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Anti-Malarial Activity of the Ethanolic Extract of *Vernonia Amygdalina* (Bitter Leave) on PCV and Parasiteamia of Experimental Mice

Yohanna Longdet Ishaya [a], Matawal Mary Mankilik [a], Obed Faven Rimzhi [a], Edward David Idoko [a, *]

[a] University of Jos, Plateau State, Nigeria

Abstract

Malaria is one of the most important infectious diseases in the tropics and sub-tropics. The search for antimalarial compounds has been necessitated by *P. falciparum* resistance to almost all antimalarial drugs. In this study, the in vivo antimalarial activities of ethanolic extracts of *Vernonia amygdalina,* a plant used by traditional healers to treat malaria and other diseases was carried out. Twenty-one (21) Albino mice were randomly divided into seven groups of three mice each (n = 3). Group 1, 2, and 3 were the experimental group, while group 4, 5, 6, and 7 were treated/untreated, normal, uninfected/treated and standard respectively. Groups 1, 2, and 3 were treated with dose of 200 mg/kg. bwt, 300 mg/kg. bwt and 400 mg/kg. bwt respectively. The average daily parasitaemia level of the *Plasmodium berghei* infected mice treated with 300 mg/kg. bwt of the ethanolic leaf extract of *Vernonia amgdalina*extract and 25 mg/kg. bwt of chloroquine significantly ($P < 0.05$) reduce when compared with negative control group. However, there is no significant ($P > 0.05$) different in the level of parasitaemia in 200 mg/kg. bwt and 400 mg/kg. bwt at the varying concentractions of the ethanolic extract's dosage, Therefore, this shows that *Vernonia amygdalina*has both curative and suppressive activities since at lower concentrations, the parasite was seen to reduce from the initial load before administration and at higher concentrations, the parasite was seen to reduce to the lowest level indicating that if treatment period is extended concentration is administered, the parasite will be cleared completely, thus, the curative activities of the *Vernonia amygdalina*leaf extract.

Keywords: Ethanolic, Parasitaemia, *Vernonia amygdalina*, PCV, *Plasmodium berghei,* extract, leaf, treatment, concentrations.

1. Introduction

Malaria is one of the most important tropical diseases and the greatest cause of hospitalization and death among children age 6 months to 5 years (Molta et al., 2006). The World Health Organization reported that there were an estimated 246 million malaria cases distributed among 3.3 billion people at risk in 2006, causing at least a million deaths. These were mostly children under five years. One hundred and nine countries were endemic in 2008 and 45 within the WHO African region (WHO, 2008). Approximately 80 % of malaria cases in the world are estimated to be in Africa where the disease is endemic (WHO, 2008). The disease is a major cause of the continent high infant mortality, killing 1 in every 20 children below 5 years of age. In Nigeria,

* Corresponding author
E-mail addresses: idoko.unijosbch@gmail.com (E.D. Idoko)

malaria transmission occurs all-year round in the South, and is more seasonal in the North. The country accounts for a quarter of all malaria cases in the WHO African region (WHO, 2008). The problem is further compounded by the upsurge in the resistance strain of the parasite. Thus, the continuous search for novel and more effective anti-malarial compounds especially from medicinal plants extracts has been of utmost importance in view of the success of artemisinin, the active principle of an ancient Chinese herbal remedy for fevers (Osamor, Owumi, 2010).

In Africa and other countries where malaria is endemic, traditional medicinal plants are frequently used to treat or cure malaria. *Vernonia amygdalina* Delile (VA), family Asteraceae or compositae are plants that are consumed locally as food and serve important ethno medicinal uses. Many parts of the plants are useful, they are used locally for the treatment of fever, stomach disorder, jaundice, worm infestation, constipation, malaria, hiccups, kidney problems, amoebic dysentery, schistosomiasis, cough, wounds, diabetes, laxative, veneral diseases and other bacterial and protozoa infection. *Vernonia amygdalina*, popularly known as bitter leaf, is an under shrub of variable height with petiolate green leaves of about 6 mm diameter. The leaves are usually bitter and are very popular soup vegetable in Nigeria (Ojiako, Nwanjo, 2006).

Malaria remains a major public health burden and resistance has emerged to every antimalarial on the market, including the frontline drug, artemisinin. Our limited understanding of Plasmodium biology hinders the elucidation of resistance mechanisms. In this regard, traditional method using plants like *Vernonia amygdalina* can facilitate the integration of existing experimental knowledge and further understanding of these mechanisms which will lead to finding cure to this parasitic disease which over the year as continue to develop resistant to existing drugs.

