IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 2 Ver. IV (Mar -Apr. 2015), PP 37-42
www.iosrjournals.org

Antiplasmodial Activity of Ethanol Extract of *Vernonia amygdalina* leaf in *Plasmodium berghei* Infected Mice: *in vivo* study

Fatima Mohammad Madaki
Department of Biochemisrty, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria

Abstract: *The antiplasmodial activity of crude ethanol extract of Vernonia amygdalina leaf in Plasmodium berghei infected mice was evaluated. A total of 20 mice were randomly distributed into five groups of four mice each. The infected mice were treated immediately after inoculation with 200, 400 and 600mg/kgbw of the extract respectively. Group 4 (Positive control) was treated with standard drug (Chloroquine, 5mg/kgbw) while the group 5 were administered normal-saline. The phytochemical constituents and acute toxicity of the plant extract were determined using standard procedure. The result showed that the extract contained alkaloid, tanin, saponin, glycoside, terpenoid, flavonoid, while the LD_{50} of the extract was 2236mg/kgbw. The parasite count was observed to decrease dose dependently. The % chemo-suppression was within the range of 17.15% for group 1 to 66.65% for the standard drug group. There was no significant difference ($p<0.05$) in the % suppression between the group administered 600mg/kgbw (58.24%) and the standard drug group. The mean survival time of mice treated with standard drug (21.33 days) was significantly ($p>0.05$) higher than those in other treatment groups and normal saline group (2.27 days). On the 5th day, the PCV of mice administered 600mg/kgbw (44.63±1.67 %) showed no significant ($p<0.05$) different compared to those in the standard drug treated group (47.92±1.75%). The ethanol leaf extract of Vernonia amygdalina exhibited antiplasmodial potential and its usage could be employed in the treatment of malaria.*
Keywords: Vernonia amygdalina, Antiplasmodial activity, Plasmodium berghei, Extract concentration, Malaria

I. Introduction

Malaria is one of the leading causes of morbidity and mortality, particularly in children under five years of age. It has remained endemic in many parts of the world, especially in the sub-Saharan Africa (Sharif and Kimani, 2010). Schofield (2007), has established that malaria is possibly the most serious infectious disease of humans, infecting 5–10% of the world's population, with 300–600 million clinical cases and more than 2 million deaths annually. Similarly, Snow et al.,(2008), reported that that there are over 500 million cases of malaria annually in the developing nations. In Nigeria, approximately 97% of the estimated 160 million inhabitants are at risk of Plasmodium infection (NMCP 2011). This translates to approximately 51 million cases and 207,000 deaths per annum, representing 25% of the total malaria burden within Africa (WHO, 2012). Malaria reportedly accounts for an estimated 60% of outpatient visits in Nigeria, 30% of hospitalization, 30% of under-five mortalities, 25% of infant mortalities and 11% of maternal mortalities (NPC, 2009).

Malaria is becoming more resistant to a number of current drugs and is on the increase because of the global warming process (Martin and Lefebvre, 1995). The alarming rate at which Plasmodium falciparum has developed resistance to chloroquine and other synthetic antimalarial drugs makes it necessary to search for more effective antimalarial compounds (Bhat and Surolia, 2001). Medicinal plants have been used since prehistoric times for the cure of different ailments (Qureshi, et al., 2009). The two main groups of modern antimalarial drugs – artemisinin and quinine derivatives are known to have their source from herbs. Many communities who live in endemic areas have started to look for malaria remedies in plants in their local environments (Miliken, 1997). It is therefore neccessary to evaluate useful medicinal plants for its anti-plasmodial activity there by forming the basis for derivation of potent, safe and affordable anti-malarial drug for the sub-saharan Africa.

