

Acute toxicity studies and evaluation of the in-vivo anti-plasmodial activities of *Ochna membranacea* (oliv) Ochnaceae leaves and stembark extracts in Swiss albino mice

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ABSTRACT

Malaria is an infectious disease with high mortality rate in Africa. Development of resistance to antimalarial drugs in current clinical use such as chloroquine and Artemisinin; along with poor health systems and funding shortfalls have elevated the importance of continuous search for novel antimalarial agents. The lead compounds from which chloroquine and artemisinin were derived were isolated from plants. Therefore, there is high potential for the discovery of new and effective treatment from plants. *Ochna membranacea* (Oliv) Ochnaceae is among several plants used traditionally against infectious diseases including malaria with no scientific validation. This study investigated the antiplasmodial effect of the crude methanol leaves extract and crude methanol stembark extract of *Ochna membranacea* coded OMCLE and OMCSE respectively against *Plasmodium berghei* chloroquine-resistant strain. Acute toxicity studies were carried out using standard protocol. The prophylactic, suppressive and curative effects of the extracts were evaluated at the doses of 500, 1000 and 1500 mg/kg using Ryley and Peters'

method. Chloroquine (5 mg/kg) and artesunate (5 mg/kg) were used as standard drugs in the suppressive and curative tests, while 1.2 mg/kg pyrimethamine was used as standard in the prophylactic test. The oral median lethal dose (LD₅₀) was estimated to be > 5000 mg/kg. All graded doses of OMCLE gave a statistically significant (P<0.05) prophylactic effect. Only the median and highest doses of OMCSE gave significant inhibition of parasitaemia. In the suppressive test, all tested doses of both extracts exhibited significant suppression of parasitaemia compared to the negative control. In conclusion, the study suggests that *O. membranacea* possesses antiplasmodial activity making it a potent candidate in the search for newer antimalarials.

Key words: *Ochna membranacea*, Antiplasmodial, Suppressive, Curative, Prophylactic, LD₅₀

Introduction

Malaria is a life-threatening, preventable and curable infectious disease caused by the protozoans of the genus *Plasmodium*

transmitted to humans through infected female anopheles mosquitoes. An estimated 241 million cases of malaria and 627,000 deaths were recorded worldwide in 2020; representing about 14 million more cases and 69000 more deaths compared to 2019 (WHO, 2021). Despite a global decline in the incidence rate of malaria between 2010 and 2018, the rate has dramatically increased in the previous year resulting from disruption of malaria control programmes due to the COVID-19 pandemic (WHO, 2021). Also, 95 % of these deaths were in the World Health Organisation African Region; with Nigeria recording the highest cases and increase in malaria deaths globally (27%) (WHO, 2021). Failure of treatment regimens due to factors such as the rising problem of parasite resistance to the current frontline antimalarial agents, recrudescence to ACT, poor accessibility to drugs necessitates the search for newer antimalarial chemotherapeutic agents with different mechanisms of action (Bloland, 2001). The use of natural products especially plants in various forms of traditional medicine and their composition of large quantities of metabolites with various structures and pharmacological properties has motivated many researchers to resort to plant as potential sources of newer antimalarial agents (Amoa et al., 2012).

Ochna membranacea (Oliv) Ochnaceae is a glabrous shrub or small tree about 8 m tall found in evergreen, galleried forests or thickets; mostly from Guinea to North and South Nigeria and across the Congo basin to Uganda, Angola, Cameroon, South Africa and some part of Asia (Hutchson et al., 1958; Burkill, 1997). Several parts of the plant are used ethnomedicinally in some parts of Northern Nigeria for the treatment of various skin infections. A decoction of the bark is also reported to be used as an aphrodisiac. Biological functions such as antimicrobial, analgesic, anti-inflammatory, cytotoxic activities of various species in this genus have been

reported (Bandi et al., 2012). *In-vitro* antimalarial activity of *O. integerrima* (Ichino et al., 2006) and *O. squarrosa* (Ndoile, 2012) have been reported, whereas the *in-vivo* antimalarial potentials of *O. schweinfurthiana* (Ibrahim et al., 2015) and *O. kibbiensis* have also been investigated.

However, the antiplasmodial efficacy of *O. membranacea* has not yet been investigated. The present study was aimed at investigating the suppressive activity in early and established infections of methanol leaves and stem bark extracts of the plant against *Plasmodium berghei* infected mice.