2. Materials and methods

Plant Identification and Authentication:

Fresh leave of *Vernonia amygdalina* were collected from Bukuru express in Gyel in 2019. It was then taken to University of Jos, Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos, Jos, Nigeria for identification and it was subsequently identified and given a Voucher number: JUHN20000304, it was subsequently air dried at room temperature for 20 days and pounded into its powdered form using mortar and pestle.

Ethical Clearance:

Ethical clearance was obtained with approval number UJ/FPS/F17- and white albino mice species which has susceptibility to *Plasmodium berghei* mosquito parasite were obtained from the University of Jos animal house.

Chemicals and Reagents Used:

Dragendorf's reagent, Ammonia solution, Acteone, lead acetate, Sodium Hydroxide, Sulphuric acid, Glacia Acetic acid, 1 % Hydrochloric acid, Distilled water, Ferric chloride, chloroform, normal saline, Giemsa stain, methanol.

The following equipment and reagents were used: Olympus microscope, hemocytometer with aspirating pipette, capillary tube, microhematocrit centrifuge, PCV reader, cover slip and slides, weighing balance, measuring cylinder, sample container, and magnetic stirrer, Centrifuge, hawksely micro-haematocrit reader, vacuum pump, porter and pestle, standard laboratory glass wares, heparinized tubes, Giemsa stain were purchased from Sigma-Andrich Chemical Company (St. Louis, USA). All other chemicals and reagents used for this study were of analytical grade.

Materials for Inoculation of Experimental Animals (White Albino Mice):

Razor blade, Butterfly needle, 2 ml syringe, Beaker, Cotton wool, Slides, Giemsa stain, and microscope.

Experimental design:

GROUP 1: 3 parasitized mice administered 200 mg/kg of extract per body weight per day.
GROUP 2: 3 parasitized mice administered 300 mg/kg of extract per body weight per day.
GROUP 3: 3 parasitized mice administered 400 mg/kg of extract per body weight per day.
GROUP 4: Infected/untreated.
GROUP 5: Neural.
GROUP 6: uninfected/ treated
GROUP 7: 3 parasitized mice administered 25 mg/kg of chloroquine per body weight per day
GROUP F: 3 uninfected and untreated mice.
Mode of administration; oral administration.

Experimental animal:

Male and female albino mice (weighing about 15.2- 30.2) were used for this experiment. They were obtained from animal farm, University of Jos, and were fed with standard commercial feed (vital feed, top feed).

Phytochemical analysis of the plant extract:

The ethanolic extract of *Vernonia amygdalina*was subjected to phytochemical screening to check for the presence or absence of plant secondary metabolites such as: Saponins, tannins, alkaloids, flavonoids, steroids and terpenes, cardiac glycosides, balsam, carbohydrates, phenols and resins according to the method of Harborne (1984) with slight modification.

Test for alkaloids: To 2 mls of extract, few drops of dragendorf's reagent was added to give an orange colouration which indicated the presence of alkaloids.

Test for flavonoids: To 2 mls of the extract, few drops of 5 % lead acetate was added to give a cream light colour which indicated the presence of flavonoids.

Test for tannins: To 2 mls of the extract, few drops of 10 % ferric chloride were added to give a deep bluish or greenish colour which indicated the presence of tannins.

Test for Saponins: To 1 ml of the extract, 4 mls of distilled water was added and shaken vigorously. Formation of froth indicated the presence of Saponins.

Test for terpens and steroids: To 1 ml of the extract, 2 mls of concentrated Sulphuric acid was added along-side of the test tube. Formation of reddish brown ring at the interphase indicated the presence of terpens and steroids.

Test for cardiac glycosides (Salkowski's test): 2 mls of the extract was dissolved in 2 mls of chloroform and Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interphase indicated the presence of cardiac glycosides.

Test carbohydrates: 5 drops of the extract were added to 2.0 mls of Benedict's reagent, placed on a hot plate for 5 minutes and was observed for the formation of brick red precipitation which indicated the presence of carbohydrates.

Test for phenol: To 2 mls of the extract, 2 mls of ferric chloride was added and observed for the formation of a deep bluish-green colouration which indicated the presence of phenol.

