Evaluation of Anti-Malarial Profile of Methanol Leaf Extract of *Jatropha curcas* Linn on *Plasmodium berghei* Infection in Mice

Abstract

The resistance of malaria parasites to anti-malarialdrugs including Artemisinin-based combination therapy is undoubtedly a huge challenge in clinical practice necessitating concerted efforts to findingnovel, safe and efficacious options. This study investigated the anti-malarialactivity of methanol leaf-extract of *J. Curcas* against chloroquine-sensitive *Plasmodium berghei* infection in mice.Acute toxicity and phytochemical analysis of the extract were carried out using standard methods.Peter's 4-day suppressive, curative and residual infection tests were used to determine efficacy. Parameters measured were parasitemia, survival time, body weight and packed cell volume (PCV).Ninety albino malemice were infected with chloroquine-sensitive strain of *Plasmodium berghei* NK65 randomly divided into three groups of thirty and separately used for suppressive, curative and prophylactic tests. A set of thirty albino mice were divided into five groups (n=6) and subjected to suppressive test. Groups 2, 3 and 4 were treated with 200, 400 and 800 mg/kg of the extract, respectively. Group I and 5 received distilled water and chloroquine (5 mg/kg) served as negative and positive controls, respectively.The remaining two sets of thirty mice were used forcurative andprophylactic tests.Data generated was subjected to descriptive statistics and levels of significance was determined at $p<0.001$ to $p<0.05$.Phytochemical analysis of the extract revealed the presence of alkaloid, carbohydrates, cyanogenic glycosides, flavonoids, saponins, steroids and tannins. The oral median lethal dose (LD_{50}) of the extract in mice was >5000 mg/kg. The extractsignificantlydecreased($p<0.05$) parasitemia and enhancedsurvival time in treated mice. The extract minimized parasite induced weight loss and improved PCV. Methanol leaf extract of *J. Curcas* is safe at ≤5000 mg/kg and exhibitedmoderate anti-malarial activity credited to the synergistic effect ofthe phytochemical constituents,hence its folkloric uses.

Keyword: Anti-malarial activity, *Jatopha curcas*, Mice, *Plasmodium berghei*, Phytochemical

1. INTRODUCTION

"Malaria is numbered among the world most devastating infectious diseases. It is a parasitic disease caused by the protozoa of the genus Plasmodium and transmitted by the female Anopheles mosquitoes. Several plasmodium species such as *P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi* are responsible for malaria in human, however, *P. falciparum*accounts for the highest number of cases and deaths" [WHO, 2023]. "The disease which occurs across the globe is most prevalent in tropical and subtropical regions especially in sub-Saharan Africa with children of five years old and pregnant women been the most vulnerable groups"[Getu et al., 2023].

"A global estimate of 245 million cases in 2020 and 247 million in 2021 with 625, 000 and 619, 000 deaths in 2020 and 2021 respectively had been reported with Nigeria accounting for the highest proportion, 27% and 31% of the cases and deaths respectively. Nigeria had also accounted for 38.5 % of global malaria death in children aged less than 5 years"[World Malaria Report, 2022]."Apart from the disturbing health challenges, the disease has caused significant

hardship and imposes a huge economic burden on the infected individuals and endemic countries through high health care cost and reduced economic output and productivity"[Alli et al., 2011].

"The various interventions put in place towards eradication of the disease have been dwarfed by the emergence of Plasmodium parasite resistance. The reduced efficacy and resistance to anti-malarial agents including Artemisinins, Artemisinin-based combination therapy and other newerdrugs have been a huge challenge in the clinical setting"[Chaniad et al., 2022]."To overcome these challenges, a total and comprehensive effort towards research for novel anti-malarial agents is needed"[Espoir et al., 2022].This study was therefore designed to investigate the *in-vivo* anti-malarial activities of methanol leaf extract of *J. Curcas* against chloroquine–sensitive*Plasmodium berghei* infection in mice.

