**Assessment of the Anti-malarial Potential of *Gmelina arborea* in suppressive and established infection study in mice**

**Abstract**: Malaria being a disease of massive burden in Nigeria and Africa has wasted income and caused death in infants and pregnant women majorly. Plants has been the main source of medicine in Africa and traditionalist has used them in the management of malaria; *Gmelina arborea* (GA) has being used in the Eastern part of Nigeria in the treatment of malaria, but there is no scientific validation on its usage.

In this study, acute toxicity test, and the antimalarial potential of GA was evaluated. *Plasmodium berghei* infected mice in a 4-day suppressive test and 7-day curative models using mice were used. Parameters assessed were post-treatment parasitemia, level of suppression, hematological indices and survival.

1. *arborea* did not show acute or delayed toxicity; the level of suppression was high at 300mg/kg and level of parasitemia was low at same dose. The extract at 300mg/kg produced elevated red blood cells (RBCs) and platelets counts, and increase in hemoglobin and lymphocytes while 900mg/kg showed only increased platelet counts in the suppressive model. In the established infection, percentage inhibition (PI) was high and parasitemia (PP) was low from day 1-7 at 300mg/kg, while at 900mg/kg PI was high only on day 1. Platelets and RBCs counts were high at 300mg/kg while lymphocytes was high at 100mg/kg.

This indicated that *G. arborea* is a potential candidate for the management of malaria and there is need for further studies to identify the bio-active principle(s) and mechanism of antimalarial action.

Key words: antimalarial, suppresive, parasitemia, *Gmelina arborea,* curative

1. **Introduction**

Malaria is a tropical disease caused by parasite (*Plasmodium sp),* it can infect human through females’ mosquitos (*Anopheles sp*.) bite; over 200 species of *Plasmodium* has been discovered but 13 of them are known to be pathogenic to human (Fikadu *et al*., 2023). According to WHO, in 2017, there are still around 219 million cases of malaria globally, with a mortality rate of 435,000 (Faloye *et al.*,2023); The rate of mortality and morbidity is still high, especially in developing countries (Irungu *et al.,* 2023). Malaria is highly lethal unless treated (Ezeani *et al.*,2022); it can be diagnose by clinical signs and symptoms, microscopic detection of parasites in the blood smear and antigen-base rapid diagnostic test (Fikadu *et al.,* 2023).

The types of *Plasmodium* that initiate malaria in humans are *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi*. The fatal *Plasmodium* type of malaria is *P. falciparum.* (Ogbole *et al.,*, 2016).

The increase in the rate of malaria infection increases the out of pocket expenses by individual thereby contributing to the decrease in economic development (Andrade *et al.*, 2022).

The partial resistance of *P. falciparum* to artemisinin component-based therapy emerged in the Southeast Asia and has spread to East Africa, this partial resistance is mediated mainly by mutation of Kelch protein K13 (PfK13) which is manifested as delayed malaria parasite clearance after therapy (Conrad *et al.*, 2023).

Antimalarial drug resistance can increase morbidity and mortality especially in population where the immunity is low and the climate change is favourable for breeding vector (Ezeani *et al.*,2022; Leal *et al.,* 2023). This led to the need to develop new antimalarial agents, which is effective and accessible for developing countries, and wherever malarial infection is prevailing.

*Gmelina arborea Roxb* is one of the important medicinal plants most widely propagated and cultivated. It is commonly known as “K*ashmarya*” and one of the herbs mentioned in all ancient scriptures of *Ayurveda* (Jajere *et al.*,2021). This medicinal plant is highly valued from time immemorial because of its vast medicinal properties. In several countries in Africa and Asia, the *Lamiaceae* family plants are widely used as a traditional antimalarial herbal medicine including *G. arborea* in the south-eastern part of Nigeria (Odoh *et al.,* 2018). Several plants commonly used to treat malaria and several other plants from the *Lamiaceae* family have been investigated for their antimalarial efficacy both *in vitro* against *Plasmodium falciparum* or *in vivo* against *Plasmodium berghei* in animal like mice, except *G. arborea.*

**2.0 Material and Methods**

**2.1 Plant material**s

About 1000g of fresh leaves of *G. arborea* was collected from Kuje LGA, Abuja, Nigeria. The leaves were washed by rinsing in distilled water and air-dried completely at room temperature (30 ± 2ºC), protected from heat and direct sunlight for about 2 weeks. The dried leaves were homogenized to a fine powder using a Laboratory Hammer mill (Zhen Chang Equipment SFS P66) and extraction of the powder of the leaves would be done using 70% ethanol for 72 hours, and dried using a rotatory evaporator. The dried extract was transferred into air-tight glass vials and stored at 40C in a refrigerator.