Test for resins: To 2 mls of the extract, 2 mls of acetic anhydride was added and drops of concentrated Sulphuric acid were added to observe for a violet colour which indicated the presence of resins.

Preparation of ethanolic extract of *vernoniaamygdalina:*

Fresh leaves of *V. amygdalina* were cut into pieces and air dried inthe laboratory. The dried pieces were pulverized using a laboratory grinder. 80 g of the dried powderedform of the plant materials were extracted with ethanol using soxlet apparatus for 72 hours. All theextracts were concentrated to dryness on a water bath and weighed. The extracts were then stored in well-closed containersand kept at room temperature to protect from light and moisture till used (Sutharson et al., 2007).

Acute Oral Toxicity Test:

Acute oral toxicity test of the solvent fractions was performed on randomly selected 3 non-infected female mice following the Organization for Economic Corporation and Development (OECD) guideline. The mice were fasted overnight and weighed before the test. A loading dose of 2000 mg/kg b. wt and 5000 mg/kg b. wt of the extract was administered to single mouse with oral gavage. Then, any sign of over toxicity and/ or mortality were observed for 24 hours with special emphasis to the first 4 hours. As no death or over toxicity was observed within 24 hours, and followed for 14 days to assess delayed toxicity of the solvent fractions. The mice were observed for any potential signs of acute toxicity such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation and/or mortality (OECD, 2001).

Mortality:

The mortality rate of infected mice was monitored during the period of the experiment according to the number of mice that died (Oche et al., 2016).

Determination of MST:

Survival time was recorded to observe the effect of the extract of *Vernonia amygdalina* on the survival of *P. berghei* infected mice. The mice were fed ad libitum and observed for about 29 days. Any death that occurred during this period was noted for each mouse in the treatment and control groups to determine the MST (Bantish et al., 2014).

MST= Sum of survival time for all mice in group (in days)

Total numbers of mice in that group

Body weight:

The body weight of each mouse was taken using a weighing balance before inoculation of parasite and after treatment (Bhat, Surolia, 2001).

Blood sample collection for *plasmodium bergheiparasiteamia* screening:

After 72 hr of infection, blood samples were collected from the infected mice to check for the presence of the parasites. Pricking the tip of the tails of the mice cause blood to flow which was collected on slides and smeared to make a thin blood film and dried under the shade for about 10 minutes and was fixed with methanol for 5 minutes. After the methanol dried completely, the blood was stained with Giemsa stain for 45 minutes and rinsed with water which was taken for microscopic examination after drying.

Immersion oil was added to the stained slides and each of the slides were viewed under the X100 objective lens. The *Plasmodium berghei* were seen in the infected blood as spherical curved or cluttered images in the stained samples. Parasitaemia count was done on Giemsa positive bodies which represents the parasitized red blood cells. Parasitaemia count was done and recorded 72 hours after infection and daily throughout the period of administration (Oche et al., 2016).

Parasite Inoculation:

Chloroquine-sensitive *P. berghei* (ANKA strain) obtained from animal house in the University of Jos maintained by subsequent passage of blood frominfected mouse to a healthy one every 5 days was used for the experiment. The anti-Plasmodial activity of *Vernonia amygdalina* was assessed. 21 male and female mice were randomly divided into 7 groups of three mice in each. Blood from a donor mouse was used to infect test mice. *Plasmodium berghei* parasitized erythrocytes were obtained from the tail of the donor mice and were diluted with 0.9 % normal saline. Mice were inoculated intraperitoneally with 0.5 mL blood suspension on day 0 and were monitored for manifestation of parasitaemia for 72 hour without treatment. The mice were randomly divided into 6 groups of three (3) mice per group and treated for 5 consecutive days with daily doses of the extracts (200, 300 and 400 mg/kg per body weight) and standard antimalarial drug (Chloroquine, 25 mg/kg per body weight) by oral route (Challand, Willcox, 2009).

Parasitaemia Count:

Parasitaemia count was carried out on group 1, 2, 3, 4, and 7 daily using light microscope. Blood was collected from the tail of the mice and dropped on a clean grease free microscopic slide. A cover-slip was used to make a thin film and allowed to air dry. After drying, the film was fixed with a methanol for five minutes, air-dried and stained with Giemsa stain for 45 minutes, and left to air-dry. After drying, drop of immersion oil was placed on the film, the microscope was connected to a light source and parasitaemia count was done using X100 (oil immersion) objective Lens (Bhat, Surolia, 2001).