V. amygdalina is commonly called bitter leaf because of its bitter taste. It is a member of the Asteraceae family and a small evergreen shrub that grows all over Africa. It can adapt to a variety of climates unlike other plants that are native to certain areas. It is grown in many countries, in savannah zones and cultivated fields (Ibrahim et al., 2010). It attains the height of 2.5m with petiolate leaf of about 6mm in diameter that is elliptic in shape (Ijeh and Ejike, 2011). The branches are brittle and break off easily. It has grey or brown coloured bark; the bark has a rough texture and is flaked. Although most popularly used for food, it has also been traditionally used for its medicinal properties (Swee et al., 2010). Its uses include treatment of jaundice, diarrhea, Hepatitis B and C, cancer; diabetes and tuberculosis (Muanya, 2013). It is also reported to be a medicinal plant for diabetes and fever (Crellin et al., 1989).

Ethanolic extracts of the stem bark and leaves are reported to have been used as purgative, antimalarial and in the treatment of eczema (Ojiako and Nwanjo, 2006). This study was focus on the in vivo antimalarial activity of V. amygdalina leaf on P. berghei infected mice. In addition, its acute toxicity, effect on Packed Cell Volume and Mean survival Period were also determined.

II. Materials And Methods

Collection of Plant

Fresh mature V. amygdalina leaves were obtained in the month of August, 2014 from Tudun Fulani area of Bosso Local Government, Minna, Niger State. The leaves were authenticated by Biological Science Department of Federal University of Technology, Minna.

Extraction of Plant material

Fresh leaves of V. amygdalina were cut into pieces and air dried in the laboratory. The pieces were then pounded using mortar and pestle followed by its reduction into powder form by Electronic blending machine. The total powdered form of leaf extract weighed 326grams on Electronic weighing machine. 70grams of the powdered leaf were extracted using 300ml of ethanol for two hours each (Reflux extraction). All the extracts were concentrated to dryness on a water bath and weighed 24.8grams after evapouration. The extracts were then stored in well-closed containers and kept in a refrigerator at 4 ºC to protect from light and moisture till used (Sutharson et al., 2007).

Experimental Animals

Healthy Swiss albino mice of either sex of about 7weeks old weighing between 20-30g were obtained from Nnena Rodents Nigeria Limited NITECO Road, Tunga, Minna, in Niger State, Nigeria and were used for the experiments. The mice were housed under standard laboratory conditions at temperature 27±2ºC, relative humidity 70% and at 12 hours day/night cycle. They had free access to rodent pellets and water. The animals were acclimatized for 14days before the commencement of the study.

Malaria Parasite

A chloroquine-sensitive strain of P. berghei (NK-65) was obtained from the National Institute For Pharmaceutical Research and Development (NIPRD), Idu. Abuja, Nigeria and maintained by re-infestation via intraperitoneal with infected blood suspension (0.2ml) containing about 1x107 suspension of P. berghei parasitized Red blood cells.

Phytochemical Tests

The phytochemical screening of aqueous extract of V. amygdalina leaf was carried out to determine the presence of the following compounds; alkaloid, flavonoids, tannins, anthraquinones, saponins, glycosides and terpenoids using standard procedures.

Acute Toxicity Test

The test was carried out in three phases. In the first phase, Twelve mice, randomly divided into three groups (A,B, and C) of four mice each, were given 10, 100 and 1000mg extract / kg/body weight orally respectively after 24hour of fasting. Signs of adverse effect and death were observed for 24hours. In the second phase, another specific doses of 1500, 1750 and 2000mg/kg were administered to another twelve mice of three groups each orally and were also observed for twenty-four hours. In the third phase, specific doses of 2500, 2750 and 3000mg/kg were administered to another twelve mice of three groups each orally. Signs of adverse effect were observed, number of deaths in each group within 24hours was recorded and the final LD_{50} values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

Antiplasmodial study

Suppresive Test

A total of twenty mice were used for the study. Each mouse was given standard intra-peritoneal inoculums of 1.0 $x10^7$ P. berghei parasites with the aid of a 1ml disposable syringe. The animals were divided into five groups of four each. Different doses of 200, 400 and 600mg/Kg/ day) were administered orally to these groups.