Materials and Methods

Ethical Approval

Ethical approval was obtained from the Animals Use and Care Ethics Committee, Ahmadu Bello University, Zaria – Nigeria (Approval No. ABUCAUC/2021/078).

Drugs and Chemicals

Chloroquine phosphate (Sigma-Aldrich), Artesunate (Mekophar), Sulphadoxine-Pyrimethamine (SwidarR) and general laboratory chemicals and reagents of analytical grade were used for the investigation.

Plant material

The whole plant material of *Ochna membranacea* was collected from Samaru, Zaria, Kaduna State-Nigeria in August 2017. The plant's identity was authenticated through comparison with herbarium specimens where reference voucher specimen (Number 387) was retained. Identification was done at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University Zaria by Mal. Musa Muhammad.

Experimental Animals

Adult Swiss albino mice of local breed (15-30 g body weight) were acquired from the National Institute of Pharmaceutical

Research and Development (NIPRD), Abuja, Nigeria. The animals of either sex were acclimatised by maintaining under standard conditions in propylene cages at room temperature and fed with growers feed as diet and water (administered *ad libitum*). All experimental procedures followed the ethical guidelines for the care and use of laboratory animals as provided by Ahmadu Bello University Research Policy (Revised, 2010) and accepted internationally (National Institutes of Health, 1985; Publication no. 85-25, Revised 1996).

Malaria parasite

Mouse-infective chloroquine-resistant strain of *Plasmodium berghei* NK-65, authenticated at the Nigerian Institute of Medical Research (NIMR) Lagos, was obtained from Department of Pharmacology and Therapeutics, Bayero University Kano. Preservation of the parasite was carried out through continuous intra-peritoneal sub passage in mice.

Preparation of Plant material

The leaves and stembark were separately removed, shade dried, pulverised into a homogenous sample, labelled and stored for further use.

The powdered materials were separately macerated exhaustively with 70% methanol. Maceration was carried out twice (5 days each) for both the leaves and stembark powders. Obtained filtrates were concentrated *in-vacuo* using a rotary evaporator at 50 °C. Extracts were further dried under air and subsequently referred to as crude leaf extract (OMCLE) and crude stembark extract (OMCSE).

Preparation of inoculum

Blood from a carrier mouse was sampled and parasitaemia was confirmed microscopically to be about 30%. The donor mouse was euthanized with chloroform and the blood was collected through cardiac puncture. The blood was diluted with normal saline until an inoculum containing approximately 10^7 infected erythrocytes was obtained. The inoculum was appropriately preserved for further use.

Acute Toxicity Studies (LD₅₀)

The oral and intra-peritoneal median lethal doses of both OMCLE and OMCSE were determined using Lorke's method (Lorke, 1983). The study was carried out in two phases. Nine mice were starved overnight and randomly divided into 3 groups of 3 mice each. In the first phase, varying doses of the extract (10, 100 and 1000 mgkg⁻¹) were administered intra-peritoneal to groups 1, 2 and 3 respectively. They were observed over a period of 24 hours for any sign of toxicity and/or mortality. In the second phase, more specific doses (depending on the result from the first phase) were administered to 4 fresh mice through the same route. Observation was also made for 24 hours post-administration.

Same groupings and procedure were adopted for determination of the LD₅₀ orally.

The geometric mean of the lowest lethal dose (for which the animals died) and highest non-lethal dose (for which the animals survived) was taken as the median lethal dose (LD₅₀).

$$LD_{50} = \sqrt{\text{minimum toxic dose} \times \text{maximum tolerated dose}}$$

Antiplasmodial Studies

Effects of OMCLE and OMCSE on Early *P. berghei* infection in mice (Suppressive Test)

The Peters 4-day test as described by (Peters, 1975) was adopted for the evaluation of the antiplasmodial effect of the extracts in early infection. Thirty-six (36) Swiss Albino mice were grouped into 6, each containing 6 mice. Parasite inoculum of 0.2 ml was given to each mouse through intra-peritoneal route.

$$\% \text{ suppression} = \frac{\% \text{ parasitaemia in control} - \% \text{ parasitaemia in treated group}}{\% \text{ parasitaemia in control}} \times 100$$

Effects of OMCLE and OMCSE on Established *P. berghei* infection in mice (Curative Test)