% parasite = Total number of parasitize cells ×10

Total RBCs number

Packed cell volume (PCV):

Blood samples were collected from the infected mice to check the effect of the extract *Vernonia amygdalina* on the blood level of *P. berghei* infected mice. Pricking the tip of the tails of the mouse causes blood to flow which was collected on heparinized capillary tube. The tube was sealed at one end with sealant and centrifuged in a haematocrit centrifuge for 5 minutes at 10000 RPM. At the end of centrifugation, the height of the packed red cells is recorded as a percentage of the total blood cell and plasma column. This was done using a haematocrit reader.

Unit of measurement; it is expressed as % of the blood (SI unit are L/L). The conversion formula to SI unit is as follows

% ÷ 100=L/L

Packed cell volume (PCV), n- The measure of the ration of the volume occupied by red blood cells to the volume of the whole blood, expressed as fraction. Note; the term “haematocrit” has been, and is often, used for this quantity (Baird, 2013).

PCV = Measure of the ration of the volume occupied by red blood cells × %

Volume of the whole blood

3. Results

Acute oral toxicity of extract:

The extract of the leaves of *V. amygdalina* did not cause mortality in mice at the level of 2000 mg/kg b. wt and 5000 mg/kg b. wt. The extract and its fractions did not induce any sign of over toxicity such as loss of appetite, hair erection, lacrimation, tremors, convulsions and salivation during the 14 days of observation. Based on the acute toxicity study, the LD50 of extract of the leaves of *V. Amygdalina* were found to be greater than 5000 mg/kg b. wt, indicating their wide safety margin. The present result is in line with the finding of Adiukwu, Amon, Nambatya (2012). Who reported that *V. Amygdalina* caused no clinical signs of toxicity at doses between 2000 and 5000 mg/kg b. wt /day for 14 consecutive days and that of Anoka et al. (2013) who reported the absence of signs of over toxicity or adverse toxicological effects at all tested dose also found no toxic effect of extracts of *V. Amygdalina in vivo* on rats. Generally, if LD50 value of the test chemical is more than three times the minimum effective dose, the substance is considered to be a good candidate for further studies in vivo assays. The LD50 has also been used for classification of chemicals. Based on WHO hazard classification system, the extract of the leaves *V. amygdalina*, to which the LD50 was greater than 5000 mg/kg b. wt, are designated as "unlikely to be hazardous." Therefore, the extract of the plant is considered to be safe at the tested doses. The aforementioned descriptive toxicological studies also support the finding (OECD, 2001).

Differences in body weight:

Effects of ethanolic leaf extract of *Vernonia amygdalina* on the body weight of albino mice infected with *Plasmodium berghei* were shown in Figure 1. The weight of the groups treated with 200 mg/kg b. wt extract, 300 mg/kg b. wt extract, 400 mg/kg b. wt, and infected/untreated reduced by 9.3 g, 4.5 g, 7.7 g and 6.0 g and 10.5 g respectively. Whereas the weight of the groups treated with 25mg/kg bwt extract and uninfected and untreated increased by 6.1 g and 3 g respectively.

Packed cell volume:

Effects of ethanol leaf extract of *Vernonia amygdalina* on packed cell volume of *Plasmodium berghei* infected mice were shown in Figure 2. The percentage packed cell volume of the groups treated with 200 mg/kg b. wt extract, 300 mg/kg b. wt extract, 400 mg/kg b. wt extract 30 %, 19 %, 11 % and untreated reduce by 15 %, on day 11, while 25 mg/kg b. Wt chloroquine increase by 8 % and neutral increase by 3 % and also treated/uninfected increase by 2 %.

Parasitaemia count:

The average daily parasitaemia level of the *Plasmodium berghei* in infected mice treated with ethanolic leaf extract of are shown in Figure 3. The average daily parasitaemia of infected mice treated with 300 mg/kg b. wt of ethanolic leaf extract of *Vernonia amygdalina* extract and 25 mg/kg b. wt of chloroquine significantly ($P < 0.05$) reduced when compared with negative control group. However, there is no significant ($P > 0.05$) difference in the level of parasitaemia in 200 mg/kg b. wt and 400 mg/kg b. Wt.