**2.2 Acute Toxicity Study**

The animals were administered 2000mg/kg body weight of leaf extract and observed for 15 minutes, 30 minutes, 1 hour, 4 hours, and a week for signs of toxicity and mortality (OECD, 2024).

**2.3 Experimental Animals**

Thirty Swiss mice, weighing 20g-35g were sourced from the Animal Facility center of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD). The animals were maintained under standard environmental conditions (temperature 24 ± 2 °C) with free access to a standard rodent diet and water. The animals were acclimatized to laboratory conditions for two weeks before the studies. Ethical approval for animal experiments was collected from the Animal Care and Ethics Committee with approval number.

**2.4 Parasite inoculation**

Eight days after parasite inoculation of a healthy mouse, a Giemsa stained thin blood film was prepared from its tail vein blood for the assessment of parasitemia. The donor mouse was anesthetized, and blood was collected by cardiac puncture. The withdrawn blood sample was diluted with physiological saline such that 0.2 ml contained approximately 107 parasitized red blood cells based on the parasitemia level of the donor (Fidock *et al.,* 2004). Each experimental mouse was inoculated intraperitoneally with 0.2 ml of the diluted infected blood.

**2.4.1 In vivo suppressive test of extrac**t

The 4-day suppressive test model was used to measure the schizonticidal activity of the extract against *P. berghei* infected mice (Ezeani *et al.,* 2023). Total of 30 mice was used in this study.

The mice was weighed and randomized by weight into five groups (I–VI) of five each. A 0.2 ml volume of dilute infected blood was used to inoculate test mice by intraperitoneal injection. After 4 h, animals received extract orally at graded doses of 100mg/kg, 300mg/kg and 900mg/kg once daily for 3 consecutive days starting from Day 0 for Groups I–V. Group I-II were negative control (distill water only), and positive control (Chloroquine only) respectively, and Group III-V were given extract. Thin blood smears was prepared from the tail vein blood of the mice on day 4 and fixed in methanol then stained using 10% Giemsa stain, counting parasite per 100 red blood cells in five fields under a microscope with immersion oil and 100 objective was done.

Percentage of parasitemia was determined by:

% parasitemia =Number of parasitized RBCs ×100

Total number of RBCs

And suppression (%) produced by test extracts was determined by:

% Suppression= % Parasitemia (control)-% Parasetemia (treated) ×100

% Parasitemia control

Based on the percentage suppression produced, antiplasmodial activity would be classified as moderate for extract that suppressed parasitemia by equal to or greater than 50%. (Nardos and Makonnen, 2017).

**2.4.2 Screening of *G. arborea* hydroethanol extract in established infection (Curative**

**Antimalarial study**)

**2.4.3 Experimental design**

The curative antimalarial activity of *G. arborea* ethanol extract was carried out using a curative test model in mice (Fidock *et al.,* 2004). The experimental mice was weighed, randomized, and inoculated on the third day after establishing parasitemia in host mice. A total of 30 mice were inoculated and divided into groups 1-5 of 6 mice each. Positive control, negative control and treated groups of *G arborea* extract with doses 100mg/kg, 300mg/kg, 900mg/kg groups. Administration was done orally. They were fed with Vital commercial feed once a day. The positive control groups received choloquine (Sigma Aldrich, Germany) orally at 5 mg/kg once daily, while negative control group received distilled water (10 ml/kg). Treatment was done for 5 days, from day 3 to day 7. Parasite load was determined on day 8 using thin smears film stained with 10 % Giemsa solution. A total of 5 fields containing 100 red blood cells per field was counted under the microscope. The percentage of inhibition of the parasite growth (% I) of each dose extract was determined according to the following formula:

% I = [(parasite count of malaria control- parasite count of extract group)/

Parasite count of malaria control] ×100

Animals was kept for monitoring survival for 30 days.

