Original

In Vivo Antiplasmodial Activity and Safety Evaluation of the Methanolic Stem-Bark Extract of Artocarpus Heterophyllus in *Plasmodium Berghei*-Infected Mice

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ABSTRACT

Background: Emergence of antimalarial drug resistance and the limited availability of new agents underscore the need to evaluate traditional medicinal plants as potential sources of novel therapies. *Artocarpus heterophyllus* stem-bark is used in ethnomedicine for febrile illnesses, but robust in vivo efficacy and safety data are limited. This study evaluated the chemosuppressive antiplasmodial activity and acute oral safety of the methanolic stem-bark extract of *A. heterophyllus* in a murine malaria model.

Methods: This randomized controlled preclinical experimental study used adult female Swiss albino mice experimentally infected with chloroquine-sensitive *Plasmodium berghei*. Stem-bark of *A. heterophyllus* was air-dried, pulverized, and extracted with methanol. Mice were randomly allocated into treatment groups (n = 5 per group) and administered graded oral doses of the extract (25–200 mg/kg) using the standard 4-day suppressive test (Peters' test). Parasitaemia, and body temperature were recorded. The median effective dose (ED_{50}) was estimated using four-parameter logistic regression. Acute oral toxicity was assessed using the OECD up-and-down/limit test to estimate the median lethal dose (LD_{50}) and therapeutic index.

Results: The extract produced dose-dependent parasitaemia suppression and significantly prolonged survival compared with untreated controls. The highest suppression (63.45%) occurred at 200 mg/kg. The ED_{50} was 95.85 mg/kg, while the LD_{50} exceeded 2000 mg/kg, yielding a therapeutic index >21. Mild hepatorenal changes were observed at high exposure on histopathologic examination.

Conclusion: Methanolic stem-bark extract of *A. heterophyllus* demonstrates significant in vivo antiplasmodial activity with a favourable safety margin in mice, supporting further bioactivity-guided isolation of active compounds and preclinical development.

Keywords: *Artocarpus heterophyllus*, antiplasmodial activity, *Plasmodium berghei*, therapeutic index, ethnopharmacology, malaria treatment.

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INTRODUCTION

Malaria remains a major global public-health problem, with an estimated 249 million cases and 608,000 deaths reported in 2022; sub-Saharan Africa bears the overwhelming share of this burden.[1] Children under five years and pregnant women are particularly vulnerable.[2] The disease is caused by protozoan parasites of the genus *Plasmodium* transmitted by infected *Anopheles* mosquitoes; *P. falciparum* remains the most lethal species.[3] The progressive emergence and spread of resistance to antimalarial drugs, including reports of delayed parasite clearance to artemisinin derivatives, has heightened the need to discover new, safe and affordable therapies.[4-5]

Historically, plant-derived compounds (e.g., quinine, artemisinin) have been critical to antimalarial chemotherapy, motivating renewed screening of ethnomedicinal plants for active leads.[6-7] *Artocarpus heterophyllus* Lam. (jackfruit) is used in traditional medicine across tropical regions for fever and febrile illnesses, including malaria.[8-10] Phytochemical surveys of *Artocarpus* species report flavonoids, stilbenoids, prenylated phenolics, saponins and other secondary metabolites with potential antiparasitic activity.[11-12]

Despite these reports, robust *in vivo* pharmacological and safety data for *A. heterophyllus* stem-bark are limited. The present study therefore evaluates the *in vivo* antiplasmodial activity of the methanolic stem-bark extract of *A. heterophyllus* using the Peters 4-day suppressive test in *P. berghei*-infected mice, and determines acute oral toxicity, ED_{50}, and histopathological effects on major organs.[13] These data provide preclinical validation of traditional use and guide subsequent fractionation and lead-isolation efforts.