The schizontocidal effect in established infection was evaluated using Rane test. Thirty-six (36) mice were inoculated with 0.2 ml standard inoculums via the intraperitoneal route. Seventy-two hours post infection; the mice were grouped into 6 groups of 6 mice each. Graded doses (500, 1000 and 1500 mg/kg) of the test drug were administered to groups 1, 2 and 3 respectively. Groups 4 and 5 taken as positive controls, were administered 5mg/kg standard chloroquine and 5 mg/kg artesunate respectively. Group 6 was taken as vehicle and was administered 0.2 ml normal saline. Administrations were done orally once daily for 4 days. On day 7, schizontocidal effect was assessed by microscopic study of Giemsa-stained thin blood smears (Ryley and Peters, 1970).

Prophylactic Effect of OMCLE and OMCSE on *P. berghei* infected mice

$$MST = \frac{\text{sum of survival time of all animals in a group (days)}}{\text{total number of animals in the group}}$$

Statistical Analysis

Doses of the extract at 500, 1000 and 1500 mg/kg were orally administered to groups 1, 2 and 3 respectively once daily as treatment for 4 days (day 0 to day 3). Parallel tests were conducted with 5 mg/kg standard chloroquine, 5 mg/kg artesunate for reference purpose and 0.2 ml distilled water as vehicle in groups 4, 5 and 6 respectively. On day 4, thin smears were made from the tail blood. The slides were fixed with methanol, stained with 10% Giemsa for 15 minutes and examined under microscope. Average suppression was calculated using the formula:

The repository effect was assessed using the method described by (Ryley and Peters, 1970). Five groups of 6 mice each were respectively administered orally with 500, 1000 and 1500 mgkg⁻¹ of the extract (test groups), 1.2 mgkg⁻¹ pyrimethamine (positive control) and 0.2 ml distilled water (negative control) once daily for 4 consecutive days. On day 5, the mice were inoculated with 0.2 ml of the standard inoculum. Seventy-two (72) hours post-infection, the parasitaemia level was assessed by studying slides with thin blood smears under a microscope.

Determination of Mean Survival Time (MST)

Mortality was monitored and the number of days from the day of inoculation to death was recorded for each animal in all groups for 30 days. Monitoring was done in all the *in vivo* models and the MST was calculated using the formula below

Data was analysed using SPSS version 23 and p-values ≤ 0.05 was considered

statistically significant. Results were presented as tables and expressed as mean \pm standard error of mean.

Results

Median Lethal Doses (LD₅₀) of Crude methanol leaves and stembark extracts of *O. membranacea*

The oral LD₅₀ of OMCLE and OMCSE in mice were found to be greater than 5000 mg/kg. Intraperitoneal LD₅₀ were 566 mg/kg and 289 mg/kg for OMCLE and OMCSE respectively.

Effects of OMCLE and OMCSE of *O. membranacea* in Early Infection in Mice (Suppressive Test)

All test groups of both extracts exhibited significant ($P < 0.05$) suppression in the level of parasitaemia compared to the negative control. Though significantly different from the control group, recorded activities were relatively low. Highest chemosuppression was observed with 1500 mg/kg of OMCSE (55.79%), chloroquine exerted 19.83% while Artesunate caused 50.21% chemosuppression (Table 1)

Table 1: Effects of *Ochna membranacea* Crude Leaves Extract (OMCLE) and Crude Stem Bark Extract (OMCSE) in Early Infection (Suppressive Test)

Treatment	Dose (mg/kg)	Average Parasitaemia \pm SEM	Percentage Chemosuppression (%)
N/saline	10 ml/kg	19.36 \pm 0.26	-
OMCLE	500	11.00 \pm 1.48*	43.18
	1000	13.42 \pm 1.58*	30.68
	1500	12.22 \pm 1.08*	36.88
OMCSE	500	14.00 \pm 1.41*	27.69
	1000	12.48 \pm 1.24*	35.54
	1500	8.56 \pm 1.91*	55.79
Chloroquine	5	15.52 \pm 0.96	19.83
Artesunate	5	9.64 \pm 1.17*	50.21

OMCLE- *O. membranacea* crude leaves extract, OMCSE- *O. membranacea* crude stem bark extract, *- statistically significant

Effects of OMCLE and OMCSE of *O. membranacea* on Established Infection (Curative/Rane Test)

With the exception of 1000 mg/kg CSE (29.46%), all tested doses of the extracts caused significant ($P < 0.05$) reduction in

parasitaemia levels. Highest percentage was recorded in the group treated with 1000 mg/kg OMCLE (57.04%), which was almost similar with the positive control Artesunate (56.60%) at 5 mg/kg. The % suppression by chloroquine was 13.06 (Table 2).