Chloroquine (5mg/Kg/day) was given as positive control and 0.2ml of normal saline as negative control for four consecutive days. (D_o to D_3). On the fifth day (D_4), thick blood and thin blood films (fixed with methanol) were made from tail blood, stained with 10% Giemsa stain for 10minutes and washed off with a phosphate buffer (pH 7.0). The slides were allowed to dry and examined microscopically with 100-x

magnification to access the parasitaemia. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of the treated mice (Knight and peter, 1980). Variation in weight and packed cell volume were monitored in the course of the study. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group.

$$\%\text{chemo-suppression} = \frac{\text{Parasitemia of Control} - \text{Parasitemia of Test}}{\text{Parasitemia of Control}} \times 100$$

III. Result

The result of the phytochemical screening of ethanol extract of V.amygdalina leaf is presented in the Table 1. The analysis revealed the presence of saponins, tannins, flavonoids, terpenoids, alkaloids and glycosides. However, anthraquinones were absent.

Table 1: **Phytochemical composition of aqueous extract of V.amygdalina leaf**

Bioactive agent	**Indication**
Alkaloids	+
Saponins	+
Glycosides	+
Terpenoids	+
Tannins	+
Flavonoids	+
Anthraquinones	-

+ = Present; - = Absent

Acute Toxicity Test

The acute toxicity of this work shows that the ethanol extract is only toxic at very high dose. The medial lethal dose of the LD_{50} was determined using the formula below.

Medial lethal dose for $LD_{50} = \sqrt{a \times b}$ (Saidu Ibrahim Ngulde et al., 2013)

Where a = least dose that killed a mice, while b = highest dose that did not kill any mice.

The ethanol extract showed sign of toxicity at dose of 2500mg/kgbw and the highest dose at which no death occurred was recorded at 2000mg/kgbw. The LD_{50} obtained was 2236mg/kgbw.

In vivo study

Percentage Suppression

The percentage suppressive activity of the standard drug and the group treated with the highest dose of the extract (600mg/kgbw) showed little or no significant difference. These two groups exhibit the highest percentage suppression when compared to the other three groups.

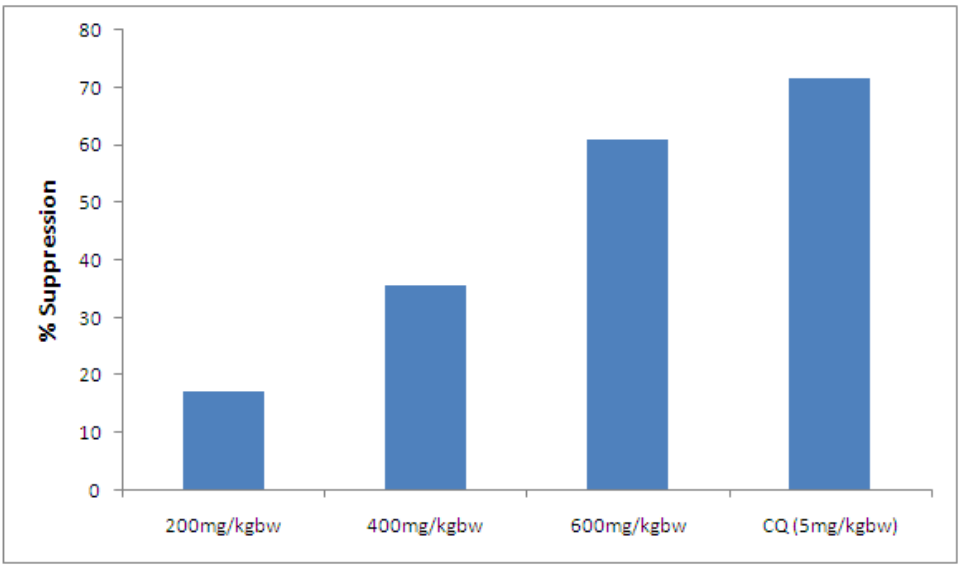

Figure 1: The Percentage Suppression activity of the Extract and the Standard drug on P. berghei infected mice.

