of the ethanolic leaf extract of *Hyptis suaveolens* leaf antiplasmodial prophylactic activity showed a dose-dependent reduction in mean parasitemia of the Swiss mice, the least parasitemia was observed in the group treated with ACT whereas the negative control group had the highest parasitemia. Hence, the mean parasitemia in relation to the treatments showed a very high significant difference ($F_{20} = 8.388$, $P < 0.000383$, Figure 1). Table 1 shows that there was a significant difference in the pairwise comparison of means between group treated with 10mg/kg/day of distilled water versus the group treated with 8mg/kg/day of ACT as well as group treated with 8mg/kg/day of ACT versus group treated with 200mg/kg/day of ethanolic leaf extract.

The in vivo study of the prophylactic activity of ethanolic leaf extract of *Hyptis suaveolens* against *Plasmodium berghei* revealed a dose-dependent activity, the highest dose administered 400mg/kg/day had the least mean parasitemia followed by 200mg/kg/day and lastly 100mg/kg/day had the highest mean parasitemia. The group treated with ACT when compared to the entire treatment group had the least mean parasitemia and the negative control had the highest mean parasitemia when compared to the mean parasitemia of all the treatment groups. This suggests that *H. suaveolens* has some prophylactic activity and it agrees with the study of Okokon *et al.* (2017) who reported that there was a significant reduction in parasitemia after their prophylactic study of antimalarial and antiplasmodial activity of husk extract of *Zea mays*. Similarly, Dawet *et al.* (2012) also reported a dose-dependent activity of ethanolic leaf extract of *Hyptis suaveolens* in a *Plasmodium berghei* infected mice. Plant leaf extract have been implicated to possess antiplasmodial activity (Bassey *et al*., 2009).

The prophylactic activity of the plant extract can be attributed to the strong presence of alkaloids in the plant extract which has been reported to be antiplasmodiac in effect (Omulokoli *et al*., 1997; Okokon *et al.*, 2017). It can also be as a result of the presence of 2-Methyl-7-phenylindole, a bioactive compound found in some plant extracts as reported by Husein *et al.* (2019) that the bioactive compound possesses Antimicrobial Activity. Plant secondary metabolites possess some bioactive and physiological activities (Christensen & Kharazmi, 2001; Aliyu *et al.*, 2022).