Mortality rate post infection:

The death rate of the experimental albino mice was monitored daily after the inoculation of *Plasmodium berghei*. Groups 1, 2, 3, and 4 lived up to day 19, 24, 22, and 15 respectively meanwhile groups 5, 6, 7 lived beyond the experimental period as shown in Table 2.

Table 1. Phytochemical profile of the ethanolic leaf extract of *Vernonia amygdalina:*

PHYTOCHEMICAL	RESULT
Alkaloid	+
Flavonoids	+
Tanins	+
Saponins	-
Terpenes and steroids	+
Cardiac glycosides	+
Carbohydrate	-

Phenol	+
Resins	+

KEY:- = absent + = present

Table 2. Mode of survival time (mst)

GROUPS	SURVIVAL RATES
200 mg/kg b. wt	19 days
300 mg/k b. wt	24 days
400 mg/kg b. wt	22 days
Infected	15 days
Uninfected	Lived beyond experimental period
Uninfected/treated	Lived beyond experimental period
25 mg/kg b. wt of chloroquine	Lived beyond experimental period

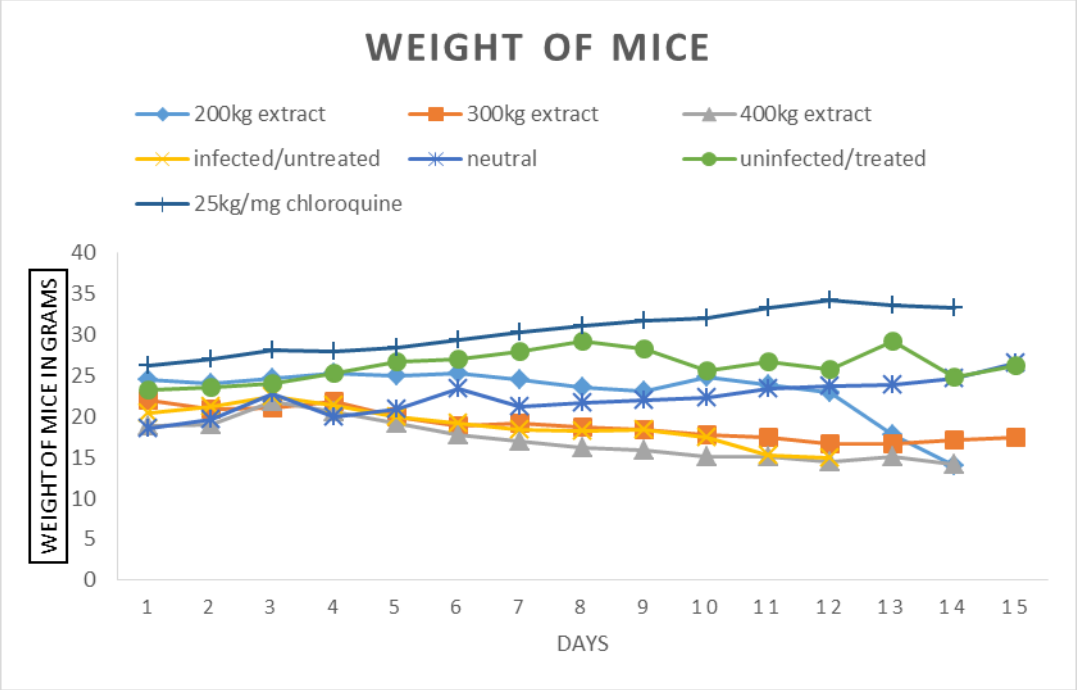

Fig. 1. The average mean of weight of mice

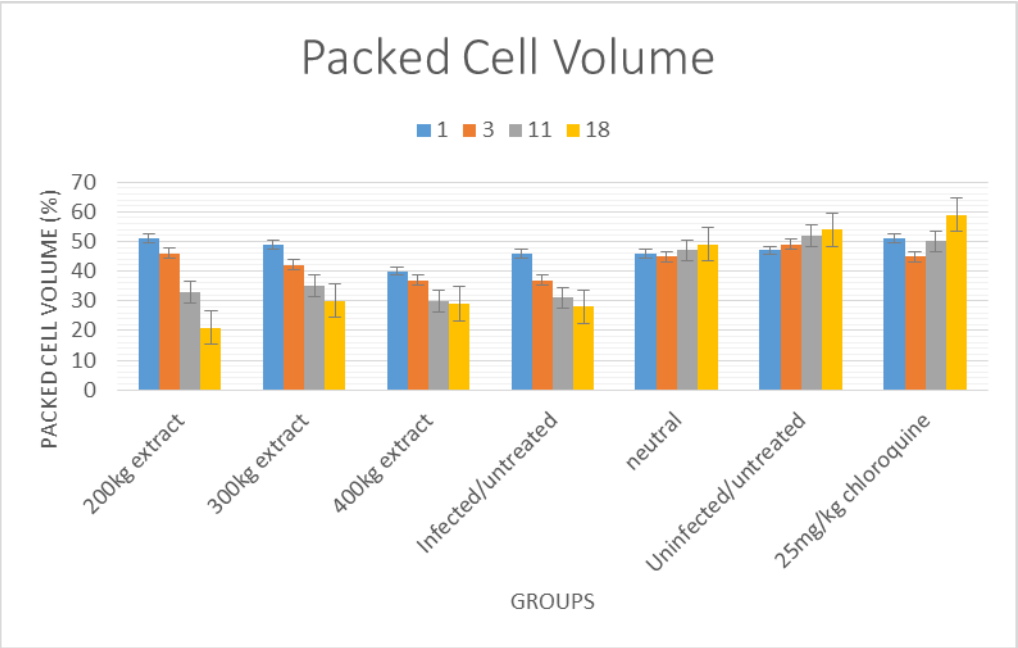

Fig. 2. Effect of the extract of *Vernonia Amygdalina* on packed cell volume of experimental mice

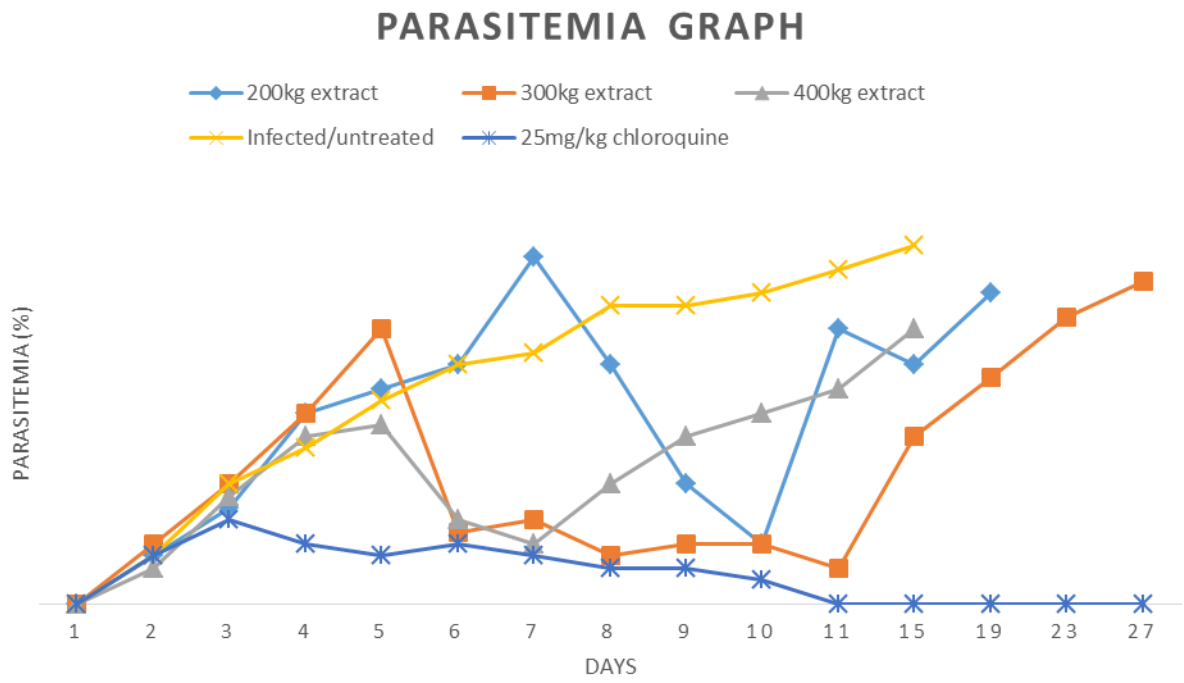

Fig. 3. The effect of the administration of the aqueous extract of *Vernonia Amygdalina* on the parasiteamia of experimental mice