Packed Cell Volume (PCV)

The PCV of the Extract, Standard drug and Normal Saline treated group was determined before, during and after the treatment. The Percentage-change in the PCV of the groups was significantly different.

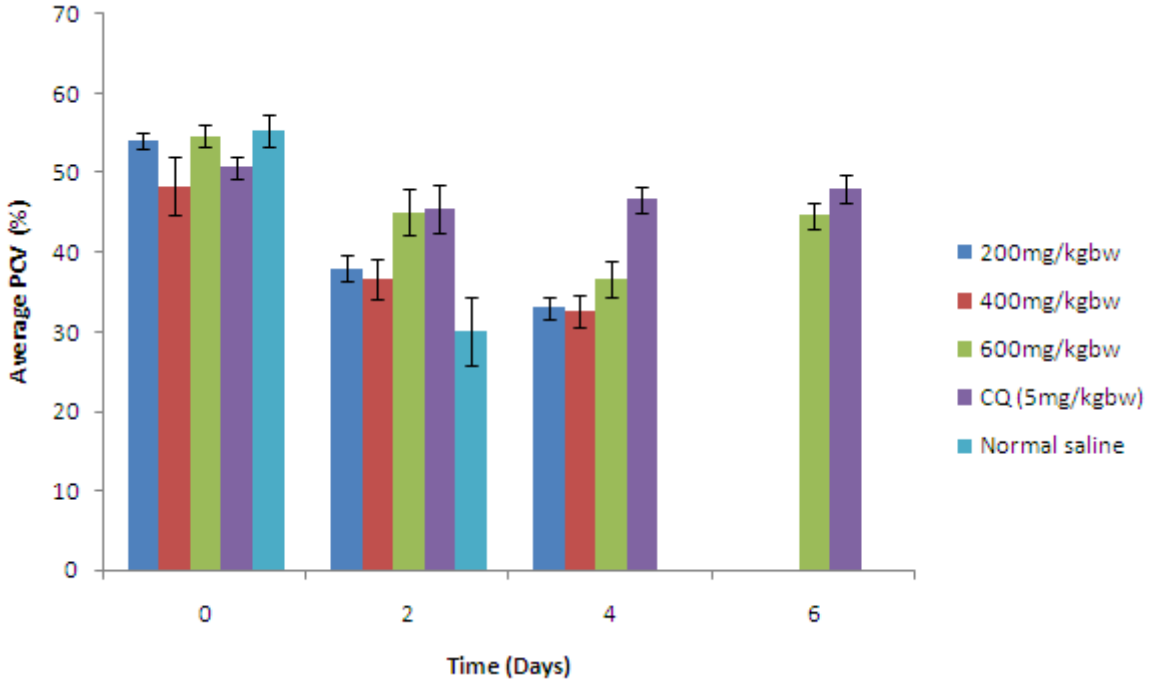

Figure 2: The Percentage-change in Packed Cell Volume of the V. amygdalina Extract, Standard drug and Normal saline treated group.

Mean Survival Time (Days)

The Mean Survival Time of the P. berghei infected mice was determined. The standard drug treated group has the highest Mean Survival Time (21 days), followed by the group treated with 600mg/kgbw (13days) while the Normal saline treated group has the lowest Mean Survival Time (2days).