Table 2: Effects of OMCLE and OMCSE of *O. membranacea* on Established Infection (Curative/Rane Test)

Treatment	Dose (mg/kg)	Average Parasitaemia ± SEM	Percentage Chemosuppression (%)
N/saline	10 ml/kg	27.56 ± 1.99	-
OMCLE	500	16.80 ± 2.85*	39.04
	1000	11.84 ± 1.61*	57.04
	1500	15.56 ± 3.62*	43.54
OMCSE	500	18.60 ± 2.82*	32.51
	1000	19.44 ± 2.09	29.46
	1500	15.60 ± 2.46*	43.39
Chloroquine	5	23.96 ± 2.58	13.06
Artesunate	5	11.96 ± 0.69*	56.60

OMCLE- *O. membranacea* crude leaves extract, OMCSE- *O. membranacea* crude stem bark extract, *- statistically significant

Prophylactic Effects of OMCLE and OMCSE on *P. Berghei* induced Malaria in mice

The results for the repository effects of graded doses of OMCLE and OMCSE of *O. membranacea* on *P. Berghei* induced malaria showed that both extracts had

significant ($P < 0.05$) activity though much less than the positive control (pyrimethamine) which caused 94.05% chemosuppression. Among the extracts, OMCLE had the highest activity with a 69.40% chemosuppression. Activity was however not dose dependent (Table 3).

Table 3: Prophylactic Effects of OMCLE and OMCSE on *P. Berghei* induced Malaria in mice

Treatment	Dose (mg/kg)	Average Parasitaemia ± SEM	Percentage Chemosuppression (%)
N/saline	10 ml/kg	31.24 ± 1.67	-
OMCLE	500	16.56 ± 2.91*	46.99
	1000	9.56 ± 0.73*	69.40
	1500	13.20 ± 1.75*	57.75
OMCSE	500	24.32 ± 2.67	22.15
	1000	17.28 ± 1.36*	44.69
	1500	16.76 ± 0.94*	46.35
Pyrimethamine	1.2	1.86 13.20 ± 0.73**	94.05

OMCLE- *O. membranacea* crude leaves extract, OMCSE- *O. membranacea* crude stem bark extract, *- statistically significant

Discussion

The Organisation for Economic Co-operation and Development (OECD) defined acute toxicity as the adverse effect occurring within a short time of administration of a single or multiple doses of a test compound given within the span of 24 hours (OECD, 2008; Ghandare *et al.*, 2013). The aim of these tests is to estimate therapeutic indices which determine the range between a safe effective dose and a harmful or lethal dose. They give information about the dosage that should be used, and how toxicity may occur (Curtis, 2006). The high oral LD₅₀ of OMCLE and OMCSE (greater than 5000 mg/kg) in mice implied that the extracts were practically safe when administered orally. An estimated intra-peritoneal LD₅₀ of 566 mg/kg implied that OMCLE was slightly toxic (range 500-5000 mg/kg), while OMCSE was moderately toxic with LD₅₀ of 289 mg/kg (range 50-500 mg/kg) intra peritoneal.

In the present study the *in-vivo* antiplasmodial effect of *O. membranacea* was evaluated. *In vivo* models are employed in antiplasmodial evaluation of plants as they cause possible prodrug effect and probably boost the immune system to aid eradication of the parasitic agent (Waako *et al.*, 2005; Nardos and Makonnen, 2015); and the most reliable parameter for the assessment of this effect is the determination of percentage inhibition of parasitaemia (Peter and Anatoli, 1998). Standard tests were used to screen the extract for suppressive, curative and prophylactic effects in laboratory animals. The result showed a statistically significant inhibition of parasite growth in all tested models.