Figure.1. *Jathropha curcas*

"*Jathropa Curcas* is a small erect branched tree with a smooth grey bark, belonging to the family, *Euphorbiaceae*.It has succulent smooth grey bark which when cut or its leaf plucked, produces whitish watery latex which causes brown stains that are difficult to remove"[Kamal et al., 2011]. "The plant which can grow up to ≥5 meters under favorable condition was thought to be native of Central America, but widely distributed in tropical regions worldwide"[Abdelgadir and Van-Staden, 2013; Mamrata et al., 2023]."The root, stem bark, leaf and fruits of the plant have been used in traditional folk medicine in many parts of West Africa"[Igbinosa et al., 2011]. "The plant has for many centuries been used in many countries around the world for its anti-malarial activities without scientific validation of such use" [Iwu, 1993; Prasad et al., 2012].

2. MATERIALS AND METHODS

2.1 Collection and Authentication of plant

The leaves of *J. curcas* were collected from Area C residential quarters of the Samaru campus of Ahmadu Bello University (ABU), Zaria, Nigeria in August 2023. The plant was identified and authenticated at the Herbarium unit of Department of Botany, ABU Zaria, Nigeria and assigned a voucher specimen number 1911.

2.2 Extraction of plant Materials

The collected leaves were air-dried to aconstant weight without exposure to direct sunlight, and subsequently crushed to a coarse powder using mortar and pestle. The plant material weighing250 g was extracted by cold maceration using 70% methanol for 72 h with continuous stirring. The mixture was filtered using Whatman's No. 1 filter paper and then concentrated on water bath at 40-50 ^{0}C and weighed.

2.3 Animals and Laboratory conditions

Ninety (90) experimental mice weighing 22 - 26 gwere obtained from the Animal House of the Department of Pharmacology and Therapeutics, ABU Zaria and housed in clean cages in a well-ventilated room, under standard conditions of temperature and relative humidity. They were fed with a standard rodent feed (vital® feeds) and allowed access to water *ad-libitum* throughout the period of the experiment. Ethical clearance for the study was obtained from the Ahmadu Bello University Zaria Research Committee and the animals were used in accordance with the principles for the care and use of laboratory animals [NIH, 1985].

2.4 Determination of median lethal dose

Acute toxicity study in mice was carried out using Lorke's method [Lorke, 1983]. Oral route was used and the experiment was carried out in two phases. In phase one, nine mice were randomly divided into three groups of three mice per group and were given 10, 100 and 1000 mg/kg of the extract. The mice were then observed for signs of adverse effects and death for 24 hours and then for 14 days. Based on the results of the phase one study, the procedure was repeated using another set of four mice randomly divided into four groups of one mouse per group. Each of the mice was given 1200, 1600, 2900 and 5000 mg/kg of the extract in group 1, 2, 3 and 4 respectively and observed for mortality rate within 24 hours. The final LD_{50} value was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose. The mice were further observed for 14 days for signs of toxicitysuch as paw licking, lacrimation, tremor, hair erection, salivation, diarrhea, stretching of entire body, rubbing of nose on the floor and wall of the cage and coma.

2.5 Phytochemical screening of the plant extract

The extract was subjected to phytochemical tests for plant secondary metabolites, in accordance with Trease and Evans [Trease and Evans, 2002]method.

2.6 Rodent parasite (*Plasmodium berghei,* NK65) and Inoculation

The chloroquine sensitive strain of *Plasmodium berghei,* NK65 was sourced from National Institute for Medical Research, Lagos, Nigeria and maintained by continuous intraperitoneal passage in mice every four days [Saiya et al., 2022]. Parasitized blood sample from donor mouse was collected using heparinised capillary tube, transferred into a sterile bottle containing normal saline in such a way that 0.2 ml of blood contained approximately 1 x 10^7infected red blood cells.

2.7 Animal grouping

Three sets of 5 groupsof 6 mice per group were used for suppressive, curative and prophylactic tests at 200, 400 and 800 mg/kg doses of the extract.