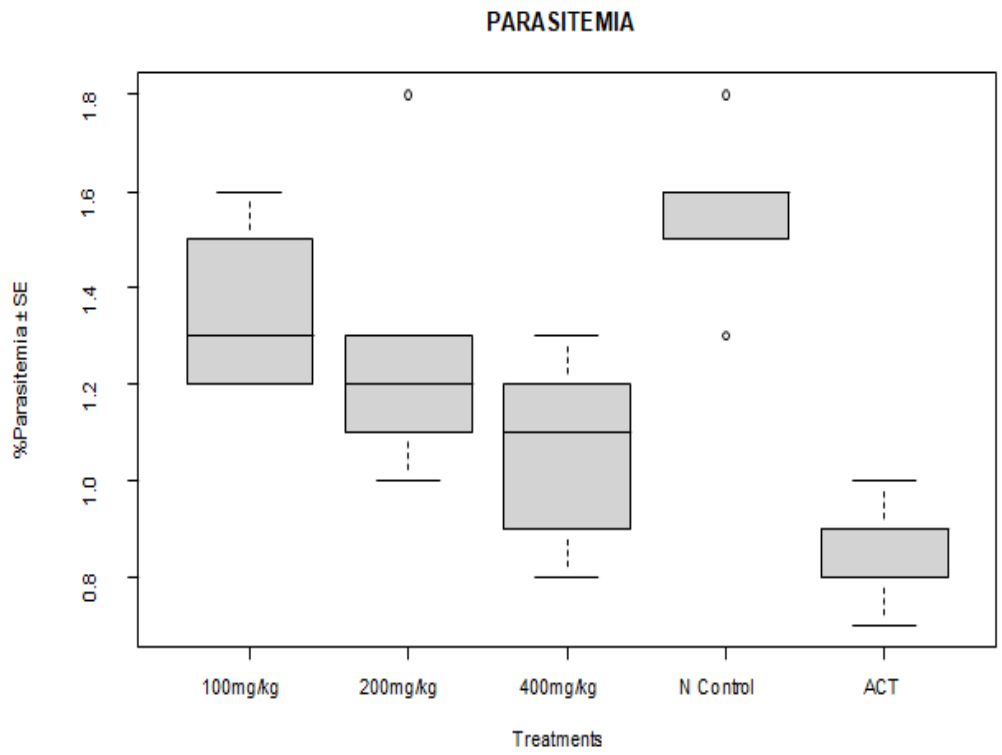

Figure 1. Mean Parasitemia in Swiss Albino Mice in Relation to Prophylactic Treatments with Ethanolic Leaf Extract of *Hyptis suaveolens* and ACT.

Table 1. Post-hoc Test on Prophylactic Treatments Mean Parasitemia in Swiss Albino Mice Treated with ethanolic leaf extract of *Hyptis suaveolens* and ACT

Treatments	Dose (mg/kg)	ParasitemiaMean±SEM
P. Control (ACT)	8	0.86 ± 0.11^{d}
N. Control (DW)	10	1.56 ± 0.18^{a}
Hyptis suaveolens	100	1.36 ± 0.18^{ab}
Hyptis suaveolens	200	1.28 ± 0.31^{bc}
Hyptis suaveolens	400	1.06 ± 0.20^{cd}

Key:
a, d, ab, bc, cd = Mean values on the same column having the same letter do not differ significantly ($p > 0.05$).
P. Control (ACT) = Artemisinin Combination Therapy (Arthemeter-lumefantrin)
N. Control (DW) = Distilled Water

Effect of Ethanolic Leaf Extract of Hyptis suaveolens on the Haematological Parameters of the Mice:
a) Mean Change in Hemoglobin (Hb) Level for the Prophylactic Test

The group treated with 100mg/kg/day of ethanolic leaf extract had the least Hb level while the group treated with 200mg/kg/day of ethanolic leaf extract had the highest Hb level. However, the mean change in Hb level of the Swiss albino mice after Prophylactic test in relation to treatments with ethanolic leaf extract of *Hyptis suaveolens* and ACT showed no significant difference ($F_{20} = 0.812$, $P = 0.533$, Figure 2).

The mean change in Hb level of the Swiss albino mice in relation to the prophylactic treatments exhibited no difference on the Hb level of the Swiss albino mice. This does not agree with the findings of Okochi *et al.* (1999) who report decrease in Hb level of rats infected with *Trypanosama bracei.* Similarly, Dawet *et al.* (2012) also reported a decrease in Hb level of *Plasmodium berghei* infected mice after treatment with ethanolic leaf extract of *Hyptis suaveolence*.