**2.5 Heamatological Parameters**

Blood was collected from the tail vein of the mice in to EDTA tubes for the evaluation of hematological parameters using automated hematology analyzer YNH7021 (Wincom Company Ltd, Hunan, China). Parameter evaluated are red blood cells, platelet, neutrophil, hemoglobin concentration, and lymphocytes.

**3.RESULTS**

**3.1 Acute Toxicity study**

There was no acute or delayed toxicity at 2000mg/kg. The mice showed no sign of morbidity and no mortality even after 30days.

**3.2 Suppressive Effects of *G.arborea* on malaria parasites**

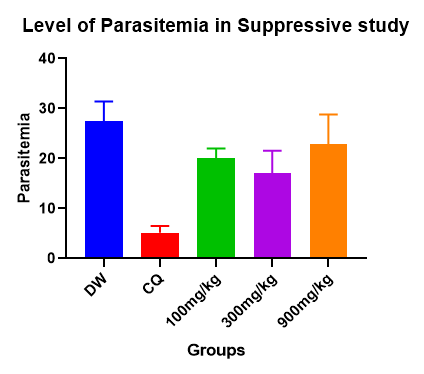
In the suppressive study, the percentage of parasitemia at 300mg/kg is low compared to 100mg/kg an 900mg/kg respectively; and the percentage of suppression is high at 300mg/kg as described in table 1 and Fig 1 respectively.

Table 1: Percentage of parasitic suppression

|  |  |  |
| --- | --- | --- |
| Groups | % Parasitemia | % Suppression |
| DW | 8.39 | - |
| CQ | 1.65 | 80.33 |
| GA 100mg/kg | 7.12 | 15.14 |
| GA 300mg/kg | 5.17 | 38.38 |
| GA 900mg/kg | 9.09 | -8.34 |

DW-Distilled water; CQ-Chloroquine; GA- *Gmelina arbroea*

Fig 1

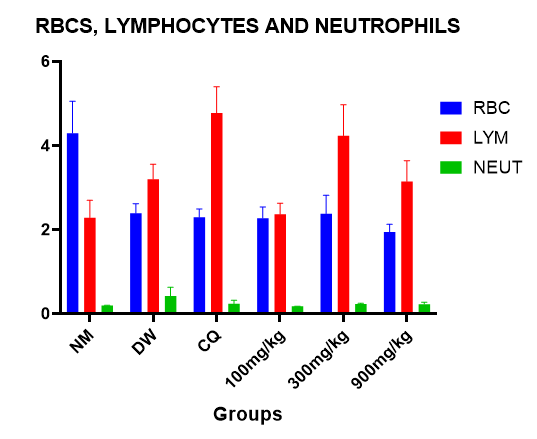


**3.4 Effects of ethanol extract of *G. arborea* on heamatological indices in suppressive study.**

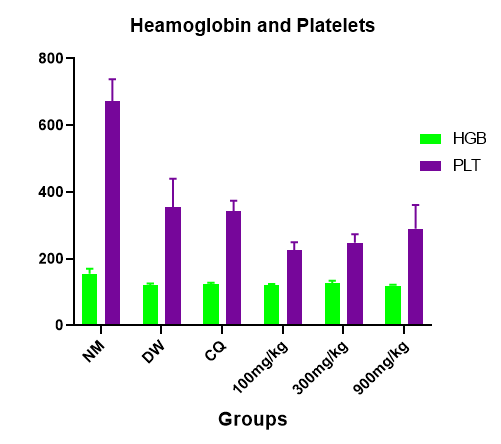
The level of RBCs at 900mg/kg is lower compared to other groups (1.94±0.18) while those of 100mg/kg and 300mg/kg showed no significant different compared to chloroquine and distilled water groups. There is increase in level of lymphocytes at 300mg/kg compared to 900mg/kg and 100mg/kg but lower to that of chloroquine group; while neutrophils level was lower in all the treated groups compared to distilled water group.

There was decrease in the level of hemoglobin and platelets in other groups compared with normal groups but 100mg/kg showed lower values as describe in Fig 2&3 below .