METHODOLOGY

Study Design: A laboratory-based randomised controlled preclinical experimental study employing *Plasmodium berghei*-infected mice to evaluate the in vivo antiplasmodial efficacy and acute oral toxicity of methanolic stem-bark extract of *Artocarpus heterophyllus.*

***Plant material and extract preparation*:** Stem-bark of *A. heterophyllus* was collected from the study region, authenticated by a botanist, air-dried, pulverized and extracted by maceration in methanol. The filtrate was concentrated under reduced pressure and stored at 4°C until use. Extraction procedures followed standard phytochemical methods.[14-15]

Animals and ethical approval: Adult female Swiss albino mice (18–22 g) were acclimatized and kept under standard conditions with food and water ad libitum. All procedures were approved by the Faculty Animal Research Ethics Committee of the Faculty of Basic Medical Sciences (FAREC-FBMS Committee Approval #: 322MBP3924) of University of Calabar on 18th September, 2024, and conformed to OECD and National Code of Health Research Ethics.[13-16]

Parasite strain and inoculation: Chloroquine-sensitive *Plasmodium berghei* (NK65) was maintained by serial passage. Donor mice with 20–30% parasitaemia were used to prepare inocula containing $\approx 1\times10^{7}$ parasitized RBCs per 0.2 mL for intraperitoneal injection.[13]

In vivo antiplasmodial assay (4-day suppressive test): Antiplasmodial activity was evaluated using the standard Peters 4-day suppressive protocol.[13] Mice were randomized into groups (n = 5) and infected on day 0. Treatments (extract at 25, 50, 100, 200 mg/kg orally once daily) began 2 h post-infection and continued for 4 days (days 0–3). Artesunate (5 mg/kg) and normal saline were used as positive and negative controls, respectively. On day 4, thin blood smears were prepared, fixed and stained; parasitaemia (% infected RBCs per 1,000 erythrocytes) was determined microscopically. Percentage chemosuppression relative to control was calculated.

Acute oral toxicity: Acute oral toxicity was conducted using the OECD 423 up-and-down/limit test: fasted mice received a single oral dose of 2,000 mg/kg extract and were monitored for clinical signs and mortality for 14 days. LD_{50} was estimated per guideline procedures.[14]

Histopathology: At study end, selected animals were euthanized, and liver, kidney and spleen excised, fixed in 10% buffered formalin, processed and stained with hematoxylin–eosin for histopathological assessment following standard protocols.[17]

Statistical analysis (expanded): Data are presented as mean ± SEM. Data normality was assessed with the Shapiro–Wilk test. Group comparisons employed one-way ANOVA followed by Tukey's post-hoc test; $p < 0.05$ was considered significant. ED_{50} (median effective dose producing 50% chemosuppression) was estimated by nonlinear logistic regression (GraphPad Prism). Survival data were analyzed by Kaplan–Meier curves and compared using the log-rank test. Pearson correlation assessed dose–response association. Statistical methods follow established recommendations for biomedical studies.[18]

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RESULTS

Table 1 shows the results of the effect of administration of *A. heterophyllus* extract on the Parasitaemia suppression in experimental animals. The lowest percentage suppression was achieved by the reference drug (artesunate) administered to the animals in the positive control group, while the highest percentage parasitaemia suppression of 63.45% was achieved by 200 mg/kg of the plant extract. The methanol plant extract also manifested a relatively concentration-dependent suppressive effect on the plasmodium parasites.

Table 1: Antimalarial activities of different doses of the methanol extract of *Artocarpus heterophyllus depicted* by average percentage Suppression of each animal group

Groups	N	% Suppression (Mean ± SEM)	95% CI of Mean	df (btw & within Groups)	F	P-value	Effect size (η^2_p)
Group 1: NC Group	5	0.00	0.00	5, 24	15.643	<0.001*	0.77
Group 2:25mg/kg	5	35.86 ± 5.72	19.954-51.761				
Group 3: 50mg/kg	5	34.48 ± 7.71	13.069-55.886				
Group 4: 100mg/kg	5	51.72 ± 3.08	43.162-60.285				
Group 5: 200mg/kg	5	63.45 ± 4.57	50.750-76.145				
Group 6: PC Group	5	29.27 ± 7.56	8.288-50.256				

Keys: NC = Control group without treatment, PC = Control group treated with artesunate, SEM = standard error of the mean; CI = confidence interval, df = degree of freedom, *p < 0.05, one-way analysis of variance followed by the Turkey Honest Significant Difference (HSD)'s post hoc test).