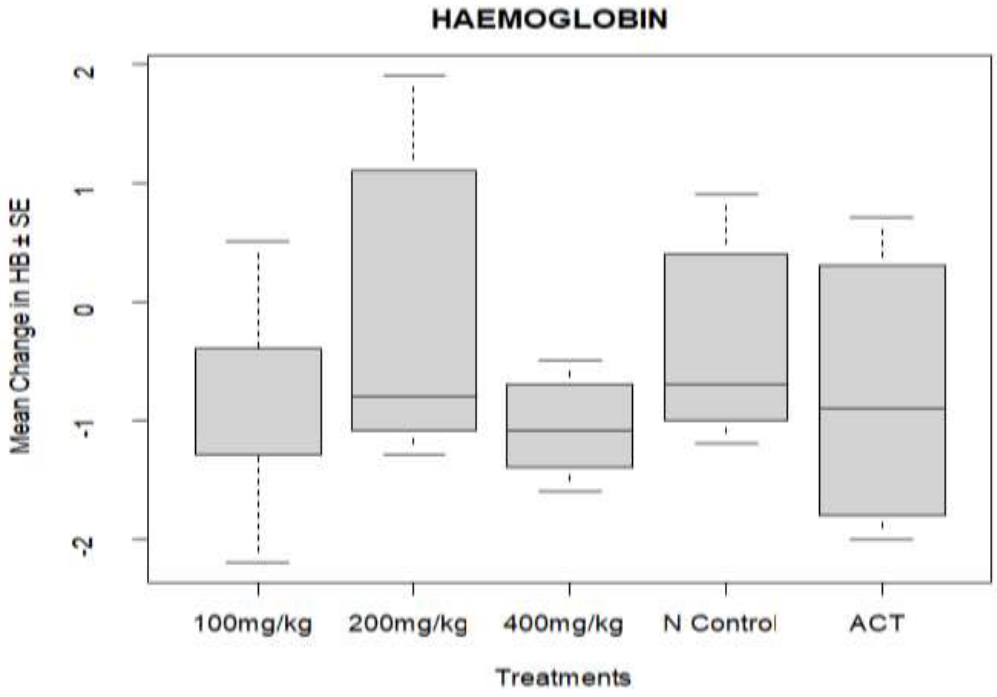

Figure 2. Mean Change in Haemoglobin Level in Swiss Albino Mice in Relation to Prophylactic Treatments with Ethanolic Leaf Extract of *Hyptis suaveolens* and ACT

b) Mean Change in Red Blood Cell (RBC) Level for the Prophylactic Test

The group treated with 10mg/kg/day of distilled water had the highest RBC level while the group treated with 400mg/kg/day of *H. suaveolens* had the least RBC level. However, the mean change in RBC level of the Swiss albino mice after Prophylactic test in relation to treatments with *H. suaveolens* crude extract and ACT showed no significant difference (F_{20} = 0.718, P = 0.589, Figure 3).

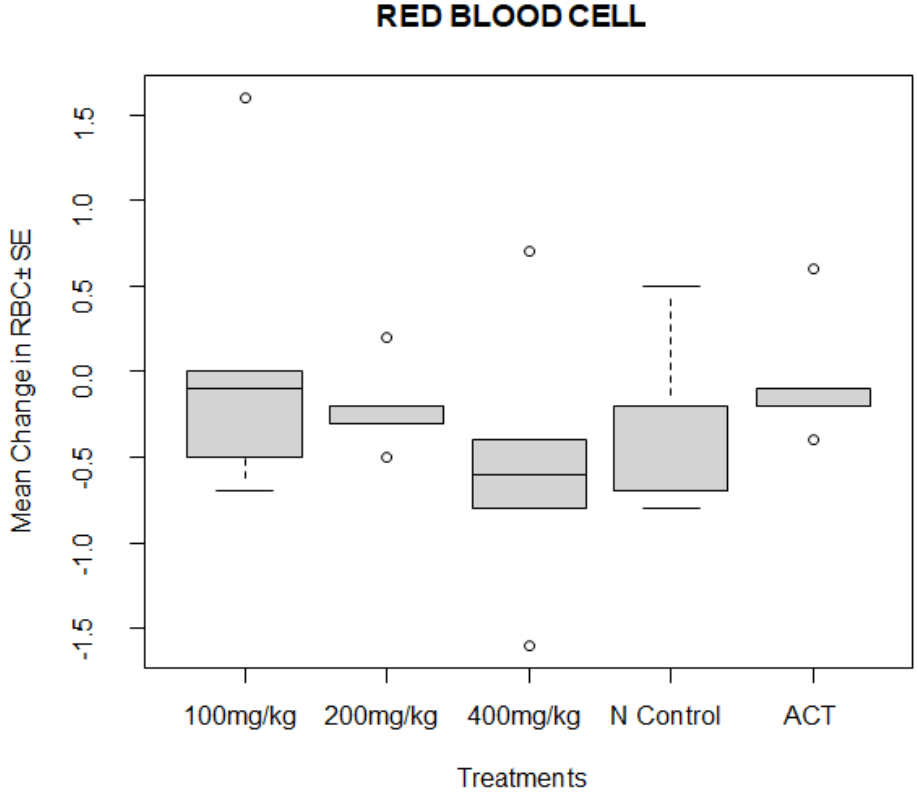

Figure 3. Mean Change in Red Blood Cell Level in Swiss Albino mice in Relation to Prophylactic Treatments with Ethanolic Leaf Extract of *Hyptis suaveolens* and ACT