4. Conclusion

Man in solving its numerous medical challenges have for ages depend on his immediate environment taking advantages of nature's provisions of its beauty for life and survival. They have learnt to depend on plants and in some cases animals in providing solutions to the myriad of their health problems (Oliver, 1960). However, the increasing use of plants for the therapeutic and medicinal use warrants an adequate scientific investigation to confirm the suitability of plants or otherwise for the purpose for which they are used. Hence, the purpose of this research which was to

investigate the *in vivo* anti-Plasmodial activity of the ethanolic leaf extract of *Vernonia amygdalina* in experimental mice. The ethanolic leaf extract of *Vernonia amygdalina* was extracted using absolute ethanol and the extract was further subjected to *in vivo* anti-Plasmodial studies compared to chloroquine, a standard anti-malaria drug which were all found to possess dose dependent anti-Plasmodial activities against the *Plasmodium berghei* species of the malaria parasite in experimental mice. The various dosage concentration of 200 mg/kg. bwt, 300 mg/kg. bwt and 400 mg/kg per body weight all showed curative properties of the ethanolic leaf extract of *Vernoniaamygdalina*and chloroquine in varying proportions. However, the 25 mg/kg b. wt of chloroquine and the 300 mg/kg b. wt dosage concentration of the ethanolic leaf extract of *Vernonia amygdalina* showed more curative activities compared to the dosage concentration of 200 mg/kg b. wt and 400 mg/kg per body weight. Anaemia, body weight loss and body temperature reduction are the general features of malaria infected mice. So an ideal antimalarial agents obtained from plants are expected to prevent body weight loss in infected mice (Bantish et al., 2014). This research study presented that the 200 mg/kg b. wt of the ethanolic leaf extract of *Vernonia amygdalina* significantly prevented weight loss associated with increase in parasitemia level the phytochemical screening of the ethanolic leaf extract of *Vernonia amygdalina* showed that the leaf contains useful phytochemicals which contributed to its anti-plasmodial activities in experimental mice. The presence of alkaloids, flavonoids, tannins,terpenes and steroids, phenol, resins, and cardiac glycosides in the ethanolic extract attributes terpenes and steroids, phenol, resins, and cardiac glycosides in the ethanolic extract attributes this anti-plasmodial activities of the ethanolic leaf extract of *Vernonia amygdalina*. Flavonoids have been reported to have exhibited significant in vitro antimalarial activity against *P. falciparum*. This could justify the antimalarial activities exhibited by the plant extract since flavonoids was found to be present in the results of the phytochemical screening.

At the varying concentrations of the ethanolic extract's dosage, the leaf of *Vernonia amygdalina* showed varying degrees of treatment of the malaria parasite. Therefore, this shows that *Vernonia amygdalina* has both curative and suppressive activities since at lower concentrations, the parasite load was seen to reduce from the initial load before administration and at higher concentrations, the parasite was seen to reduce to the lowest level indicating that if treatment period is extended concentration is administered, the parasite will be cleared completely, thus, the curative activities of the *Vernonia amygdalina* leaf extract.

This study shows that the presence of alkaloids, flavonoids, tannins, terpenes and steroids, balsam, phenol, resins, and cardiac glycosides in the ethanolic leaf extract of *Vernonia amygdalina* has been shown to possess anti-plasmodial activities which can be developed into Anti-Malarial Combination Therapy (ACT) could help in dealing with the malaria cases. The result of this study also verifies the folk use of *Vernonia amygdalina* leaf for the treatment of malaria, and other infectious diseases that affects the health of people leaving in the tropical areas. Even though the study was carried out using the ethanolic leaf extract of *Vernonia amygdalina* on *Plasmodium berghei* species, this has given new insight into developing anti-malarial drugs that have high efficacy against the resistant strain of *Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale* and *Plasmodium malariae* that affect humans.

5. Recommendations

Since the ethanolic leaf extract of *Vernonia amygdalina* showed high levels of anti-plasmodial activities, further work on *Vernonia amygdalina* is required on this promising plant since most of the folks in the rural areas have put this plant into use for several years. Development of an appropriate anti-malarial drug using this plant will also help in treatment of malaria since the present Anti-Malarial Combination Therapy are faced with the problem of resistance of the malaria parasites due to constant exposure and subsequent mutation of the mosquito parasite to adapt to the active compounds present in the malaria drugs and hence its resistance to the drug.