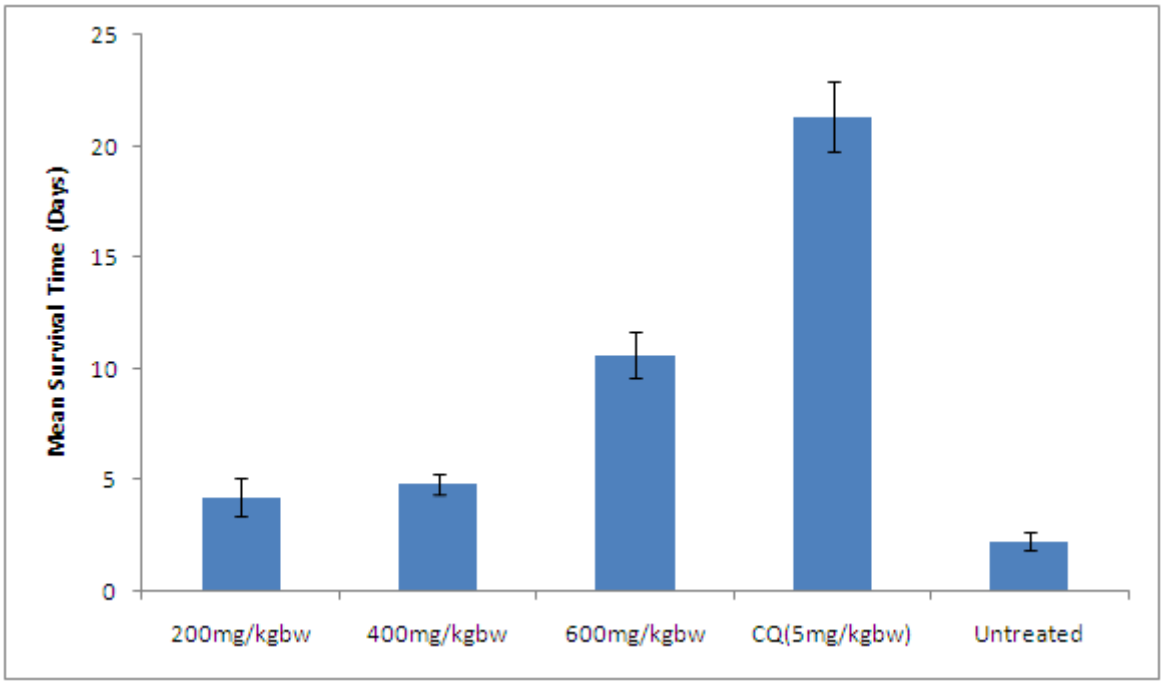

Figure 3: The Mean Survival Time of the P. berghei infected mice treated with V. amygdalina Extract, Standard drug and Normal saline treated group.

IV. Discussion

In this study, the in vivo antimalarial activity of ethanol etract of V. amygdalina leaf was evaluated. The group administered the highest dose(600mg/kgbw) of the extract showed a significant progressive reduction in parasitemia level with time when administered daily for four days (Suppressive Test). It produced 61% suppression of parasitemia which is almost similar to the percentage suppression showed by the standard drug (70%). This is in agreement with the work of Abosi and Raseroka (2003), who earlier recorded 67% parasitemia suppression in an in vivo antimalarial activity test of the plant against resistant P. berghei.

The acute toxicity of this work shows that the ethanol extract is only toxic at very high dose (orally given). The ethanol extract which showed sign of toxicity at dose of 2500mg/kgbw also had an LD_{50} of 2236mg/kgbw. The LD_{50} of this study seems to disagree with Ojiako and Nwanjo (2006) who recorded an LD_{50} of 500mg/kgbw in rats administered intraperitoneally, (The wide difference could be mode of administration of the extract or any other determinants), but agrees with Sha'a et al. (2013) work who recorded an LD_{50} of 1950mg/kgbw having shown sign of toxicity at dose of 3000mg/kgbw.

The administration of the extract to the P. berghei infected mice for four days in this study showed a significant increase in the value of the Packed Cell Volume (PCV) as the doses increases. This could be as a result of destructive antiplasmodial effect of the extract against the parasitized Red blood cell and the causative parasite, thereby sustaining the availability of the new red blood cells produced in the bone marrow. The group treated with the highest dose of the extract (600mg/kgbw) and the group administered Standard drug (Chloroquine) showed a high PCV on the fifth day. This could simply mean that the extract at high dose can reverse the manifestation of the low PCV that occurred during the early stage of infection of the mice as the treatment continues i.e with time. The significant decrease in the PCV of the Negative control (Normal saline administered group) could be an indication of anaemic condition which may be attributed to the malarial infection. This could also be as a result of increase in the rate at which red blood cells are destroyed and decrease in the rate at which new ones are produced. However, evaluation of body weight showed little or no significant difference before and after treatment.