Though statistically significant, all tested doses of OMCLE and OMCSE produced low chemo-suppressive effect in early infection in mice. Highest activity was recorded at an administered dose of 1500 mg/kg OMCSE. Likewise in the curative test, very low activities were exhibited by

all tested doses of both extracts. Highest activity was recorded with 1000 mg/kg OMCLE. It has been reported that antimalarial drugs with suppressive and curative effects exert their effect through the formation of heme-drug complex that caps hemozoin molecules thus preventing further biocrystallisation of toxic heme produced in the digestive vacuole of the parasite. This complex causes lysis and eventual auto-digestion of the parasite cell (Geary *et al.*, 1986). Furthermore, inhibition of DNA and RNA biosynthesis and induction of rapid degradation and dissimilation of ribosomes and their RNA respectively has been observed as a secondary effect of chemo-suppressive antimalarials (Saifi *et al.*, 2013). As such, observed chemo-suppression by the extracts may be through any of the above mechanisms.

Furthermore, in the repository studies, a statistically significant prophylactic effect was exhibited by various doses of both OMCLE and OMCSE. Prophylactic effect of OMCSE was dose-dependent. Activity was also low compared to the effect recorded in the positive control. Prophylactic antimalarials act at the pre-erythrocytic stage (tissue level) of the parasite's life cycle (Bruce-Chwatt, 1962). Proposed mechanisms of this prophylaxis include prevention of parasite invasion through modulation of the erythrocytes' membrane (Hansen, 2012) or a direct cytotoxic effect on the parasites thereby causing their death (Golenser, *et al.*, 2006). Observed repository effects of the extracts might thus be through any of these processes.

Ability to prolong survival time of test animals is an important parameter for evaluating the antimalarial property of plant extracts (Peters, 1975). A test compound that has the ability to extend Mean Survival Time beyond 12 days post inoculation is regarded as having good parasite suppressing activity (Peter and Anatoli, 1998). In this study, survival time of the

mice was prolonged for more than 12 days at all tested doses of both extracts in all tested models, further indicating that *O. membranacea* has good potential as an antiplasmodial agent. Response of the host's immune system against foreign pathogens through processes such as activation of phospholipase cascade series, generation of free radicals, free fatty acids and prostaglandins in the presence of the parasite cause disorder in the body (Salawu *et al.*, 2010; Nureye *et al.*, 2021). Plant phytochemicals such as saponins, terpenes, flavonoids, alkaloids, etc have been reported to have a counteractive effect on the above processes (Okokon *et al.*, 2008). Thus, prolongation of survival time by the extracts may be through their selective anti-oxidant activity against cellular oxidative damage caused by high parasitaemia level or an entirely different pathway.

The antiplasmodial activity of test drugs can be classified as moderate, good and very good if they display percentage parasitaemia suppression $\geq 50\%$ at a dose of 500, 250 and 100 mg/kg b.w /day respectively (Munoz *et al.*, 2000). On this basis, none of the tested doses of both OMCLE and OMCSE displayed high inhibition of parasite growth in all models; that is both extracts can only be classified as slightly active. Observed low antiplasmodial effect of *O. membranacea* extracts as against other species of same genus including *O. schweinfurthiana* leaves extract which had 92.2% suppressive effect and 100% curative effect at the lowest dose of 50 mg/kg (Ibrahim *et al.*, 2015) may not be unrelated to the resistant strain of parasite used.

Parasite inhibitory effect of plant extracts might be attributable to the individual or synergistic antiplasmodial activity of group of phytochemicals present in the plant (Matur *et al.*, 2009). The genus *Ochna* has been reported to contain several phytochemicals including fatty acids, triterpenes, anthranoids, flavonoids and saponins (Bandi *et al.*, 2012), which have

been revealed in several researches to possess antimalarial activity (Francois *et al.*, 1999; Rudrapal and Chetia, 2017; Nafiu *et al.*, 2022). Hence, exhibited chemo-suppressive and chemoprophylactic effect may be due to individual or synergistic effect of these phytochemicals.

Conclusion

Based on the results from this study, we were able to establish *O. membranacea* as orally safe and a potential source of phytoconstituents with inhibitory activity against chloroquine-resistant strain of *P. berghei*, though none of the extracts

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exhibited high activity at the tested doses. However, chronic toxicity study to ascertain its safety in prolonged use and evaluation of parasite inhibitory effect at other doses are required. Additionally, further antimalarial studies should be conducted using human-infective *Plasmodium falciparum*. *In-vitro* antimalarial studies and bio-assay guided isolation of the active principles are currently being conducted.

Conflict-of-Interest Disclosure

No conflict of interest is reported.

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