ANTIMALARIA AND HEMATOLOGICAL PROPERTIES OF ETHANOL LEAF EXTRACT OF *PENNISETUM PURPUREUM* ON *PLASMODIUM BERGHEI* INFECTED MICE

Abstract:

Through bite from a female Anopheles mosquito, Malaria is transmitted by infection with single-celled parasites of the genus *Plasmodium.* Studies have shown it to be characterized by periodic bouts of severe chills, accompanied with high fever. It has been suggested that *Pennisetum purpureum* possess antiplasmodia effects, however, no scientific record(s) yet exist(s) to validate this claim. This study was therefore undertaken to determine the anti-malaria and haematological properties of ethanol leaf extract of *Pennisetum purpureum* in *Plasmodium berghei* -infected mice. Thirty-Five (35) albino mice (20 – 20g) were procured, acclimatized (for two weeks) and assigned to five groups of 7 mice each. With group I receiving standard rat feed ad-libitum (control), Groups II through V were respectively infected with *Plasmodium berghei* (malaria infected, untreated), *Plasmodium berghei* infected + treated with 5mg/kg body weight of Artesunate (malaria infected, Artesunate treated), infected with *Plasmodium berghei* + treated with 200mg/kg body weight of *Pennisetum purpureum* (malaria infected, low dose extract treated), and infected with *Plasmodium berghei* + treated with 400mg/kg body weight of *Pennisetum purpureum* (malaria infected, high dose extract treated). After 21 days of administration, mice were sacrificed, blood samples collected, centrifuged for 10 minutes at 300g, and resulting supernatant biochemically analysed for hematologic changes. Result showed a significant increase in initial parasite count across groups except control. Administration of Artesunate also caused a significant ($p < .05$) reduction in parasite counts upon comparison with control. More so, administration of low and high dose extract caused a significant ($p < .05$) reduction in parasite count following comparison with control. Administration of 200mg/kg caused the highest parasitemia suppression than high dose. We recommend for further evaluation of the plant in other to identify active ingredients responsible for the observed antimalarial activity.

Keywords: Malaria, Haematological Properties, *Plasmodium berghei, Pennisetum purpureum*

Comment [IJN1]: antiplasmodial

Introduction

Being one of the most common infectious diseases and a great public health problem globally, malaria is reportedly the world's deadliest disease, particularly affecting people in tropical and sub-tropical regions of the world, especially in sub-Saharan Africa and Southeast Asia[1-4]. It remains a major health care challenge in Nigeria with high morbidity and mortality. According to WHO report, the country is one of the two countries that accounts for 40% of all deaths associated with the disease[5]. The disease reportedly accounts for an estimated 60% of outpatient hospital visits in Nigeria, 30% of hospitalizations, 30% of under-five mortalities, 25% of infant mortalities and 11% of maternal mortalities[5-7].

In the past few years, available reports posit that tremendous gains have been made in the fight against malaria. Between 2000 and 2012 for instance, the malaria incidence rate has reportedly reduced by 25% globally, and by 31% in West African Region[5]. The estimated malaria mortality rates have also fallen by 42% in all age groups, and by 48% in children under 5 years of age[5]. This success has been attributed to the adoption of the artemisinin combination therapy (ACT) as first line drug treatment in malaria endemic regions and also the scale - up of intervention efforts such as the use of long lasting insecticide nets (LLIN), intermittent prevention treatment (IPT) for pregnant women, vector control measures and more importantly increased funding[5]. Though cheap and accessible solutions have been made to prevent malaria and its life-threatening complications, overtime, the cost of anti-malaria, undesirable and adverse effects associated with some of these ACT drugs has caused sufferers to resort to the use of suitable herbs with minimal effects as such for malaria management. Over 50% of known local herbs now serve trado-medical practitioners in treatment and management of malaria; some examples are; *Artemisia annua*[7], the leaves of *Guinesis* unripe fruit of *capsicum frutescenee*, stem bark of *chrysophyllum albidum*[8], *kaya grandifolia*[9], *Azadirchta indica (Dogon yaro)* [10], *Zingiber officinale*[8], *Vernonia amygdalina*[11], and *Garcina kola*[12].