All the animals that were given 2000mg/kg doses of the plant extract were subjected to necropsy at the end of 14 days. The figures (Figs 1 & 2) below show the findings from the animals administered the 2000mg/kg dose of the extract. The results showed that the extracts had some toxic effect on the liver and kidney cells.

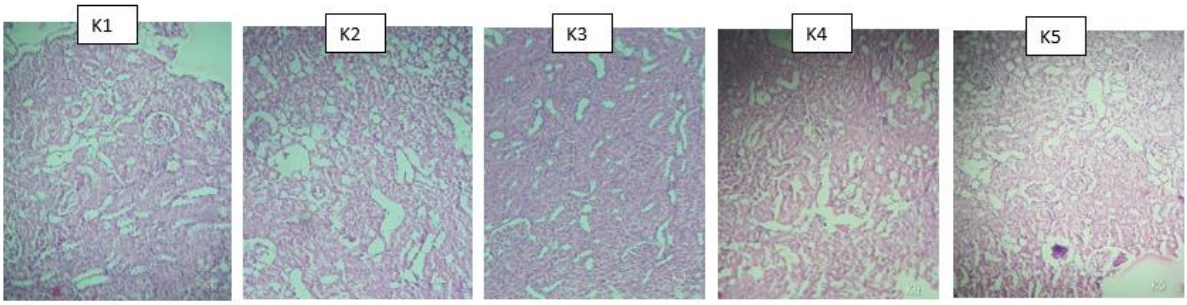

Fig 1: Toxicity/pathologic effects of the *Artocarpus heterophyllus* stem-bark extract on the kidneys of the animals treated with 2000 mg/kg plant extract. K1-K5 kidney: acute tubular injury characterised by patchy luminal dilatation, simplification of epithelial lining with areas of tubular cell sloughing. K3 also shows glomerular membrane prominence.

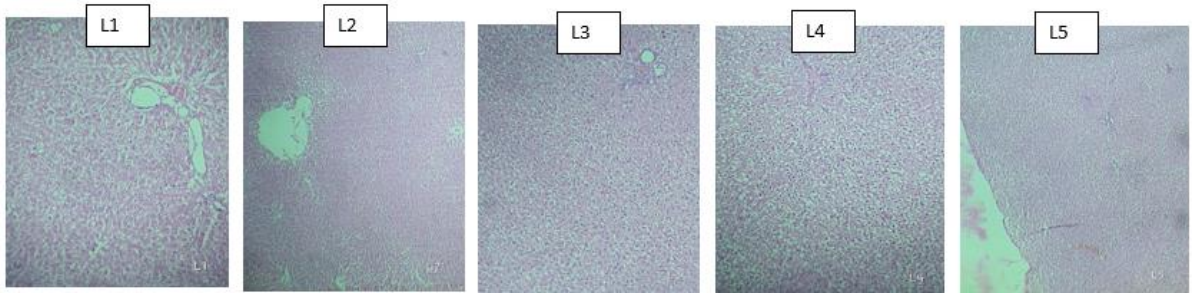

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Fig 2: **Toxicity/pathologic effects of the *Artocarpus heterophyllus* stem-bark extract on the livers of the animals treated with 2000 mg/kg plant extract.** L1-l5 liver: dilated venules and sinusoids; microvesicular vacuolation of hepatocytes in diffuse pattern.

Table 2 shows the results of the effect of administration of *A. heterophyllus* extract on the observed temperature in experimental animals. There was no statistically significant difference in mean body temperature between NC group and 25 mg/kg group (p=0.88), 50 mg/kg group (p=0.22), 200 mg/kg (p=0.17) or between NC group and PC group (p=0.74). These findings indicate that animals that were infected but later treated with the doses of the plant extract were more likely to have normal body temperature

Table 2: Antimalarial activities of different doses of the methanol extract of *Artocarpus heterophyllus depicted* by average body temperature of each animal group.