2.8 Suppressive test (4-day early infection)

Peter's 4-day suppressive test against chloroquine-sensitive *P. berghei* (NK65) infection in mice was employed [Peters, 1965]. Each mouse received standard inoculums of $1x10^7$ *P. berghei* infected erythrocytes through the intraperitoneal route at the commencement of the experiment (day 0). Three (3) hours post-infection, 200, 400 and 800 mg/kg daily oral doseof the extractwere given to the test mice in groups 2, 3 and 4, respectively. Group 5 received chloroquine (5 mg/kg) for four consecutive days, whilegroup 1 which served as negative control was given 10 ml/kg of distilled water.On the fifth day, drops of blood samples were taken from the tail of each mouse. Thin blood films were made on a slide, air-dried, fixed in absolute methanol and stained with 3 % Giemsa solution at pH 7.2.The slides were examined under the microscope and mean percentage parasitemia was determined by counting the number of parasitized erythrocytes in random fields and calculated using the formula:

$$\text{The percentage parasitemia} = \frac{\text{number of parasitized erythrocytes}}{\text{Total RBC in the random fields}} * 100\%$$ [Eyob et al., 2022]

$$\text{Percentage chemosuppression} = \frac{\text{Average parasitemia in control group} - \text{Average parasitemia in treated group}}{\text{Average parasitemia in control group}} * 100\%$$

Body weight, rectal temperature and packed cell volume were measured on day 0 and day 4. Survival time after treatment was also monitored for each mouse for 28 days.

2.9 Evaluation of schizontocidal activity (Rane test)

Evaluation of the curative potential of the extract was carried out according to the method described by Ryley and Peters [1970]. Each mouse was inoculated with 1 x 10^7*P. berghei* infected erythrocytes through intraperitoneal route. Seventy two hours (72 hours) after the animals were infected with parasites, groups 2, 3 and 4 animals were treated with 200, 400 and 800 mg/kg per day of extract respectively, using oral route daily for five days. Group one received 10 ml/kg per day of distilled water, while group 5 received 5 mg/kg of chloroquine daily for 5 days. On the sixth day after treatment, drops of blood samples were taken from the tail of each mouse. Thin blood films were made on a slide, air-dried, fixed in absolute methanol and stained with 3% Giemsa solution at pH 7.2. Percentage parasitemia reduction relative to the negative control was determined for each dose. The mean survival time for each group was also determined arithmetically over a period of 28 days and death(s) occurring during this period was noted and used to determine the mean survival time. Body weight, rectal temperature and PCV were also monitored and recorded.

2.10 Evaluation of prophylactic activity (repository action)

The prophylactic activity of the extract was evaluated using the residual infection procedure described by Peters [1965] and Alehegn et al.[2020]. Groups 2, 3 and 4 received 200, 400 and 800 mg/kg of the extract respectively daily for three days, while 10 ml/kg/day of distilled water and 1.2 mg/kg/day of Pyrimethiamine were administered to group 1 and 5, respectively, for three days. On the fourth day, each of the animals was inoculated with $1x10^7$ *P. berghei*-infected erythrocytes and observed for 72 hours. Thin blood films stained with Giemsa stain was prepared from tail blood of each mouse and reduction in percentage parasitemia calculated. The mice were weighed again on day seven and the differences between the pre-treatment and post-treatment body weights recorded. The mean survival time for each group was determined over a period of

28 days.Blood was collected after tail-snip of each animal into a heparinized capillary tube pre-treatment and post-treatment, centrifugedand the packed cell volume (PCV) determined using the formula.

$$PCV=\frac{Height\ of\ Red\ Blood\ cell\ column}{Total\ height\ of\ blood\ column} * 100\%$$

2.11 Statistical analysis

Results obtained were analyzed using the statistical software, Statistical Package for the Social Sciences (IBM-SPSS version 21).Statistical analysis were carried out using One Way Analysis of Variance (ANOVA) followed by Bonferroni *post hoc test* for multiple comparison and repeated measure ANOVA as applicable. Data were expressed as Mean ± Standard Error of Mean (SEM) and percentages where applicable. Values of $p<0.001$ to $p<0.05$ were considered as significant.

3. RESULTS

3.1 Phytochemical constituent and acute toxicity of methanol leaf extract of *J. curcas*

The preliminary phytochemical screening of the extract showed the presence of alkaloid, carbohydrates, cyanogenic glycoside, flavonoids, saponins, steroids and tannins (Table 1).The oral median lethal dose studies indicated that methanol leaf extract of *J. curcas* caused no mortality at the 5000 mg/kg dose used within the first 24 hours and for the next 14 days in the mice. The physical and behavioural observations of the experimental animals also revealed no visible signs of acute toxicity.