The plant *Pennisetum purpureum* is allegedly used as a diuretic in anuria or oliguria and also as a source of medicinal salt[2]. *Pennisetum purpureum* is a robust grass with perennial stems. The plant produces short, creeping rhizomes of between 15 to 25 cm long with fine roots at the nodes and culms of about 2 to 8 m in high, 2.5 cm in diameter, and a solid centre. Older culms may branch several times. Leaf blades are 50 to 90 cm long and 1 to 3cm wide, flat, and have a white midrib. Leaves of new, vigorous growth have wide, robust leaves; older culms have finer, narrow leaves. Leaf margins are rough (fine-toothed). The inflorescence is a compact, erect, bristly tawny or purplish spike 8 to 30 cm long and 1.5 to 3 cm wide[6].

Although, it has been suggested that *Pennisetum purpureum* possess antiplasmodia effects, no scientific record(s) yet exist(s) to validate this claim[13]. Hence, current study was designed to evaluate the antiplasmodia effect of ethanol leaf extract of *Pennisetum purpureum* on *Plasmodium berghei*-infected mice. Specifically, study examined the antimalaria activities of ethanol leaf extract of *Pennisetum purpureum* on packed cell volume (PCV), in *Plasmodium berghei* -infected mice. Study also investigated the antimalaria activities of ethanol extract of *Pennisetum purpureum* at different doses on body weights and red blood cell counts in *Plasmodium berghei* -infected mice. Lastly, study compared the

Comment [IJN2]: antiplasmodial.

Effect this change in any subsequent places where this word appears

efficacy of the extract at different doses with known drugs (Artesunate) in the therapy malaria.

Materials and Methods

Scope of Study

This Study was conducted at the Departments of Pharmacology and Human Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria. Albino mice rats were used as choice of experimental model.

Comment [IJN3]: There's nothing like mice rats. It is either mice or rats.

Study Design

Thirty-Five (35) albino mice (20 – 20g) of the Swiss variant were assigned into five (5) groups of seven rats each (n=7). *Pennisetum purpureum* extract was orally administered for 21 days as follows;

Comment [IJN4]: Rats????????????

The title says mice.
Correct this

Group 1:	Normal control
Group II:	Infected mice not treated
Group III:	Infected mice + Artesunate (5mg/kg)
Group IV:	Infected mice + Extract low dose (200mg/kg)
Group V:	Infected mice + Extract High dose (400mg/kg).

Resources and Sources

Hand glove, Oral cannula, Test tubes, Feeds (grower mash), Syringes and needle (1ml, 2ml and 5ml), Plain container, Dissecting kit, Dissecting board, Whiteman filter papers (size/circles 110mm, cat no. 1001110), Cotton wool, Masking tape, Nose mask, Soap, Tissue paper, Distilled water, Sample bottle, Measuring cylinders, Petri dish, lancet, universal Container, Lithium Heparin tube, Spatula, Stop watch, Animal cage and plate, Mortal and pistle, Microlux MicroPipette (Range: 0-100µ), Remi Micropipette (Range 20-200µ)and Lab Tec Drying Oven (Drying cabinet). Other apparatus include; Weighing balance, heating mantle, Optima Centrifuge (model number: REM-R-24), Thermocool Refrigerator (model number: NX-275C China), Spectrophotometer, Electronic weighing balance (Model number: 3A 3003) and Incubator (Model number: SM 801A).

Collection and Identification of Plant Sample

Fresh leaves of *Pennisetum purpureum* were procured from local markets within the University environment. They were then authenticated by renowned taxonomists from the

Herbarium Unit of the Department of Botany, Faculty of Science, Delta State University, Abraka, Nigeria.