Groups	N	Temperature (°C) Mean ± SEM	95% CI	df (btw & within Groups)	F	P-value	Effect size (η^2_P)
Group 1: NC	5	37.36 ± 0.05	37.218-37.502	5, 24	2.947	0.033*	0.38
Group 2:25mg/kg	5	37.20 ± 0.15	36.779-37.621				
Group 3: 50mg/kg	5	37.02 ± 0.11	36.711-37.329				
Group 4: 100mg/kg	5	36.86 ± 0.13	36.502-37.218				
Group 5: 200mg/kg	5	37.00 ± 0.06	36.824-37.176				
Group 6: PC	5	37.160 ± 0.07	36.972-37.348				

Keys: NC = Control group without treatment, PC = Control group treated with artesunate, SEM = standard error of the mean; CI = confidence interval, df = degree of freedom, *p < 0.05, one-way analysis of variance followed by the Turkey Honest Significant Difference (HSD)'s post hoc test).

Table 3 below is the result of the effect of administration of *A. heterophyllus* extract on mean survival time and percentage survivor of the treated and controlled experimental animals. Though the mean survival time and percentage survivor of the animals in the group treated with herb extract were increased more than those in the negative control group, however, the values were not statistically significant F(5, 24) = 0.533, p < 0.735. There was no animal deaths among the animals treated with 200 mg/kg of the plant extract and in those of the positive control group (treated with 15 mg/kg of artesunate). The mean survival time and percentage survivor of the animals treated with 25 mg/kg, 50 mg/kg, and 100 mg/kg of the extracts were the same.

Table 3: Survival time and percentage survivor (in parenthesis) of mice treated with stem-back of *Artocarpus heterophyllus* using a-4-day test.

Groups	N	Mean Survival time (Mean ± SEM) & (% Survivor)	95% CI	df (btw & within Groups)	F	P-value	Effect size (η^2_P)
Group 1: NC	5	26.00 ± 1.27 (60)	22.49-29.51	5, 24	0.553	0.735	0.10
Group 2:25mg/kg	5	26.20 ± 1.8 (80)	21.20-31.20				
Group 3: 50mg/kg	5	25.20 ± 2.8 (80)	17.43-32.97				
Group 4: 100mg/kg	5	23.20 ± 4.8 (80)	9.87-36.53				
Group 5: 200mg/kg	5	28.00 ± 00 (100)	28.00-28.00				
Group 6: PC	5	28.00 ± 00 (100)	28.00-28.00				

Keys: NC = Control group without treatment, PC = Control group treated with artesunate, SEM = standard error of the mean; CI = confidence interval, df = degree of freedom, *p < 0.05, one-way analysis of variance followed by the Turkey Honest Significant Difference (HSD)'s post hoc test).

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No mortality was observed within the follow-up period of carrying out oral toxicity testing (Limit test method) at a test dose of 2000mg/kg, it means that the 50% lethal dose (LD_{50}) was greater than 2000mg/kg, which implies that the extract is orally safe. There were also no signs of toxicity except that about 4 hours after administration of the test dose, few of the animals started feeling sleepy, however, this observation disappeared after approximately another 4 hours (Table 4). Based on the result of the LD_{50} which is > 2000 mg/kg, and ED_{50} which was 95.85 mg/kg. The therapeutic index for the extract of *A. heterophyllus* was estimated to be > 21.