The mean change in the RBC of the Swiss albino mice after the in vivo prophylactic treatments with the crude extract and ACT was observed to have no variation, although there was decrease in the RBC level of the mice after the test. This is in accordance with the findings of Dawet *et al.* (2012) who reported decrease in RBC level of *Plasmodium berghei* infected mice after treatment with ethanolic leaf extract of *Hyptis suaveolens*. *Plasmodium* parasites damages the RBC of their host organisms which results to anemia (White, 2018).

c) Mean Change in the Packed Cell Volume (PCV) Level for the Prophylactic Test

The group treated with 8mg/kg/day of ACT had the highest PCV level while the group treated with 100mg/kg/day of *H. suaveolens* had the least PCV level. However, the mean change in PCV level of the Swiss albino mice after Prophylactic test in relation to treatments with *H. suaveolens* crude extract and ACT showed no significant difference (F_{20} =1.604, P = 0.212, Figure 4).

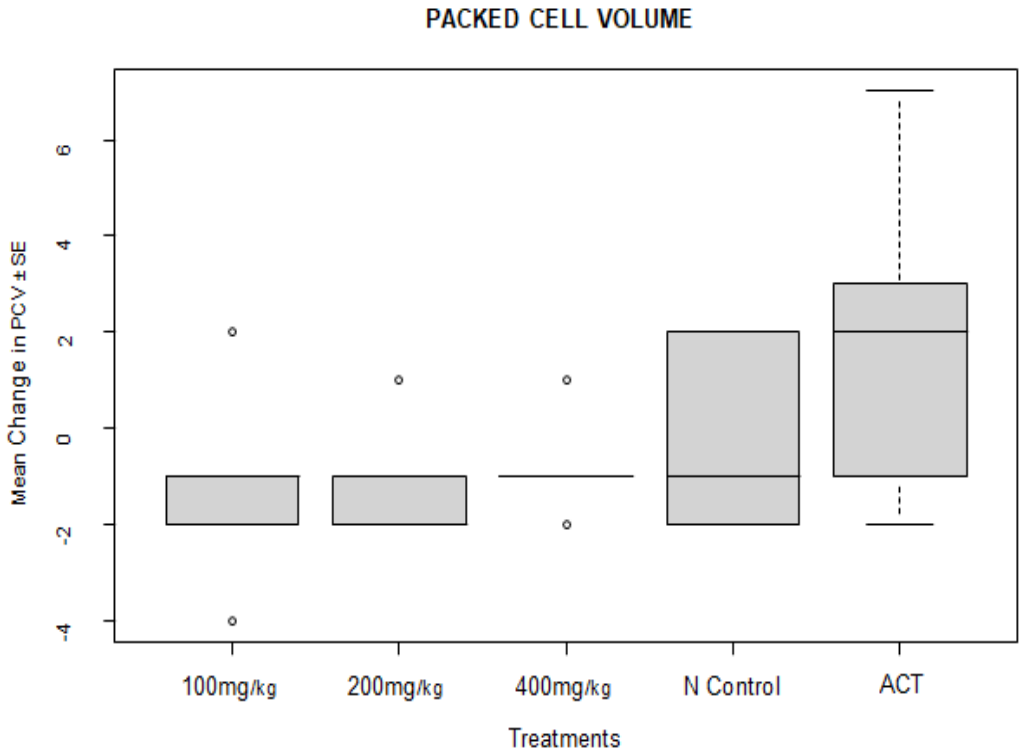

Figure 4. Mean Change in Packed Cell Volume Level in Swiss Albino Mice in Relation to Prophylactic Treatments with Ethanolic Leaf Extract of *Hyptis suaveolens* and ACT

The PCV level mean change in the Swiss albino mice after the prophylactic treatments with the crude extract and ACT was observed to have no variation; the lack of variation observed might be as a result of the repository process which involves administration of the extract before inoculation of the parasite. Although this agrees with the findings of Ayim *et al.* (2021) who reported that toad venom was able to inhibit the effect of *P. berghei* in Swiss albino mice PCV. Okochi *et al.* (1999) reported decrease in the PCV level of rats infected with *Trypanosoma brucei.* The PCV level of Mice infected with *Plasmodium berghei* was observed to decrease after treatment with *Hyptis suaveolens* (Dawet *et al.*, 2012).