Preparation of Plant's extract

After proper authentication by certified taxonomists, obtained leaves (*Pennisetum purpureum*) were washed in tap water and sun-dried for 5 days at room temperature. With the aid of an electric blender, the crispy dried leaves were then blended to powder. 164g of the powder was then macerated (after weighing) in 60ml of 70% ethanol for 72 hours. Next, the mixture was filtered with the aid of a filter paper to obtain some clear filtrate. Obtained filterate was further concentrated to dryness with the aid of a heating mantle at 50^0C, and then, placed inside a fume hood. Final weight of extracts' *Pennisetum purpureum* was weighed after concentration. The extract was then stored in refrigerator for 24 hours and thereafter stock solution was prepared from it.

Chemical and Reagents

Normal Saline, chloroform, Ethanol, and Giemsa stain.

Procurement and Inoculation with *Plasmodium berghei*

Chloroquine-sensitive *Plasmodium berghei* (NK65) was obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. Experimental mice were then infected by obtaining parasitized blood (3-4 drops) from the cut tail tip of another infected mice, which acted as donor. Following was the dilution of infected blood in 0.9ml of phosphate buffer with a pH of 7.2 and the inoculation of the mice with 0.1ml of the parasitized blood intraperitoneally. This contained about twelve thousand (12000) parasites.

Ethical Issues

Ethical clearance (RBC/FBMS/DELSU/142/1160) was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State. Animals were handled in accordance with approved guidelines of the institutional animal ethics committee (IAEC), as adopted by the Faculty of Basic Medical Sciences, Delta State University, Abrake, Nigeria.

Comment [IJN5]: Abraka

Inoculation of Experimental Malaria

At the end of acclimatization, the parasite was maintained in mice (weighing 20-25g) by serial passage of infected blood to uninfected mice in the animal house (0.2ml of diluted blood sample from infected mice) intraperitoneally. Parasitized red blood cells used for inoculation in the experiment were obtained by cardiac puncture from an infected donor mouse. The blood was diluted to desired parasite density in a 9 Molar solution of NaCl (1ml of blood sample was dissolved in 9ml of Normal saline). After 72 hours, a test was carried out to confirm mice that were infected with malaria.

Procedure

Determination of Body Weight

Body weight of experimental animals was determined at week 0 (before administration) and subsequent weeks and the last day of experiment. Percentage change in weight (weight gain/loss) was later calculated using;

$$\frac{\textit{Final weight-initial weight} \times 100}{\textit{Initial weight}\ 1}$$

Preparation of Stock Solutions

Artesunate (5mg/kg)

One tablet of Artesunate (50mg) was dissolved in 100mls of distilled water. The final concentration (0.5mg/ml) was administered at a standard dose of 5mg/kg.

Norormal **saline**

Comment [IJN6]: Normal

0.9g of NaCl (sodium chloride) was dissolve in 100ml of distilled water.

Extract low dose (200mg/kg)

200mg (0.2g) of extract was measured using analytical weighing balance and was dissolve in 5mls of distilled water. The final concentration 20mg/ml was administered to the animals at a dose of 200mg/kg.

Extract High dose (400mg/kg)

400mg (0.4g) of extract was measured using analytical weighing balance and was dissolve in 5mls of distilled water. The final concentration 40mg/ml was administered to the animals at a dose of 400mg/kg.

Sample Collection

At the end of administration (21 days), animals were anesthetized in a desiccator containing cotton wool soaked with chloroform. After they had attained deep anaesthesia, animals were brought out of the desiccator and a laparotomy was carried out (by making V-shape incision in abdominal region with the aid of surgical scissor) and the organs were exposed. With the aid of needle attached to 5ml syringes, blood samples were collected from the inferior vena cava into a well labelled plain bottle. Blood samples were centrifuge for 10 minutes at 300g. Cleared supernatants were aspirated into well labelled plain bottles and stored in the freezer at 4^{o}C prior to biochemical analysis.

Statistical Analysis

Results from this study are expressed as mean ± SEM, and analysed using one way analysis of variance (ANOVA) with statistical package for social science (SPSS, 16). Differences between means were tested with post Hoc-Turkey's test for multiple comparison, and significance was considered at $p < .05$.