Table 4: Acute oral toxicity data sheet for control and extract treated rats

No of animals	Toxicity			ANS & CNS Observations											Behavioural Observations	
	Onset	Stop	Time of death	Skin & fur	Eye Lacri	Sali	Dia	Res	Let	Sleep	Con	Coma	Ste	Stu	Retr	
(5)	Nil	Nil	0	x	x	x	x	x	x	√	x	X	x	x	x	

Keys: Test: tested animals; Lacri: lacrimation; Sali: salivation; Dia: diarrhoea; Res: respiratory distress; Let: lethargy; Con: convulsion; Ste: stereotypy; Stu: Stupor; Retr: retropulsion; x: absence of signs; √: presence of signs

DISCUSSION

This study evaluated the in vivo antiplasmodial efficacy and safety profile of the methanolic stem extract of *Artocarpus heterophyllus*, and the findings provide important insights into its potential as a source of antimalarial agents. In this study, the antiplasmodial activity of *Artocarpus heterophyllus* was evaluated by comparing parasitaemia levels in animals treated with the extract, untreated controls, and those receiving a reference drug. The extract's antiplasmodial effect was dose-dependent, with higher doses leading to lower parasitaemia. Notably, the group treated with 200 mg/kg of the extract exhibited the lowest parasitaemia on the fifth day.

The percentage of growth inhibition (or suppression) of *Plasmodium berghei* was derived from a formula that inversely relates it to percentage parasitaemia. The doses of 100 and 200 mg/kg achieved more than 50% parasitaemia suppression in infected animals. According to Bantie et al., any plant extract that achieves ≥50% suppression at doses of 500, 250, and 100 mg/kg body weight per day can be classified as moderate, good, and very good respectively[19]. Based on this classification, *A. heterophyllus* extract in this study fits well into the "good" antiplasmodial activity category.

Another classification by Munoz et al. proposes that an extract be considered active if its inhibition is ≥30%.[20] In this study, even the lowest dose (25 mg/kg) achieved suppression over 30%, confirming *A. heterophyllus* as an active antiplasmodial agent.

A previous study in Indonesia demonstrated that *Artocarpus* plant extracts displayed very good antiplasmodial activity[21]. Other studies have also recorded *Artocarpus* extracts as excellent antiplasmodial agents[22-24]. Hafid et al. conducted both in vitro and in vivo tests evaluating the antiplasmodial activities of extracts from three different *Artocarpus* species[23]. Both methods revealed excellent activity against *P. falciparum* (in vitro) and *P. berghei* (in vivo), with an ED_{50} of 10.35 mg/kg body weight—much lower than the 95.85 mg/kg observed in this study.

A prenylated flavonoid, heteroflavanone C, isolated from the stem bark of *Artocarpus champeden*, has previously been reported to exhibit strong antiplasmodial activity, even surpassing that of Chloroquine[25]. Consistent with these observations, the present study demonstrated substantial parasite suppression across all extract doses, with the treated groups exhibiting greater parasitaemia reduction than the reference antimalarial, Artesunate, used as the positive control.

In Nigeria, a study evaluated various fractions of *Artocarpus altilis* extracts and found that certain partitioned fractions were more potent than the crude extract. Importantly, the high LD_{50} value reported for the crude extract indicated a favourable safety profile, underscoring its non-toxic nature.[26] These findings

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reinforce the potential of *Artocarpus* species as effective and safe antimalarial agents, complementing the observations of the current study. Similarly, another Nigerian study investigating combinations of ethnomedicinal plants used for malaria treatment showed that the stem bark of *Artocarpus altilis* exhibited significant chemosuppressive activity against *Plasmodium berghei* in infected mice, supporting its traditional use in malaria therapy.[27]

Furthermore, investigations of other *Artocarpus* species have also demonstrated promising antimalarial properties, with bioactive fractions from the stem bark of *Artocarpus sericicarpus* showing strong inhibitory activity against *Plasmodium falciparum*, attributed largely to flavonoid and polyphenolic constituents[28]. Collectively, these findings reinforce the growing body of evidence supporting the potential of *Artocarpus* species as promising and relatively safe sources of antimalarial agents, thereby complementing the observations of the present study.

One of the cardinal signs of malarial infection in rodents is a change in body temperature.[29] The temperature recordings in this study did not show a consistent pattern. Artesunate and the 200 mg/kg dose of the extract elicited body temperatures significantly different from those treated with 100 mg/kg, 25 mg/kg, and the untreated control. The former displayed normal temperatures, while the latter had slightly elevated readings.