Fig 2-



**Fig 3-**



**3.5 Curative Effects of *G. arborea* on malaria parasites**

In the curative study, percentage inhibition was high at 900mg/kg on day 1 only, while at 300mg/kg the percentage inhibition was high on day 3 and 7 in extract treated groups as described in table 2.

Table 2: Percentage inhibition of *G. arborea* on malaria parasites

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | % inhibition day 1 | % inhibition day 3 | % inhibition day 7 |
| DW | 18.2 | 31.20 | 7.60 |
| CQ | 94 | 82.40 | 57.89 |
| GM 100mg/kg | 57.1 | 0.64 | -8.55 |
| GM 300mg/kg | 54.9 | 58.30 | 47.40 |
| GM 900mg/kg | 65.9 | -21.70 | 23.68 |

**Level of parasitemia in established infection**

On day 3 and 7, the level of parasitemia was low at 300mg/kg and high at 900mg/kg among the extract treated groups described in Fig 4&5 below.

Fig 4

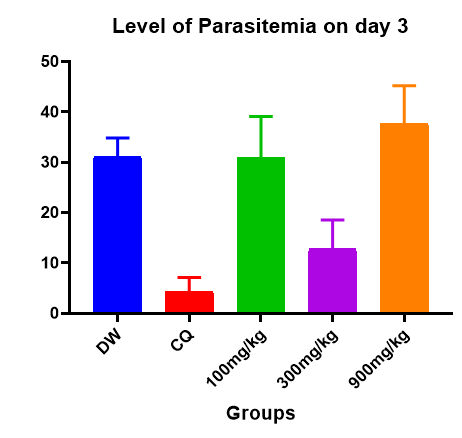
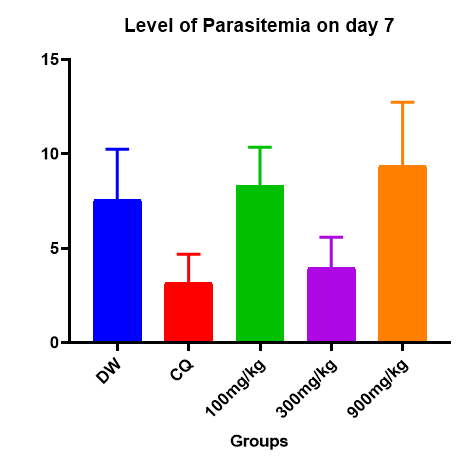


Fig 5



**Effects of ethanol extract of *G. arborea* on heamatological indices in curative study**

The hematological analysis in the established infection study showed that the level of platelets and lymphocytes was high at 100mg/kg, while the level of RBC was high at 300mg/kg, but there was no significant different in hemoglobin level in extract treated groups as shown in fig 6 and 7 below.

Fig 6

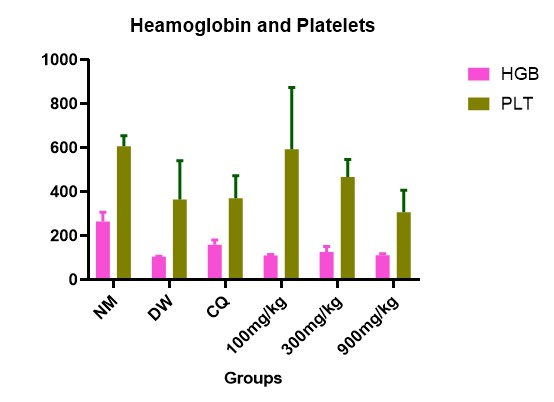
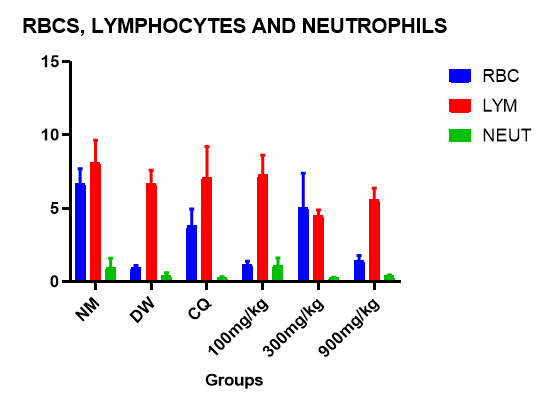