References

Adiukwu et al., 2012 – *Adiukwu, P.C, Amon, A., Nambatya, G.* (2012). Acute toxicity, antipyretic and antinociceptive study of the crude saponin from an edible vegetable: *Vernoniaamygdalina* leaf. *Int J BioChemistry Science*. 6: 1019-1028.

Anoka et al., 2006 – *Anoka, A. Njan, BulusAdzu, Amon G. Agaba, Dominic Byarugaba, Silvia Díaz-Llera, Cameron, A., Read, J., Tranter, R., Winter, V.J., Sessions, R.B.* (2006). Identification and activity of a series of azole-based compounds with lactate dehydrogenase-directed anti-malarial activity. *Journal of Biological Chemistry*. 279(30): 31429-31439.

Baird, 2013 – *Baird, J.K.* (2013). Evidence and implications of mortality associated with acute Plasmodium vivax malaria. *Clinical Microbiology. Rev*. 26, 36.

Bantish et al., 2014 – *Bantish, L., Assefa, S., Teklehaimanot, T., Engidawork, E.* (2014). *In vivo* antimalarial activity of crude leaf extract and solvent fraction of Croton macrosachyusHocsht.(Euphoribiaceae) against *Plasmodium berghei*in mice. BMC *Complement Alter medical*. 14: 79. DOI: 10.1186/1472-688-14-79

Bhat, Surolia, 2001 – *Bhat, G.P., Surolia, N.* (2001). *In vitro* antimalarial activity of extracts of three plants used in the traditional medicine of India. *The American Journal of Tropical Medicine and Hygiene*. 65(4): 304-308. Publisher: The American Society of Tropical Medicine and Hygiene. DOI: https://doi.org/10.4269/ajtmh.2001.65.30

Challand, Willcox, 2009 – *Challand, S., Willcox, M*. (2009). A clinical trial of the traditional medicine *Vernoniaamygdalina* in the treatment of uncomplicated malaria. *Journal Alternative Complement Medicinal*. 15: 12311237.

Harborne, 1984 – *Harborne, J.B.* (1984). Phytochemical Methods. Chapman and Hall, Londond-New York. 120 p.

Molta et al., 2006 – *Molta, N.B.S., Oguche, S.D., Pam, I.C., Omalu, V.P., Gyang, J.O., Dabit, L.* (2006). Efficacy of single-dose amodiaquine co-administered with sulfadoxine/pyrimethamine against falciparum infection in BarkinLadi, an area of multi-drug resistant malaria, ajol.info. *Journal of Pharmacy and Bioresources*. 3(1): 1-6.

Oche et al., 2016 – *Oche, O., Nathan, H., Joseph, I., Vincent, A. Upev, Stanley, I.R. Okoduwa., Omiagocho, T.I.* (2016). Antimalarial Potential of Carica papaya and Vernoniaamygdalina in Mice Infected with Plasmodium berghei. *Journal of Tropical Medicine*. Article ID 8738972, 6 p. DOI: http://dx.doi.org/10.1155/2016/8738972

OECD, 2001 – Organization of Economic Cooperation and Development (2001). Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 24.

Ojiako, Nwanjo, 2006 – *Ojiako, O.A., Nwanjo, H.U.* (2006). Is *Vernoniaamygdalina* hepatotoxic orhepatoprotective? Response from biochemical andtoxicity studies in rats. *African Journal of Biotechnology*. 5(18): 1648-1651. [Electronic resource]. URL: http://www.academic journals.org/AJB

Oliver, 1960 – *Oliver, B.* (1960). Medicinal plants in Nigeria. Ibadan College of Arts and Sciences and Technology, Ibadan. P. 358.

Osamor, Owumi, 2010 – *Osamor, P., Owumi, B.* (2010). Complementary and alternative medicine in the management of hypertension in an urban Nigerian community. VL-10. BMC complementary and alternative medicine. DOI: 10.1186/1472-6882-10-36

Sutharson et al., 2007 – *Sutharson, L., Lila, K.N., Prasanna, K.K., Shila E.B., Rajan, V.J.* (2007). Anti-inflammatory and anti-nociceptive activities of methanolic extract of the leaves of *Fraxinus floribunda Wallic. African Journal of Traditional, Complementary and Alternative Medicines*. 4(4): 411-416.

WHO, 2008 – World Health Organization (WHO). Guide name for assessment of herbal medicine programmed and traditional medicine WHO/TRIM914 Geneva, 2008.