Table 1: Phytochemical groups in methanol leaf extract of *J. curcas*

Parameter	Observation
Alkaloid	+
Carbohydrates	+
Cyanogenic glycoside	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+

Key: += phytochemical grouppresent

3.2 Parasite suppressive effect of methanol leaf extract of *J. curcas* and survival time *in P. berghei* infection in mice

The extract in 4-day suppressive test causeddecrease in parasitemia relative to the administered dose as shown(Table 2). The mean percentage chemo suppression produced by the extract at 800 mg/kg was 72.92 % and significantly higher ($p<0.05$) compared 200 and 400 mg/kg. Percentage chemo suppression at 800 mg/kg was comparable to 89.68% produced by the positive control, chloroquine at 5 mg/kg.The mean survival time (MST) of experimental mice at graded doses of methanol leaf extract of *J. curcas* is presented in Table 2.The MST at highest dose of 800 mg/kg

was 17.50 ± 1.50 days and significantly lower ($p<0.001$), relative to MST of 24.83 ± 1.05 days produced by the positive control.

Table 2: Effects of methanol leaf extract of *J. curcas* on parasitemia and mean survival time *in P. berghei* infection in the suppressive test in mice

Treatment	Dose (mg/kg)	% ParasiteSuppression	MST (days)
DW (10 ml/kg)		0	4.83 ± 0.60
	200	24.87	13.67 ± 0.92[a,b]
MLE	400	41.96	15.17 ± 1.56[a,b]
	800	72.92	17.50 ± 1.50[a]
CQ	5	89.68	24.83 ± 1.05[a]

Key: Data presented as mean ± SEM; n=6, superscript [a] and [b] = significantly different at $p<0.001$ or $p< 0.05$, DW = distilled water, CQ = Chloroquine, MLE = methanol leaf extract, MST= mean survival time

3.3 The effect of methanol leaf extract of *J. curcas* on weight and PCV in mice infected with *P. berghei* in suppressive test

The result showed decreased changes in body weight at all dose levels including the positive and negative controls. The decrease were not dose-related however, there was a significant ($P< 0.05$) difference in weight loss only in the negative control mice between day 0 and day 4. There was no significant ($P> 0.05$) difference between the PCV in groups 2 and 3 and the positive control mice that received chloroquine(Table 3).However, there was no decrease in PCV in the group of mice treated with 800 mg/kg *J. curcas* extract.

Table 3: Effect of methanol leaf extract of *J. curcas* on weight, temperature and PCV in mice infected with *P. berghei* in 4-day suppressive test

Treatment	Dose (mg/kg)	% loss in body weight	% decrease in PCV
DW(10 ml/kg)		8.55*	5.62*
	200	4.79	2.60
MLE	400	6.19	1.40
	800	4.30	0
CQ	5	1.92	1.39

Data presented as mean ± SEM; n=6,*= significantly different at $p< 0.05$ from distilled water and positive control, respectively, DW = distilled water, CQ = Chloroquine, MLE = methanol leaf extract

3.4 Schizontocidal effects of methanol leaf extract of *J. curcas* and survival time in parasite infected mice

The extract significantly decreased ($P<0.05$) parasitemia in mice at 400 and 800 mg/kg relative to the untreated negative control. The mean percentage chemo-suppression ranged from 30.07-71.48%.The positive control, chloroquine at 5 mg/kg body weight produced 83.85% chemo suppression. The mean survival time of treated infected mice steadily increased from 10.33 to

18.83 dayscorresponding to increase in the dose of the extract. However, the positive control produced longer survival times of 23.67days, respectively (Table 4).