The standard drug treated group and the 600mg/kgbw treated group exhibited high mean survival time (21 and 13days respectively) when compared to the group treated with Normal saline (2days). This is in agreement with the work of Trager and Jesen (1976) which showed that the group treated with the highest dose of the extract and the group administered the standard drug exhibited prolong mean survival time when compared to the Negative control group.

This could mean that the 600mg/kgbw and standard drug administered groups are capable of sustaining the P. berghei infected mice for longer period of time before death. The death of these two groups in the later days could be as a result of recurrent malaria or any other determinants.

Though the malaria model, P. berghei is not similar to that of human plasmodium parasite, however, the antiplasmodial activity demonstrated as the dose increases by the extract against P. berghei infected mice in this study could be a potential indication of active antiplasmodial effect against the human malaria parasite (Audualem Getie (2010).

V. Conclusion

In this study, ethanol extract of V. amygdalina leaf appeared to be toxic only at a very high dose. This is in agreement with the work of Sha'a et al. (2013) who also observed extract of V. amygdalina leaf to be toxic only at a very high dose.

The ethanol extract of V.amygdalina leaf exhibited antiplasmodial potential and its usage can be employed in the treatment of malaria.

References

[1]. Abosi, A. O. and Raseroka, B. H. (2003). In vivo antimalarial activity of Vernonia amygdalina. British Journal of Biomedical Science, 60(20): 89-91.
[2]. Bhat, G. P. and Surolia, N. (2001). In vitro antimalarial activity ofextracts of three plants Used in the traditional medicine of India. American Journal of Tropica Medicine and Hygiene, 65(4): 304-308.
[3]. Ijeh. I.I. and Ejike, C.E.C. (2011). "Current perspectives on the medicinal potential of Vernonia amygdalina Del". Journal of Medical Plant Research, 5 (7): 1051–1061.
[4]. Lorke, D (1983), "A new approach to practical acute toxicity tests", Archives Toxicology journal, 54(2), 275-287
[5]. Miliken, W. (1997). Malaria and anti-malarial plants in Roraima, Brazil. Tropical Doctor, 27, 20-25.
[6]. Ngulde, S.I., Tijjani, M.B., Ihopo, J.M and Ya'uba, A.M (2013). Antitrypanosomal potency of methanol extract of Cassia arerehdelili root bark in albino rats. International Journal of Research and Technology, 3(1)1-7
[7]. Qureshi, R., Waheed, A., Arshad, M. and Ambreen, T. (2009). Medicoethnobotanical Inventory of Tehsil Chakwal, Pakistan. Pakistanian Journal of Botany, 41(2), 529-538.
[8]. Ojiako, O.A. and Nwanjo, H. U. (2006). Is Vernonia amygdalina hepatotoxic or Hepatoprotective? Response from biochemical and toxicity studies in rats. African Journal of Biotechnology, 5(18):1648 – 1651.

[9]. Sharif, S.K. and Kimani .F. (2011). National Cancer Control Strategy (2011-2016), Ministry of Public Health and Sanitations and Ministry of Health Services. Nairobi.
[10]. Sha'a, K.K., Oguche, S., Watila, I.M and Ikpa T.F (2011). In vivo antimalarial activity of the extracts of Vernonia amgdalina commonly used in traditional medicine in Nigeria. Science World Journal, 6(2):5-9
[11]. Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y. and Hay, S.I. (2005). The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature 434, 214–217.
[12]. Swee, K., Wan, Y., Ho, B., Boo, K.H., Woon, S.L., Huyunh, K., Abdul, H., Naoman, Y. and Noorjaham, B.A. (2010). V. amygdalina: an ethnoveterinary used green vegetables with multiple bioactivities. Journal of Medicinal Plant Research, 4(25):2787-2812.
[13]. Trager, W. and Jensen, J. B. (1976). Human malaria in continuous culture. Journal of Science, 193:673–675.