Fig 7-



**Discussion**

Malaria is the highest cause of mortality due to the level of parasites in Africa especially in infants and pregnant women (Anyasodor *et al.,*2023; Ezenyi *et al.*, 2020); the causative agent (*Plasmodium* sp.) has developed resistance to drugs, and this makes the management or treatment of malaria a challenge (Igwenyi *et al.,* 2024). *G. arborea* is a medicinal plant that has been used as diuretic, laxative, anti-haemorrhoids and anti-helmintic (Idowu *et al.*, 2020), and was documented to be used in the management of malaria in the southeast of Nigeria (Odoh *et al.,* 2018). In this study, the anti-malaria effects in suppressive and curative infection are being evaluated.

Previous research showed that 300mg/kg of *Azadirachta indica* with chloroquine (20mg/kg) has antimalarial effects but this study demonstrated that only *G. arborea* at 300mg/kg has antimalarial potential (Zuleta-Castro, 2021); previous study showed that *Chukrasia tabularis* also has suppressive effects on *Plasmodium falciparum* at high doses of 800mg/kg (Ogbole *et al.,* 2016) unlike *G. arborea* which exhibited suppression at 300mg/kg; though plants like *Fuerstia africana* and *Ludwigia erecta* has been demonstrated to also have suppressive effects against *Plasmodium falciparium* at low dose (100mg/kg) (Irungu *et al.*, 2023) but this is the first work done on *G. arborea* indicating its antimalarial suppressive effects at 300mg/kg.

The curative antimalarial effects of *G. arborea* was observed at 300mg/kg also, with decrease level of parasitemia and high inhibition percentage from day 1-7; even-though the percentage inhibition was high on day 1 at 900mg/kg but could not be sustained for prolong days, this implies that 900mg/kg as a single doses may have antimalarial effects and continuous usage may be overdose because hematological indices showed low RBCs and Hemoglobin; and the level of parasitemia was high on days 3 and 7; related work done by Zuleta-Castor (2021) and Ogoble (2016) showed that high dose of *A. indica* (1000mg/kg) and *C. tabularis* (800mg/kg) elicit antimalarial activities but not for prolong use.

The heamotological indices revealed increase in RBCs, at 300mg/kg compared to 100mg/kg and 900mg/kg which implies that there was no aneamia; the level of lymphocytes and platelets showed no significant difference when compare with that of chloroquine that shows there was no leucopenia and thrombocytopenia, this corroborates with previous study that showed that 300mg/kg of *Cucurbita pepo* reduced same parameters when evaluating its antimalarial potentials in an established infection (Ezeani *et al.,* 2022); *Antrocaryon micraster* was also discovered to prevent thrombocytopenia, leucopenia and anemia at 400mg/kg while evaluating its curative effect in malaria ( *P. berghei*) infected mice (Kumatia *et al.,* 2021). Study done by Njidda and Co showed that feeding large animals like ram and goats with *G. arborea* instead of Soy bean did not affect the blood picture of the animals and they were normal (Njidda *et al.,* 2020).

The antimalarial activities shown by *Gmelina arborea* can be related to its phyto-compositions such as saponins, tanins, terpenoids, flavonoids, phenolics, triterpens, and steroids, and most importantly iridoid glucosides (El Sayed *et al.,* 2023, Kumari *et al.,* 2024), because the antimalarial effects of the combination of *Azadirachta paniculata,* *Mangifera indica* and *Morinda lucida* was reported to be due to the phytochemical constituents like terpens, flavonoids and others in the plants (Abdulai *et al.,* 2023). *A. paniculata* dichloromethane fraction has high antiplasmodial effect, and its potency has been attributed to the present of terpenes (Olanlokun *et al.,* 2024); these reports showed that the phytochemical constituents of these plants are responsible for their antimalarial efficacy; and the potentials showed in this study by GA may have been conferred by the phyto-constitutents.

**Conclusion**

This study showed that ethanol extract of *Gmelina arborea* has antimalarial effect at 300mg/kg as a suppressant and curative potential in established infection which justifies its usage by traditional healers in the southeast of Nigeria; and this is reported herein for the first time. Further studies to isolate and characterize the bioactive constituents and identify the antimalarial mechanism are therefore required.

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