Table 4: Effect of methanol leaf extract of *J. curcas* on parasitemia and Mean survival time in the established infection in mice

Treatment	Dose (mg/kg)	% parasitemia Suppression	MST (days)
DW (10 ml/kg)		-	4.83 ± 0.75
	200	30.07	10.33 ± 0.62[a,b]
MLE	400	40.72	14.17 ± 0.60[a,b]
	800	71.48	18.83 ± 1.08[a]
CQ	5	83.85	23.67 ± 1.15[a]

Data presented as mean ± SEM; n=6, superscript [a] and [b] = significantly different at $p<0.001$ or $p< 0.05$ from distilled water and positive control, respectively, DW = distilled water, CQ = Chloroquine, MLE = methanol leaf extract

3.5 Effects of methanol leaf extract of *J. curcas* on body weight and PCV in established *P. berghei* infection in mice

The extract significantly ($p> 0.05$) minimised body weight loss associated with the increase in parasitemia at 200 and 400 mg/kg doses relative to the untreated controlas presented in Table 5.At 800 mg/kg, the effects of the extract on percentage loss in body weight and % decrease in PCV post-infection were not significantly ($p<0.05$)different relative to the standard drug, chloroquine(Table 5).

Table 5: Effect of methanol leaf extract of *J. curcas* on body weight and packed cell volume in established *P. berghei* infection in mice

Treatment	Dose (mg/kg)	% loss in body weight	% decrease in PCV
DW(10 ml/kg)		13.27*	3.88
	200	5.38	3.28
	400	4.11	2.77
MLE	800	2.93	1.61
CQ	5	2.67	1.52

Data presented as % of mean values of three repeated measurements, * = significantly different at $p< 0.05$, DW = distilled water, CQ = Chloroquine, MLE = Methanol leaf extract, PCV= packed cell volume

3.6 The prophylactic effect of methanol leaf extract of *J. curcas* on parasitemia and survival time in the repository test in mice

The extractconsistently decreased percentage parasitemia that were significantly ($p<0.05$) higher in 400 and 800 mg/kg treated groups relative to the untreated negative control, but lower thanthe percentage reduction in parasite by the positive control pyrimethamine (91.38 %)as shown in Table 6. Methanol leaf extract of *J. curcas* increasedthe survival time of infected mice corresponding to the dose. The mean survival times of infected mice that received the extract at 400 and 800 mg/kg were11.83 ± 1.82 and 14.66 ± 1.31 days and were significantly ($p<0.001$) highercompared to 4.33 ± 0.62 days in the untreated control group(Table 6).

Table 6: The prophylactic effect of methanol leaf extract of *J. curcas* on parasitemia and Mean survival time in the repository test in mice

Treatment	Dose (mg/kg)	% Parasite Suppression	MST (days)
DW(10 ml/kg)		0	4.33 ± 0.62
	200	33.68	8.33 ± 1.59[b]
MLE	400	45.38	11.83 ± 1.82
	800	65.09	14.66 ± 1.31[a]
PYR	1.2	91.38	20.83 ± 1.08[a]

Data presented as mean ± SEM; superscripts [a] and [b] = significantly different at p<0.001 0r p<0.05, DW = distilled water, PYR = Pyrimethamine, MLE = methanol leaf extract

3.7 The effect of methanol leaf extract of *J. curcas* on body weight and PCV in mice infected with *P. berghei* in the prophylactic test

Methanol leaf extract of *J. curcas* minimised weight loss and reduction in packed cell volumeinduced by parasitemia (Table 7). Percentage loss in body weight was 5.16 and 10.49 in 200 mg/kg and untreated control groups, respectively. There was no significant (P> 0.05) difference between loss in body weightamong animals treated with 800 mg/kg of the extract and the positive control. Similarly, there was no significant (P> 0.05) difference in percentage decrease in PCV of mice that were treated with the extract and those treated with pyrimethamine (Table 7).

Table 7: Effect of methanol leaf extract of *J. curcas* on weight and PCV in mice infected with *P. berghei* infection in prophylactic test

Treatment	Dose (mg/kg)	% loss in body weight	% decrease in PCV
DW(10 ml/kg)		10.49*	2.77
	200	5.16	2.42
MLE	400	4.54	2.41
	800	1.57	1.58
PYR	1.2	0.96	1.17

Data presented as mean values of three repeated measurements, * = significantly different at p< 0.05, DW = distilled water, PYR = Pyrimethamine, MLE = Methanol leaf extract