Normal mouse temperature ranges between 35.5–38°C, with a median of 37°C[30]. Rodents are known to have poor heat regulation. Larson et al. found that at 21°C, mice had an average body temperature of 38.1°C, and at 36.7°C, their temperature rose to 40°C[31]. Malaria infection in rodents, specifically by *P. berghei*, is associated with hypothermia rather than fever.[32-34] The exact mechanism is unclear, but increased levels of serotonin (5-HT), a neurotransmitter known to reduce food intake and body temperature, have been implicated[33]. The absence of hypothermia in this study may be due to the timing of temperature measurement - day five post-treatment may have been too early to observe significant reductions.

The untreated control group recorded the highest mortality rate (40%) and the lowest percentage survivor rate (60%), though not the lowest mean survival time. The 200 mg/kg and artesunate-treated groups had the highest survival times and 100% survival rates. The 25, 50, and 100 mg/kg groups had 80% survival rates, though with varying mean survival times.

Mean survival time is an important parameter for evaluating antiplasmodial activity, although some studies correlate parasitaemia suppression with percentage survivors rather than survival time.[35-37] Adebajo et al. recorded prolonged survival times among mice treated with *Artocarpus altilis* extract in an in vivo study evaluating ethnomedicines.[38] However, Odediran et al. found that combining *Artocarpus* with other ethnomedicines did not enhance survival times.[39]

The result of the acute toxicity testing of *A. heterophyllus* in this study showed that there was no mortality observed up to the maximum dose level of 2000 mg/kg body weight of the crude plant extract administered orally. There were also no behavioural changes noted viz: lacrimation; salivation, diarrhoea, respiratory distress, lethargy, convulsion, etc., except that the rats felt sleepy the first few hours after administering the extracts. According to the limit test recommendation, the 2000 mg/kg plant extract was safe, indicating that the approximate median lethal dose (LD_{50}) of the extract in the experimental rodents was higher than 2000 mg/kg body weight. This finding of acute toxicity in this study agrees with the findings in previous study where the extracts from a genus *Artocarpus* plant were subjected to toxicity tests.[26] The absence of mortality or overt organ toxicity at 2000 mg/kg suggests the extract's constituents are well tolerated in vivo. Histopathological findings corroborate biochemical safety, showing only mild reversible hepatic changes at high doses—possibly due to transient metabolic adaptation.[17]

Limitations of the study

This study like most others has its areas of strength and limitation. *In vivo* testing of plant extracts for medicinal activity has some notable limitations - the issue of complexity of biological systems. The use of whole animals that exhibit complex interactions and responses that makes interpretation difficult. Actually, the activities or effects observed in this study may not be solely due to the methanol plant extract. Furthermore, there is the problem of biological variability that do exist among test subjects such as genetic differences, health status, etc., that could lead to inconsistence in outcomes, this could pose a challenge in drawing definitive conclusions. Additionally, there is problem of limited predictive value – animal-model study results may not always translate to

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humans due to differences in physiology and metabolism, which could lead to failure in clinical trials. Overall, the present findings align with existing literature confirming that *Artocarpus* species possess promising antiplasmodial activity and good safety profiles. The observed dose-dependent efficacy, favourable survival outcomes, and high LD_{50} together support *A. heterophyllus* stem-bark extract as a potential source of lead compounds for new antimalarial therapies.

CONCLUSION

The methanolic stem-bark extract of *Artocarpus heterophyllus* exhibited significant dose-dependent antiplasmodial activity achieving over 50% suppression at 100–200 mg/kg and demonstrating good safety margins and minimal histopathological alterations in *P. berghei*-infected mice. These results confirm its ethnomedicinal use against malaria and suggest further studies focusing on isolation and characterization of active constituents, as well as mechanistic and chronic toxicity assessments.

Declarations

***Contributions of authors*:** AAI, GIO, and COO conceptualized this study. AAI, UEE, ABO designed the study. OME, CJI, PAO, and BEE participated in fieldwork and data collection. AAI performed data analysis and interpretation. All authors contributed to the development of the final manuscript and approved its submission.

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