Results

Table 1: Effect of Ethanol Leaf Extract of *Pennisetum purpureum* on Parasite Count of *Plasmodium berghei*- infected mice.

Groups	Parasite count (%) before treatment	Parasite count (%) after treatment	Parasitemia suppression
Normal control	0.00±0.00	0.00±0.00	-
Infected control	21.80±1.32	26.80±1.74	0
Artesunate (5mg/kg)	21.80±1.66	21.60±1.57	19.40
***P. purpureum* (200mg/kg)**	15.80±1.43	9.20±0.37*[a]	65.67

***P. purpureum* (400mg/kg)**	24.60±1.89	14.60±1.63*[a]	45.52

*Values are presented as mean ± Standard error of mean (SEM), n=5. *p < .05: Significantly different from parasite count in group II. [a]p <.05: Significantly different from parasite count in group III.*

Table 2: Effect of Ethanol Leaf Extract of *Pennisetum purpureum* on packed cell volume and Red blood cell count of *Plasmodium berghei* infected mice.

Groups	PCV (%)	RBC (Cell count × 10^{12}/L)
Normal control	50.00±4.32	4.52±0.37
Infected control	44.20±4.08	4.00±0.36
Artesunate (5mg/kg)	42.20±2.03	3.51±0.33
***P. purpureum* (200mg/kg)**	46.00±2.00	4.16±0.18
***P. purpureum* (400mg/kg**	49.00±0.84	4.42±0.07

Values are presented as mean ± Standard error of mean (SEM), n=5.

Discussion

Malaria is caused by the parasite *plasmodium*, of which four species are known to cause human infection; *P. falciparum, P. vivax, P. malariae* and *P. ovale. P. falciparum* infection may be associated with life threatening complications such as cerebral malaria, severe anaemia, acidosis, respiratory distress and acute renal failure (ARF) [14]. Increase in parasite count has been observed in patients suffering from malaria infection and this is due to the invasive nature of the causative agent (*Plasmodium*) into the blood and organs of the patient[15].

From this study (Table 1), *Plasmodium berghei* infected (control) mice showed a significant increase in parasite count (26.80±1.74) as compared to other groups. The increase in parasite count is as a result of *Plasmodium berghei* that was injected into the mice. Even though oral administration of Artesunate (5mg/kg), *Pennisetum purpureum* ethanol leaf extract (200mg/kg and 400mg/kg) caused a dose-dependent reduction in parasite count (21.60±1.57, 9.20±0.37 and 14.60±1.63) in the *Plasmodium berghei* infected treated mice, however, administration of 5mg/kg of Artesunate caused only a significantly mild reduction

in parasite count upon comparison with the infected (control) group. Hitherto was oral administration of *Pennisetum purpureum* ethanol leaf extract (200mg/kg and 400mg/kg) found to produce a statistically significant decrease ($p < .05$) in parasite count (9.20±0.37 and 14.60±1.63) for the *plasmodium berghei* infected (group II) mice as compared to the standard drug (Artesunate, 5mg/kg); returning an average value of 21.60±1.57. This finding concurs with those of Adeleye *et al.* (2012)[15&16], who reported that RBC counts are significantly reduced in malarial infection, with an accompanying increase in parasite count that ultimately reduces the packed cell volume (PCV) levels of *plasmodium berghei* infected mice. However, Adeleye *et al* showed that administration of Artesunate caused a significant restoration in RBC count, tending the PCV levels towards normal.

Parasite count after treatment:

From table 1 above, there was reduction in parasite count of all animals in all groups (except infected control) after treatment. Administration of Artesunate caused a non-significant ($P > .05$) reduction in parasite count (21.60±1.57) when compared to parasite count in infected control group (26.80±1.74). However, administration of extract 200mg/kg and 400mg/kg caused a significant ($P < .05$) reduction in parasite count (9.20±0.37 and 14.60±1.63) when compared to parasite count in infected control group (26.80±1.74).