4. DISCUSSION

"This study investigated the acute toxicity and anti-malarial profile of the 70% methanol leaf extract of *J. curcas* against *P. berghei* infection in mice. The determination of LD_{50} of any substance is critical in the assessment of its toxicity and an important component of evaluation protocol of a novel drug. Thus, the LD_{50} was conducted to determine the dose that might cause mortality or serious toxicity in 50% of the animals used in the experiment within a specific time"[Amira et al., 2021]. "The result of LD_{50} of the extract showed no mortality at the 500 mg/kg dose within the first 24 hours and up to 14 days in mice suggesting that the oral LD_{50} of the extract was greater than 5000 mg/kg in mice. This implies that the orally administered methanol leaf extract of *J. curcas* is practically nontoxic"[Alli et al., 2011]."Although the LD_{50}

may not be directly extrapolated from the experimental animals to human, it however produced the first clue to the toxicity profile of extract"[Kagbo and Ejebe, 2010]. In addition,the experimental animals did not show visible behavioural signs of acute toxicity such as paw licking, lacrimation, tremor, hair erection, salivation, diarrhea, stretching of entire body, rubbing of nose on the floor and wall of the cage and coma.

P. berghei have beenextensivelyused in evaluatingsuspected anti-malarial properties of plants becauseit is a suitable animal model parasite that has higher accessibility and can sequester within the blood microcirculation [Plirat et al., 2022]. It has also been reported to produce in rodent disease features similar to those of human plasmodial infection [Olusegun et al., 2020]. This therefore stressed its suitability for usage in this study.

Substances that ease parasite multiplication in the host were considered to possess anti-malarial activities [Ryley, 1970]. In this study, the inhibition of parasitemia and parasitemia reduction parameters were used as indicators of activities in each of the three models [WHO, 2023]. The results which showed statistically significant ($p < 0.05$) graded dose-dependent reduction in parasitemia count which is an indication ofpotential suppressive, curative and prophylactic anti-malarial activities of the plant.

One important index used to measure anti-plasmodial activity of potential anti-malarial agent in *P. berghei* model of animal infection is the mean survival time (MST). *Plasmodium berghei* is reported to decreaseMST of infected mice if untreated[Saiya et al., 2022]. The extract exhibited a dose-related increase in MST which is an indication of anti-plasmodial activity which prevented the parasite from overwhelming increase in parasite density in the RBC of infected mice and thus prevented early death of mice treated with the extract[Christiana et al., 2020].

Mitigation of body weight loss and rectal temperature reduction are some of the positive features of plant materials with anti-plasmodial action inmice infected with Plasmodium species[Eyob et al., 2022; Getnet et al., 2022].Althoughall the parasite infected mice exhibited gradual decrease in body weight however, the extract significantlymitigated the loss of weight associated with the increase in parasitemia at all doses. Body weight loss could also be an expression of metabolism disturbance, appetite suppression and hypoglycemic effects which are often induced by malaria parasite [Fentua et al., 2023]. Plants with anti-malarial potential should is expected to correct or prevent such anomalies.

The PCV was measured to predict the effectiveness of the test extract in preventing haemolysis resulting from increased parasitemia associated with malaria. The extract which significantly prevented the reduction in PCV of parasite infected mice implies that the extract is effective in reducing malaria induced haemolysis. Its failure to completely prevent or reverse PCV reduction could probably associated with the presence of saponins in the extract which have strong hemolytic effects [Mekonnen, 2015]. Surprisingly, neither the extract nor the standard drug completely cleared the parasitemia in all the three models implying limited anti-malarial activities.

J. curcas has been shown to be rich in phytochemicals such as alkaloids, cyanogenic glycosides, flavonoid, saponins, steroids and tannins supported the finding of Igbinoa *et al*.[2009].Although the mechanisms of anti-malarial actions of the extract have not been elucidated, anti-plasmodial

effects of natural plant products are often attributed to synergistic effects of some of these pharmacologically active phytochemical compounds [Eyob et al., 2022; Plirat et al., 2022].

5. CONCLUSION

The result of this study showed that methanol leaf extract of *J. curcas* was safe and has promising anti-malarial activities against *P. berghei*. Its anti-malarial activities are suggested to be probably due to the presence of some bioactive phytochemical constituents. This justifies its folkloric long usage for the management of fever.

Ethical Approval:

Ethical clearance for the study was obtained from the Ahmadu Bello University Zaria Research Committee and the animals were used in accordance with the principles for the care and use of laboratory animals [NIH, 1985].

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

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