The Gain/Loss in Body Weight of Albino Mice after Prophylactic Treatments

The group treated with 400mg/kg/day of *H. suaveolens* had the highest body weight while the group treated with 200mg/kg/day of *H. suaveolens* had the least body weight. However, the mean change in body weight of the Swiss albino mice after Prophylactic test in relation to treatments with *H. suaveolens* crude extract and ACT showed no significant difference (F_{20} =1.147, P = 0.363, Figure 5).

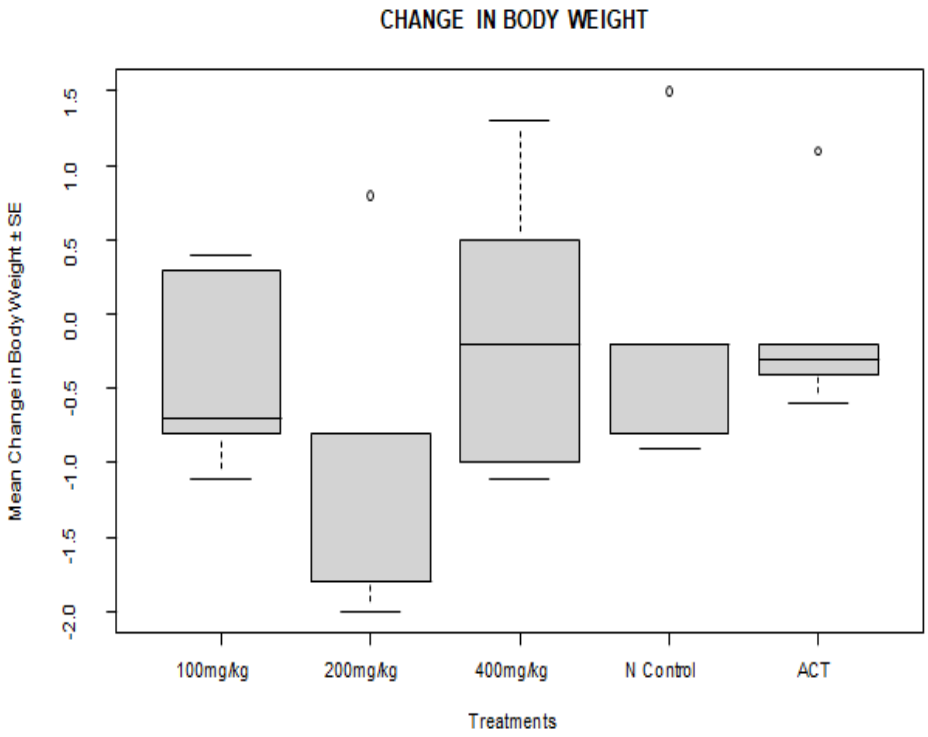

Figure 5. Mean Change in body Weight of Swiss Albino Mice in Relation to Treatment with Ethanolic Leaf Extract of *Hyptis suaveolens* and ACT

The mean change in the Swiss albino mice body weight analyzed after the prophylactic test had no variation, which is in agreement with the report of Zemicheal and Mekonnen (2018) that there was no significant difference in the body weight of the Swiss albino mice after their study on the antiplasmodial activity of *Vernonia adoensis* aqueous, methanol and chloroform leaf extracts against chloroquine sensitive strain of *Plasmodium berghei*.

CONCLUSION

The prophylactic result in this study shows that the ethanolic leaf extract of *Hyptis suaveolens* possesses some effective antiplasmodial properties that can act against *P. berghei* most especially when administered at very high concentration. These findings justify the traditional use of *Hyptis suaveolens* for treatment of malaria.

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