Parasitemia Suppression

From table 1 above, extract 200mg/kg showed the highest parasitemia suppression (65.67%) followed by extract 400mg/kg (45.52%) and was least in Artesunate group (19.40%) when compared to infected control group (0%).

Effect on packed cell volume (PCV)

From table 2 above, there was non-significant reduction in PCV of animals in infected control (44.20±4.08) when compared to normal control (50.00±4.32). Administration of extract 200mg/kg and 400mg/kg caused non-significant increase in PCV of animals (46.00±2.00 and 49.00±0.84) when compared to PCV in infected control group (44.20±4.08).

Effect on Red Blood cell (RBC):

From table 2 above, there was non-significant reduction in RBC of animals in infected control (4.00 ± 0.37) when compared to normal control (4.52 ± 0.37). Oral administration of

Artesunate, 5mg/kg caused non-significant (P>005) reduction in RBC (3.51 ± 0.33) when compared to infected control. However, administration of extract 200mg/kg and 400mg/kg caused non-significant increase in RBC of animals (4.82 ± 0.36 and 4.50 ± 0.39) when compared to RBC in infected control group (4.00 ± 0.37).

Antiplasmodial activity has been related to a range of several classes of secondary plant metabolites including alkaloids, sesquiterpenes, triterpenes, flavonoids, limonoids, quassinoids, xanthones, quinines and phenolic compounds of which alkaloids have been the most important and have shown very interesting activities[16]. Indeed, quinine is the first antimalarial drug that belongs to the class of alkaloids[17]. *Pennisetum purpureum* generally contain diterpenoids, triterpenoids, alkaloids, flavonoids, lignoids and proanthocyanidins[18], which have strong antiplasmodial activity. Therefore, the antiplasmodial activity observed in this study may be attributed to the presence of these bioactive compounds. This result is similar with that obtained from previous publications[18].

Both high, 400mg/kg and low dose, 200mg/kg of *Pennisetum purpureum* ethanolic leaf extracts exhibited parasitemia suppressive effect on *P. berghei* (45.52% and 65.67%). However, only the low dose (200mg/kg) of the ethanolic leaf extract shows a significant suppressive effect on *P. berghei* infected treated mice. Thus, the result of this study may justify the traditional use of the plant for antimalarial therapy in the rural area[19]. Hematological abnormalities are considered a hallmark of malaria[20]. As reported by Taylor[20], *Plasmodium berghei* increases erythrocyte fragility and significantly reduces packed cell volume in mice. But, from this study, there was no significant reduction in red blood cell (RBCs) and packed cell volume in *Plasmodium berghei* infected control and treated mice as compared to the normal control mice. From result obtained from previous publications[18] there was a significant reduction in red blood cells (RBCs) and PCV in *Plasmodium berghel* infected mice. The possible explanation why the reverse is the case for this present study might be due to atmospheric factors such as; temperature and humidity or genetic composition of the mice which act to resist the action of the parasite from affecting these haematological parameters in the mice.

Benefit of Study

Study will enhance the establishment of pharmacological basis for the use of ethanol extract of *Pennisetum purpureum* as a possible antimalarial agent, especially in improving packed cell

volume and Red blood cell count and decreasing parasite count in patient suffering from malaria.

Conclusion

Ethanol leaf extract of *Pennisetum purpureum* (in high doses) can be said to pose antiplasmodial effect(s) on *P. berghei* infected mice. This may partly justify the claim by traditional practitioners about the use of this plant against malaria. *Pennisetum purpureum* ethanol leaf extract shows non-significant alteration in red blood cell and packed cell volume in treated *P. berghel* infected mice at the dosage use for this study.

Recommendations

Though the ethanol leaf extract of *Pennisetum purpureum* was found to possess antiplasmodic activities on infected mice, the active phytochemical composition of the plant that elicit this effect is yet to be fully known. Hence, further evaluation of the plants is recommended to identify the active components responsible for its observed antimalarial activity.

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